

**The Role of HIV-1 in the Pathogenesis of Cerebral Cortical
Changes in HIV-positive Patients: A study using Morphometry,
Immunohistochemistry and the Polymerase Chain Reaction.**

by

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ABSTRACT

The human immunodeficiency virus (HIV-1) is believed to cause neurological dysfunctions, including dementia. Subcortical lesions were originally thought to underlie these problems; however, the recent application of immunohistochemical and morphometric methods has revealed that cortical changes, consisting of a glial cell reaction and a loss of nerve cells, are also present in AIDS. It has even been suggested that these cortical changes are a more likely correlate of dementia, especially since the association between subcortical lesions and dementia is not entirely convincing. As yet, the existence of HIV-1 within these cortical lesions has not been demonstrated; indeed, viral antigen and nucleic acid are found more often in the white matter than in the cortex.

This study attempts to correlate the presence of HIV-1 in the cerebral cortex, with changes in astrocyte and microglial cell density and morphology. The frontal cortex of AIDS patients with and without immunohistochemical evidence of HIV-1 cerebral infection and HIV-1-seropositive non-AIDS brains were compared with normal controls. However, immunohistochemical localisation of HIV-1 may not be optimal. A more sensitive detection technique, the polymerase chain reaction (PCR), was therefore also employed, to detect HIV-1 proviral DNA from the cortex of AIDS and HIV-1-seropositive non-AIDS cases.

HIV-1 antigens were found in the cortex of a small proportion of AIDS brains, despite being present in the white matter of many cases. In fact, when antigen was

detected in the cortex and white matter of the same section, antigen was invariably more abundant in the white matter. In contrast, HIV-1 proviral DNA can be detected in both cortex and white matter of the majority of AIDS patients using PCR. The amount of proviral DNA seems to be equal in both regions, while there is a clear difference in antigen detection. These findings suggest that although both regions are infected, replication of HIV is more restricted in the cortex, than in the white matter. HIV-1 antigen was not detected in any of the HIV-1-seropositive non-AIDS brains; however proviral DNA was detected in 2 out of 8 cases, confirming that HIV-1 can enter the brain at a relatively early stage of disease.

An astroglial reaction was observed in the anterior frontal cortex of the majority of AIDS patients, but not in most of the HIV-1-seropositive non-AIDS. A more subtle microglial cell reaction was also present in many AIDS cases whilst the density of microglial cells was significantly higher in the HIV-1-seropositive non-AIDS cases than the AIDS. However, it was not determined whether this was due to the different risk factors or the stage of disease.

The presence of HIV-1 proviral DNA in the cortex of AIDS brains was associated with an increased density of GFAP positive astrocytes and the presence of reactive microglial cells. However these changes also existed, although less severely, in cases from which HIV-1 proviral DNA was not detected. These results suggest that HIV-1 contributes to the glial reaction observed in the cerebral cortex, but is not the only factor involved.

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Glossary of Abbreviations

AIDS	acquired immunodeficiency syndrome
ADC	AIDS dementia complex
CMV	cytomegalovirus
CMVE	CMV encephalitis
CNS	central nervous system
CSF	cerebrospinal fluid
DIG	digoxigenin
DPD	diffuse poliodystrophy
GFAP	glial fibrillary acidic protein
HIV	Human immunodeficiency virus
HIV-lep	HIV leucoencephalopathy
HIVC+	HIV antigen or proviral DNA detected in the cortex
HIVC\pm/-	Indeterminate or negative result for PCR detection in the cortex
HIVE/lep	HIV encephalitis and/or HIV leucoencephalopathy
HIVE	HIV encephalitis
HSE	Herpes simplex encephalitis
IFN	interferon
IL	interleukin
JBE	Japanese B encephalitis
MFMT	most frequent morphological cell type (microglia)
MFNS	most frequent nuclear score (astrocyte)
MGC	multinucleated giant cell

MGN	microglial nodule
MHC	major histocompatibility complex
NMDA	N-methyl-D-aspartate
PCR	polymerase chain reaction
PML	progressive multifocal leucoencephalopathy
PBMC	peripheral blood mononuclear cell
QUIN	quinolinic acid
RCA-120	<i>Ricinus communis</i> agglutinin
ssDNA	salmon sperm DNA
SSPE	subacute sclerosing panencephalitis
TFG	transforming growth factor
TNF	tumour necrosis factor
VIP	vasoactive intestinal peptide

HIV-1 genes and proteins

<i>gag</i>	}major structural genes
<i>pol</i>	
<i>env</i>	
LTR	long terminal repeat sequences
gp120	envelope protein
gp41	envelope protein
p24	core protein
p17	core protein
tat	regulatory protein
nef	regulatory protein

**CHAPTER ONE:
REVIEW OF THE LITERATURE**

1.1 Historical overview

The Acquired Immune Deficiency Syndrome (AIDS) was first recognised in 1981, following reports of opportunistic infectious disease and unusual neoplasms in previously healthy homosexual men (CDC, 1981a,b; Gottlieb et al., 1981). During the past eleven years the incidence of AIDS has continued to increase at an alarming rate, both in developed and third world countries. By July of this year 501,272 cases of AIDS had been reported world wide to the WHO, 5,451 from the UK (AIDS information, 1992). At present time there is no evidence to suggest that the incidence of this disease will decline in the near future. It is now almost universally accepted that infection with the human immunodeficiency viruses (HIV-1 and HIV-2) initiates a slow progressive dysfunction of the immune system leading to AIDS in the majority of infected individuals (Matthijs and Miedema, 1990). A severe depletion of CD4 lymphocytes is now known to underlie this immune dysfunction. Thus patients are extremely susceptible to a wide range of opportunistic infectious agents, unusual neoplasms and neurological disease.

Neurological symptoms were recognised early in the AIDS epidemic as important causes of morbidity and mortality (Snider et al., 1983). Around 25-30% of AIDS patients develop neurological abnormalities during the course of their illness, and 10% of cases present with neurological symptoms (Bredesen and Messing, 1983; Levy et al., 1985a). At post-mortem the incidence of neuropathological lesions may be higher, and are identified in up to 90% of patients (Petito et al., 1986). Neurological complications of HIV-1 infection, commonly seen in AIDS patients, include myelopathy, myopathy, peripheral neuropathy, meningitis and a number of

opportunistic infections and lymphoma of the CNS (Harrison, 1992). However the most commonly occurring CNS dysfunction of AIDS patients is a syndrome involving cognitive and motor deficits, originally called "subacute encephalitis" by Snider et al. (1983). Although symptoms such as mental dulling, intellectual impairment and memory loss were attributed at first to depression, delirium or "adjustment disorders" (McArthur, 1987), it was soon recognised that a unique constellation of cognitive and motor deficits was occurring in AIDS patients, and was renamed the AIDS Dementia complex by Navia et al. (1986a&b).

The neuropathology of AIDS probably encompasses the most diverse group of lesions hitherto encountered by the neuropathologist in association with a single disease. A number of opportunistic infections as well as lymphoma and cerebrovascular disease are found, in addition to changes that are now considered to be caused directly by HIV-1 itself, with the presence of more than one type of lesion in the brain being common. This multiplicity of pathological lesions has made it difficult to invoke correlations between neurological symptoms and specific neuropathological lesions. Indeed Snider et al. (1983) originally proposed that cytomegalovirus (CMV) invasion of the brain was responsible for the neurological changes he described as "subacute encephalitis", since viral inclusion bodies, characteristic of CMV, and microglial nodules were found within some of these brains. However, a number of observations suggest that CMV may not be involved in the pathogenesis of "subacute encephalitis". Firstly, a similar disorder was observed in HIV-1 positive children who are less prone to opportunistic infection (Belman et al., 1985). Secondly, the clinical symptoms of AIDS patients with "subacute encephalitis" were distinct from those

produced by pre-AIDS cases of adult CMV encephalitis (Dorfman, 1973). This problem was addressed by Navia et al., (1985) who carried out a clinico-pathological analysis of AIDS patients suffering from dementia. No association between the presence of microglial nodules and dementia was found. As CMV did not seem to be responsible for this disorder, the search for another pathogen in the brain began.

Shortly after the discovery of HIV-1 (Barré-Sinoussi et al., 1983; Gallo et al., 1984), this virus was detected in autopsy brain samples from patients who had died from AIDS (Shaw et al., 1985). It was already known that animal lentiviruses closely related to HIV-1, such as the visna-maedi agent in sheep (Nathanson et al., 1985) and caprine arthritis-encephalitis virus (Narayan et al., 1980) could infect the CNS. The first suggestion that HIV-1 may also be neurotropic, came from the successful transmission of this virus to chimpanzees following inoculation with brain tissues of AIDS patients (Gajdusek et al., 1985). In the same year Shaw et al., (1985) ^{demonstrated} ~~isolated~~ HIV-1 ⁱⁿ ~~from~~ the brains of AIDS patients by Southern blot analysis, and *in-situ* hybridisation. They found that the brain contained higher levels of viral nucleic acids than had previously been found in blood or lymph nodes (Shaw et al., 1984). Shaw et al. (1985) therefore proposed that HIV-1 was neurotropic, and that the brain may act as a reservoir for HIV-1. Further studies closely followed, and confirmed that HIV-1 was present in the brain and CSF of AIDS patients (Ho et al., 1985; Levy et al., 1985b).

Neuropathological examination of the same brains from which Shaw et al. (1985) had detected HIV-1 nucleic acids followed, and revealed the presence of multinucleated

giant cells (MGCs) scattered haphazardly throughout the brain (Sharer et al., 1985). These cells were often associated with microglial nodules, gliosis or neuronal loss. Although MGCs had been described previously in the brains of AIDS patients (Snider et al., 1983; Moskowitz et al., 1984), little importance had been attributed to them. Moskowitz suggested they may be a result of opportunistic measles infection of the brain, as they had previously been observed in this condition. However, Sharer et al. (1985) noted a striking resemblance between these cells and the multinucleated giant cells that form in permissive T cell cultures infected with HIV-1 (Popovic et al., 1984); they therefore suggested that MGCs in the brains may be a marker for HIV-1 infection. In subsequent studies MGCs were found in brains of children dying from AIDS (Sharer et al., 1986), and were also described in European studies (Kleihues et al., 1985; Budka, 1986). Sharer's suggestion that MGCs may harbour HIV-1 was confirmed by the localisation the virus within these cells (Epstein et al., 1984; Wiley et al., 1986; Koenig et al., 1986). It is now well established that MGCs are infected by HIV-1, and these cells are recognised as the hallmark of HIV-1 infection of the brain (Budka, 1986).

Following the demonstration that HIV-1 can infect the CNS, the possibility that HIV-1 underlies the clinical symptoms of the AIDS dementia complex was investigated by Navia et al., (1986a&b), in a clinico-pathological study of a large series of brains from patients who had died of AIDS. Diffuse pallor of the white matter, infiltration by lymphocytes, macrophages and MGCs were all found in patients with dementia. This study confirmed that there was some association between MGCs and dementia. Since then, the AIDS dementia complex has been attributed to direct HIV-1 infection

of the brain, and is believed to result from the predominantly sub-cortical pathology that was frequently associated with MGCs (Michaels et al., 1988; Price et al., 1988). However, the lack of cerebral cortical pathology has led some authors to question whether these pathological changes alone could account for the occurrence of dementia (Sharer, 1992). Furthermore, the correlations between the clinical symptoms and pathological findings have never been overwhelmingly convincing. Indeed in the original study by Navia et al., (1986b) one third of the dementia cases had relatively minor histopathological findings, whilst mild changes occurred in some of the non-demented patients. In addition, MGCs were observed in only 18 out of the 46 patients with dementia, while CMV inclusions were found in 12 of these cases.

The AIDS dementia complex remains clinically over-diagnosed; patients with symptoms of AIDS dementia frequently show none of the expected pathological changes, or indeed any evidence of HIV-1 infection at post-mortem (Rosenblum, 1990; Budka, 1991). This may be due to the fact that clinically, HIV-1 involvement is often a diagnosis of exclusion rather than the result of direct evidence for the presence of HIV-1 in the CNS (Shaunak et al., 1990). In addition, a number of HIV-1 encephalitis cases are undiscovered until post-mortem.

Although early reports suggested that cortical damage is seen only in patients with severe white matter disease (Navia et al., 1986b; de la Monte et al., 1987), it has recently been recognised that cortical changes do frequently occur in the brains of AIDS patients, even in the absence of severe encephalitis (Budka et al., 1987; Ketzler et al., 1990; Ciardi et al., 1990; Everall et al., 1991; Wiley et al., 1991). These

studies offer an alternative explanation for the development of dementia in AIDS; although detailed clinico-pathological correlations have not yet been performed, cortical damage may be a more likely explanation for dementia than the subcortical changes of HIV-1 encephalitis. The aetiology of cortical changes in AIDS has not yet been established. However, *in vitro* and animal studies have demonstrated that HIV-1 is toxic to some neuronal cell lines, and have also demonstrated that it can affect astrocytes and microglial cells (Lipton, 1991, 1992; Brenneman et al., 1988; Giulian et al., 1990; Pulliam et al., 1991).

Nine years on from the initial discovery that HIV-1 infects the central nervous system, the pathological effect of viral infection remains unclear, whilst our understanding of the clinical manifestations of HIV-1 infection of the brain is far from complete.

In this study, the astrocyte and microglial cell changes occurring in the cortex of AIDS patients were investigated by immunohistochemical and morphometric methods, in an attempt to establish whether a specific pattern of cellular changes occurs in the cortex of brains with cerebral HIV-1 infection. Immunohistochemistry and the polymerase chain reaction (PCR) were both employed to detect HIV-1, and the presence of viral antigen and nucleic acid in the cortex was correlated with the astrocyte and microglial cell changes. The rationale and aims of the present study are detailed in section 1.10, following the literature review. Various aspects of the virology of HIV-1 will be considered, followed by a discussion on the means by which HIV-1 gains access to nervous system and the cells that subsequently become

infected within the CNS. This will be followed by a description of the neuropathological changes believed to occur in response to HIV-1 infection in the brain and the proposed clinical counterparts of these lesions. Finally the role of astrocytes and microglial cells is considered in the normal brain, in pathological reactions and in response to HIV-1 infection, before discussing possible mechanisms for the cortical damage shown by HIV-1 infected patients.

1.2 Aspects of HIV-1 virology

The Human Immunodeficiency Viruses, types 1 and 2 (HIV-1 and HIV-2) belong to the lentivirus subfamily of the *Retroviridae* family (Weiss, 1987). The genome of all retroviruses is encoded in RNA and replicated via a DNA intermediate, thus reversing the normal flow of genetic information. They share a common virion structure, genomic organisation and have a similar replication cycle. Moreover, they produce a wide spectrum of disease in their hosts (Shaunak and Weber, 1992). As well as the *Lentivirinae*, two other subfamilies of *Retroviridae* exist: the *Oncovirinae*, which include HTLV I and HTLV II, and the *Spumavirinae* or foamy viruses.

The *Lentivirinae* typically cause disease with a prolonged symptom-free incubation period followed by a protracted symptomatic phase (Fenjö et al, 1989). They are responsible for a variety of neurological and immunological disorders but have not been found to be directly transforming (oncogenic), a characteristic of the *Oncovirinae*. HIV-1 and -2 are the only pathogenic lentiviruses that have been identified in humans. Other viruses in this group include simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV) and the "slow viruses" visna/maedi,

equine infectious anaemia virus and caprine arthritis/encephalitis virus (Shaunak and Weber, 1992).

1.2.1 *Genome*

The HIV-1 genome is 9-10 kb long and encodes three main structural genes, *gag*, *pol*, and *env*, flanked at both the 5 and 3 prime ends by the Long Terminal Repeat sequences (LTRs) (O'Brien et al., 1990a, Shaunak and Weber, 1992; Weber, 1992).

The *gag* gene encodes the internal structural proteins p24 and p18(p17) and two RNA binding proteins, p7 and p8. The *pol* gene encodes the enzymes; reverse transcriptase, an integrase and the *gag* proteases. The *env* gene encodes the membrane associated glycoproteins gp41 and gp120. The LTR is a non-coding region involved in the initiation of transcription and replication, and contains promoter and regulatory gene product, acceptor region sequences which respond to viral and cellular factors. By shifting the reading frame, additional genes are encoded, at least six of these "open reading frames" have been identified, some of which encode regulatory genes. Individually, or through interaction with one another and host factors, these additional genes have been found to specify the conditions of quiescence, steady and moderate growth or explosive replication.

Considerable variation, as much as 25% (Wain-Hobson, 1989), has been found between the genomes of different HIV-1 strains, particularly in the *env* gene. Although the *pol* and *gag* sequences are more conserved, the variation in these regions is greater among HIV-1 strains than in any other sequenced retrovirus. Genomic variation is found not only between isolates from different patients, but also

between isolates from the same individual obtained over a period of time (Hahn et al., 1986; Cheng-Mayer and Levy, 1988). Furthermore, genetic variation is reflected in the biological characteristics of different HIV-1 isolates (Fenyö et al., 1989). Those from individuals with advanced HIV-1 related disease, "rapid/high" viruses, replicate quickly to a high titre, in both T cells and macrophages, and are highly cytopathic *in vitro*. "Slow/low" isolates from HIV-seropositive, non-AIDS individuals replicate in macrophages, but not in T cells and have different cytopathic characteristics.

1.2.2 Overview of the life cycle of HIV-1

1.2.2.1 Infection

CD4 lymphocytes are depleted in HIV-1-infected patients, and following observations of HIV-1 infection of lymphocytes *in vitro* (Klatzmann et al., 1984), it was proposed that these cells were infected via the CD4 receptor (Dalglish et al., 1984). It is now well established that HIV-1 infects cells that express the CD4 molecule; this receptor binds the envelope glycoprotein gp120 with a high affinity (Sattentau, 1988). Cross-linking studies have shown that a physical interaction occurs between gp120 and CD4 (McDougal et al., 1986) and that HIV-1 can be prevented from infecting CD4 expressing cells by various factors that competitively inhibit gp120-CD4 binding. These include: soluble CD4 (Fisher et al., 1988; Hussey et al., 1988; Deen et al., 1988; Trauncker et al., 1988), soluble surface *env* protein (Lasky et al., 1986) and monoclonal antibodies (Dalglish et al., 1984; Klatzmann et al., 1984). Furthermore, some CD4 negative cells become capable of sustaining HIV-1 after transfection with a CD4 gene (Maddon et al., 1986;

Mitrani et al., 1987).

Following attachment of gp120 to the CD4 molecule, events are initiated that probably result in fusion of the viral and host cell membranes (Steffy and Wong-Staal, 1991), and allow the viral core to enter the host cell. Fusion of viral and cell membranes follows, by a process that is as yet not fully understood (Sattentau, 1988).

However, both competitive inhibition studies and site directed mutagenesis aimed at regions outside the CD4 binding domain of gp120, can block viral infection (Ho et al., 1988; Rusche et al., 1988; Wiley et al., 1988). O'Brien (1990b) proposed that regions of gp120 outside this region are important in facilitating HIV-1 entry into mononuclear phagocytes, which can also be infected by HIV-1, but express lower levels of CD4 than T4 lymphocytes. In addition, infection of some CD4 negative cell lines by HIV-1 has been reported (glial, fibroblasts, liver and neuroblastoma cell lines; Cheng-Mayer, 1990). HIV-1 may be able to infect cells by several alternative routes, dependent on the type of host cell (Kowalski et al., 1987). Indeed, HIV-1 infection can occur independently of CD4 in cells that express FC receptors, provided that virions are opsonized by non-neutralising antibodies (Takeda et al., 1988).

Macrophages and microglial cells are the principal type of infected cell within the

brain. Although these cells are believed to express CD4, some studies show the level of CD4 expression on microglial cells to be very low, leading to suggestions that HIV-1 may infect these cells by an alternative route. However, Jordan et al. (1991) have demonstrated that infection by HIV-1 of human brain derived microglial cells by can be blocked by anti-CD4 antibodies or soluble CD4. Nevertheless, different strains of HIV-1 differ in their ability to infect T lymphocytes and mononuclear phagocytes. Meltzer (1990) has shown that whilst monocyte cell lines can become infected and support HIV-1 taken from patients at any stage of infection, infection of T cells becomes more successful with virus isolated from patients in the later stages of disease. These findings suggest that macrophages may be important as host cells early in the course of infection, supporting "slow/low" type variants that have a relatively poor ability to infect T4 lymphocytes.

1.2.2.2 Integration and latency

Once inside the host cell, the viral ~~core~~ is uncoated; transcription of the genome into a double stranded DNA copy, the provirus, and degradation of the original RNA molecule, are catalysed by reverse transcriptase (Goff, 1990; Gilboa et al., 1979; Davies et al., 1991). The provirus is transported to the nucleus where it is inserted into the host cell genome, by a viral integrase, at apparently random sites (Battula and Temin, 1987; Shimotohno and Temin, 1980), and becomes a stable genetic component for the lifetime of the host cell. The process of integration seems to occur independently of host cell mechanisms; reverse transcription and integration of the provirus are carried out by viral enzymes. However, once integrated, viral replication is heavily dependent on host cell mechanisms.

Retroviral DNA is not always integrated into the host cell genome; the visna/maedi provirus remains in the host cell cytoplasm (Haase, 1986). In addition, large quantities of proviral DNA, can accumulate in the cytoplasm of host cells in some avian and feline retrovirus infections. This process, known as "superinfection", is due to repeated infection by subsequent generations of virions. High levels of unintegrated HIV-1 proviral DNA have been found in both lymphoid (Shaw et al., 1984) and brain tissue (Pang et al., 1990). However, it has not been established how the unintegrated DNA in lymph nodes and in the brain is distributed within cells, i.e. whether a few cells are superinfected with high levels of proviral DNA, or if many cells contain a single unintegrated provirus as occurs in visna infection.

The ability of HIV-1 to establish latent infection within immune cells of the host is the hallmark of all the human T-lymphocyte retroviruses (Steffy and Wong-Staal, 1991). Although HIV-1 can only infect activated T4 lymphocytes, which are permissive for viral replication, some of these cells have a transient phase of activation and will revert back to the G⁰ phase of the cell cycle, to form memory cells (Cullen and Greene, 1989). HIV-1 cannot replicate in cells in this phase of the cell cycle, and thus will remain latent until the host cell becomes reactivated. However, although individual cells may remain inactive for prolonged periods, it now seems that even during the symptom free period following infection, there is continued production of HIV-1, which at first is kept under control by the immune system (Tersmette and Miedema, 1990).

1.2.2.3 Transcription

The control of HIV-1 transcription is unusually complex and is influenced by cellular as well as viral regulatory factors. The production of new viral proteins can be increased enormously, kept to a low level, or completely inhibited by the interaction of these regulatory factors (O'Brien et al., 1990a). For example, activation by interleukin of CD4 positive lymphocytes, causes release of the host cell nuclear factor NF- κ B, an enhancer of transcription (Sen and Baltimore, 1986). This protein also recognises initiation sequences present in HIV-1 LTRs and initiates production of HIV-1 transcripts in addition to those of the host cell. Furthermore, HIV-1 has a duplicated NF- κ B acceptor region in the LTR (Cullen and Greene, 1989), and may be especially sensitive to this cellular activation factor, responding at levels not sufficient to activate host cell transcription. HIV-1 replication seems to be dependent upon host cell regulatory factors, in addition to NF- κ B. Hence the host cell factors, Sp1, TFID and NFAT-1 may also be involved in the expression of HIV-1 sequences (Cullen and Greene, 1989).

1.2.2.4 Assembly and release

Following the production of HIV-1 structural proteins, they are transported to the host cell membrane, where new virion particles are assembled. The core proteins and enzymes are packaged together with two full length HIV-1 RNA transcripts, the genome of the new virus, to form the viral core. Completed viral cores enclose themselves in patches of the host cell membrane that have already been embedded with viral glycoproteins, as they bud from the cell.

Meyenhofer et al., (1987) compared the ultrastructural morphology of HIV-1 virions

produced by H9 lymphocyte cultures and those found in the brain of a child with AIDS. While the appearance of viral particles was similar, there were differences in their localisation. In T-cells viral particles were found within intracellular vacuoles or were extracellular, whereas in macrophages, within the infected brain, viral particles were found free in the cytoplasm, not within vacuoles. In addition, intracellular budding from unidentified membranes was seen. These observations suggested that the release of HIV-1 from lymphocytes and macrophages is different. Virion release from macrophages may be suppressed, sequestering virions from the immune system; virions that do not bud through the plasma membrane, but instead bud into the cytoplasm from an intracellular membrane may have different host cell and viral proteins embedded in their membranes. However, HIV-1 particles have been observed budding from the surface of a multinucleated giant cell (Koenig et al., 1986), which demonstrates that HIV-1 can be released by these cells. The importance of different mechanisms of viral release remains to be determined.

1.3 HIV-1 infection of the nervous system

The most likely route of entry, by HIV-1, into the CNS is via the circulatory system. Free viral particles, from the blood, may infect cerebral endothelial cells, which may then allow entry into the brain; indeed HIV has been demonstrated within endothelial cells by *in situ* hybridisation (Wiley et al., 1986). Alternatively, HIV-1 may be carried into the brain by latently infected monocytes or lymphocytes. Entry into the brain via infected monocytes has previously been proposed by Peluso et al. (1985) for another "neurotropic" lentivirus, visna virus. Currently this idea, the "Trojan horse" theory, is the most favoured for HIV-1 infection of the brain, as macrophages are frequently found in infected brains.

found in infected brains. In addition, monocytes readily cross the blood-brain barrier in response to an inflammatory stimulus (Wisniewski and Lossinsky, 1991).

Recruitment of monocytes into the brain in AIDS may be encouraged by the expression of FC receptors on endothelial cells, induced by opportunistic infective agents within the brain (Nelson et al., 1988; M^cKeating et al., 1990). On encountering opportunistic infections, monocytes become transformed into macrophages, thus facilitating HIV-1 replication in infected cells. The expression of FC receptors by endothelial cells may also allow infection of these cells by free viral particles (M^cKeating et al., 1990).

The alternative route of viral entry into the CNS, via nerve axons, as employed by Herpes viruses and rabies virus to infect the CNS (Mims and White, 1984), seems unlikely. There is no firm evidence to suggest that HIV-1 infects either nerve cells, or their peripheral processes.

HIV-1 replication, within the brain parenchyma, may also be encouraged by regulatory elements of other viruses that are present in the CNS. For example, HTLV-I expresses a regulatory protein, rex, which has been shown to functionally mimic the HIV-1 rev protein (Nelson et al., 1990). In addition, a number of viruses found in AIDS patients, including CMV, Epstein-Barr virus, human herpes virus-6 and papovavirus encode transactivator proteins which are capable of increasing expression of the HIV-1 LTR (Nelson et al., 1990). However, for transactivation of one virus by another to occur, it is likely that both would have to infect the same cell. There is only limited evidence to suggest that HIV-1 and CMV can infect a single cell

in vivo (Nelson et al., 1988) and the importance of these events has not been established. Furthermore, HIV-1 infection of, and replication within the CNS, is frequently found in the absence of any other detectable pathogen. In such instances, morphological evidence of blood brain barrier breakdown, such as mural thickening of the microvasculature and presence of haemosiderin-laden macrophages, associated with perivascular and intramural macrophages infected with HIV-1, may be found (Smith et al., 1990). However, whether this is a primary event, or occurs secondary to HIV-1 infection of the CNS, is not known.

HIV-1 may also gain access to the CNS within infected CD4 lymphocytes, as both activated and non-activated lymphocytes can cross an intact blood brain barrier, in response to CNS infection or in the course of immune surveillance of the CNS, (Juhler and Neuwelt, 1989). Lymphocytes have been given less consideration than monocytes as potential vehicles of HIV-1 spread into the CNS as these cells are rare in brains of patients with AIDS. However, in the brains of patients that die prior to developing AIDS, perivascular cuffs of lymphocytes are frequently seen (Gray et al., 1992) and HIV-1 proviral DNA has been detected, by PCR, in frontal, temporal, occipital lobe and basal ganglion tissue samples from one HIV-1-seropositive non-AIDS patient (Sinclair et al., 1992). HIV-1 has also been isolated from the CSF of infected non-AIDS patients (Resnick et al., 1988; Goudsmit et al., 1986; Shaunak et al., 1990; Goswami et al., 1991) and Ho et al. (1985) has isolated the virus from the CSF of a patient with acute aseptic meningitis, which is associated with seroconversion. The stage at which HIV-1 enters the CNS is of importance in the treatment of HIV-1 related CNS dysfunction, if virus infects the CNS during the

asymptomatic stage, early therapeutic intervention may be appropriate.

1.4 Localisation of HIV-1 within the brain

HIV-1 antigens, nucleic acids and retroviral particles have been consistently identified within microglia, macrophages and multinucleated giant cells in the CNS of AIDS patients (Epstein et al., 1984; Shaw et al., 1985; Sharer et al., 1986; Wiley et al., 1986; Stoler et al., 1986; Koenig et al., 1986; Gabuzda et al., 1986; Gartner et al., 1986; Pumarola-Sune et al., 1987; Vazeux et al., 1987; Budka et al., 1987; Meyenhofer et al., 1987; Mirra and del Rio, 1989; Porwit et al., 1989; Budka, 1990; Kure et al., 1990; Eilbott et al., 1989; Weiser et al., 1990). Reports of endothelial cell (Pumarola-Sune et al., 1987; Wiley et al., 1986) astrocyte (Pumarola-Sune et al., 1987; Stoler et al., 1986; Gyorkey et al., 1987; Mirra and del Rio, 1989) oligodendrocyte (Pumarola-Sune, 1987; Stoler et al., 1986; Gyorkey et al., 1987; Esiri et al., 1991) and neuronal (Pumarola-Sune et al., 1987; Stoler et al., 1986) infection by HIV-1 remain controversial. For example the ultrastructural identification of infected cells as astrocytes and oligodendrocytes (Gyorkey et al., 1987) was questioned by Budka (1988), who interpreted these infected cells as being compatible with macrophages. In addition it is impossible to distinguish, at the light microscope level, endothelial cells from pericytes, a population of vascular macrophages. In most studies where HIV-1 was localised to cells other than microglia, macrophages or MGCs, the HIV-1 containing cells were identified on the basis of their morphological appearance alone. When double labelling has been employed to identify HIV-1 infected cells, only cells expressing macrophage markers are labelled by HIV-1

antibodies or nucleic acid probes (Koenig et al., 1986; Budka, 1990; Kure et al., 1990). However, Pumarola-Sune et al. (1987) identified HIV-1 antigen in large cells that contained Nissl substance, some of which were also labelled by neurofilament antibodies. They therefore suggested that HIV-1 can infect neurons, although the double labelled cells were not illustrated in their report. In addition, this group also discovered HIV-1 antigen in cells identified as endothelial cells, on the basis of alkaline phosphatase staining. However, in most reports claiming HIV-1 localisation to neuroectodermal cells, staining of these cells was rare, and occurred in only a few cases, while the majority of positive cells were identified as microglia or macrophages. It therefore seems likely that microglia and macrophages are the major host cells of HIV-1 within the CNS, although other cells may be occasionally infected. The inconsistency of such findings makes it unlikely that the infection of neuroectodermal cells is an important factor in the production of HIV-related pathology.

The quantity of HIV-1 antigen found in infected brain is often low in comparison with other viral infections of the CNS, such as Herpes simplex encephalitis (HSE) and subacute sclerosing panencephalitis (SSPE), where large amounts of viral antigen are readily detectable within infected cells (personal observation). This may be attributable to poor preservation of HIV-1 antigens in fixed tissues, low affinity of available antibodies to HIV-1 proteins, or to the fact that HIV-1 may replicate at a low rate. Another possibility, is that production of new HIV-1 virions may be blocked before the production of the viral proteins that are detected by immunohistochemistry. However *in situ*-hybridisation, which detects HIV-1 messenger RNA and proviral

DNA, has proved to be no more sensitive than immunohistochemistry, suggesting that the low levels of HIV-1 antigen seen in many brains reflects the low level of replicating HIV-1 (Wiley, 1992). However, abundant HIV-1 antigen has been reported in some cases (Budka, 1991), and in frozen material, in which antigens are well preserved (Esiri, 1992). Large amounts of antigen have also been observed in this laboratory, but only in cases with lesions of both HIV-1 and PML, where HIV-1 and JC virus infected cells exist in the same region.

1.5 Neuropathology of HIV-1

As outlined above, HIV-1 has been firmly associated with the predominantly subcortical pathological lesions thought to underlie the dementia and motor deficits that occur in AIDS. However, HIV-1 may also be involved in some other pathological changes for which no cause has been established. In particular, HIV-1 has been proposed as the aetiological agent for: changes in the cerebral cortex, subacute meningitis, vacuolar myelopathy and mineralization of blood vessels. The pathological findings of all these conditions have recently been reviewed (Scaravilli et al, 1992): HIV-1 related subcortical pathology and the changes in the cerebral cortex, which may be related to HIV-1, are discussed below.

HIV-1 encephalitis, HIV-1 encephalopathy and MGC encephalitis are among the many names ascribed to the same pathological appearance, which led to confusion amongst both clinicians and pathologists. However, a recent consensus report by over 50 neuropathologists from Europe and the USA (Budka et al., 1991) has

established a new terminology in an attempt to improve the current situation. Various HIV-1-related lesions and those possibly related to HIV-1 were described, assigned a working name and their clinical counterpart briefly discussed. These terms have been adopted for the discussion below and will be used throughout.

1.5.1 Subcortical pathology (HIV-1 encephalitis and HIV-1 leukoencephalopathy)

1.5.1.1 Pathology

Although originally regarded as variants of a single pathological process, the nomenclature has been revised to take into account the different features present in these two lesions which may reflect differences in pathogenesis (Budka, 1991). Common to both are MGCs, the hallmark of HIV-1 infection of the brain (Budka 1986). The presence of these cells has for some time been considered a necessary feature for the diagnosis of HIV-related pathology. However, it is now considered that a diagnosis of HIV-1 encephalitis (HIVE) or HIV-1 leukoencephalopathy (HIV-lep) can be made in the absence of MGCs, providing that considerable amounts of HIV-1 antigen or nucleic acids are found associated with the other typical pathological features of these conditions (Budka et al., 1991), as described below. The main difference between the two pathological patterns is their distribution, one being focal, the other diffuse. In HIV-1 encephalitis (HIVE), multiple scattered foci of macrophages, MGC, microglia, reactive astrocytes and occasional lymphocytes occur throughout the white matter, deep grey nuclei and more rarely, the cortex. These nodules contain many macrophages and some MGCs but are notable for their lack of lymphocytes. Within these foci, circumscribed myelin loss is revealed by myelin

stains. In severe cases, necrosis may develop within these inflammatory foci and occasionally necrotising lesions containing HIV-1 infected macrophages and MGCs may be widespread. The diffuse pattern, HIV-lep, is characterised by a widespread myelin pallor and increased cellularity of the white matter throughout both the cerebral and cerebellar hemispheres, without usually extending into the white matter of the gyri, corpus callosum, internal capsule, optic pathways or cerebellar peduncles. Myelin loss of variable severity is associated with proliferation of glial cells and/or perivascular infiltration by macrophages, and MGCs (Budka et al., 1987; Gray et al., 1988a&b; Lang et al., 1989). The quantitative involvement of CNS myelinated fibres in HIV-1 infection has been reported in a morphometric study (Kauss et al., 1991). The breakdown products of myelin can be seen within macrophages and numerous HIV-1 infected macrophages and multinucleated cells are found in these regions. Vacuolation of myelin with intra-vacuolar macrophages can occasionally be seen (Budka, 1987, 1991). Mural thickening of small blood vessels is another common feature, and these may be surrounded by foci of haemosiderin-laden macrophages, indicating breakdown of the blood-brain barrier (Smith et al., 1990). A diffuse myelin pallor, and gliotic reaction in the absence of macrophages and multinucleated cells has been described frequently in AIDS brains; although it is tempting to speculate that this appearance represents the early changes of HIV-lep, when MGCs or other evidence of HIV-1 infection of the brain cannot be found, it must be regarded as non-specific.

1.5.1.2 Incidence

The incidence of HIVE and/or HIV-lep (HIVE/lep) is highly variable from one

reported series to another, ranging from 3 to 65% (Levy et al., 1985a; M^cArthur, 1987; Navia et al., 1986a & b; Snider et al., 1983; Petito et al. 1986; Hénin et al., 1987; Budka et al., 1987; Lang et al., 1989; Gray et al., 1988a&b). A number of reasons have been suggested for these discrepancies and include geographical variations (Lang et al., 1989), differences in patient and brain sampling and the lack of strict diagnostic criteria (American Academy of Neurology AIDS Task Force, 1989). In the series of Navia et al., (1986b), in which 65% of cases were reported to have HIV-1 related pathology, all the patients involved had been referred for neurological examination. Another series of brains collected over a three year period suggested that these differences may also be due to an increased incidence of HIV/leprosy (Petito et al., 1986). This changing yearly incidence may reflect the lengthening survival times resulting from improved diagnosis and treatment of some previously lethal pathogens (*Pneumocystis carinii*, *toxoplasmosis*, *CMV*). Indeed, in central African areas, where patients tend to die at an earlier stage of disease (Lucas, personal communication), the incidence of neurological involvement seems to be much lower (3.2%, Bëlec et al., 1989) with the exception of Tanzania, where clinical features of dementia were found in 54% of cases (Howlett et al., 1989). More recently, there has been some evidence to suggest that the incidence of HIV-1 encephalopathy has declined, since the introduction of zidovudine treatment (Portegies et al., 1989). Other factors, such as differences in numbers of females and children included in different series and differences in the major risk groups patients belong to, may be of importance. However, differences in terminology and diagnostic criteria also account for discrepancies between some reports. In some early published series, the presence of MGCs was not considered a necessary feature for a diagnosis

of HIV-1 related pathology. In these reports the number of cases that contain MGCs, rather than the number quoted by the author to represent HIV-1 induced damage, gives a more representative estimate of the incidence of HIVE/lep. For example, de la Monte (1987) claimed that 90% of patients in their series had "subacute encephalitis", and in some reviews (Kliehues et al., 1991) this figure has been quoted as representative of HIVE/lep. However a careful examination of de la Monte's data reveals that MGCs were present in only 30% of cases. Although as mentioned above, it is now recognised that HIVE/lep may occur in the absence of MGCs, the presence of antigen or nucleic acid is still considered a prerequisite for diagnosis. In the absence of techniques to detect these molecules, the presence of MGCs, in association with other appropriate pathological changes, still provides the most satisfactory criterion for a diagnosis of HIVE/lep.

1.5.2 Cortical pathology

1.5.2.1 Pathology

In contrast to subcortical lesions it is comparatively recently that the more subtle changes of the cortical grey matter in AIDS have been evaluated. Use of morphometric methods and immunohistochemical staining techniques has revealed changes in the numbers of nerve cells, astrocytes and microglial cells in this area (Budka et al., 1987; Ketzler et al., 1990; Ciardi et al., 1990; Everall et al., 1991; Wiley et al., 1991), the extent of which could not easily be determined in routinely stained histological sections. De la Monte et al. (1987) were the first to report a gliotic reaction in the cerebral cortex, which was associated with severe HIV-1 related

white matter lesions. Budka et al. (1987) also described a diffuse glial reaction which was frequently associated with gyral atrophy; he called this appearance "diffuse poliodystrophy" (DPD). In a previous semi-quantitative morphometric study carried out in this laboratory (Ciardi et al., 1990), we assessed the striking cortical gliosis found in a number of AIDS brains with HIVE or HIV-lep. In addition immunohistochemical staining with GFAP for astrocytes, and lectin histochemistry with RCA-120 for microglial cells, revealed a degree of cortical abnormality in some cases, that was unexpected on the basis of clinical data and routine histological features. In order to determine whether this reaction was related to HIV-1, three groups of AIDS brains were examined: patients with severe HIVE/lep lesions who had a history of dementia, patients with no history of neurological symptoms and milder HIVE/lep lesions and patients with neither neurological symptoms nor obvious pathological abnormalities. The astrocyte and microglial cell reactions in AIDS brains were compared with cellular changes in age-matched HIV-1-seronegative brains with no abnormalities, and also with a number of brains with other types of pathological lesion. It was found that in patients with HIVE/lep, both the volume and density of GFAP-positive astrocytes were increased in the sub-pia, mid and deep cortical regions as well as in the white matter, including the U-fibres (as compared to HIV-1 negative controls with no pathological abnormalities). Unexpectedly, there was also increased numbers of GFAP-positive astrocytes in the cortex of AIDS patients with otherwise normal brains. In addition, the microglial cell reaction was also increased in AIDS brains, although this reaction was not as notable as the astrogliosis. When the severity of the gliotic reaction in the cortex was related to the degree of HIV-1 associated white matter pathology, mild cortical changes were found

in brains with no obvious pathological abnormalities, moderate cortical changes in the HIVE/lep group with no neurological symptoms, whilst the most severe cortical lesions were seen in the HIVE/lep group of patients with dementia. We suggested that HIV-1 may be the cause of these pathological changes in the cortical grey matter.

This cortical reaction has recently been defined as diffuse poliodystrophy in AIDS by the international consensus report on HIV-1 neuropathological terminology (Budka et al., 1991). Although gliosis of both grey and white matter is commonly found in AIDS brains it was suggested in this consensus report that DPD may be distinct from the HIV-1 related white matter pathology.

Nerve cell loss has also been reported in a number of studies. It was first noted by Navia et al., (1986b) in cases where the white matter was severely affected. More recently, morphometric studies have revealed that neuronal loss may be common in AIDS. Ketzler et al. (1990) showed a 18% reduction in the neuronal density and a decrease of the perikaryal volume fraction by 31% in an unselected group of AIDS patients. Everall et al. (1991) found a 38% reduction in neuronal density in a group of AIDS patients with either HIV-1 related pathological changes, or cases with minimal changes. Wiley et al., (1991) reported cortical thinning and a reduction in nerve cell density in patients with "HIV-1 encephalitis". In addition, the latter found a reduction in synaptophysin levels in the same patients. At present it is not known whether neuronal loss and microglial or astrocyte reactions occur in the same brains. In one study, of a single case with severe cortical atrophy and loss of nerve cells, a moderate astrocyte and microglial cell reaction was also found in the cortex (Gray et al., 1991). Interestingly, HIV-1 antigen was also located in the cortex of this case

by immunohistochemistry.

In addition to DPD neuronal loss, spongiform changes of the type seen in prion-disease have been occasionally reported (Schwenk et al., 1987; Gosztonyi et al., 1988; Artigas et al., 1989); HIV-1 DNA and RNA and MGCs were found in some of these cases; however the rarity of these reports makes their significance uncertain. Diffuse necrotising cortical encephalopathy with HIV-1 antigens and HIV-like particles has also been described in one case (Clague et al., 1988).

1.5.2.2 Incidence

Two initial reports found DPD-like changes in 50% of the cases they examined (de la Monte et al., 1987; Budka et al., 1987). However, the incidence may be higher if cases are examined with immunohistochemistry for astrocytes (Ciardi et al., 1990). Neuronal counting has not been performed routinely on any large series of brains, making its incidence difficult to evaluate. It may, however, be present in the majority of cases as Everall et al. (1991) found no difference in the severity of neuronal loss between cases with and without HIV-1.

1.6 Clinical manifestations of HIV-1 infection and correlations with pathological changes due to HIV-1

Cognitive impairment, personality or behavioural changes and motor dysfunctions are frequent developments in patients infected with HIV-1. These complications may result from opportunistic infections or neoplasia, although in some cases with these

neurological complications, HIV-1 is the only identifiable pathogen in the brain (Navia et al., 1986a&b; M^cArthur, 1987). Therefore it has been assumed that HIV-1 contributes to neurological dysfunction.

The constellation of neurological symptoms, attributed to HIV-1 were defined as the AIDS dementia complex (Navia, 1986a&b) and have been described as a subcortical dementia. The earliest changes, consist of a subtle slowing of verbal and emotional responses. Progression to mental slowing and forgetfulness with loss of libido and personality may occur, in addition to headaches, motor problems and seizures. Deterioration may progress to global dysfunction with dysphasia and dyspraxia. Cognitive changes become more prominent late on in the course of disease. Eventually patients may become severely disabled, and ultimately paraplegia and decorticate posturing may be seen (Harrison, 1992).

The first large clinico-pathological study, investigating the relationship between clinical symptoms of the AIDS dementia complex and brain pathology, was carried out by Navia et al.(1986b), as already mentioned, on a large series of brains from patients who had died of AIDS. When cases with macroscopic lesions or sustained metabolic disorders were excluded, 46 of the remaining 70 cases displayed clinical signs of ADC and the remaining 24 were without any neurological symptoms. While the majority of patients (29) with dementia already had AIDS, in 17 cases neurological symptoms developed during the AIDS related complex, or were the presenting symptoms. The main neuropathological abnormalities in the demented patients were located in the hemispheric white and subcortical grey matter, with

relative sparing of the cortex; obvious neuronal loss was noted only in brains with severe white matter disease. The most common finding in patients with overt dementia was a diffuse pallor of the white matter accompanied by infiltration by lymphocytes, macrophages and, in cases with the most severe white matter lesions, MGCs. This study confirmed for the first time that there was some association between multinucleated giant cells and dementia and the authors suggested that dementia may result from the presence of HIV-1 in the CNS. These findings were confirmed in latter studies (Petito et al., 1986; M^cArthur, 1987).

An ADC staging system was subsequently devised by Price and Brew (1988) to classify patients according to the severity of cognitive, behavioural or motor deficit. Recently, however, the use of this system has attracted some criticism as patients may be classified as having severe ADC, due to motor deficits without any cognitive impairment (Burgess and Riccio, 1992). As a result the American Academy of Neurology AIDS Task Force (1991) has recently proposed an operational system of categorisation. The term AIDS dementia complex is replaced by "HIV-1-associated cognitive/motor complex" which includes patients with a prominence of either cognitive or motor symptoms. The term "HIV-1-associated dementia complex" is used where cognitive impairment predominates and "HIV-1-associated myelopathy" for patients with mainly motor dysfunction. Further subdivisions, to take into account patients with cognitive and motor function but no behavioural changes, and those with cognitive and behavioural changes but no motor dysfunction, have also been outlined. In addition, "HIV-1 minor cognitive/motor disorder" is applied to patients with mild neurological changes.

The advantage of this system is that the descriptive definitions may allow more accurate clinico-pathological correlations to be made. For example, it is as yet unknown whether variations in cognitive and motor symptoms are related to the various types or distributions of HIV-1 induced lesions. One problem with this system of definition is that terms such as HIV-1-associated cognitive motor complex, suggests that HIV-1 has been definitely established as the aetiological agent. However, it has not yet been demonstrated that all cases with these symptoms, have cerebral HIV-1 infection. Indeed, in the original study by Navia et al. (1986b), MGCs were only found in only 18 out of the 46 patients with dementia and one third of the demented cases had relatively minor histopathological findings.

As already discussed, the occurrence of pathological changes in the cerebral cortex of AIDS patients is now well established. As yet, there has been no systematic study of the relationship between these changes and the occurrence of neurological symptoms, though it has been repeatedly suggested that cortical changes may underlie dementia in AIDS. If cortical pathology does manifest itself clinically, then it is important for treatment, that the cause of these changes be established. HIV-1 is the most favoured candidate for this role at present, but an association between this virus and cortical pathology remains to be proved.

1.7 Reaction of astrocytes and mononuclear phagocytes to HIV-1 infection of the brain

Astroglial reaction, microglial proliferation and mononuclear cell infiltration are

common responses to pathological stimuli in the CNS (Duchen, 1992). However, the timing and localisation of the response of these cells is variable, and may provide pathogenic clues. For example, a florid reactive astrogliosis may occur in prolonged cerebral oedema with only a mild microglial cell reaction, while the microglial cell reaction tends to be prominent in infectious conditions (Esiri, 1992).

1.7.1 The role of astrocytes in the CNS

Traditionally, two morphologically distinct types of astrocytes are recognised, protoplasmic and fibrous (Duchen, 1992). Both have round to oval nuclei with fine evenly dispersed chromatin. Protoplasmic cells have an irregular outline and abundant branching process giving a bush-like appearance. Fibrous astrocytes have a smoother outline and long processes with fewer branches than protoplasmic cells. Both cell types are commonly seen around vessels; in addition, protoplasmic cells are often in close proximity to neuronal cell bodies. Traditional methods for demonstrating these cells, capricious metal impregnation and hazardous histochemical methods, have largely been replaced by immunohistochemistry to detect glial acidic fibrillary protein (GFAP). This intermediate filament protein was first isolated by Eng et al. (1971), and is biochemically and immunologically distinct from other intermediate filament proteins. Antibodies to GFAP are considered to be a specific marker for astrocytes, although a number of other cell types may be labelled. In particular, developing and neoplastic oligodendrocytes and ependymal cells, as well as some peripheral nervous and non-neural types of cells (Perentes and Rubinstein, 1987). However, in the mature nervous system, GFAP antibodies are a reliable

marker for astrocytes in non-neoplastic tissues. In the normal brain, fibrous astrocytes are distributed in the subpial region, the white matter, some parts of the basal ganglia including pallidum and around vessels, and are strongly positive for both GFAP and S100. Most cortical and putamen astrocytes have protoplasmic morphology and show sparse or absent immunoreactivity for GFAP in contrast to strong immunoreactivity for S100 (Kimura and Budka, 1986). Following initial descriptions of astrocytes, it was believed that they merely provided a support system for neural cells. It is now, however, clear that they perform many functions in the CNS. Astrocyte processes are often seen surrounding synaptic surfaces of neurons (Peters et al., 1976) where they may have an insulating function and be involved in the mopping up and recycling of the neurotransmitters glutamate and GABA. They are the only cells in the CNS to contain the enzyme glutamine synthetase (Martinez-Hernandez et al., 1977) which suggests they may recycle glutamate taken up from synaptic clefts and return it to neurons. Astrocytes may also have a role in the maintenance of neuronal ionic balance, essential for neurotransmission; neuronal activity results in an increase in extracellular K^+ , and astrocytes, which are permeable for this ion, and actively take it up (Hertz, 1978), may regulate its extracellular concentration. Evidence from some tissue culture studies suggests that astrocytes may also perform immune functions (Fontana et al., 1987; Frei et al., 1987) For example, they have been shown *in vitro* to express HLA-DR and present antigen, as well as release cytokines such as interleukin-6 (IL-6, Vitkovic et al., 1991). Occasional reports of HLA-DR expression *in vivo* exist (Hickey et al., 1985; Frank et al., 1986), however, most studies have failed to find evidence of MHC II expression by astrocytes *in vivo* (Sobel and Ames, 1988; M^cGeer et al., 1988; Sasaki

and Nakazato, 1992; Graeber et al., 1992).

Astrocyte gliosis consisting of hyperplasia and hypertrophy or degeneration may occur in response to injury (Duchen, 1992). Degenerate astrocytes are commonly seen in areas of infarction: the cell nucleus becomes pyknotic, undergoes karyorrhexis and eventually disappears, while the cytoplasm swells, processes disintegrate and lipid droplets may also appear. Collewijn and Schadé (1965) found that following 10 minutes of experimentally induced cardiac arrest, astrocyte bodies shrink to about 1/4 of their original size. Nuclear degenerative changes have also been observed, following experimentally induced hypoglycaemia (Anderson et al., 1967). A second type of degenerative astrocyte, Alzheimer type II cells, may be seen in metabolic encephalopathies such as Wilson's disease (Sobel et al., 1981; Kimura and Budka, 1986). These cells occur in the cortex and basal ganglia, have an indented vesicular nucleus, and are unstained with conventional stains and GFAP. However in experimentally induced metabolic encephalopathy two types of Alzheimer type II cells were identified, showing either well developed or shrunken nuclei (Ma et al., 1991). The authors interpreted the well developed nuclei as an attempt to cope with the metabolic insult, whilst the latter were characteristic of a dying cell.

Hypertrophy and hyperplasia of astrocytes is a common reaction to injury and is often identifiable by an increased number of GFAP-positive cells. However, due to the rarity with which mitotic figures are seen in astrocyte nuclei in human post mortem material, it cannot be certain whether the increased number of GFAP-positive cells represents an actual increase in cell number, or an increase in the number of cells

expressing GFAP. Mitotic figures can be seen in human biopsy material (Duchen, 1992) and have also been observed in some animal studies (Bignami and Railston, 1969). Hypertrophy of astrocytes can be observed in both acute and chronic lesions. In acute lesions such as oedema, reactive or gemistocytic astrocytes can be seen (Duchen, 1992). These cells become enlarged and appear rounded, their cytoplasm is homogeneous and stains strongly with eosin and with GFAP; granules and vacuoles are sometimes present. The cell nucleus is enlarged and is usually eccentrically placed to one side of the cell. In older resolved lesions astrocytes form a scar tissue, filling gaps left by previous damage; these cells have less cytoplasm than reactive astrocytes and extensive glial fibres.

1.7.2 The role of microglia and macrophages in the CNS

Cells in the brain of the mononuclear phagocyte series derive from two sources: endogenous microglial cells and circulating blood monocytes (Jordan and Thomas, 1988). The former are a resident CNS cell population, the latter enter the CNS either in response to injury, or in the course of immune surveillance. Microglial cells were originally described by del Rio Hortega (cited by Perry and Gordon, 1988) who visualised them with a silver carbonate impregnation method. He distinguished three types of morphologically distinct microglia, "amoeboid", "ramified" and "reactive". His proposal, that these cells were derived from mesodermal elements that entered the brain from the blood early in development, was accepted for some time. More recently, there has been some controversy over whether these cells originate from the mesoderm or neuroectoderm, and whether they in fact exist as a separate class of

cell; these issues have been discussed in recent reviews on microglial cells (Jordan and Thomas, 1988; Perry and Gordon, 1988; Esiri, 1992). Most evidence suggests that microglia are a distinct group of cells and it seems likely that they are derived from monocytes that enter the brain before birth (Hickey and Kimura, 1988; Choi, 1981).

The "amoeboid" cells of Rio de Hortege are involved in histogenesis and angiogenesis within the CNS, and may be attracted into the brain by chemotactic signals released by dying cells which amoeboid cells phagocytose (Perry and Gordon, 1988). Amoeboid cells have the morphological appearance of small macrophages and are believed to develop into ramified microglial cells, present in the adult human brain (Perry and Gordon, 1988).

Ramified microglia form a network throughout the cortical and subcortical grey and white matter of the CNS (Esiri, 1992; Graeber and Streit, 1990). These cells have small dense nuclei and very fine processes with short spine-like extensions that branch at right angles and extend in all directions. Although ramified microglial cells have also been called "resting" microglia it now seems that they are far from quiescent; they may be involved in the metabolism of some neurotransmitters (Murabe and Sano, 1982; Perry and Gordon, 1988) and in synaptic remodelling and repair (Esiri, 1992). Although they lack some of the enzymes present in other tissue macrophages, the main function of microglial cells is probably the initiation of an immune response within the CNS, and therefore, they may be analogous to fixed tissue macrophages such as Kupfer cells of the liver and Langerhans cells of the skin (Esiri, 1992).

Microglial have been shown to express low levels of MHC II in normal human brain (Graeber et al., 1992; Hayes et al., 1987) which is greatly increased in a number of pathological conditions (Sasaki and Nakazato, 1992; M^CGeer et al., 1988). These cells may therefore present antigen to T helper cells, stimulating an immune response, which may be further enhanced by the secretion of interleukin-6 (IL-6) by microglia (Dickson et al., 1991).

Two other populations of mononuclear cell are resident in the normal adult CNS; perivascular macrophages and pericytes. The former occupy perivascular spaces, have more abundant cytoplasm and are less ramified than parenchymal microglia (Hickey and Kimura, 1988). These cells are more similar to monocytes, in terms of the surface antigens they express, than to brain macrophages (Peudenier et al., 1991) and in the normal human brain express much higher levels of MHC II, than microglia (Graeber et al., 1992; Sasaki and Nakazato, 1992) Pericytes are a separate population of mononuclear phagocytes enclosed within the basal lamina of vessels.

Almost any pathological disturbance in the CNS induces a response from microglial cells, which seems to consist of increases in both the number of cells and cell volume (Duchen, 1992; Dolman, 1985). Both the size and length, as well as the number of microglial cell processes are increased (Esiri, 1992). These cells, known as reactive microglia, vary in their morphological appearance from elongated rod shaped cells with long oval nuclei and uni- or bipolar cytoplasmic processes, to small rounded cells with few or no cytoplasmic processes. From their morphological appearance the rod shaped cells seem likely to derive from microglial cells. The small macrophage

like cells often have a few processes suggesting that they are also derived from ramified microglial cells. This is, however, difficult to prove, since it is not possible to differentiate between microglial cells and macrophages derived from circulating monocytes that have entered the CNS in response to injury. Resident microglial cells may differentiate into macrophage-like cells in response to injury, while monocytes entering from the blood may develop processes similar to microglial cells (Esiri, 1992). Indeed, various intermediate morphological stages between ramified cells and macrophages can be seen in many pathological reactions. Unlike ramified microglial cells, reactive microglial cells possess lysozyme, acid phosphatase and non specific esterase activity (Esiri, 1992), and have complement Type 3 and Fc receptors (Perry and Gordon, 1988; Graeber and Streit, 1990), as do other tissue macrophages.

Microglial cells can be demonstrated by enzyme histochemistry or metal impregnation, and are identifiable by electron microscopy, although more recently immunohistochemical markers have superseded these older methods (Streit et al., 1988). Although many tissue macrophage markers fail to demonstrate microglia and macrophages within the CNS well, or at all (Esiri, 1992), antibodies (HAM 56, LN1) and lectins (RCA-120) are now available that visualise these cells in both normal and diseased CNS tissue.

The microglia/macrophage response to injury varies in different pathological conditions. In response to tumours and infarcts, macrophage like cells are seen which may phagocytose cellular debris, while aggregations of more elongated cells form microglial nodules around many infective agents. In some infective conditions the

microglia reaction tends to be more prominent than the astrocytic, particularly in the early stages. (Esiri, 1992) In inflammatory conditions such as viral and immune-mediated encephalitides, reactive microglia are a prominent feature. The most striking reactions of microglial cells in human pathology are seen in subacute and chronic infective conditions such as subacute sclerosing pan encephalitis and general paralysis of the insane due to syphilis (Dolman, 1985).

1.7.3 Astrocyte and Microglial cell reaction to HIV-1

Reactions of astrocytes and microglial cells to HIV-1 can be studied in cases where this virus is the only detectable pathogen within the CNS. Extensive astrogliosis occurs throughout the cortex and white matter of cases with HIV-1 and HIV-1ep, but in addition, a diffuse increase in the number of GFAP-positive cells can be seen at early stages of disease before the appearance of pathogens in the brain (Ciardi et al., 1990). The increased numbers of GFAP-positive cells and the increase in cell size may be a specific response to HIV-1 or a generalised response to injury, since astrogliosis is also seen in AIDS brains with opportunistic infective agents and other lesions. In the white matter, these changes are often associated with pathological reactions attributable to HIV-1 or HIV-1 antigens. It has been suggested that the glial reaction in the cortex may be an independent process, as it often appears in the absence of evidence of HIV-1 infection (Budka, 1991). This, however, remains to be established.

Microglial cell reaction is most noticeable in AIDS cases with opportunistic infection

or HIVe/lep. A slight microglial cell reaction can also be seen in cases that have no specific neuropathological abnormalities (Ciardi et al., 1990) and in HIV-1-seropositive cases dying from their haemophilia before developing AIDS (Esiri, 1992). In these instances, an uneven but diffuse, mild reaction is present, microglial cell numbers are increased as are the thickness and number of processes.

In cases where HIV-1 can be identified, the number of activated microglia may be increased and microglial nodules may be present. These nodules are similar to those produced by CMV infection of the brain, though a number of differences can be seen. CMV induced foci are small and dense, contain lymphocytes in addition to microglial cells, and frequently contain a central astrocyte in which a cytomegalic inclusion may be present. They are scattered throughout the cortex, white and subcortical grey matter. In contrast, foci of HIV-1 encephalitis are seen much more frequently in subcortical regions; they contain mainly loosely arranged microglia, multinucleated giant cells, very few lymphocytes and can be quite extensive.

HIV-1 antigens are often visualised within microglial cells and it has been suggested that infection of these cells is dependent on the CD4 receptor (Jordan et al., 1991). However, CD4 antigen is not expressed on microglial cells isolated from normal adult human brain (Peudenier et al., 1991; Williams et al., 1992), although this antigen is expressed by cells isolated from human embryonic CNS tissues and may be expressed by reactive microglial cells (Perry and Gordon, 1988).

Expression of CD4 ^{which has only be found on reactive cells,} may be necessary before these cells can be infected by HIV-1. Alternatively, HIV-1 may infect microglial cells by a different

mechanism; Peudenier et al. (1991), found that CD4 negative microglial cells could not support either lymphocytic or monocytic adapted HIV-1 strains. In contrast, Watkins et al. (1990) found that two macrophage-adapted strains of HIV-1 (AD87 and Bal) could productively infect microglial cells in mixed glial cultures from adult temporal lobe tissue, whilst two lymphocytic adapted strains (LAV and HTLV.IIIB) could not. Although the expression of CD4 on microglia, was not investigated in the latter study, Jordan et al. (1991) have demonstrated that HIV-1 infection of microglia can be blocked by CD4 antibodies and soluble gp120.

The most characteristic morphological feature of HIV-1 infection of the brain, as mentioned above, is the presence of MGCs. Various morphological forms can be seen: 'macrophage-like' MGCs, have eosinophilic cytoplasm, often more densely stained at the centre and vacuolated at the periphery with numerous, up to about 20, round or elongated basophilic nuclei which may be arranged in a horseshoe pattern; whilst smaller 'microglia-like' MGCs have less cytoplasm and elongated nuclei often bunched together in a grape-like fashion. In SIV infected monkeys, similar cells have been observed in the liver and gastro-intestinal tract, lymph nodes, spleen and brain, although they have only rarely been observed outside the CNS in humans (Gray et al., 1990). Immunohistochemical staining of these cells has confirmed that they belong to the monocyte/macrophage group of cells, but it is still not clear if they originate from macrophages, microglial cells or both. MGCs are seen in other brain infections such as immunosuppressive measles encephalitis (Esiri et al., 1982). The microglial hyperplasia and infiltration of the brain by monocytes increases the mononuclear phagocyte population in the brain during AIDS. The initial stimulation

for their increase remains obscure, but early infection of the brain with HIV-1 may be responsible. The expansion of this cell population within the CNS has important implications for the progression of disease. This expansion is in contrast to the drastic reduction in T4 lymphocytes produced by HIV-1.

1.8 Immune response within the CNS, in HIV-1 infection

The frequent occurrence of opportunistic infective agents in the CNS of AIDS patients suggests that the immune reaction within the CNS is deficient, and the response to HIV-1 infection of the CNS may also be lacking. Expression of major histocompatibility complex (MHC) molecules within the brain parenchyma may be necessary to recruit immune cells from the peripheral circulation into the brain (Achim 1991a&b) and there is evidence that these molecules are expressed in the CNS, during AIDS. Levels of β -2-microglobulin (major histocompatibility class I fragment) in CSF, have been found to correlate with the stage of dementia (Brew et al., 1992). A study by Achim et al (Achim et al., 1991a) found increased expression of MHC I on all endothelial cells and some microglia, macrophages and MGCs in patients with HIV-1 in areas of myelin pallor while no expression was detected on astrocytes. In addition, Kennedy and Gairns (1992) found increased expression of both MHC I and II on monocytes and macrophages in some cases of HIV-1. These findings suggest that the cellular reaction within the CNS is appropriate for initiation of a specific immune response (Achim et al., 1991b). In addition, complement activation, which has been proposed as a marker for macrophage activation within the brain, has been reported in AIDS (Schüller, 1990) and in some HIV-1-seropositive

asymptomatic patients, levels of intrathecal antibody to HIV-1 seem to be raised in the CSF (Zimatore et al., 1989). However, there is a notable absence of lymphocytic cells within the CNS of AIDS patients, except for occasional T cells ⁱⁿ microglial nodules formed in response to opportunistic infection (Achim 1991a). In contrast, in the brains of HIV-1-seropositive patients who die without developing AIDS, meningitis and perivascular cuffing are seen (Gray et al., 1992), which are typical responses to viral infection of the CNS (Esiri and Kennedy, 1992). This suggests that the progressive peripheral depletion of lymphocytes accounts for the absence of an inflammatory reaction to the presence of HIV-1 in the brain tissue of patients with AIDS.

1.9 Possible mechanisms of damage to the cortex

Although a definite association has been established between the morphological features of HIV-1 and HIV-1ep and the presence of viral molecules (Budka, 1991), it is far from clear how these specific features arise, although a number of mechanisms have been suggested. Even more speculative are the numerous theories now being put forward, following *in vitro* experiments, as to how HIV-1 may induce neuronal damage, since it has not been firmly established that HIV-1 is a consistent presence in the cortex of these patients. Neuropathological features that have been proposed as correlates of HIV-1 infection include myelin damage, neuronal loss and astrogliosis; however the evidence for infection of astrocytes, oligodendrocytes and neurons is tentative (as described in section 1.4). Other viral infections of the nervous system can cause damage in two ways. Direct damage to cells can result

from cytolytic infection or from the toxicity of viral products, whilst indirect toxicity resulting from the immune response of the host can destroy either infected cells or surrounding tissues, arising from the "bystander effect" of inflammatory agents (Dal canto, 1989). Progressive multifocal leukoencephalopathy (PML) is an example of direct viral cytotoxicity; oligodendrocytes are infected by JC virus and productive infection of these cells leads to cytolysis with consequent disruption of myelin. Astrocytes are also infected, although not permissively and show morphological transformation. An example of damage caused by the bystander effect is provided by Theiler's murine encephalomyelitis virus, which causes a chronic demyelinating disease, not due to a primary viral attack on myelinating cells. Large amounts of viral antigen are found within macrophages, and demyelination is thought to be a secondary effect dependent upon the host immune response (Dal canto & Rabinowitz, 1982).

1.9.1 HIV-1 cytotoxicity

In vitro studies have provided some evidence to suggest that HIV-1 or components of the virus (gag and tat proteins, proviral DNA) may be directly toxic to nerve cells, or may exert a toxic effect on nerve cells, mediated by astrocytes or microglia (Lipton 1991, 1992; Brenneman et al., 1988; Sabatier et al, 1991; Giulian et al., 1990; Pulliam et al.,1991).

1.9.1.2 Direct infection of nerve cells

Although HIV-1 has not been consistently detected in nerve cells of brain tissues *in*

in vitro, the possibility remains that the virus may be present below the limits of current *in situ* detection techniques. Mizrachi et al. (1991) demonstrated that HIV-1 can enter neuronal cells via the viral envelope glycoprotein gp120 in a manner similar to infection of T4 cells, despite the fact that nerve cells lack the CD4 receptor. The authors suggest that fusion of viral and neural cell membranes may disrupt cell function by interfering with conductivity. However, no evidence for cell death was presented in this study, and it remains to be determined whether HIV-1 can infect neurons *in vivo*.

Pang et al. (1990) found high levels of unintegrated proviral DNA in the brains of patients with HIV-1 encephalopathy. This type of DNA has been associated with cytotoxicity in other retroviral infections (Hoover et al., 1987). Weller et al. (1980) proposed the following mechanism to explain the cytopathic effect of avian leukosis virus. Following infection of a cell, progeny virions are rapidly produced and released. These new virions infect surrounding cells, but also reinfect the original host cell and previously infected cells. This so-called "superinfection" leads to the accumulation of large amounts of unintegrated proviral DNA. Accumulation of these molecules ^{or the products they encode} may be toxic to the host cell. However, although Pang et al. (1990) found high levels of unintegrated proviral DNA, little or no HIV-1 antigen was detected in some cases. The superinfection mechanism proposed by Weller et al. (1980) requires the production of new HIV-1 virions, therefore HIV-1 proteins should be detectable in high quantities if superinfection is occurring as proposed. Pang et al. (1990) proposed that unintegrated HIV-1 proviral DNA may be able to replicate itself without an RNA intermediate, and that DNA may

accumulate in this way without new HIV-1 virions being produced. There is to date, however, no evidence for the replication of HIV-1 proviral DNA in the absence of intermediate HIV-1 virions. In addition, it was not established in Pang's study which cell type was infected. If nerve cells do contain large amounts of proviral DNA, it is likely that they would be easily detectable by *in situ* hybridisation; however, where nucleic acid containing cells have been identified, HIV-1 nucleic acids have only been found in monocyte/macrophage type cells.

1.9.1.3 HIV-1 proteins

Most interest has focused on the HIV-1 envelope glycoprotein gp120, which is reported to be neurotoxic to fetal rodent hippocampal neurons (Brenneman et al., 1988) and postnatal retinal ganglion cells (Lipton 1991, 1992). In rat pups, intraventricular injections result in dystrophic hippocampal pyramidal cells and behavioral deficits (Pert et al., 1989). In addition, application of gp120 to human fetal brain aggregates has been reported to produce neuronal vacuolation and loss of astrocyte cytoplasmic fibrils (Pulliam et al., 1991). One problem with these studies is that they employ fetal or immature nervous tissue which cannot be expected to represent an accurate model of the adult human brain. Nevertheless, some interesting possible mechanisms by which gp120 may exert a neurotoxic effect have been proposed, and these are discussed below.

Gp120-mediated nerve cell damage is preceded by a rise in intracellular calcium, which is thought to represent a final common pathway for a diverse group of neurological abnormalities that involve nerve cell death, including some infectious

agents, ischemia hypoglycaemia and some dementias (Beal, 1992). This kind of neuronal cell death was first described by Olney (1986), and is believed to be the direct consequence of excessive excitatory depolarisation via the N-methyl-D-aspartate (NMDA) receptor, activation of which allows an influx of calcium into the cell. The NMDA receptor is voltage dependent, and will not fire unless the normal resting potential across the cell membrane is reduced. A reduction in the membrane resting potential or, an increase in the amount of glutamate may increase the likelihood of the NMDA receptor allowing calcium entry into the cell (Beal, 1992). The maintenance of the normal resting potential across the nerve cell membrane and the removal of glutamate from the synaptic cleft, are both energy dependent processes and any insult that is likely to cause a depletion of energy may increase the likelihood of excitotoxic damage (Beal, 1992).

The neurotoxic effect of gp120 can be blocked by antagonists of the NMDA receptor, suggesting that gp120 may act as a NMDA agonist. However, in patch clamp studies, gp120 does not appear to increase NMDA-elicited electrical responses in retinal ganglion cells, suggesting this hypothesis is too simplistic (Lipton, 1992). In addition to NMDA antagonists, toxicity can also be blocked by antagonists to voltage dependent L-type calcium channels (Dreyer et al., 1990). Neurotoxicity therefore seems to require the activation of more than one calcium channel, since antagonists to either L-type voltage dependent calcium channels, or to the NMDA receptor protect against gp120 induced toxicity. In addition, the degradation of endogenous glutamate also prevents neurotoxicity, although addition of excessive amounts of glutamate and the addition of antagonists to non-NMDA receptors have no effect

(Lipton, 1991,1992). Lipton has therefore suggested that both gp120 and glutamate are necessary for neuronal damage, and may act as synergists. Gp120 may directly or indirectly sensitise nerve cells to the lethal effects of glutamate. There are several pathways through which this type of sensitisation may occur. Gp120 may bind to another receptor on nerve cells, thus possibly reducing the resting potential of the cell and increasing its vulnerability to neurotoxic damage. Alternatively gp120 may bind to a receptor on astrocytes or microglial cells interfering with the neurotrophic functions of these cells.

Excitotoxic damage in AIDS patients may also occur as a result of raised levels of quinolinic acid (QUIN). This weak NMDA agonist has been found in raised levels in the CSF. Furthermore, the levels of QUIN correlate with the progression of dementia (Heyes et al., 1989, 1991). Therefore in AIDS the effects of increased levels of the excitotoxin QUIN may be enhanced by the action of gp120 in patients.

Gp120 neurotoxicity can also be blocked by the neuropeptide vasoactive intestinal peptide (VIP), and peptide T (an analogue of VIP). Both of these peptides share some sequence homology with gp120 and competitively inhibit the neurotoxic effect of gp120 in hippocampal cell cultures (Brenneman et al., 1988). It is presumed that VIP prevents the binding of gp120 to VIP receptors which are found on astrocytes, as well as on some nerve cells. VIP has been shown to increase calcium oscillations in astrocytes and may influence the release of neurotrophic factors such as protease nexin 1 (Festoff et al., 1990). Hence gp120 may be the indirect cause of nerve cell damage by competing for VIP receptors on astrocytes and preventing release of

neurotrophic factors essential for neuronal survival.

Evidence for astrocyte damage, by HIV-1, has been provided by Pulliam (1992) by the application of recombinant gp120 to astrocyte cultures. These cells showed a loss of GFAP immunoreactivity and a dose dependent loss of a 66KDa protein. Puillam concluded that gp120 directly damages astrocytes which may then indirectly affect neurons.

A toxic role has also been proposed for one of the HIV-1 regulatory proteins, *tat*. Studies by Sabatier et al. (1991) have shown that this regulatory protein specifically binds to rat synaptic membranes, and modifies the cell permeability of some invertebrate nerve cells. Furthermore, culture of murine neuroblastoma and rat glioma cells in the presence of 0.7 to 70 μg of synthetic peptide, from the *tat* sequence, resulted in cell damage, inhibition of cell proliferation, and cell death. Intracerebroventricular injection of various synthetic *tat* peptides into mice produced neurological effects similar to symptoms induced by some snake neurotoxins and had an LD50 of 18 to 180 μg depending on the peptide sequence. This work by Sabatier et al (1991) remains to be confirmed, and demonstration of cytotoxicity to human cell lines would provide firmer evidence. Like gp120 neurotoxicity, *tat* toxicity appears to be mediated through a glutamate dependent calcium channel. In addition, it has recently been shown that *nef* shares sequence similarities with scorpion peptides and increases the K^+ in chick dorsal root ganglion cells (Werner et al., 1991).

1.9.1.3 Indirect HIV-1 toxicity

Macrophages and microglial cells, the main reservoir of HIV-1 in the CNS, produce a range of cytokines and other potentially toxic substances (Dickson et al., 1991),

which in excessive quantities may interfere with various nerve cell functions. Several studies have shown that HIV-1-infected macrophages/microglia can produce soluble factors toxic to neural cells (Giulian et al., 1990) and brain explants (Pulliam et al., 1991). As with gp120 neurotoxicity, the neurotoxic effect in Giulian's study was averted by NMDA antagonists. Although, to date, the molecules involved have not been identified, filtration studies have shown that gp120 is too large to be this toxin, although a small part of the molecule is a possibility (Giulian et al, 1990). However, the NMDA receptor is involved in many neurotoxic interactions, and macrophages are known to secrete a wide range of toxic factors. The possibility that macrophages may release QUIN in response to gp120 or another HIV-1 protein is particularly interesting (Lipton, 1992), though in Giulian's study QUIN was not detected in the macrophage supernatant. In addition, biochemical analysis showed that the macrophage toxin is not one of the known NMDA toxins or free radicals. More recently, Giulian (1992) found that the toxic factors released from HIV-1 infected macrophages were not released from HIV-1 infected lymphocytes, which suggests that a macrophage toxin, rather than a product of HIV-1, may be responsible.

1.9.2 Alternative mechanisms of cortical damage

Other macrophage products, that may cause damage in the CNS are the cytokines. Tumour necrosis factor (TNF) damages both myelin and oligodendrocytes, producing a non-delayed-onset oligodendrocyte necrosis and non-reversible myelin damage (Selmaj & Raine, 1988). This cytokine has been found to be raised in the serum of AIDS patients, and the levels detected correlate with the stage of disease (Lahdevirta

et al., 1988). Other cytokines that may be involved are interleukin-1 which stimulates astrocyte growth, and transforming growth factor- β (TGF- β) which is expressed abnormally in the brains of HIV-1 positive patients (Whal et al., 1991). TGF- β expression by HIV-1 infected macrophages induces non-infected cultured astrocytes to release TGF- β . It has been suggested that release of TGF- β in the CNS may initiate a positive feedback cycle; recruitment of HIV-1 infected cells into the CNS, in response to this factor may induce further release of TGF- β from astrocytes, facilitating viral spread and encouraging the release of other neurotoxic cytokines from astrocytes (Matsuyama et al., 1991).

Chronic macrophage stimulation may also interfere with the biochemical pathway responsible for the production of S-adenosylmethionine (SAM) the major methyl group donor, necessary in the CNS for a number of functions, but in particular, the maintenance of myelin (Surtees et al., 1990). Reduction in SAM can be caused by vitamin B¹² deficiency which produces subacute combined degeneration of the cord, the pathological appearance of which is very similar to vacuolar myelopathy seen in adults with AIDS and the myelopathy that is seen in HIV-1 positive children. Although HIV-1 positive patients have not been found to have significantly reduced vitamin B¹² levels (Keating et al., 1991), levels of other components involved in methyl metabolism are reduced in HIV-1 seropositive children (Smith et al., 1987; Surtees et al., 1990) and adults (Keating et al., 1991). It has been suggested that SAM may be involved in the detoxification of some macrophage products leading to its depletion, or may be diverted into an inappropriate cycle, as occurs following chronic alcohol consumption (Keating et al., 1991).

1.10 Rationale and aims of the present study

The lack of correlation between clinical symptoms of the AIDS dementia complex and the presence of HIV-1-associated white matter pathology has led to the proposal of alternative explanations for the occurrence of dementia in AIDS. Recent studies have shown that the cerebral cortex as well as subcortical regions can be abnormal in AIDS; changes include loss of nerve cells and of synaptic complexes, and increased numbers of astrocytes and microglial cells (Budka et al., 1987; Ketzler et al., 1990; Ciardi et al., 1990; Everall et al., 1991; Wiley et al., 1991). In addition, a number of *in vitro* and animal studies have suggested that HIV-1 is neurotoxic (Lipton, 1991, 1992). Together, these findings have led to proposals that dementia in AIDS may result from HIV-1 related damage of the cerebral cortex. There is to date, however, little pathological evidence to support the suggestion that HIV-1 is related to the cortical damage now recognised to occur in AIDS. This study is therefore an attempt to correlate the presence of HIV-1 in the cortex with the astrocytic and microglial cell reactions previously described in this region.

The Department of Neuropathology at the Institute of Neurology has been collecting autopsy brain material from patients dying of AIDS since 1987; the majority of cases are from the London homosexual population who have suffered greatly from this epidemic. This material was provided by Dr Harcourt-Webster of Westminster Hospital (formerly of St Stephen's) and Dr Sebastian Lucas of University College Hospital.

The study of AIDS autopsy brains has contributed a great deal to our understanding

of neurological disease in AIDS, to the biology of HIV-1 and pathogenesis of AIDS. Identification of various opportunistic agents has led to improvements in diagnosis of neurological deficits due to cytomegalovirus, toxoplasmosis, lymphoma and PML. Studies aimed at localising HIV-1 in the brain have helped to formulate theories about how HIV-1 enters the central nervous system, the cell types it infects and how it induces damage in the CNS. The examination of autopsy AIDS brains has been instrumental in establishing the now widely accepted view, that HIV-1 acts as a pathogen in the central nervous system (Budka, 1991).

This study is an attempt to apply morphological and molecular biology techniques to brain tissues of AIDS patients in order to investigate the relationship between, the presence of HIV-1 antigens and nucleic acids, and the astrocytic and microglial cell changes that occur in the frontal cerebral cortex. The frontal lobe was chosen for study as frozen material from this region was available from a relatively large group of post-mortems. Initial studies related immunohistochemical staining of HIV-1 with responses of microglial, macrophages and astrocytes within the brain (Ciardi et al., 1990). It was demonstrated that these cells were increased in number in the cerebral cortex and basal ganglia of AIDS patients with HIV-1 encephalitis. Morphometric study revealed increases not only in brains with HIV-1 encephalitis but in other cases where no morphological or immunohistochemical evidence of HIV-1 infection of the brain could be found. The pattern of staining was compared to that found in other neuropathological lesions, but the reaction of glial cells in AIDS seemed distinct from reactions observed in hypoxia, multiple sclerosis, PML and Japanese C cell encephalitis. In the cortex of AIDS brains a diffuse astrocyte reaction was observed

throughout the cerebral cortex, which appeared distinct from the cellular arrangements in the non-AIDS lesions.

Since changes in astrocyte and microglial cells are non-specific, the morphology, distribution and density of these cells, in different groups of AIDS brains was examined, in the first part of this study (chapter 2), to determine whether a particular pattern of cellular reaction might be associated with cerebral HIV-1 infection. The presence of other co-existing cerebral disease commonly seen in AIDS cases, such as opportunistic pathogens, lymphoma or cerebrovascular disease, also produce a microglial and astrocyte reaction. Therefore, the AIDS cases selected for study had either HIV-related changes only or, in cases without HIV-related changes, a single identifiable pathology, as described below. AIDS brains with immunohistochemical evidence of HIV-1 were compared with brains from AIDS patients with no apparent neuropathological abnormality and no immunohistochemical evidence of HIV-1, in order to determine which cellular changes occurred more frequently or whether they were more severe in cases with cerebral HIV-1 infection. HIV-1-seronegative cases lacking any identifiable neuropathological changes (normals) provided a base line control. The specificity of cellular changes to HIV-1 cerebral infection was examined by comparing changes in these cases with cellular changes in AIDS brains with neuropathology not associated with HIV-1, in which no HIV-1 antigens were detected (CMVE and cerebrovascular lesions).

In addition, brains of HIV-1-seropositive cases who had died without developing AIDS were examined to determine whether glial changes can occur early in the

course of disease. Brains in this group were compared with both HIV-negative and AIDS cases.

Due to the comparative rarity of HIV-1 antigen in the cortex of AIDS brains, a more sensitive detection method, the polymerase chain reaction (PCR, Saki et al., 1985) was used, in the second part of the study (chapter 3), to detect HIV-1. PCR amplifies specific nucleic acid sequences using two oligonucleotide primers that hybridise to opposite strands, flanking the target region within the template DNA sequence (the region of DNA being tested) (Erlich, 1989). Repeated cycles of heating and cooling are applied to denature the template DNA and allow annealing of the oligonucleotide primers. New DNA strands are produced by a thermal stable form of DNA polymerase which extends the annealed primers on each strand. The primer extension products of each cycle can serve as templates for the next, thus at the end of each cycle the amount of target DNA should in principle be doubled. During the first heat cycle the annealed primers will be extended far beyond the target region; in subsequent cycles the termini of the extended fragments are defined by the 5' ends of 'short templates' produced in the previous cycle.

Due to powerful amplification of target DNA sequences, PCR can be used to detect small quantities of virus below the resolution of either immunohistochemistry or *in-situ* hybridisation. In addition, since HIV-1 may remain latent within its host cell, PCR can detect HIV-1 sequences that are not being replicated. Following observations of microglial and astrocytic changes in the cerebral cortex and the immunolocalisation of HIV-1 to relatively few cells in the cortex, PCR was used to

amplify HIV-1 proviral DNA from the frontal cortex and white matter of AIDS patients to address two questions: 1) since the number of cases with cortical abnormalities exceeds those in which HIV-1 can be detected in the cortex, with immunohistochemistry, this more sensitive technique was employed to look for an association between the presence of HIV-1 in the cortex and glial changes; 2) to determine whether, HIV-1 infects subcortical regions of the brain more readily than cortical regions, or if there is a difference in the rates of viral replication in these two regions. If the former were true, using PCR, HIV-1 should be detected more frequently in the cortex than in the white matter, as found by immunohistochemical localisation. On the other hand if HIV-1 infects both regions of the brain, proviral DNA should be present in both the cortex and white matter of cases in which HIV-1 was immunolocalised, regardless of whether antigens were found in the white matter only or in both regions.

A non-isotopic PCR method was employed to amplify HIV-1 proviral DNA sequences from DNA extracted from both cortical and white matter samples. Three regions of the HIV-1 genome were detected using primers designed to amplify conserved regions of the *gag*, *pol* and *env* genes (Simmonds et al., 1990).

CHAPTER TWO:

**IMMUNOHISTOCHEMICAL AND MORPHOMETRIC ANALYSIS OF
THE RELATIONSHIP BETWEEN
ASTROGLIOSIS, MICROGLIAL CELL ACTIVATION AND
THE LOCALIZATION OF HIV-1.**

2.1 Preliminary observations on astrocyte and microglial cell changes in the cerebral cortex of AIDS cases and comparison with cellular reaction in other pathological lesions.

In a previous study we found that astrocyte and, to a lesser extent, microglial cell densities were increased in AIDS brains (Ciardi et al., 1990). However, in addition to changes in cell density reported by Ciardi et al., (1990), alterations in the morphological appearance of astrocytes and microglial cells were commonly seen. In the present study, the method designed to examine these changes is based on initial observations of GFAP-positive astrocytes and RCA-120-positive mononuclear cells in the frontal cortex of AIDS cases, as presented below. These observations were also compared with cellular changes in the cortex of cases in a number of well defined pathological lesions in non-AIDS brains, and in AIDS brains with lesions other than those directly attributable to HIV-1.

The observations described below were carried out on paraffin sections stained for GFAP and RCA-120, as described in section 2.2.2. GFAP-positive astrocytes and RCA-120-positive mononuclear cells were examined in the cerebral cortex of the following cases.

2.1.1. Cases selected

2.1.1.1 Normal

Three brains from cases that were not in any of the risk groups for HIV infection,

and that lacked any pathological abnormalities were selected. The age of these cases ranged from 32 to 53, two were male and the other female.

2.1.1.2. AIDS

Fourteen brains from AIDS cases with HIVE, HIV-lep, diffuse gliosis, and no detectable abnormalities were selected. The age of these cases ranged from 30 to 49 and all were male.

2.1.1.3. Pathological lesions

One case of each of the following pathological lesions: subacute sclerosing panencephalitis (SSPE), Japanese B encephalitis (JBE), herpes simplex encephalitis (HSE), progressive multifocal leucoencephalopathy (PML), cryptococcosis, toxoplasmosis and hypoxia were examined. The cryptococcosis, toxoplasmosis and PML lesions were all from AIDS cases.

2.1.2 Observations on GFAP-positive astrocytes and RCA-120-positive mononuclear cells in the anterior frontal cortex.

2.1.2.1 Comparison of AIDS brains with normal controls

2.1.2.1.1 GFAP-positive astrocytes

In the cortex of all normal brains examined a discrete, but distinct perivascular accumulation of astrocytes was present (**fig. 2.1a**). This perivascular distribution was not obvious in many of the AIDS cases, indeed, in some, there appeared to be a loss of GFAP immunoreactivity, especially around vessels (**fig. 2.1b**). In other

AIDS cases, there was a diffuse increase in GFAP expression throughout the parenchyma (fig 2.1c). In the normal brains, perivascular astrocytes had small dense nuclei and abundant, fine, branching, GFAP-positive processes (fig 2.2a). Vessels, but especially arterioles were surrounded by a network of GFAP-positive staining, consisting of numerous astrocyte processes. In AIDS cases where perivascular GFAP immunoreactivity was reduced, this was due to a reduction in immunoreactive processes, rather than to a reduction in the number of GFAP-positive astrocytes (fig. 2.2b). GFAP-positive astrocyte processes could be seen in contact with vessels (fig 2.2c), in cases with an increased density of GFAP-positive astrocytes. The perivascular distribution of astrocytes was therefore examined in this study as described in section 2.24.

In normal brains, occasional positive cells were seen in the parenchyma; these cells had a small dense nucleus, some with abundant branching processes, but most had a small amount of ill-defined cytoplasm. In the AIDS cases the morphological appearance of astrocytes varied considerably. In some cases their nuclei were enlarged with a watery appearance and marginalised chromatin, while GFAP expression was minimal (fig. 2.3a). In other cases reactive astrocytes were seen (fig. 2.3b), or more rarely the nuclear changes were minimal while the cytoplasmic volume was increased and numerous GFAP-positive branching cell processes were present (fig. 2.3c). These variations made reactive cells difficult to define. For the present study, it was therefore considered more appropriate to examine the density of GFAP-positive processes and the nuclear morphology independently, as described in section 2.24.

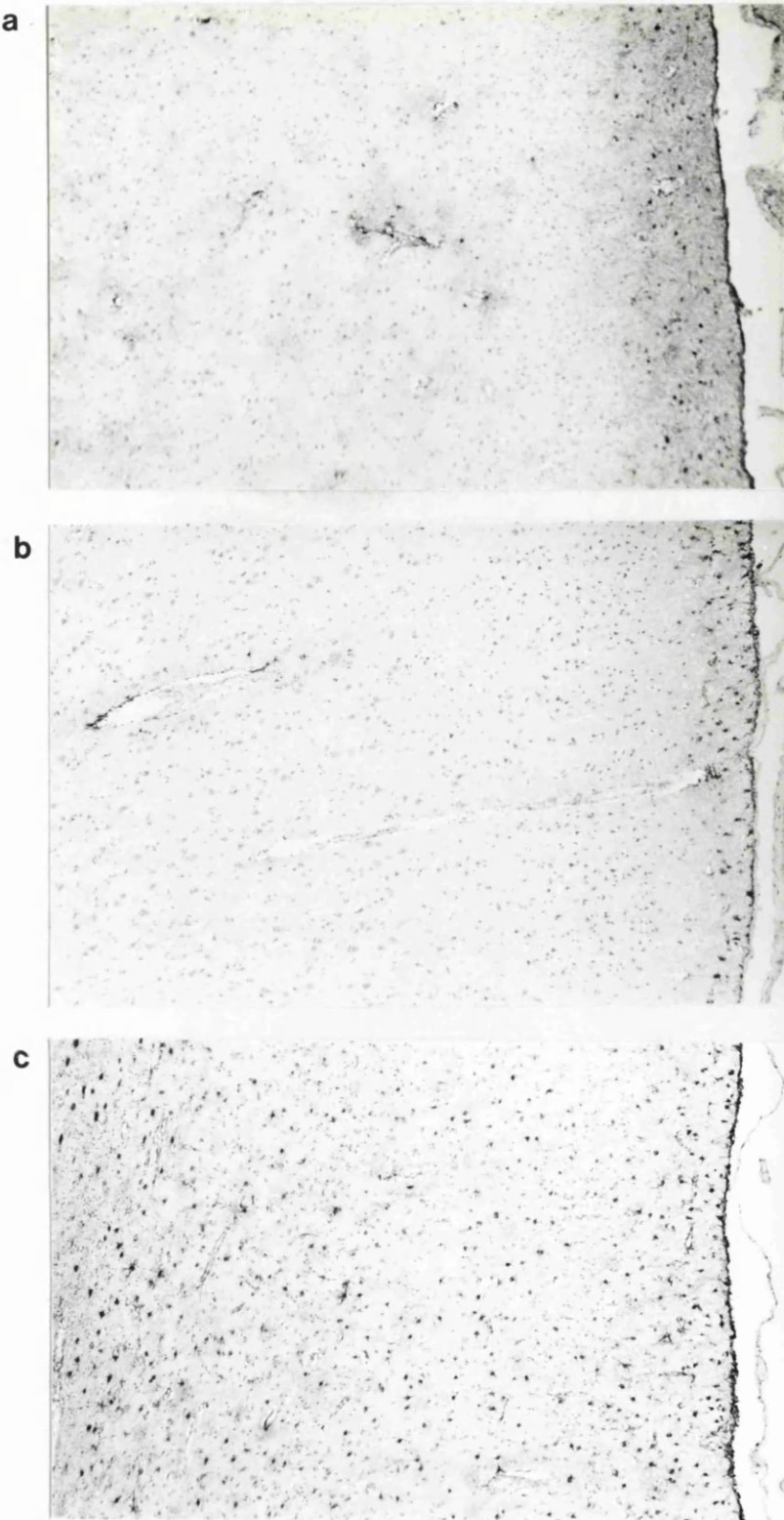


Figure 2.1 Photomicrographs showing the distribution of astrocytes in the anterior frontal cortex of a normal brain (a), an AIDS brain with no obvious neuropathological abnormalities (b) and an AIDS brain with HIV-1ep (c). The astrocyte distribution is predominantly perivascular in (a), whereas in (b) the perivascular staining is reduced, especially around small vessels. In (c) there is an increased density of GFAP positive cells and the perivascular distribution is less evident, in comparison with (a). GFAP and haematoxylin (x 48).

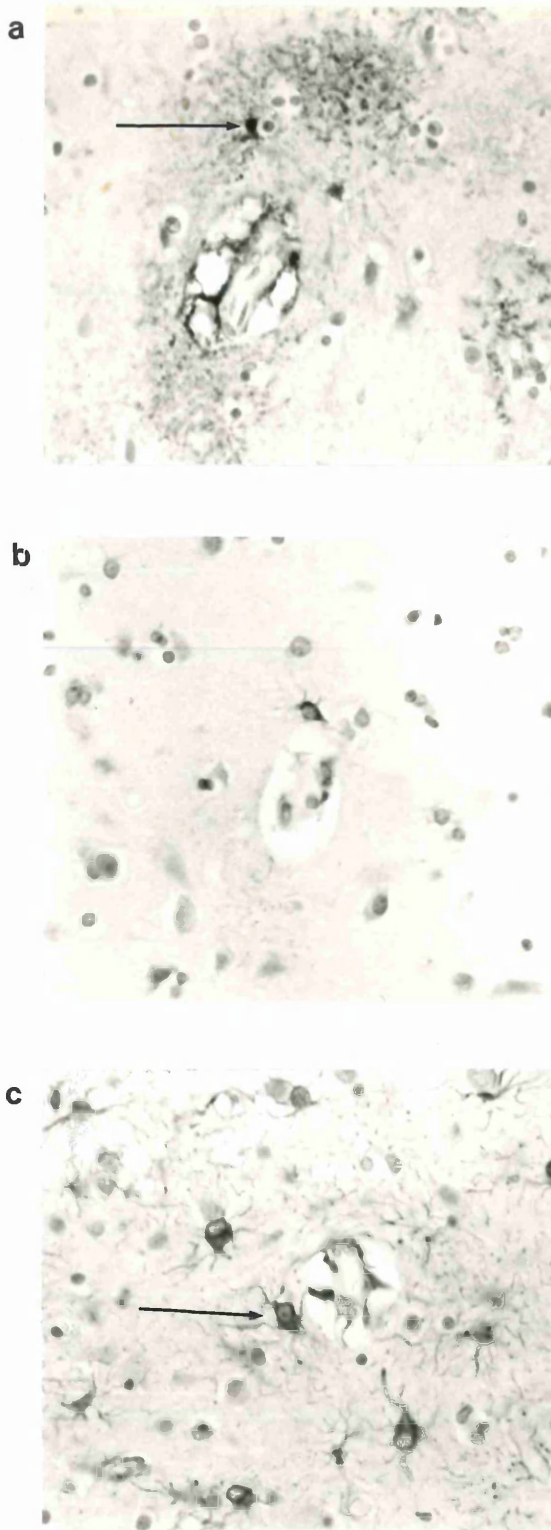


Figure 2.2 Photomicrographs showing small blood vessels from the same cases illustrated in figure 2.1 In (a) perivascular astrocytes have small dense nuclei (arrow) and abundant branching processes. In (b) a GFAP positive astrocyte can be seen adjacent to the vessel, but the amount of GFAP immunoreactivity is reduced as compared with (a). In (c), astrocytes with enlarged nuclei and increased cytoplasmic volume are present; one cell (arrow) appears to be in contact with the vessel. GFAP and haematoxylin (x310).

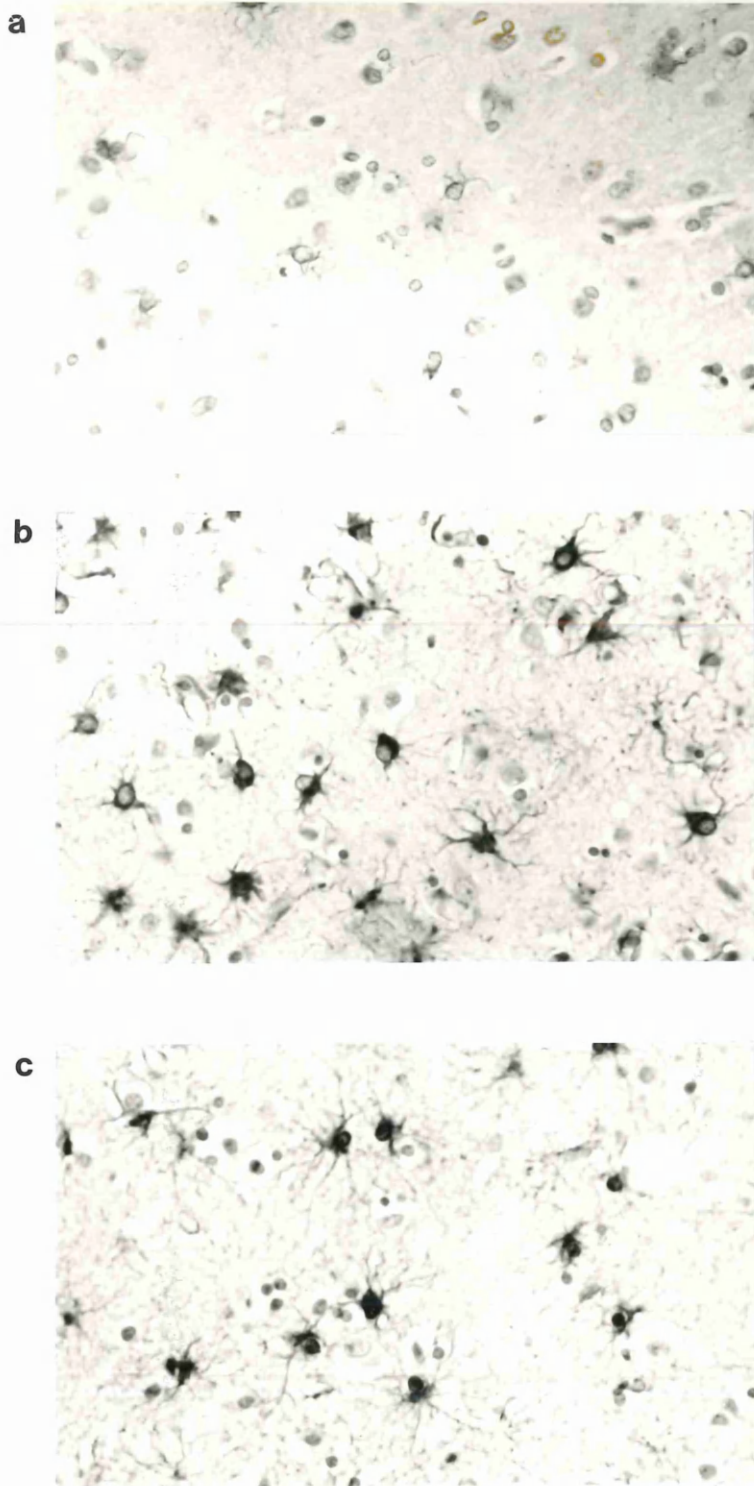


Figure 2.3 Photomicrographs showing the morphological appearances of astrocytes in the anterior frontal cortex of AIDS cases with no obvious neuropathological abnormalities (a) and HIV-lep (b and c). In (a), GFAP positive cells have enlarged nuclei but few cell processes. In (b), numerous reactive astrocytes have both enlarged nuclear and cytoplasmic volumes; the nuclei are pale while the cytoplasm is strongly GFAP positive and cells have a few short thick processes. Similarly in (c), GFAP positive cells have strongly stained GFAP positive cytoplasm, with numerous thick processes whilst the nuclei are smaller with densely stained chromatin. GFAP and haematoxylin (x310).

2.1.2.1.2 RCA-120-positive cells

In normal brains the lectin RCA-120 labelled only microglial cells with a ramified type morphology. These cells had a small dense nucleus and very fine branching processes (**fig 2.4a**). In some instances, processes were so fine that stained cells could only be identified at high power. Microglia are reported to form a continuous, non-overlapping diffuse network throughout the cerebral cortex of normal brains (Esiri, 1992); however in paraffin sections examined in this study, the distribution was not continuous although cells did appear to be spread evenly throughout the cerebral cortex. In AIDS cases, large numbers of microglia and macrophages were frequently observed in the white matter, and their density was emphasised by staining with the lectin RCA-120. In the cortex, the density of these cells was not so remarkable; however they were considerably more obvious than in normal brains, due to an increase in the number, length and thickness of RCA-120-positive processes (**fig 2.4b**). In addition to these enlarged ramified RCA-120-positive cells, other morphological types were seen in AIDS cases. Rod cells with elongated nuclei and long thick uni- or bipolar, non-branching processes were often seen (**fig 2.4c**). Small macrophage like cells with rounded densely staining cytoplasm and occasional small processes were seen in a few cases (**fig 2.4d**), as were small and large multinucleated cells (**fig 2.4c**). To examine changes in microglial morphology, cells were classified according to the morphological features they displayed as described in section 2.24.

2.1.2.1.3 Focal lesions

Nodular aggregates of RCA-120-positive cells were found in some cases and consisted of densely packed microglial cells and macrophages (**fig 2.5a**). GFAP-positive

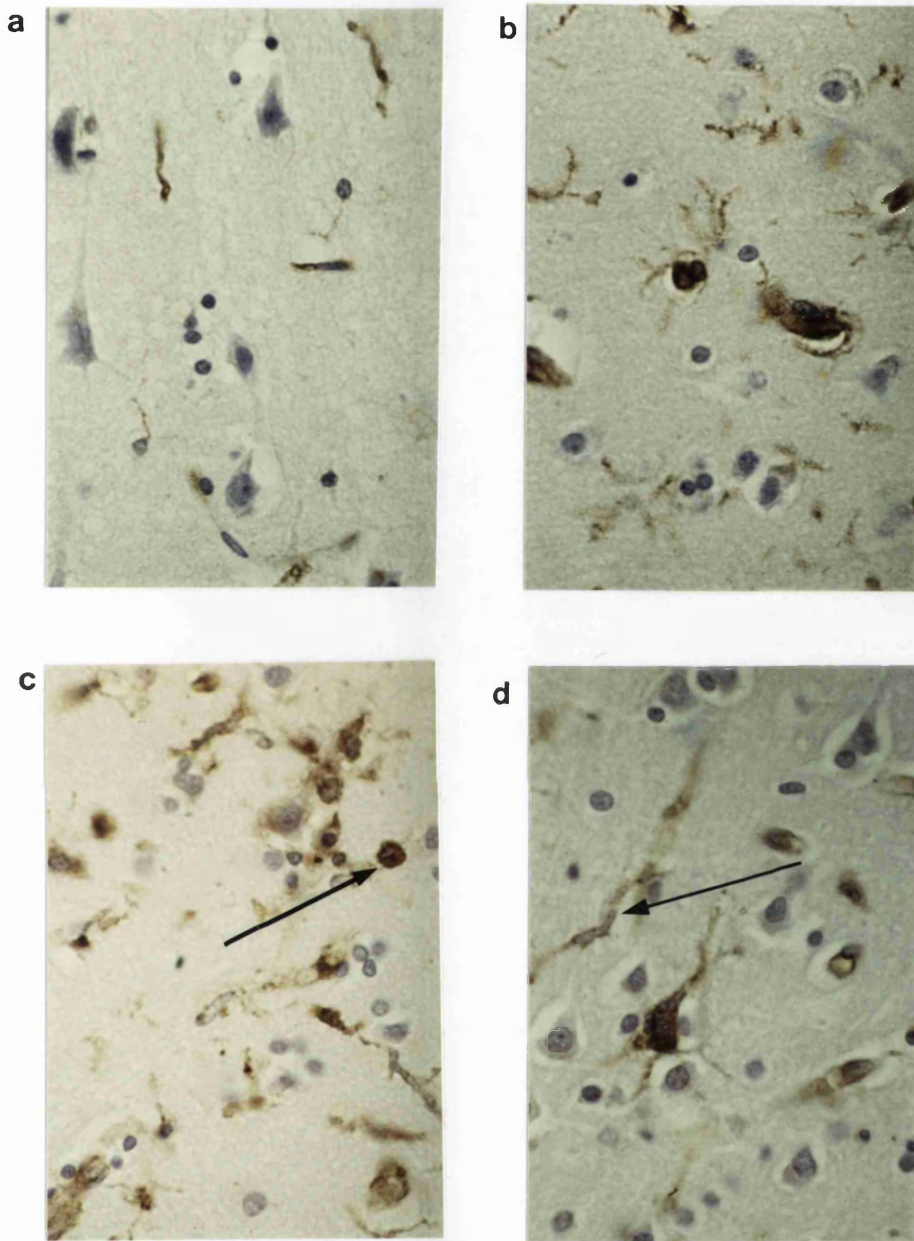


Figure 2.4 Photomicrographs showing the morphological appearances of microglia in the anterior frontal cortex of a normal brain (a) and AIDS brains with HIV or HIV-lep (b,c and d). In (a), microglia have very fine processes; in (b) processes are increased in thickness and number. In (c) small macrophage like cells (arrow) are present; in (d) a multinucleated cell and a rod cell (arrow) have been stained. RCA-120 and haematoxylin (x 400).

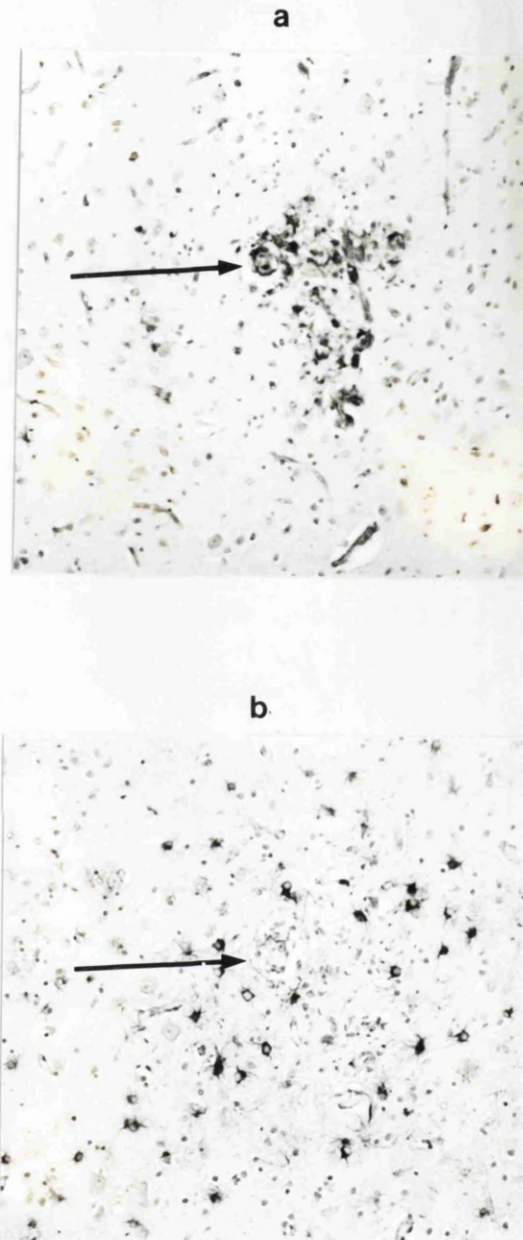


Figure 2.5 Focal lesion with microglial cells, macrophages and astrocytes in the anterior frontal cortex of an AIDS case with HIV. Microglial cells and macrophages surround a small vessel (arrow) (a). Astrocytes are also present around this vessel (arrow) (b). RCA-120 and haematoxylin (a);GFAP and haematoxylin (b), (x 120).

astrocytes were not associated with these nodules to any significant extent but were occasionally seen surrounding them (fig 2.5b). In addition, loose foci of microglial cells and macrophages were also seen, and in some of these foci astrocytes were also seen.

2.1.2.2 Astrocyte and microglial cell reactions in other pathological lesions

2.1.2.2.1 Viral infections

In SSPE, there was a diffuse astrogliosis throughout the cortex (fig 2.6), which was similar to that seen in the most severely affected AIDS cases (fig 2.1c); many of the astrocytes were reactive and were distributed throughout the parenchyma (fig 2.7a). A remarkable mononuclear cell reaction was also seen in the SSPE case, in which enlarged ramified cells, rod cells and macrophages were present in large numbers throughout the cortex (fig 2.7b); this reaction was considerably more intense than that observed in any of the AIDS cases.

In the HSV case few GFAP-positive astrocytes were seen, and a loss of GFAP immunoreactive processes were evident, especially around vessels (fig 2.8). Perivascular accumulations of macrophages were seen surrounding vessels with perivascular cuffs of lymphocytes, in some areas macrophages spread into the surrounding parenchyma.

MGNs were seen in the cortex of the JBE case; these focal accumulations contained enlarged ramified cells and rod cells stained with RCA-120 (fig 2.9a). Reactive



Figure 2.6 Photomicrograph showing astrocytes in the anterior frontal cortex of a SSPE case. There is a diffuse increase in the density of GFAP positive astrocytes throughout the cortex; this appearance is similar to that observed in figure 2.1c. GFAP and haematoxylin (x48).

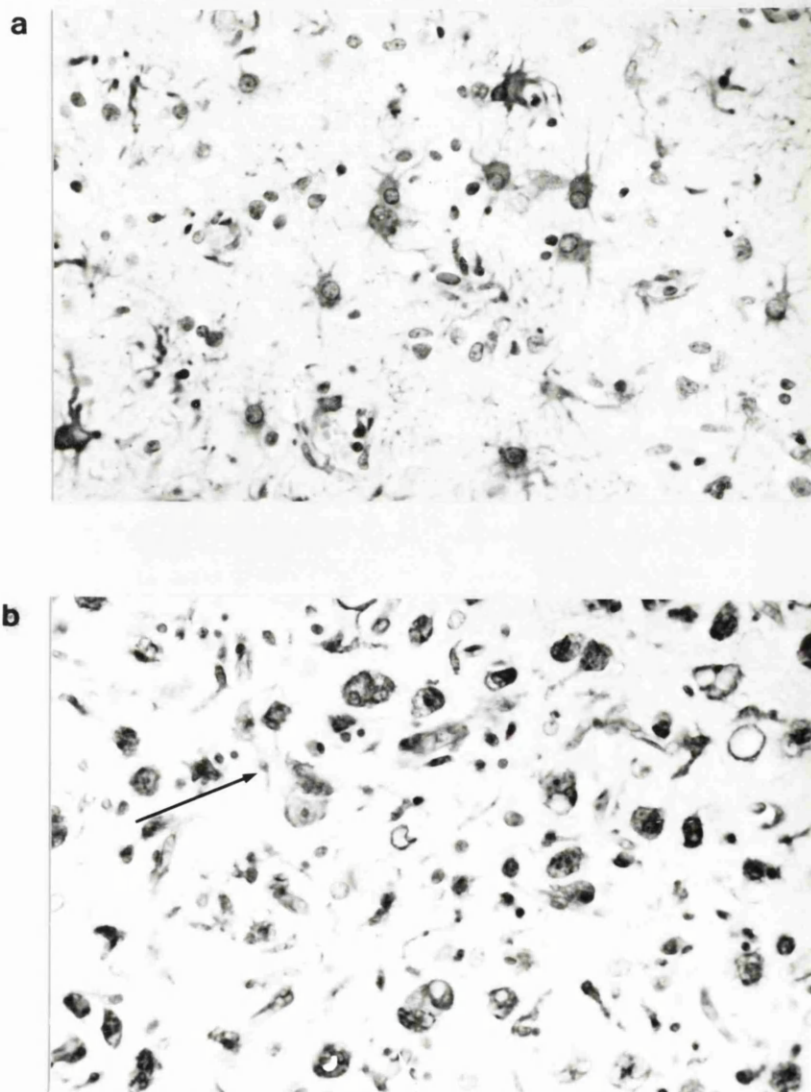


Figure 2.7 Photomicrographs showing the morphological appearances of astrocytes (a) and microglia (b) in the anterior frontal cortex of a SSPE case. A high density of both GFAP positive astrocytes (a) and RCA-120-positive cells (b) is evident. In (a) astrocytes have enlarged cytoplasmic and nuclear volumes; in b, most of the positive cells are macrophage-like but microglial cells are also present (arrow). GFAP and haematoxylin (a); RCA-120 and haematoxylin (b) (x300).

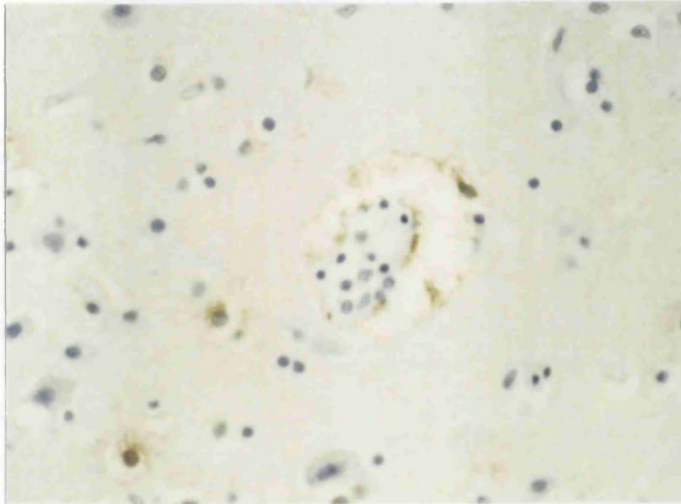


Figure 2.8 Photomicrograph showing a small blood vessel in the temporal cortex of a HSE case. A few astrocytes with a small amount of cytoplasm are present, but none are obviously in contact with the vessel. GFAP and haematoxylin (x400).

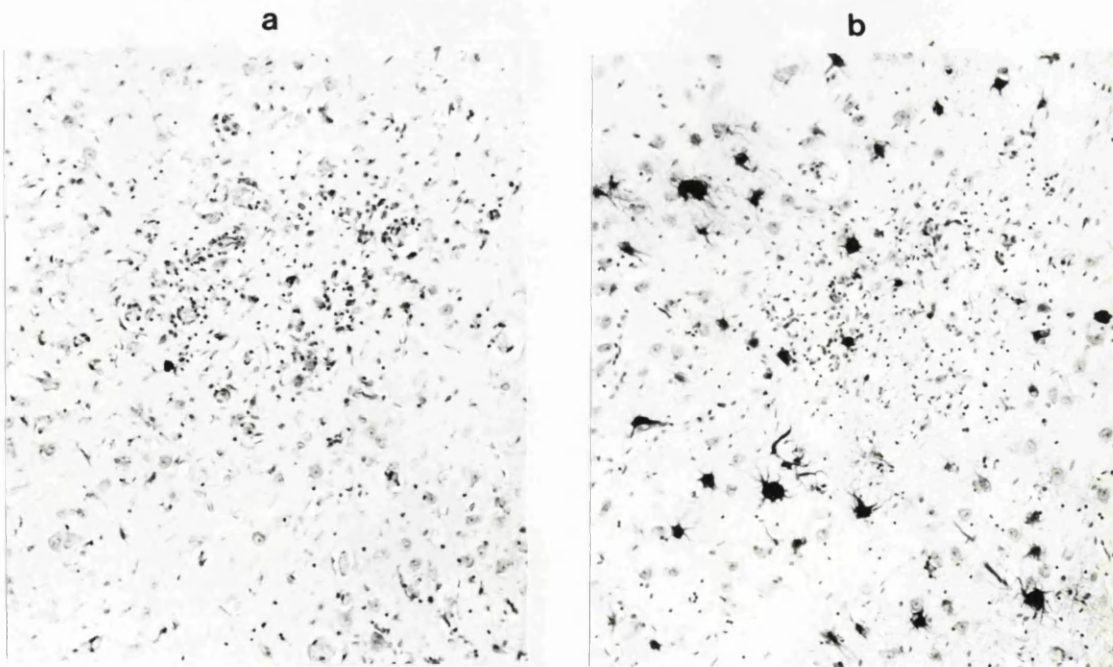


Figure 2.9 Photomicrographs showing a microglial nodule in the temporal cortex of a JBE case. Macrophages and microglial cells are closely aggregated in (a), whilst in (b), astrocytes are present around the nodule. RCA-120 and haematoxylin (a), GFAP and haematoxylin (b)(x 120).

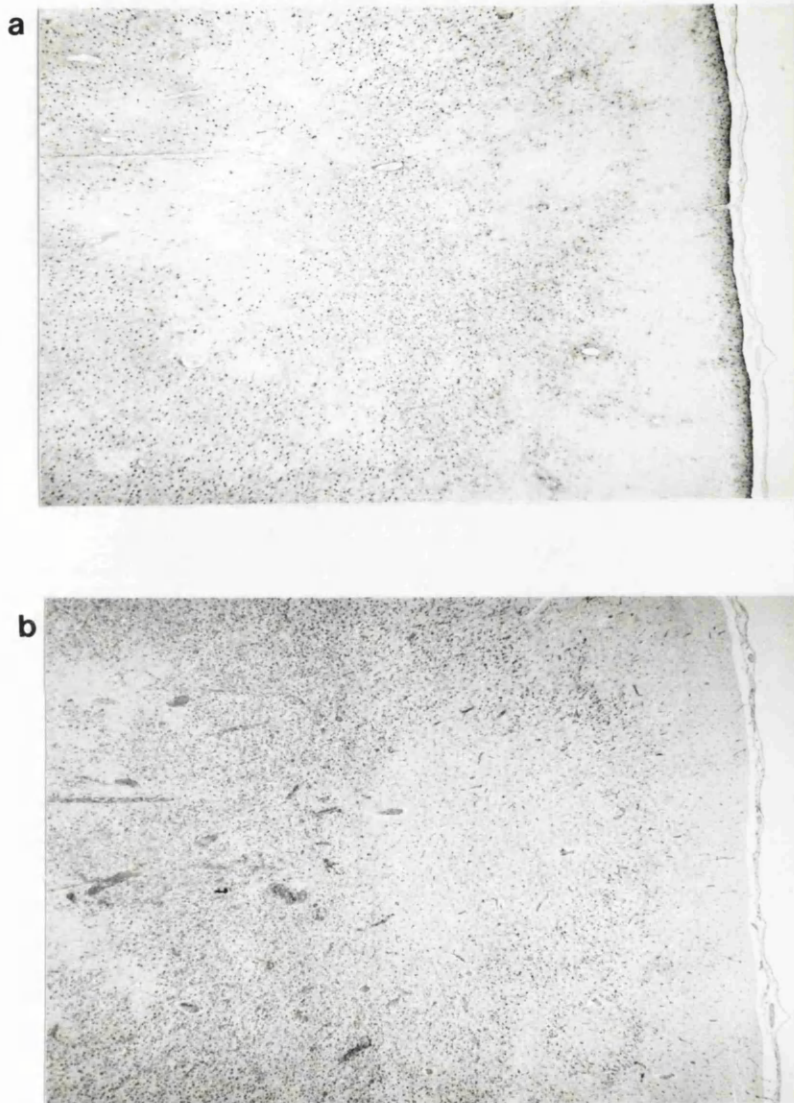


Figure 2.10 Photomicrographs showing astrocytes and microglia in the frontal cortex of a PML case. The density of both astrocytes (a) and microglial cells (b) is increased. The distribution of both types of cell is uneven throughout the cortex, in contrast to the diffuse increase in GFAP positive astrocytes in severely affected AIDS (figure 2.1c) and in SSPE (figure 2.6a). GFAP and haematoxylin (a) and RCA-120 and haematoxylin (b), (x12).

astrocytes and gliofibrillary astrocytes, with long thick processes, were present around the microglial nodules. (fig 2.9b).

In PML, loose foci of reactive astrocytes were seen in the cortex, usually adjacent to white matter lesions. In severely affected areas, sheets of reactive astrocytes were seen throughout the cortex in a patchy distribution (fig 2.10a), GFAP-positive cells being more abundant in the lower layers of the cortex. Numerous reactive microglial cells and macrophages were also present in this region and had a similar distribution to the GFAP-positive astrocytes (fig 2.10b).

2.1.2.2.2 Fungal infection

Lesions in the cortex of an AIDS case infected by *Cryptococcus neoforms* were variable in the appearance of astrocytes and microglial cells. RCA-120 revealed numerous microglial cells and macrophages within areas containing numerous organisms, and microglial cells were also evident around these foci (fig 2.11a). Reactive astrocytes visualised with GFAP, are also present around these lesions, but not within them (fig 2.11b). In older lesions, in which fungi were reduced or absent, few macrophages or microglia were seen (fig 2.11c), although a few rod cells were sometimes seen in these lesions. In addition there were fewer astrocytes, although the density of GFAP immunoreactive processes was increased, and they formed a gliofibrillary network (fig 2.11d).

2.1.2.2.3 Hypoxia

In the hypoxic brain, reactive astrocytes were observed predominantly in the cortex,

in the depth of the sulci (Fig. 2.12) and in the watershed areas. Microglial^{cells} and macrophages, labelled with RCA-120 were seen in the same areas.

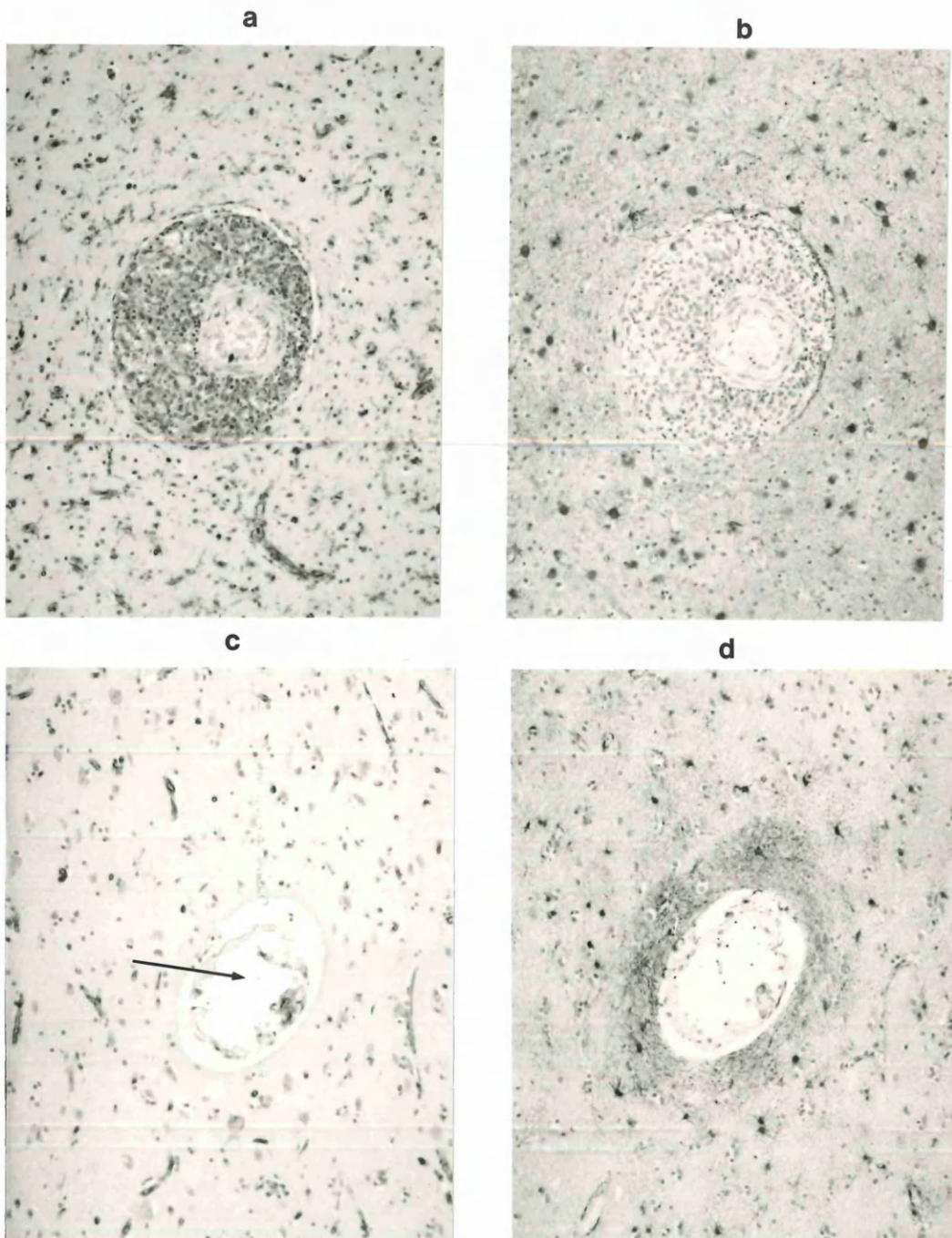


Figure 2.11 Photomicrographs showing lesions in the frontal cortex of an AIDS case, in response to *Cryptococcus neoforms*. Numerous fungi can be seen surrounding a vessel (a and b). In (a), RCA-120-positive cells are intermingled with these fungi, while microglial cells surround the lesion. In addition GFAP positive reactive astrocytes (b) are seen around the lesion. In contrast the RCA-120 reaction is non-existent within a lesion that contains only a few fungi (arrow)(c), while there is a gliofibrillary reaction around this lesion (d). RCA-120 and haematoxylin (a and c); GFAP and haematoxylin (b and d), (x120).



Figure 2.12 Photomicrograph of the frontal cortex of a hypoxia case. A large aggregate of reactive astrocytes is situated at the base of a sulcus. GFAP and haematoxylin (x12).

2.2 Materials and Methods

2.2.1 Selection of Cases

Cases were selected from the Institute of Neurology series of AIDS brains provided from post mortems carried out at the Westminster (formerly at St Stephens) and Middlesex-University College Hospitals; a full neuropathological examination had previously been carried out on all cases by Professor Scaravilli. These brains, were fixed in 10% buffered formalin for periods ranging from 1 to 12 months. In all cases, following macroscopic examination of the brain, 15 blocks were taken from various regions, according to the MRC AIDS protocol, and processed to paraffin.

All AIDS and control cases, selected for morphological examination, were subject to the same processing schedule on the same tissue processor. Routine histological examination of these blocks included staining with haematoxylin and eosin and van Gieson methods in all cases. Luxol fast blue/cresyl violet, Glees and Marsland's silver stain, histological and immunohistochemical methods for opportunistic infections were carried out on selected blocks from each case, to examine for signs of other diseases that might not have been morphologically obvious. Cases with no neuropathological abnormalities, with a neuropathological diagnosis of HIVE, HIV-lep, CMV encephalitis, or with cerebrovascular lesions in which no other pathological reaction was seen were selected for study.

Tissue from HIV-1-seropositive cases that had died prior to the development of

AIDS were obtained from the Edinburgh satellite of the MRC AIDS Brain Bank (Edinburgh Western Infirmary, courtesy of Dr Bell) and from Professor Gray's French AIDS series (Hopital Henri Mondor, Créteil, France). Some of these cases were symptomatic, but none had developed full-blown AIDS.

Non-AIDS cases, that were not from any of the risk groups for HIV-1 infection, with no neuropathological abnormalities were selected as normal controls. These were, of a similar age to the AIDS cases and brains had been fixed for times comparable with those of the AIDS cases. However, normal brains which could be matched for age, sex and fixation time with AIDS cases were not readily available. The three most suitable cases consisted of one case of muscular dystrophy, one unexpected cardiac arrest and one from a patient who died suddenly while recovering from an unconfirmed measles infection. In addition, samples from two HIV-seronegative cases that had died of drug overdose were provided by Dr Bell. On routine neuropathological examination, all five cases appeared normal, apart from very occasional inflammatory cells in the brains of the two drug users (**table 2.1**). These five cases made up a "normal control" group for the comparison of astrocyte and microglial cell changes.

The neuropathological diagnosis and the cause of death for each case are shown in **table 2.1**; cases were divided into three clinical groups: 1) Normal controls, 2) HIV-1-seropositive, non-AIDS and 3) AIDS cases. The AIDS group was further divided into cases with no obvious neuropathological abnormalities (3a); cases with neuropathology related to HIV-1, and positive with p24 antibody (3b); cases with

CMV encephalitis (3c) and cases with cerebrovascular pathology (3d).

The age, sex, cause of death or PM diagnosis and relevant neurological details of all cases selected for this study are shown in **tables 2.2-2.7**.

2.2.2 Immunohistochemistry

The modified avidin-biotin method (Sobel et al., 1984) was used initially with all antibodies, but was further modified for use with HIV-1 antibodies (Scaravilli et al., 1992), as described below. For detection of the lectin RCA-120, an avidin-biotin method was also used. A five-layer avidin-biotin method, recommended by Esiri et al. (1991), to increase the sensitivity of HIV-1 p24 antigen detection, was also assessed; however we found no difference between the sensitivity of this method and the three-stage avidin-biotin method. The antibodies and the lectin used in this study are listed in **table 2.8**, together with optimal dilutions and other technical details. For GFAP and RCA-120 detection, the optimal section thickness included a large proportion of the cell bodies and their processes, was 10 microns.

Anterior frontal lobe blocks were selected from each case; all sections were cut at 10 microns, on the same microtome, mounted on poly-L-lysine coated slides and air dried for 1 hour at 37°C, coded, then stored at 37°C until stained. All sections were examined blind. Each GFAP stained section was examined three times to assess: 1) cell density and morphology, 2) the size and number of GFAP-positive cell processes and 3) the distribution of cells. RCA-120 stained sections were examined twice to assess 1) and 3) (above).

Table 2.1 Neuropathological diagnosis and cause of death in cases selected

Case	Group and (HIV-1 status)	Neuropathology
C1-C3	1	No abnormalities
C4	1	Oedema Very occasional perivascular macrophage and lymphocyte
C5	1 (-)	Oedema Very occasional perivascular macrophage and lymphocyte
1	2 (+)	No abnormalities
2	2 (+)	Moderate meningitis Perivascular infiltrate
3	2 (+)	Occasional perivascular infiltrate Occasional perivascular macrophages
4	2 (+)	White matter gliosis Perivascular macrophage infiltration Slight pallidal sclerosis
5	2 (+)	Discrete meningeal infiltrate Perivascular macrophage infiltrate Cortical foci of hypoxia
6	2 (+)	Slight intraventricular haemorrhage White matter gliosis Occasional perivascular macrophages and micro-mineralisation
7	2 (+)	White matter oedema Slight sub-arachnoid haemorrhage Discrete perivascular and leptomeningeal infiltrate
8	2 (+)	White matter oedema perivascular and leptomeningeal infiltrate foci of perivascular macrophages
9-14	3a AIDS	No abnormalities
15-16	3b AIDS	Diffuse non-specific gliosis
17-22	3b AIDS	HIVE/lep
23-27	3d AIDS	CMVE
28-31	3e AIDS	Cerebrovascular lesions

Table 2.2 Age, sex, post-mortem diagnosis and salient neurological details in group 1: HIV-1-seronegative cases.

Case	Age	Sex	PM diagnosis	Salient Neurological details
c1	45	♀	Muscular Dystrophy	none recorded
c2	32	♂	Sudden death during recovery from measles like infection	none recorded
c3	53	♂	Cardiac arrest	none recorded
c4	27	♂	Butane inhalation	none recorded
c5	19	♂	Drug overdose	none recorded

Table 2.3 Age, sex, post-mortem diagnosis and salient neurological details in group 2: HIV-1-seropositive cases that died prior to developing AIDS.

Case	Age	Sex	PM diagnosis	Salient Neurological details
1	31	♀	Heroin overdose	none recorded
2	30	♂	Heroin overdose	none recorded
3	31	♂	Heroin overdose	none recorded
4	29	♂	Died after fit Pneumonitis	Recent onset of fits
5	28	♀	Drug overdose Chest infection	none recorded
6	31	♂	Died 3 days after accidental intra-arterial drug injection	none recorded
7	36	♂	Sudden death from drug overdose	none recorded
8	35	♂	Pneumonia and chronic obstructive airway disease	none recorded

Table 2.4 Age, sex, post-mortem diagnosis and salient neurological details in group 3a: HIV-1-seropositive cases with AIDS that lacked any obvious neuropathological changes.

Case	Age	Sex	PM diagnosis	Salient Neurological details
9	32	♂	PCP Myocarditis Liver failure	left sided epileptiform seizure meningism
10	49	♂	Kaposi's sarcoma Possible PCP	Dizziness Postural Hypotension
11	N/A	♂	N/A	N/A
12	44	♂	PCP Kaposi' sarcoma	None recorded
13	30	♂	Kaposi's sarcoma Pulmonary oedema	Grand mal seizure
14	29	♂	Acute respiratory distress syndrome Bronchopneumonia Septicaemia	None recorded

N/A = not available

Table 2.5 Age, sex, post-mortem diagnosis and salient neurological details in group 3b: HIV-1-seropositive cases with AIDS, HIV-related neuropathology and HIV-1 antigens in the brain.

Case	Age	Sex	PM diagnosis	Salient Neurological details
15	38	♂	Kaposi's sarcoma Pneumonitis	Headache CT - frontal atrophy
16	40	♂	Bronchopneumonia	Hypomania
17	31	♂	PCP	Hypermania and delusions CT - generalised atrophy
18	33	♂	PCP	None recorded
19	49	♂	bronchopneumonia	Confusion Dementia
20	40	♂	PCP Myocarditis	Overall intellectual deterioration
21	30	♂	Bronchopneumonia	None recorded
22	N/A	♂	Bronchopneumonia	None recorded

N/A = not available

Table 2.6 Age, sex, post-mortem diagnosis and salient neurological details in group 3c: AIDS cases with CMV neuropathology and no HIV-1 antigen in the brain.

Case	Age	Sex	PM diagnosis	Salient Neurological details
23	33	♂	Kaposi's sarcoma Pneumonia Bronchopneumonia	No neurological symptoms
24	40	♂	Cardio-respiratory arrest	Intermittent episodes of Hypomania
25	39	♂	Septicaemia Multiple abscesses Haemorrhagic infection of small intestine	Progressive intellectual impairment leading to dementia
26	32	♂	N/A	N/A
27	51	♂	Bronchopneumonia	Memory loss Unsteady gait Dementia

N/A = Not available

Table 2.7. Age, sex, post-mortem diagnosis and salient neurological details in group 3d: AIDS cases with cerebrovascular neuropathology and no HIV-1 antigen in the brain.

Case	Age	Sex	PM diagnosis	Salient Neurological details
28	26	♂	Acute renal failure Disseminated necrotic lesions	None recorded
29	27	♂	Pneumonia	Stroke - left sided speech and personality disorder epileptic fit
30	47	♂	Bronchopneumonia	Not specified
31	28	♂	Lobular pneumonia Raised intracranial pressure Brain haemorrhage	Grand mal fits

2.2.2.1 HIV-1 antibodies

Four antibodies were assessed for their ability to label HIV-1 infected cells in formalin fixed paraffin embedded tissue sections. Titration of each antibody to give optimal staining was performed using 3 cases with HIV-lep that contained numerous multinucleated giant cells. Antibody dilutions that gave maximum signal and minimal background staining are shown in **table 2.8**. All antibodies stained more strongly when no trypsin pretreatment was used. The specificity of these antibodies was then assessed by staining pathological lesions in brains that contained high numbers of macrophages, from cases which were not from any of the risk groups for HIV-1 infection. These included two cases of multiple sclerosis (one acute and one chronic) and three cases of cerebral infarcts. The sensitivity of three of these antibodies was compared by staining brains from AIDS patients. The effect of formalin fixation on the immunoreactivity of p24 was tested by staining 22 cases of HIVE and HIV-lep that had been fixed for variable periods of time ranging from 1 to 15 months. The delay between death and post-mortem was known in 16 of these.

2.2.2.2 Cellular markers

Three antibodies for the detection of microglial cells and macrophages in the brain were assessed before the lectin RCA-120 was selected for this study. KP1 stained only rare cells in these brains, and LN1 stained a large number of cells, but also neuroectodermal cells. HAM 56 (Enzo Biochem) had a very similar staining pattern to RCA-120; it stained large numbers of both macrophages and microglia, however RCA-120 labelled small ramified cells more consistently than HAM 56. Both HAM 56 and RCA-120 labelled endothelial cells, in addition to microglial cells and

macrophages; however, endothelial cells are easily recognised and excluded from the count of RCA-120-positive cells.

Table 2.8 HIV-1 antibodies and cellular markers.

Antibody/ lectin	Reactivity	Source	Clonality	Optimal dilution
LAV gp41.1	HIV-1 envelope protein	Genetic Systems	Monoclonal	1:250
NEA p17	HIV-1 core protein	Dupont	Monoclonal	1:50
NEA p24	HIV-1 core protein	Dupont	Monoclonal	1:80
KAL p24	HIV-1 core protein	Dako	Monoclonal	1:20
GFAP	Astrocyte intermediate filament	Dako	Polyclonal	1:400
RCA-120	Mononuclear phagocyte Endothelial cells	Vector labs	Lectin	1:1500

Fixation - An initial study to examine the effects of fixation on the density of GFAP-positive astrocytes and RCA-120-positive microglia was performed, as some cases in the AIDS brain series were fixed for long periods of time. This study used multiple blocks from three cases, two with AIDS and one HIV-1-seronegative, which were fixed in 10% formalin for periods between 1 week to 10 months; sections were prepared as described above and stained with GFAP and RCA-120 antibodies. Sections were coded and examined blind, counts of immunostained cells with an identifiable nucleus were made within cortical layers 4 and 5, as described in section 2.2.3.

2.2.3 *Quantitative assessment of astrocyte and microglial cell density*

Quantitative measurements of cell density can be obtained by simply counting cells in a defined area on a tissue section. A graticule is inserted into a light microscope eye piece to define an area over the section, within which cells are counted. The area of the field defined by the graticule can be calculated, or measured using a graduated microscope slide. Cells are usually counted in multiple fields (between 4 and 25) and the mean of these counts expressed as the number of cells per mm². It is usual to count the number of recognisable cells with a well defined nucleus, rather than the stained cells; this may increase the accuracy of the count as nuclei are usually of a more uniform size and shape than the cells themselves. Fields can be selected at random, or according to well defined criteria. When the tissue is homogenous, and the cells to be counted are distributed evenly throughout the tissue, fields can be selected randomly. However, in tissues with a complex structure, such as the cerebral cortex, well defined criteria should be established in advance, and applied consistently to each section, as the count can be biased if fields are not selected consistently in each section examined. This basic method has been applied to a large number of studies for counting changes in astrocyte number, examples of this being: following stab wounds to the cerebral cortex of mice (Miyake et al., 1988), in hepatic encephalopathy (Kimura and Budka, 1986), in aging (Mandybur et al., 1989) and in close head injuries (Crooks et al., 1991).

Counts of GFAP and RCA-120-positive cells were made in cortical layers 4 and 5. Ten areas were marked for counting; three were located at the top of the gyrus, three

at the base of the sulcus and the remaining four counts were positioned midway between the top of one gyrus and the sulcus the next. Only regions where the cortex had been cut perpendicular to the meninges were used for counting, since the width of different cortical layers can be distorted in tangential section. Sections were examined under a Zeiss light microscope, with a x25 objective and a square grid eyepiece graticule. The field defined by the graticule was measured using a graduated microscope slide. Immunoreactive cells with a clearly recognisable nucleus that fell within the area of the grid were counted in ten fields. The scores were adjusted to mm^2 and the mean score from ten counts was calculated for each case.

A more sophisticated method of assessing cell density using a Context Vision AB image analyzing computer was also investigated. The major disadvantage of this system, was the amount of time required to study each slide. This method was therefore considered too time-consuming to justify the marginal increase in accuracy it may have provided.

2.2.4 Assessment of cellular morphology and distribution

The system described below is based on the initial observations of GFAP and RCA-120 stained sections, as described in section 2.1. Ten observations at high magnification (x400) were made within cortical layers 4 and 5.

2.2.4.1 Astrocyte morphological classification

Astrocyte nuclear and cytoplasmic appearances did not appear to change in a

consistent manner in preliminary observations (section 2.1). Therefore, for assessment of morphological changes to be made, these two elements were graded separately. In addition, the presence of reactive astrocytes was also recorded.

2.2.4.1.2 Nuclear score

The nuclear appearance of GFAP-positive astrocytes was graded according to the size and staining pattern. Small nuclei with darkly stained homogenous chromatin were scored as 0, while higher scores indicated increasing nuclear size and reduced chromatin density, as described in **table 2.9** and illustrated in **figure 2.13 (a-d)**. As cell counts were being made, as described above (section 2.2.3), the number of nuclei falling into each score, in each field, was also recorded. The mean of each nuclear type was calculated after ten counts, and the highest mean score was designated as the astrocyte "most frequently observed nuclear score", (MFNS).

2.2.4.1.3 GFAP density

An estimation of the number and size of GFAP-positive processes per cell (GFAP immunoreactivity) was estimated, and each section assigned a score of +, ++, or +++ as described in **table 2.10**, and illustrated in **fig 2.14**. Scores indicated the appearance of the majority of the GFAP-positive astrocytes in a section. A few cases in which astrocytes had very little GFAP-positive staining were assigned the score of \pm . In addition, the presence of reactive astrocytes was recorded as these cells did not fit into the criteria described above. Reactive astrocytes were defined as hypertrophic astrocytes with densely GFAP-positive cytoplasm with or without a few cell processes and enlarged pale nuclei.

2.2.4.2. Microglia/macrophage morphological classification

RCA-120-positive cells were classified according to their cellular morphology described in **table 2.11** and illustrated in **figure 2.15**, and defined as; small ramified cells (ram), enlarged ramified cells (ram+), rod cells (rod), macrophages (mac) or multinucleated cells. All cells with a macrophage like morphology were classified as macrophages irrespective of the fact that they may have been derived either from reactive microglia or from blood monocytes. As microglial cells were being counted, as described above (section 2.2.3), the number of cells with each morphological appearance, in each field was also recorded. The mean number of each morphological type of microglial cell was calculated after ten counts, and the highest mean score was designated as the "most frequently observed morphological type", (MFMT).

2.2.4.3 Assessment of astrocyte distribution

GFAP-stained sections was examined at low power to assess the distribution of immunoreactive cells. The distribution of GFAP-positive astrocytes was designated as "perivascular", "diffuse", "patchy" or "focal". A perivascular distribution indicated that most of the GFAP-positive cells were situated around vessels, as observed in normal control brains. Diffuse indicated that cells were evenly distributed throughout the cortex, and patchy indicated that astrocyte density was variable in different areas of the cortex. Focal lesions consisted of small local concentrations of between approximately 5-50 astrocytes.

2.2.4.4 Assessment of microglial cells/macrophage distribution

The distribution of RCA-120-positive cells was defined as "diffuse", "patchy", "rare" or "focal". Diffuse indicated a fairly even distribution of microglial cells throughout the cortex, patchy indicated a widespread but uneven distribution and rare indicated that RCA-120-positive cells were difficult to locate. Focal groups of RCA-120-positive cells were described as "microglial nodules" when tight aggregations of cells occurred or "loose" when cells were less closely associated.

Table 2.9 Scoring system for astrocyte nuclear morphology

Score	Astrocyte Nuclear Morphology
0	Small with dense homogenous chromatin
1	Slightly enlarged with pale chromatin
2	Enlarged with unevenly dispersed chromatin
3	Enlarged with watery appearance and irregular outline

Table 2.10 Scoring system for GFAP immunoreactivity: appearance of the majority of GFAP-positive astrocytes

Score	GFAP immunoreactivity
±	GFAP-positive cells rare.
+	A small amount of ill-defined cytoplasm or a few short processes.
++	Moderate amount of cytoplasm or processes of moderate length.
+++	Moderate densely stained cytoplasm and thick processes.

Table 2.11 Scoring system for microglial cell morphology

Classification	Microglial cell morphology
ram	Ramified microglia with slender processes
ram +	Ramified microglia with enlarged nucleus and thickened processes
rod	Elongated nuclei and thickened bipolar processes
mac	Rounded amoeboid type microglia/macrophages or multinucleated cells

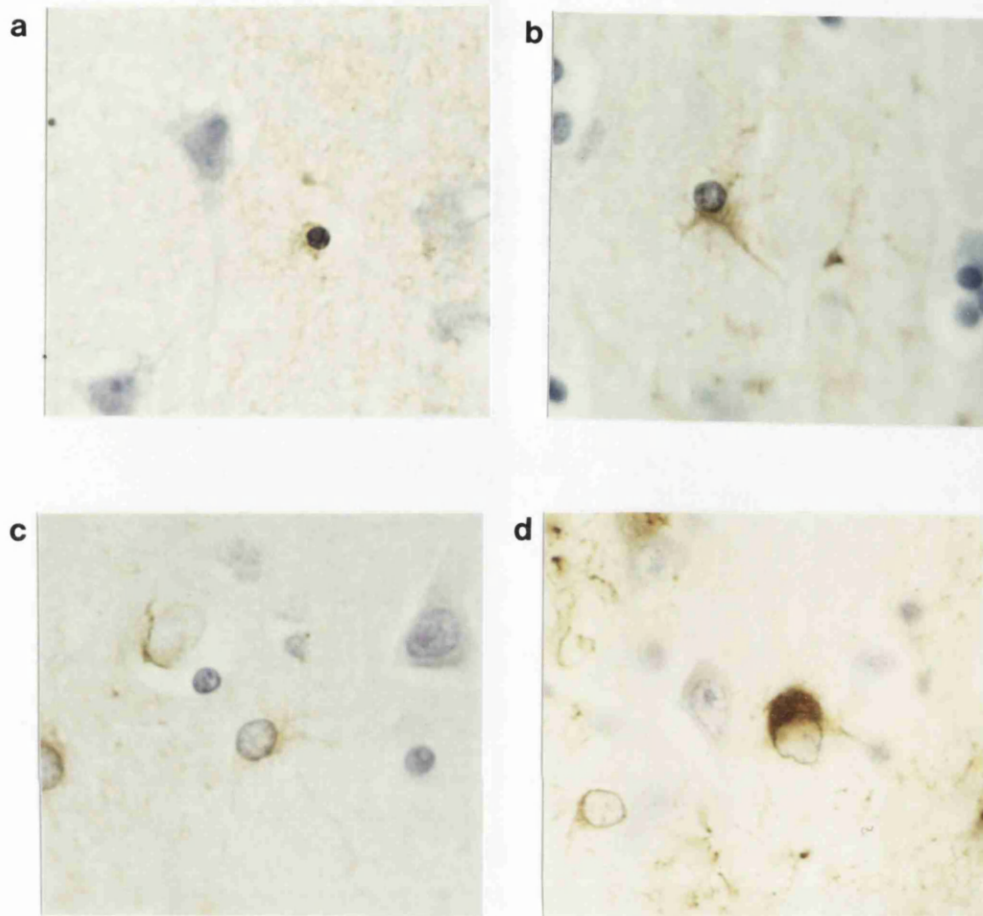


Figure 2.13 Photomicrographs showing GFAP-positive astrocytes with the various nuclear scores: 0 - small and dense with evenly stained chromatin (a); 1 - slightly enlarged and paler than in a (b); 2 - enlarged with uneven very pale chromatin (c) and 3 - enlarged with a watery appearance and an irregular outline (d). GFAP and haematoxylin (x640).

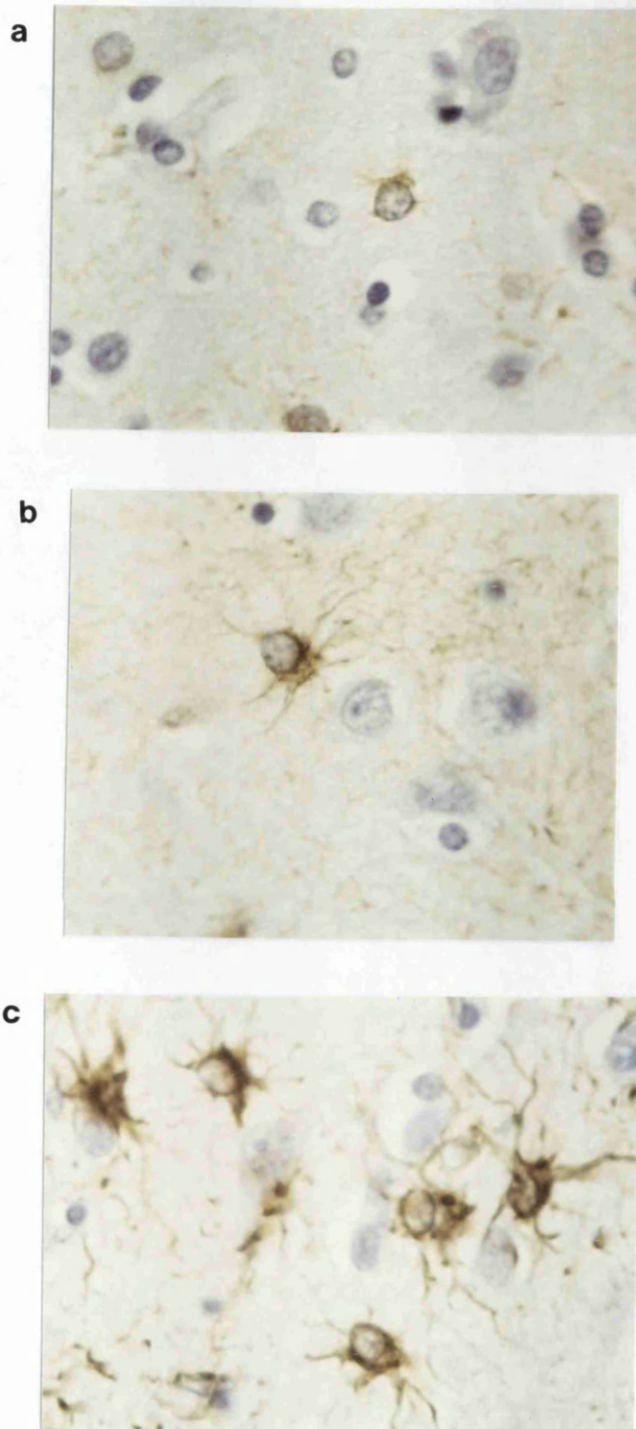


Figure 2.14 Photomicrographs of GFAP-positive astrocytes with the various GFAP immunoreactive scores: + - a few thin GFAP positive processes surround the nucleus (a); ++ - a moderate amount of GFAP positive cytoplasm and a few short processes surround the nucleus (b); +++ - densely stained GFAP positive cytoplasm with thick processes. GFAP and haematoxylin (x640).

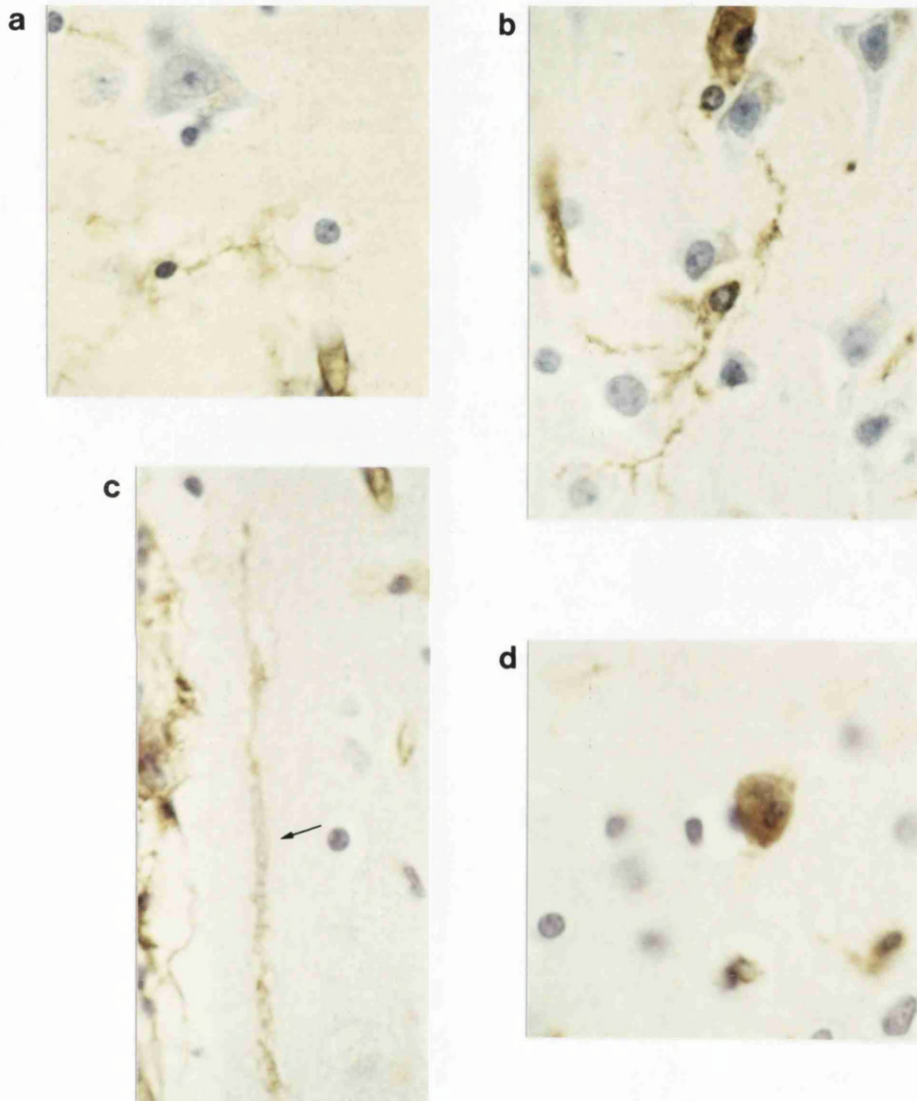


Figure 2.15 Photomicrographs showing the various types of microglia classified according to their morphology: ram - small ramified microglial cell with slender processes (a); ram+ - ramified microglial cell with an enlarged nuclei and thickened processes (b); rod - elongated nucleus (arrow) and bipolar processes and mac - small rounded macrophage like cell. RCA-120 and haematoxylin, (x640).

2.3 Results

2.3.1 *HIV-1 antibody assessment*

2.3.1.1 False positive staining

As shown in **table 2.12** all the antibodies except NEA p24 gave false positive staining in HIV-seronegative pathological lesions. In the infarcts and the chronic MS lesions, macrophages were often stained, although the reaction product was distinct from the typical granular appearance of the immunoreaction seen in AIDS cases (**fig 2.16a**), and consisted of wisps of non-granular material (**fig 2.16b**). In the acute MS lesion the gp41.1 antibody also stained astrocytes. Modification of the method to reduce false positive staining had variable results. Blocking with bovine serum albumin (Boehringer Mannheim), or normal rabbit serum (Dako) had little effect; however fetal calf serum (FCS, Sigma) eliminated background staining produced by NEA p17 and KAL p24, and reduced considerably that produced by LAV gp41.1.

2.3.1.2 Comparative sensitivities of HIV-1 antibodies

LAV gp41.1, even with preincubation in FCS remained capricious, while the other three antibodies gave consistent results and were therefore compared for sensitivity. The two p24 antibodies from different sources had similar sensitivities, but the DAKO antibody (KAL p24) was used at a higher concentration. NEA p17 was sometimes negative when the p24 antibodies were positive, although the reverse was never the case. NEA p24 was therefore considered to be the best choice of antibody, and was used to detect HIV-1 in all cases included in this study.

Table 2.12 Specificity of HIV-1 antibodies: Immunoreactivity for HIV-1 in HIV-1-seronegative brains

Pathological lesion	Antibody			
	LAV gp41.1	NEA p17	NEA p24	KAL p24
Infarct	+	+	-	+/-
Infarct	+	+	-	+/-
Chronic Multiple Sclerosis	+	+	-	+
Chronic Multiple Sclerosis	+/-	+/-	-	-
Acute multiple sclerosis	+	+/-	-	-

+ false positive staining of macrophages
 +/- weak false positive staining of macrophages
 - no false positive staining

2.3.1.3 Fixation time and post-mortem delay

Of the twenty-two HIVE and HIV-lep cases examined, immunoreactivity for p24 was present in seventeen. There was no correlation between length of fixation time and absence of immunoreactivity (Table 2.13), since two of the seven cases with numerous positive cells had been fixed for longer than eleven months. Furthermore, one case in which no antigen was detected had been fixed for less than one month. Information on the delay between death and post mortem was available in sixteen of these twenty-two cases. In three cases, in which no p24 antigen was detected, the post-mortem delay was between 3 to 6 days. On the other hand, positive results were obtained in cases in which it had been delayed for 5 and 7 days. In conclusion, since this antibody could label p24 antigens following post-mortem delays of 7 days and fixation times of 15 months, the factors responsible for lack of reaction in 5 cases with MGCs could not be identified.

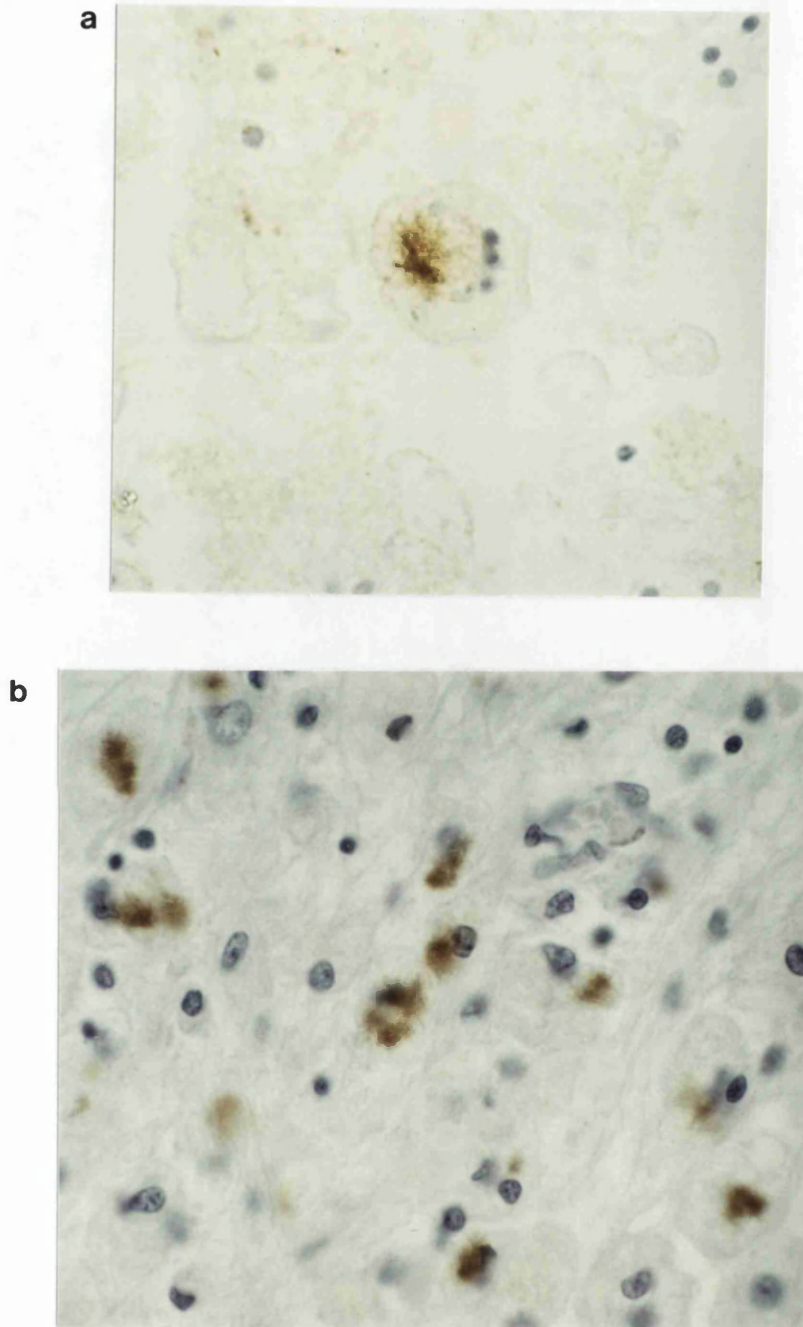


Figure 2.16 Photomicrographs showing positive (a) and false positive (b) staining, with HIV-1 antibodies. In (a), a granular reaction product is seen within a multinucleated giant cell, whilst in (b), wisps of immunoreactive product are seen within macrophages. NEA p24 and haematoxylin (a), (x400); Kal p24 and haematoxylin (b), (x640).

Table 2.13 Detection of HIV-1 core proteins with NEA p24 following formalin fixation

Fixation time (months)	Number of p24 positive cells (++, +, +/-, -) and post-mortem delay (PMD)			
	++	+	+/-	-
1 or less				PMD ?
1-2	PMD ? PMD ? PMD 2 days		PMD 4 days	
2-3	PMD 3 days	PMD ?	PMD 7 days	
3-4		PMD 1 day PMD ?	PMD 2 days	
4-5	PMD 4 days			
5-6				PMD 3 days PMD ?
6-7				
7-8				
8-9			PMD 4 days	PMD 6 days
9-10				
10-11				
11-12	PMD 4 days		PMD 7 days	
12-13	PMD 5 days			
13-14				PMD 5 days
14-15			PMD 5 days PMD 2 days	

++ > 20 positive cells per section
 + 5-20 positive cells per section
 + < 5 positive cells per section
 - no positive cells

2.3.2 Immunohistochemical detection of HIV-1 p24 in cortex and white matter of anterior frontal lobe

NEA p24 antigen was detected only in cases assigned to the HIV-1 antigen positive group. In the 2 cases with diffuse gliosis, HIV-1 antigen was found within microglial cells that showed a ramified morphology and were scattered throughout the white matter. In the six cases with HIVE/lep, antigen was located within microglial cells, macrophages and MGCs. Positively stained cells were scattered throughout the white matter in cases of HIV-lep, and were frequently seen in loose foci in cases of HIVE. In three of these cases, p24 antigen was also detected in the cortex, within ramified microglial cells and MGCs (**figure 2.17**). In cases 18 and 21, these cells were rare, though they were more frequent in case 19; however, in all these cases, the number of positive cells was higher in the white than in the grey matter. Occasionally, antigen was detected within the walls of a vessel, most often in case 21; however it was not possible to decide whether infected cells were endothelial cells or pericytes. No NEA p24 positive cells with morphology suggestive of neurons, astrocytes or oligodendrocytes were observed.

2.3.3 Effects of fixation on GFAP immunoreactivity and RCA-120 lectin reactivity

Ten counts of GFAP-positive and RCA-120-positive cells were made in sections from blocks that were fixed in formalin for various lengths of time. These data are illustrated in scatter graph form in **figures 2.18 and 2.19**. It can be seen from these

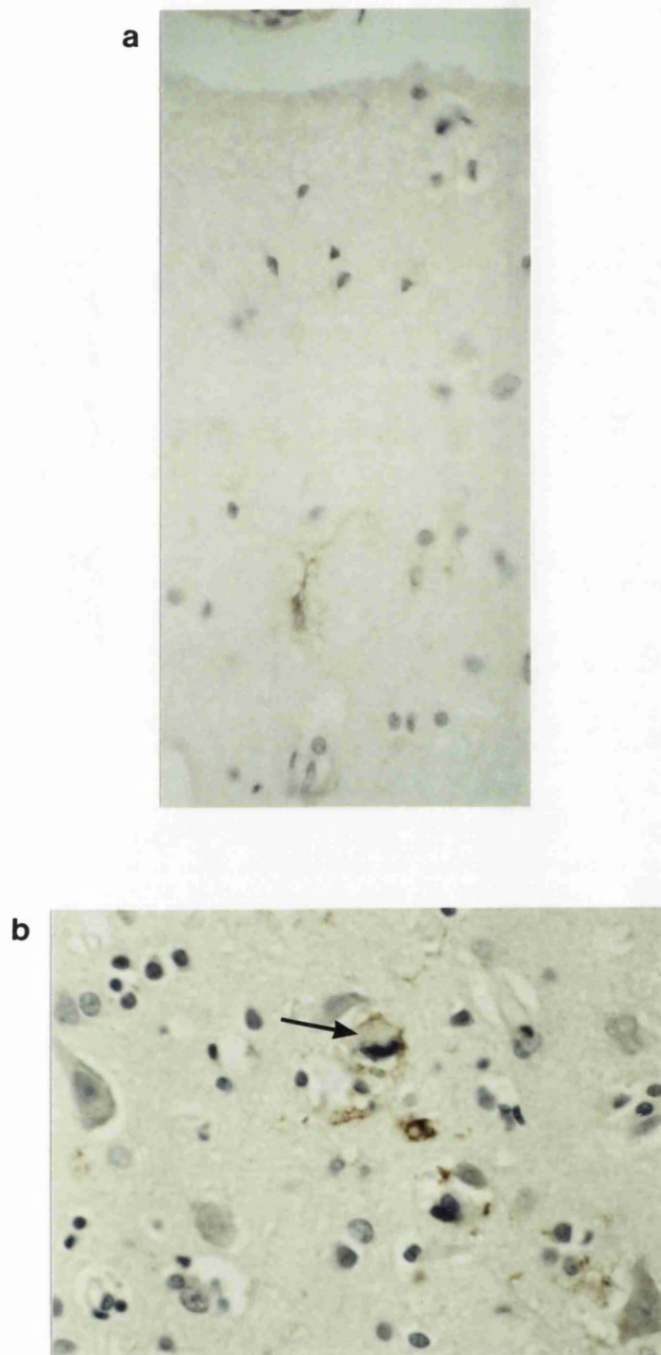


Figure 2.17 Photomicrographs of anterior cerebral cortex of cases 18 (a) and 19 (b), showing a microglial cell in the molecular layer infected with HIV (a) and a multinucleated cell (arrow) in the deep cortex which appears to have HIV antigens associated with the cell membrane (b). In (b) there also appears to be staining of cell processes or membranes which are not obviously associated with a cell nucleus. This appearance was regarded as specific for HIV as it was not observed in any of the HIV negative controls. Immunostaining with NEA p24.(x400)

graphs that the number of cells in each of the ten counts varied in some cases. The scores for both astrocyte and microglial cell density at some time points were not normally distributed.

Linear regression was calculated, in order to test for a change in cell density with increasing fixation time. Since the data, illustrated in **figures 2.18** and **2.19**, were not normally distributed they were converted to log scores and a scatter graph of cell density over fixation time plotted for each case in order to calculate the correlation coefficient. GFAP-positive astrocyte cell densities in the three cases tested showed a variable change with fixation time. In case A, which had no neuropathological abnormalities and was not from any of the HIV risk groups, a small number of astrocytes were detected at each stage and there was no significant relationship between cell density and fixation time ($p=0.141$). In case B, which had severe DPD, there was a significant reduction in cell density as fixation time increased ($p < 0.001$). Finally in C, a case of HIVE, there was no relationship between cell density and fixation time ($p=0.504$). The numbers of RCA-120-positive cells detected at each fixation time in these cases was much lower than the number of astrocytes, and in case A, very few cells were seen at any fixation time. The number of cells in each count was variable in cases B and C. However, there was no relationship in any of these cases between fixation time and loss of microglial ^{cell} immunoreactivity (case A, $p=0.455$; case B, $p=0.289$; case C, $p=0.494$), suggesting that although variable, RCA-120 lectin histochemistry is resistant to prolonged fixation.

The mean cell density of GFAP-positive astrocytes and RCA-120-positive microglial

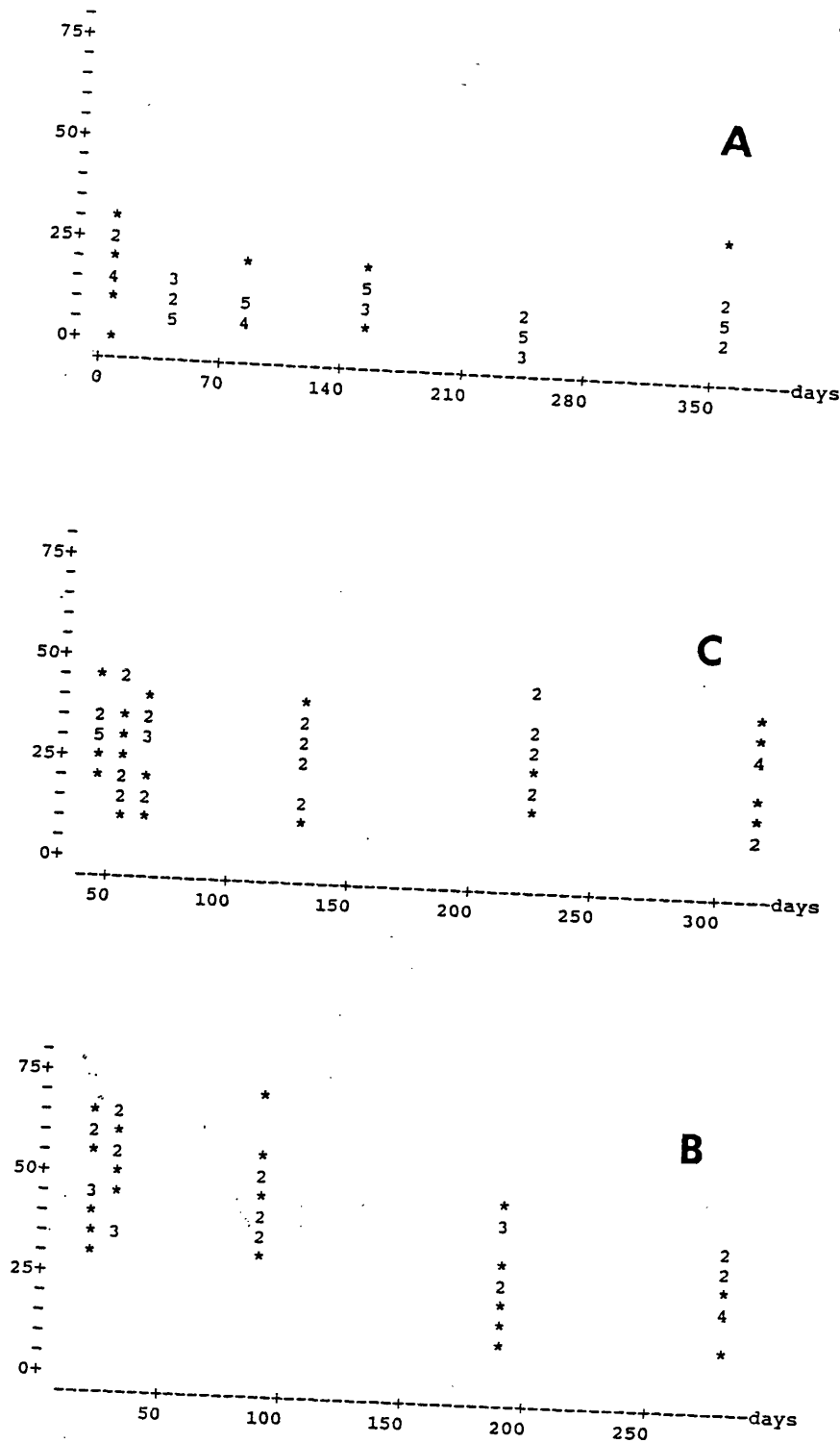


Figure 2.18 Scatter graphs illustrating the number of GFAP positive astrocytes counted in ten fields, then converted to cells per mm², in the three fixation controls, for each fixation time. Each asterix indicates a single data point, numbers indicate the number of data points plotted at the same position.

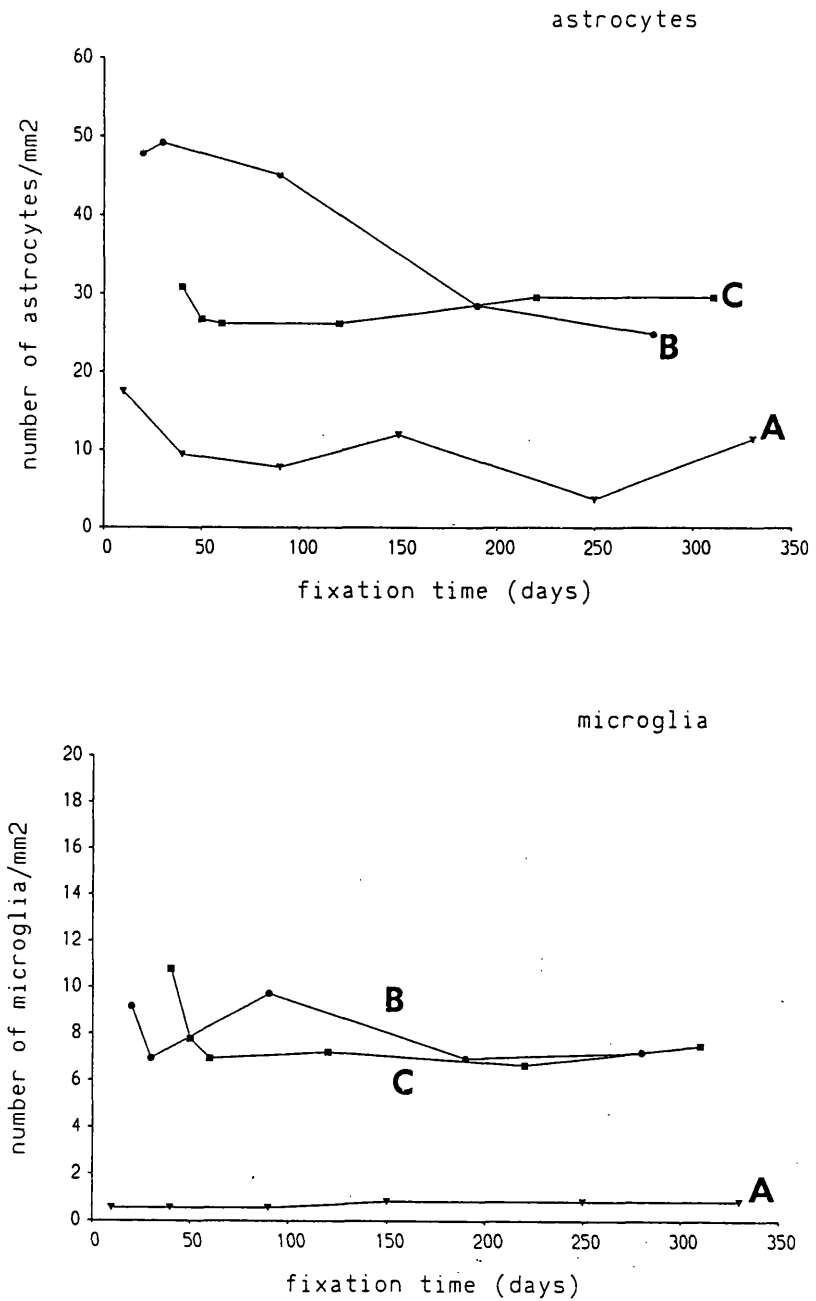


Figure 2.20 Mean cell density per mm² of GFAP positive astrocytes and RCA-120-positive microglial cells, in fixation controls, plotted against fixation time.

cells at each fixation time is plotted over fixation time for cases A, B and C in **figure 2.20**.

However, following 4 months fixation (approximately 120 days), the mean cell density of ten counts is sufficiently different to allow distinction between these two pathological cases and the normal control. All immunohistochemical staining in this study was therefore carried out on blocks that had been fixed for less than four months. The mean number of RCA-120-positive microglial cells in case A is clearly different from case B at all fixation times. In addition, the differences between mean RCA-120-positive microglial cell density in cases B and C, is very small at all fixation times.

2.3.4 Astrocyte density morphology and distribution

Tables 2.14 - 2.16 summarises the results of GFAP immunohistochemistry. For each case; the following data are presented: 1) the mean GFAP-positive astrocyte cell density of ten counts, expressed as cells per mm²; 2) the astrocyte nuclear score that was most frequently observed. The mean density of each astrocyte nuclear score over ten counts was calculated, and the score that had the highest mean value was designated as, the most frequently observed nuclear score (MFNS). Where there was little difference between two scores, both scores are given; 3) the GFAP immunoreactivity score; 4) the presence of reactive astrocytes; 5) the most predominant distribution of GFAP-positive cells.

2.3.4.1 Group 1 - Normal controls

Four of the five control cases had a similar appearance after GFAP immunohistochemistry, although the density of GFAP-positive astrocytes showed some variability (mean 14.96, range 7.51-23.07). Most GFAP-positive cells were situated perivascularly, their nuclei were small and dense and they had abundant GFAP-positive branching processes. In case c2, some slightly enlarged nuclei were present, both perivascularly and throughout the parenchyma, in addition to smaller nuclei. The GFAP-positive processes of perivascular astrocytes were less prominent in this case than in the other four; the distribution of GFAP-positive cells was therefore designated as diffuse/PV. Occasional parenchymal astrocytes, with a small amount of ill-defined GFAP-positive staining, were present in all cases; GFAP immunoreactivity, in these cases, was therefore designated as +.

2.3.4.2 Group 2 - HIV-1-seropositive, non-AIDS

In three cases (2,3,7) the nuclear appearance was similar to that seen in the controls, and astrocyte density was within the control range. In the remaining five the MFNS was higher; in these cases, GFAP-positive astrocyte density was similar to the normal controls in cases 1 and 4, lower in cases 5 and 6 and higher in case 8. The astrocyte density was therefore very variable within this group (range 3.06-42.23), while the mean density for the group was similar to the normal controls (14.34). Perivascular astrocytes with a normal appearance were evident in all except case 4, in which astrocyte nuclei were the most enlarged. The distribution of cells was perivascular in cases 1 and 2, while in cases 3, 7 and 8, where the distribution was designated as diffuse/PV, there was a reduction in the GFAP positivity around vessels. Cases 5

and 6 had very low numbers of GFAP-positive cells both in the parenchyma and around vessels, and the distribution was described as rare (diffuse/PV). GFAP immunoreactivity was also very sparse in these cases and designated \pm . In the remaining cases, GFAP immunoreactivity of parenchymal astrocytes was similar to that seen in the normal controls.

2.3.4.3 Group 3a - AIDS: no neuropathological abnormalities

The mean astrocytic density (28.91, range 15.29-39.75) was higher than in both the above groups. In addition, most nuclei were enlarged and pale in all cases in this group, although mildly so in case 13. In all six cases, the distribution of GFAP-positive cells was predominantly diffuse, although in cases 12 and 14 a distinct perivascular accumulation of astrocytes was also evident. In the other four cases, there was a distinct loss of perivascular GFAP immunoreactivity as compared with the normal controls. The density of GFAP immunoreactive processes in this group was similar to the normal controls, except in case 13 where the GFAP density was designated as ++; reactive astrocytes were also observed in this case.

2.3.4.4 Group 3b - AIDS: antigen positive (HIVE/lep & diffuse gliosis)

The mean astrocyte density in this group of 35.63 (range 22.24-50.60) was higher than all the above groups. The MFNS was high in 6 cases (15-20) while in cases 21 and 22 nuclear changes were not so pronounced. GFAP immunoreactivity was increased in cases 15, 18, 19, 20 and 21, most noticeably so in cases 19 and 21. The distribution of cells was predominantly diffuse in cases 17, 18, 19, 21 and 22. In the two cases with non-specific gliosis (15, 16) and in case 20 perivascular astrocytes with

extensive branching processes were prominent although these cells had enlarged pale nuclei. Focal accumulations of astrocytes were seen in cases 17, 20 and 21. In case 17 and 20, they consisted of reactive astrocytes; however in case 21 foci consisted of astrocytes similar to the diffusely distributed cells. Typical reactive astrocytes were present in 4 of the 6 HIVE/lep cases (17-20) while none were seen in the non-specific gliosis cases.

2.3.4.5 Group 3c - AIDS: CMVE

Astrocyte density in this group was similar to that seen in the HIVE/lep group (mean=39.23, range 25.30-49.49), whilst the MFNS and GFAP immunoreactivity were variable. One case with small dense nuclei (case 24) and one with mildly enlarged nuclei (case 23) had extensive GFAP immunoreactive branching processes, which were designated as + + +. The other three cases had more reactive nuclei and fewer GFAP-positive processes. The distribution was predominantly diffuse although a distinct perivascular population of cells with reactive nuclei was observed in case 24. No foci of GFAP-positive astrocytes were seen; in cases with MGNs, astrocytes were present around these lesions, but not within them. Typical astrocytes were present only in case 26.

2.3.4.6 Group 3d - AIDS: cerebrovascular lesions

Mean GFAP-positive astrocyte density was lower in this group (16.64; range-8.90-27.19) than in the other AIDS groups, and came within the range of the normal controls, whilst in all four cases the astrocyte MFNS was high. GFAP immunoreactivity was low in case 28 (+), but the other three cases scored + + +.

Table 2.14 Group 1 - Astrocyte density, nuclear reactivity, GFAP immunoreactivity and distribution of GFAP-positive astrocytes in the frontal cortex of normal control cases.

Case	Astrocyte Density Cells/mm ² (Mean of 10 counts)	Most frequent astrocyte nuclear score	GFAP immunoreactivity	Diffuse Reactive astrocytes	Predominant distribution of GFAP-positive astrocytes	Loose foci of GFAP-positive cells
c1	11.46	0	+	-	perivascular	-
c2	11.42	0-1	+	-	diffuse/PV	-
c3	21.41	0	+	-	perivascular	-
c4	23.07	0	+	-	perivascular	-
c5	7.51	0	+	-	perivascular	-

Table 2.15 Group 2 - Density, nuclear reactivity, GFAP density and distribution of GFAP-positive astrocytes in the frontal cortex of HIV-1-seropositive non-AIDS cases.

Case	Astrocyte Density Cells/mm ² (Mean of 10 counts)	Most frequent astrocyte nuclear score	GFAP immunoreactivity	Diffuse reactive astrocytes	Predominant distribution of GFAP-positive astrocytes	Loose foci of GFAP-positive cells
1	8.37	1	±	-	perivascular	-
2	14.12	0	++	-	perivascular	-
3	9.45	0	++	-	diffuse/PV	-
4	15.85	2	+	-	diffuse	-
5	3.06	1	±	-	rare: diffuse/PV	-
6	3.06	1	±	-	rare: diffuse/PV	-
7	18.63	0	+++	-	diffuse/PV	-
8	42.23	1	+	-	diffuse/PV	-

Table 2.16 Group 3 - Density, nuclear reactivity, GFAP density and distribution of GFAP-positive astrocytes in the frontal cortex of AIDS cases.

Case	Astrocyte Density Cells/mm ² (Mean of 10 counts)	Most frequent astrocyte nuclear score	GFAP immuno-reactivity	Diffuse Reactive astrocytes	Predominant distribution of GFAP-positive astrocytes	Loose foci of GFAP-positive cells
No Neuropathological Abnormalities						
9	38.65	2-3	+	-	diffuse	-
10	20.57	2	+	-	diffuse	-
11	39.75	3	+	-	diffuse	-
12	23.36	2	+	-	diffuse/PV	-
13	35.86	1	++	+	diffuse	-
14	15.29	2	+	-	diffuse/PV	-
HIV-1 p24 positive (HIVE/lep and diffuse gliosis)						
15	22.24	2	++	-	diffuse/PV	-
16	27.80	2	+	-	diffuse/PV	-
17	22.72	2	+	+	diffuse	+
18	50.60	2-3	++	+	diffuse	-
19	43.93	2	+++	+	diffuse	-
20	40.31	2	++	+	diffuse/PV	+
21	48.65	1-2	+++	-	diffuse	+
22	29.19	1	+	-	diffuse	-
HIV-1 p24 negative (CMVE and cerebrovascular lesions)						
23	25.30	1	+++	-	diffuse/PV	-
24	26.69	0	+++	-	diffuse	-
25	49.49	2	++	-	diffuse	-
26	30.02	2	++	+	diffuse	-
27	32.25	2	++	-	diffuse	-
28	27.19	3	+	+	diffuse	+
39	18.91	3	++	+	diffuse	+
30	10.87	2	++	-	diffuse	-
31	8.90	2	++	-	diffuse	-

The distribution of cells was diffuse in all four cases, and a reduction in perivascular astrocytes was seen in all cases. Cases 28 and 29 had focal accumulations of reactive astrocytes.

2.3.5 Microglial cell density, reactivity and distribution

Tables 2.17 - 2.19 summarises the results of RCA-120 lectin histochemistry. For each case, the following data are presented: 1) the mean RCA-120-positive microglial cell density of ten counts, expressed as cells per mm²; 2) the microglial morphological cell type (ram, ram+, rod and mac) that was most frequently observed (MFMT). The mean density of each microglial morphological cell type was calculated and the morphological type that had the highest mean value designated as the MFMT. Where there was little difference between two or more mean values, all frequently present cell types are listed; 3) the predominant distribution of RCA-120-positive microglial cells.

2.3.5.1 Group 1 - Normal controls

Small ramified cells were most numerous in 4 cases in this group (c1,c3-c5). However in case c2 enlarged RCA-120-positive microglial cells were also frequently observed and the density of cells was highest in this case. The mean density for the whole group was variable (range 0.83-9.46; mean density = 5.06). Microglial cells were diffusely distributed in c1, c4 and c5, while in c2 the distribution was patchy, and only rare cells were seen in case c3.

2.3.5.2 Group 2 - HIV-1-seropositive, non-AIDS

In four cases small ramified cells (ram) were the MFMT, whilst in the other 4, enlarged ramified cells (ram+), and in one (case 8), rod cells more frequent. The distribution of cells was predominantly diffuse in all cases except case 3, in which a more patchy distribution of cells was observed. The mean density of positive cells was twice that seen in the HIV-negative group (11.01; range 4.45-16.68).

2.3.5.3 Group 3a - AIDS: no neuropathological abnormalities

In 4 cases (9,11,12,14) in this group only small ramified cells were seen, while in the other 2 (10 and 13) rod cells were the MFMT. A diffuse distribution of cells was observed in cases 9 and 10; in case 10, loose foci of rod cells were also observed. The distribution was patchy in case 11 and only rare cells were observed in the remaining cases. Microglial cell density (3.75, range 1.11-9.17) was similar to group 1 and lower than that in the HIV-1-seropositive non-AIDS group.

2.3.5.4 Group 3b - AIDS: antigen positive (HIVE/lep & diffuse gliosis)

In the two cases with non-specific gliosis and two of the HIVE/lep cases, (15,16,17,21) small non-reactive ramified cells were the MFMT. Of the remaining cases, rod cells were the MFMT in cases 18 and 19; both enlarged ramified cells and rod cells in case 22 and all three in case 20. The mean density (6.22, range 1.11-15.29) was close to that found in the normal controls. The distribution of cells was variable, being diffuse in 3 cases, patchy in 3, while only rare cells were seen in the other two cases. In addition, foci, present in 3 cases (17, 20 and 21), consisted of loosely associated rod cells, macrophages and multinucleated cells. Patches of

macrophages and multinucleated cells were also present in case 18.

2.3.5.5 Group 3c: CMVE

In all cases, only small ramified microglia were seen throughout the parenchyma; their density was low (mean 2.27, range 1.11-3.89) and the distribution of cells was patchy or cells were rare. However, in four out of five cases (23-26) MGNS consisting of tightly packed aggregations of ramified and rod cells were seen. In one case (27) loose foci similar to those seen in group 4 cases were present in addition to MGNs.

2.3.5.6 Group 3d - AIDS: cerebrovascular lesions

In two cases (30 and 31), only small ramified cells were observed in the parenchyma, while more enlarged ramified cells and rod cells were the MFMT in the other two cases. The mean density (6.60, range 3.34-8.33) was similar to the HIV-1 p24 positive group (group 4). Cells were diffusely distributed throughout the parenchyma in 3 cases (28,30,31) and patchy in case 29. Loose foci of reactive ramified cells, rod cells and macrophages similar to those seen in group 4 were seen in 2 cases (28 and 29) and MGNs similar to those present in CMVE cases (group 5) were seen in one case (32).

Table 2.17 Group 1 - Density, reactivity, cellular processes and distribution of RCA-120-positive microglial cells in the frontal cortex of normal controls.

Case	Density of RCA-120 + microglial cells/mm ² (Mean of 10 counts)	Most frequent morphological appearance of RCA-120 + cells	Predominant distribution of RCA-120 + cells	Foci of RCA-120 + microglial cells
c1	5.00	ramified	diffuse	-
c2	9.46	ramified +	patchy	-
c3	0.83	ramified	rare	-
c4	4.73	ramified	diffuse	-
c5	5.01	ramified	diffuse	-

Table 2.18 Group 2 - Density, reactivity, cellular processes and distribution of RCA-120-positive microglial cells in the frontal cortex of HIV-1-seropositive non-AIDS cases.

Case	Density of RCA-120 + microglial cells/mm ² (Mean of 10 counts)	Most frequent morphological appearance of RCA-120 + cells	Predominant distribution of RCA-120 + cells	Foci of RCA-120 + microglial cells
1	9.45	ramified	diffuse	-
2	4.45	ramified	diffuse	-
3	12.79	ramified	patchy	-
4	16.68	ramified +	diffuse	-
5	11.96	ramified +	diffuse	-
6	12.78	ramified +	diffuse	-
7	8.61	ramified	diffuse	-
8	11.40	ramified + /rod	diffuse	-

Table 2.19 Group 3 - Density, reactivity, cellular processes and distribution of RCA-120-positive microglial cells in the frontal cortex of AIDS cases.

Case	Density of RCA-120 + microglial cells/mm ² (Mean of 10 counts)	Most frequent morphological appearance of RCA-120 + cells	Predominant distribution of RCA-120 + cells	Foci of RCA-120 + microglial cells
No Neuropathological Abnormalities				
9	4.45	ramified	diffuse	-
10	9.17	rod	diffuse	loose foci (rod)
11	5.29	ramified	patchy	-
12	1.11	ramified	rare	-
13	1.40	ramified/rod	rare	-
14	1.67	ramified	rare	-
HIV-1 p24 positive (HIVE/lep and diffuse gliosis)				
15	5.01	ramified	diffuse	-
16	1.11	ramified	rare	-
17	3.61	ramified	diffuse	loose foci (macs, MGCs)
18	15.29	rod	patchy	-
19	9.18	rod	patchy	-
20	8.34	ram/ram + /rod	diffuse	small perivascular foci (mac, MGC)
21	1.39	ramified	rare	large loose foci (rod,mac)
22	5.84	ram/ram +	patchy	-
HIV-1 p24 negative (CMVE and cerebrovascular lesions)				
23	1.39	ramified	rare	MGNs (Ram +)
24	3.05	ramified	patchy	MGNs (Ram +)
25	3.89	ramified	patchy	MGNs (Rod, Macs)
26	1.94	ramified	rare	MGNs (Ram +) & loose foci (ram +)
27	1.11	ramified	rare	-
28	8.33	ramified +	diffuse	loose foci (ram + ,rod)
29	6.95	rod	patchy	loose foci (ram + ,rod)
30	7.79	ramified	diffuse	-
31	3.34	ramified	diffuse	MGN (ram + ,rod,mac)

2.3.6 Variation in astrocyte and microglial cell density

The densities of GFAP-positive astrocytes and RCA-120 microglial cells in each clinical group and within each AIDS group are summarised in **tables 2.20 and 2.21**. The mean density of ten counts was calculated for each case, and these scores were used to calculate the mean, standard deviation (SD) and range for each group. However, since scores were not normally distributed about the mean in some groups, the median value was also calculated to allow statistical comparisons, the median cell density in each group is illustrated in **figure 2.21** . The Mann-Whitney test with a 95% confidence level was used to test for differences between groups **table 2.22**.

2.3.6.1 Comparison of clinical groups

The median density of GFAP astrocytes in all AIDS cases (group 3) was significantly higher than the median of the normal controls (group 1)($\alpha=0.014$); however the median microglial cell density was not significantly different between these two groups ($\alpha=0.834$). In contrast, although there was no significant increase in median astrocyte density of the HIV-1-seropositive non-AIDS cases (group 2) as compared with the normal controls (group 1)($\alpha= 0.608$), the density of RCA-120-positive microglia was significantly higher in the non-AIDS than in normal controls ($\alpha=0.048$). The median astrocyte density was significantly higher ($\alpha=0.004$) in the AIDS cases (groups 3-6) as compared with non-AIDS cases (group 2); while the density of RCA-120 microglia was significantly lower ($\alpha=0.001$) in the AIDS cases (groups 3-6) than in the non-AIDS group (group 2).

Table 2.20 Summary of GFAP-positive astrocyte density in clinical and pathological groups.

GFAP-positive astrocyte density/mm ²					
Clinical groups:	N	Mean	SD	Range	Median
1. Normal control	5	14.96	6.86	7.51-23.07	11.46
2. HIV + Non-AIDS	8	14.34	12.60	3.06-42.23	11.78
3. AIDS	23	29.94	11.88	8.9-50.60	27.80
AIDS groups:					
3a. No neuropathology	6	28.91	10.45	15.29-39.75	29.61
3b. HIV-1 p24 antigen +	8	35.63	11.55	22.24-50.60	34.75
3b i. HIVE/lep	6	39.23	11.09	22.72-50.60	42.12
3c. CMVE	5	32.75	9.75	25.30-49.49	30.02
3d. Cerebrovascular	4	16.47	8.36	8.90-27.19	14.89

Table 2.21 Summary of RCA-120-positive microglial density in clinical and pathological groups.

RCA-120-positive microglia density/mm ²					
Clinical groups:	N	Mean	SD	Range	Median
1. Normal control	5	5.06	3.05	0.83- 9.46	5.00
2. HIV + Non-AIDS	8	11.01	3.60	4.45-16.68	11.68
3. AIDS	23	4.81	3.63	1.11-15.29	3.89
AIDS groups:					
3a. No neuropathology	6	3.75	3.22	1.07- 9.17	3.06
3b. HIV-1 p24 antigen +	8	6.22	4.68	1.11-15.29	5.42
3b i. HIVE/lep	6	7.27	4.88	1.39-15.29	7.09
3c. CMVE	5	2.27	1.17	1.11- 3.89	1.94
3d. Cerebrovascular	4	6.60	2.25	3.34-8.33	7.37

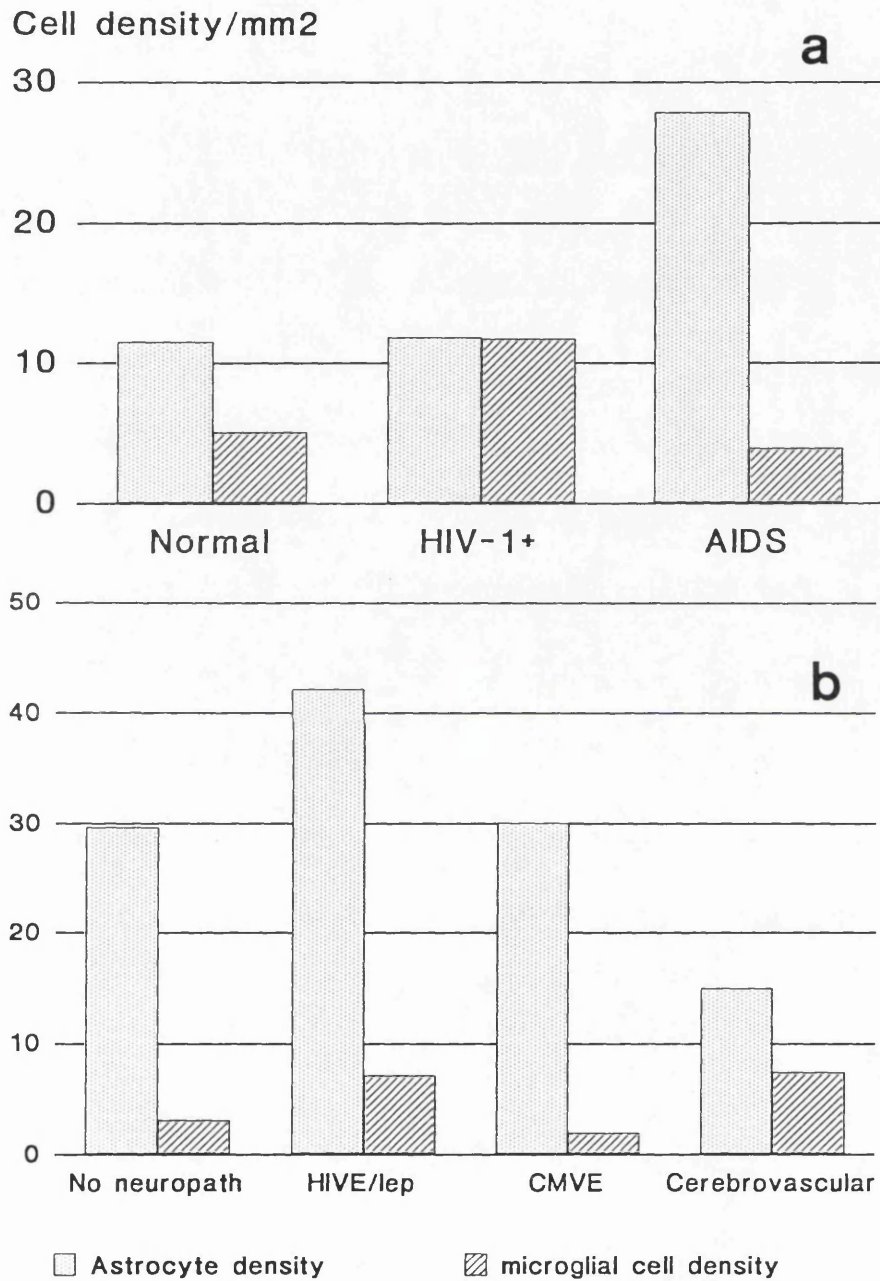


Figure 2.21 Median GFAP positive astrocyte and RCA-120-positive microglial cell densities per mm² for each clinical group (a) and each AIDS group (b)

Table 2.22 Mann-Whitney U test

Comparison, by Mann Whitney U test (95% confidence level)			GFAP-positive astrocytes $\alpha =$	RCA-120-positive microglia $\alpha =$
Control	vs	Non-AIDS	0.608	0.048
Control	vs	AIDS	0.014	0.834
Non-AIDS	vs	AIDS	0.004	0.001
No abnormalities	vs	HIV-1 p24 positive	0.220	0.230
No abnormalities	vs	HIVE/lep	0.093	0.229
No abnormalities	vs	CMVE	0.648	0.465
No abnormalities	vs	Cerebrovascular	0.110	0.241
HIVE/lep	vs	CMVE	0.523	0.068
HIVE/lep	vs	Cerebrovascular	0.025	0.749

2.3.6.2 Comparison of AIDS groups

The median density of GFAP astrocytes was highest in the HIV-1 p24 antigen-positive cases (group 3b), although the difference was not significant between this group and that with no neuropathological abnormalities (group 3a) ($\alpha=0.220$). Even when cases with only non-specific gliosis were discounted from group 3b, to form an HIVE/lep group (group 3b i), the median density was not significantly higher ($\alpha=0.093$) than that of group 3a. Similarly, no significant differences in RCA-120-positive microglial cell density were found between groups 3b or 3b i and group 3a ($\alpha=0.230$, $\alpha=0.229$). In addition, there was no significant difference in median astrocyte densities of CMVE cases (group 3c), and HIVE/lep cases (group 3b) ($\alpha=0.523$), and although the median density of microglia was much lower in group 3d than in group 3b, this difference did not reach significance ($\alpha=0.068$). In contrast, there was a significantly lower median astrocyte density in cerebrovascular

cases (group 3d) than in HIVE/lep (group 3b) ($\alpha = 0.025$), whilst the density of microglia was not significantly different between these two groups ($\alpha = 0.749$). The cerebrovascular group had the lowest density of GFAP-positive astrocytes but the highest density of RCA-120-positive cells.

3.3.7 Variation in astrocyte nuclear scores and microglia cell morphology.

The astrocyte MFNS and microglial cell MFMT were compared between clinical and between AIDS pathology groups. The number of cases in each group was too small to allow for statistical comparisons to be made; therefore the data were converted to percentages and plotted to allow visual comparisons between the groups. A comparison of the MFNS between clinical and AIDS pathology groups is illustrated in **figure 2.22**, cases with split scores (i.e 0-1, 1-2, 2-3) assigned to the higher category. Microglial MFMT, is compared between clinical and AIDS pathology groups in **fig 2.23**; cases with split scores (e.g ram/rod; ram/ram+/rod) were assigned as fractions, (0.5 or 0.33) to each morphological category.

2.3.7.1 Comparison of clinical groups

The proportion of cases with a high astrocyte MFNS increases with the severity of disease (**fig 2.22**). A MFNS of 0, was observed most often in the normal controls (group 1) and least often in the AIDS cases (group 3). In one of the normal controls, half of the HIV-1-seropositive non-AIDS cases and in a few of the AIDS cases the MFNS was 1. Scores of 2 and 3 were not observed in the normal control group; and only one of the non-AIDS cases had a MFNS of 2. In contrast, for nineteen out of

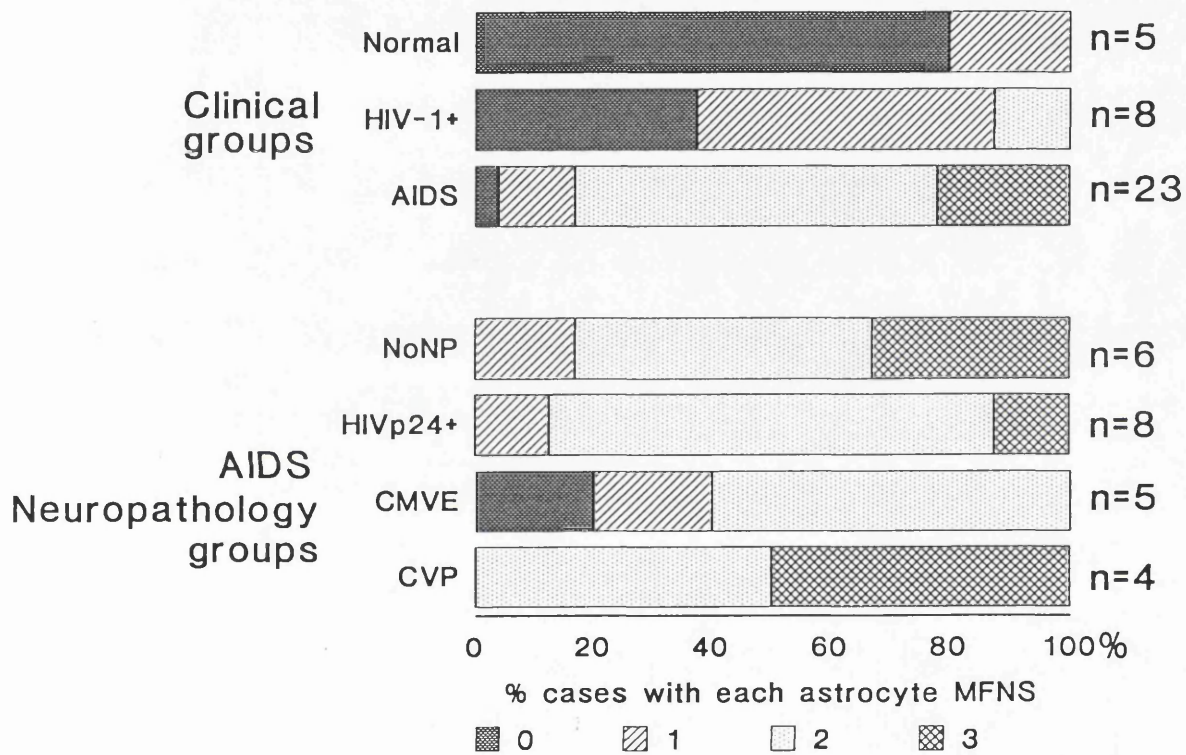


Figure 2.22 Comparison of the percentage of cases in each category of astrocyte "most frequent nuclear score" (MFNS) between clinical and AIDS pathology groups.

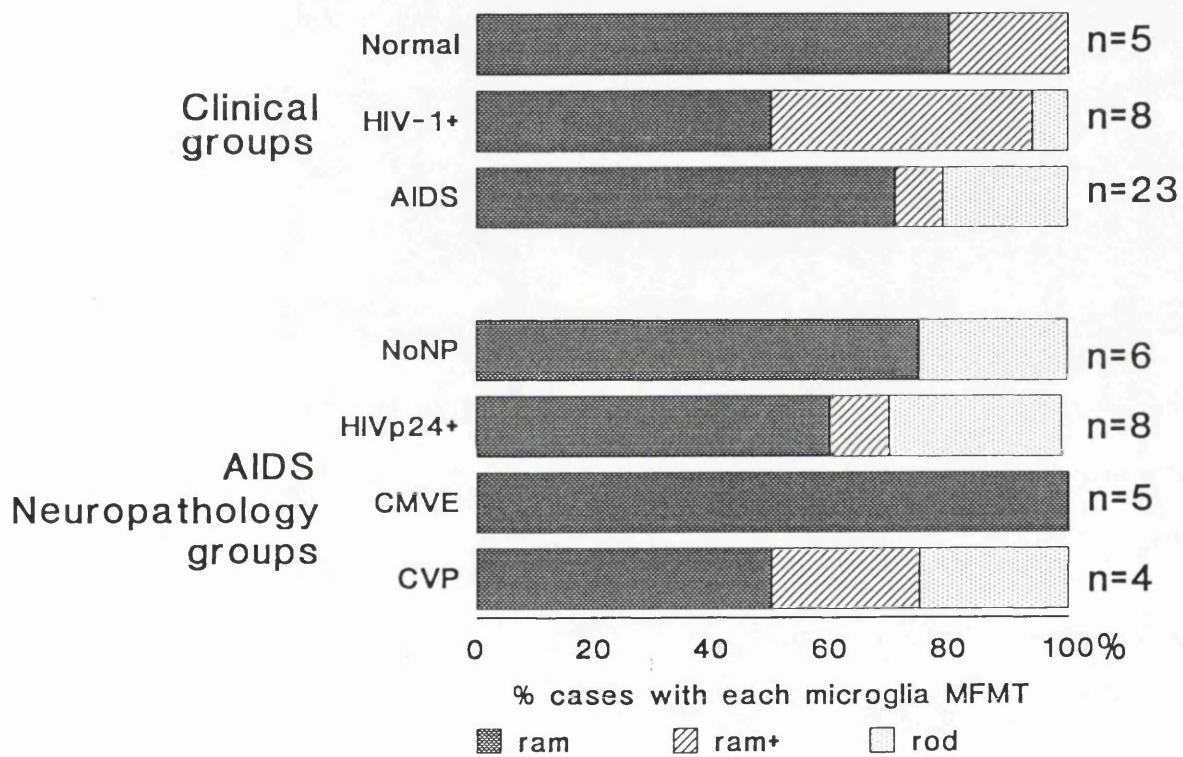


Figure 2.23 Comparison of percentage of cases in each category of microglial cell "most frequent morphological type" (MFMT) between clinical and AIDS pathology groups.

twenty-three AIDS cases the MFNS was 2 or 3.

Small ramified cells were the MFMT in most of the normal controls (**fig 2.23**); half of the HIV-1-seropositive non-AIDS cases and most of the AIDS cases. Enlarged ramified cells were the MFMT in a greater proportion of the HIV-1-seropositive non-AIDS than either of the other two groups. However, rod cells were the MFMT in a greater proportion of the AIDS cases than in the HIV-1-seropositive non-AIDS.

2.3.7.2 Comparison of AIDS pathology groups

An astrocyte MFNS of 2 was seen in the majority of cases in all of the AIDS pathology groups (**fig 2.22**), except for the cerebrovascular group where in two cases the MFNS was 2, and in the other two cases was 3. Type 3 astrocyte nuclei were also the MFNS in a small number of cases in the HIV-1 p24 antigen group, and in the no neuropathology group.

The microglial cell MFMT, was the small ramified cell type in the majority of cases in all AIDS pathology groups (**fig 2.23**). The enlarged ramified cell type was the MFMT in only a few cases whilst rod cells were more frequently observed in a slightly higher proportion of the HIV-1 p24 antigen positive cases, than in any of the other groups.

2.3.8 Variation in GFAP immunoreactivity and reactive astrocytes

The number of cases falling into each GFAP immunoreactivity category were

compared between clinical groups and between AIDS pathology groups. Since the number of cases in each group was small the data were treated as above and are illustrated in **figure 2.24** The number of cases in each group in which reactive astrocytes were observed are compared in **figure 2.25**.

2.3.8.1 Comparison of clinical groups

All normal controls (group 1) had a GFAP immunoreactive score of + for diffusely distributed astrocytes (**fig 2.24**). In the HIV-1-seropositive cases (group 2) the scores ranged from \pm to +++; in 3 cases there was a reduction in GFAP immunoreactivity as compared with the normal controls, and in 3 there was an increase. Scores ranged from + to +++ in the AIDS cases (groups 3-6), and GFAP immunoreactivity was increased in 60% of cases. Reactive astrocytes were not observed in any of the HIV-seronegative or non-AIDS cases, but were present in eight of the twenty-three AIDS cases (35%).

2.3.8.2 Comparison of AIDS pathology groups

Density of GFAP immunoreactivity was increased (++ or +++) in the majority of cases in all AIDS pathology groups (**fig 2.24**), with the exception of the no neuropathology cases, where density of GFAP immunoreactivity was increased in only one case. Reactive astrocytes were present in 50% of the HIV-1 antigen positive group and the cerebrovascular group (**fig 2.25**). These cells were observed in fewer cases from the other two AIDS groups.

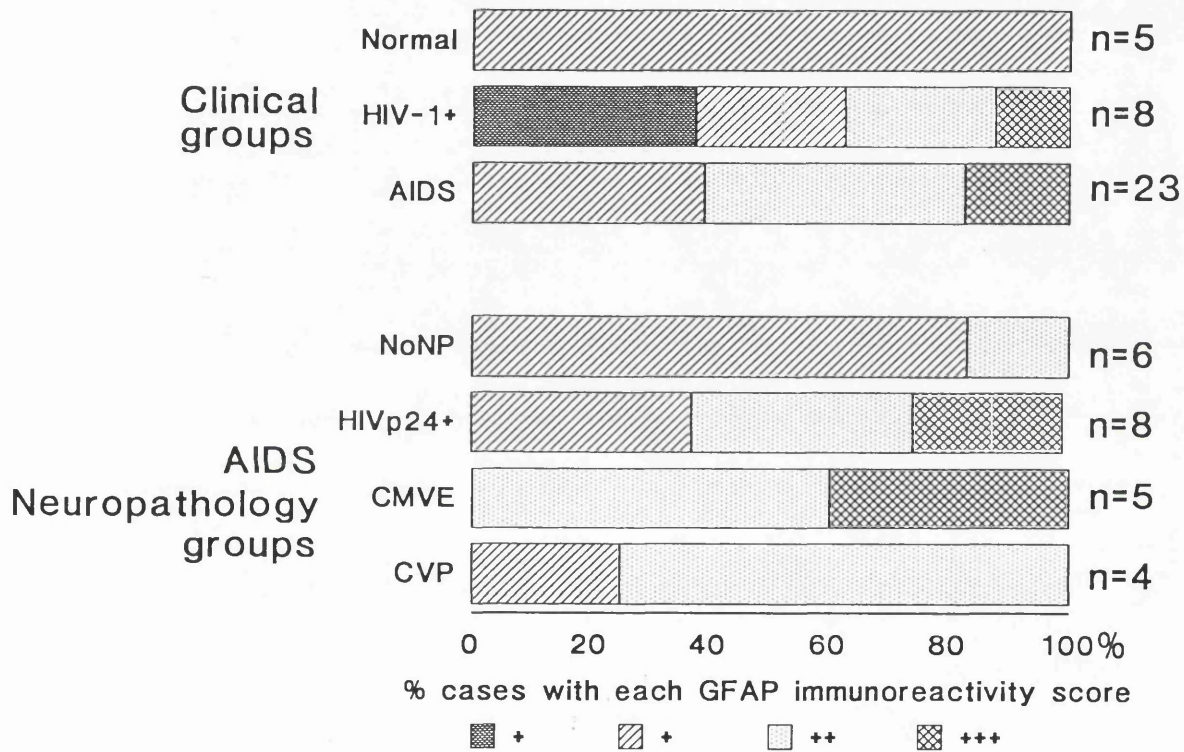


Figure 2.24 Comparison of percentage of cases in each category of "GFAP immunoreactivity" between clinical and AIDS pathology groups.

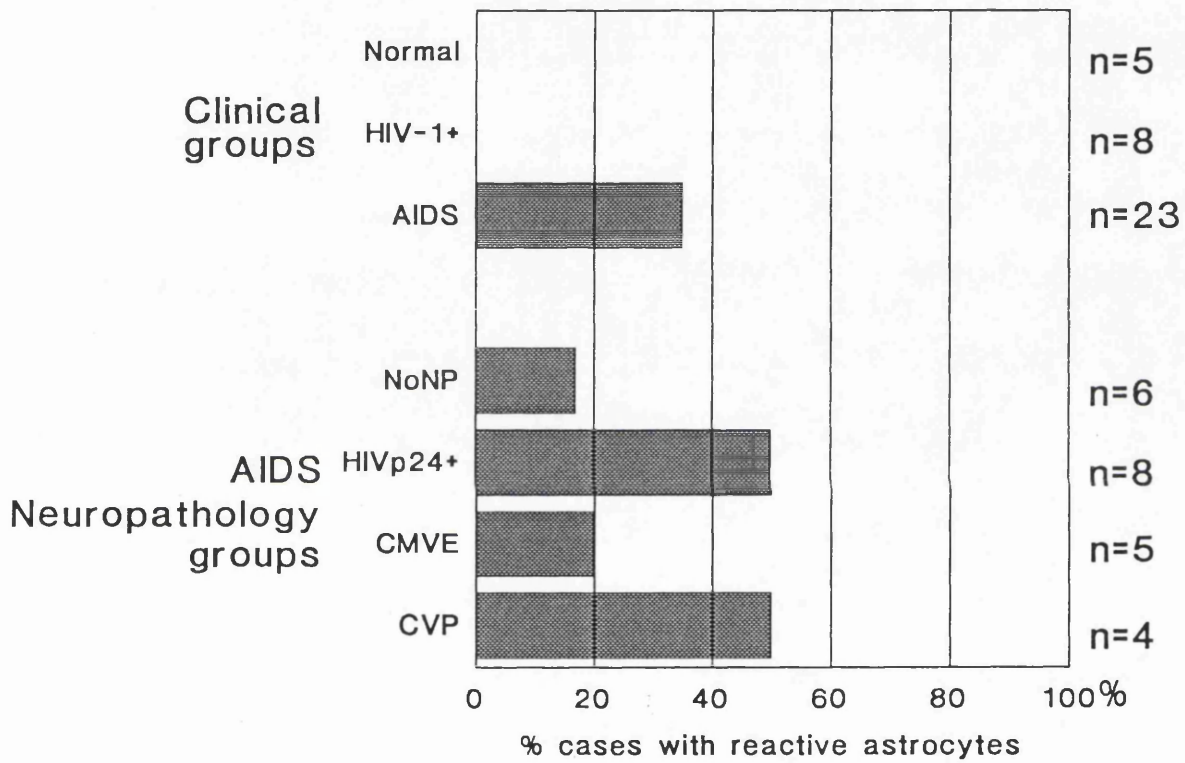


Figure 2.25 Comparison of the number of cases in which reactive astrocytes were observed between clinical and pathological groups.

2.3.9 *Variation in astrocyte and microglial cell distribution*

Three types of distribution of GFAP-positive astrocytes were observed: perivascular in which the majority of GFAP-positive cells surrounded vessels; diffuse, where there was an even distribution of astrocytes throughout the parenchyma, and diffuse/PV, in which a diffuse distribution of astrocytes was present but an obvious perivascular distribution of these cells was also evident. Lack of perivascular staining was either due to a reduction in GFAP expression by perivascular astrocytes or masking of the perivascular distribution in cases where there was a large increase in the density of cells in the parenchyma. The proportion of cases in each clinical and AIDS pathology groups showing each pattern of distribution is illustrated in **figure 2.26**.

Three types of distribution of RCA-120-positive microglial cells were observed; diffuse, patchy and rare. The term diffuse was applied to cases with an even distribution of positive cells throughout the cortex; 'patchy' to sections with a diffuse but uneven distribution of cells; and 'rare', when positive cells were difficult to locate. The proportion of cases in clinical and AIDS pathology groups that show each pattern of distribution is shown in **figure 2.27**.

Two types of foci were distinguished: The term 'MGN' was used to define tight clusters of RCA-120 cells and 'loose foci' was used to describe loose clusters of RCA-120-positive cells. Foci were only observed in the AIDS groups; the percentage of cases in each AIDS group with focal lesions is shown in **figure 2.28**.

2.3.9.1 Comparison of clinical groups

GFAP-positive astrocytes

The most frequently observed distribution of GFAP-positive astrocytes (**fig 2.26**) was perivascular in the normal controls (group 1), diffuse/PV in the HIV-1-seropositive non-AIDS cases (group 2) and diffuse in the AIDS cases (group 3). Foci of astrocytes were not observed in any of the normal control or non-AIDS cases, while they were observed in five (22%) of the AIDS cases.

RCA-120-positive microglial cells

The most frequently observed distribution of microglial cells (**fig 2.27**) was diffuse in both the normal controls and HIV-1-seropositive non-AIDS cases. However, there was an equal number of AIDS cases in which each of the three distributions were observed. Foci of microglial cells were observed in 11/23 of the AIDS cases; MGNs were observed in 5 cases and loose foci of microglial cell in 7 cases.

2.3.9.2 Comparison of AIDS pathology groups

GFAP-positive astrocytes

In all cases, the observed distribution of GFAP astrocytes (**fig 2.26**) was either diffuse/PV or diffuse. The distribution in the majority of cases in all groups was diffuse. Loose foci of astrocytes were present in 3 cases of the HIV p24 antigen positive group and 2 cases of the cerebrovascular group (**fig 2.28**).

RCA-120 positive microglial cells

There were no remarkable differences between the numbers of cases with each

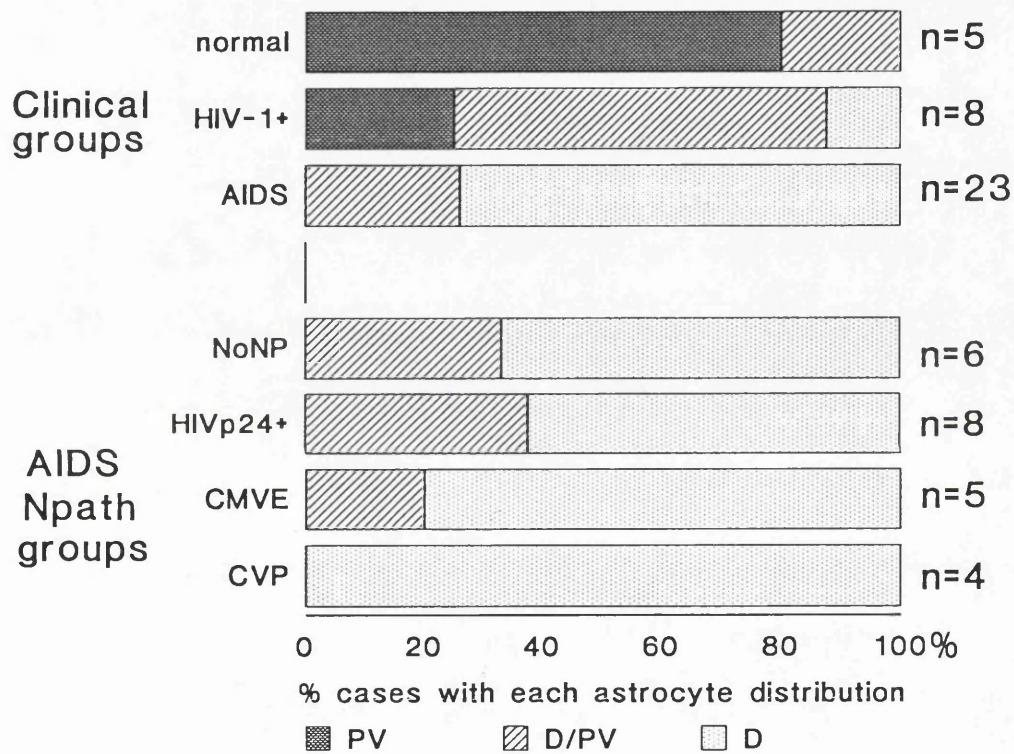


Figure 2.26 Comparison of percentage of cases in each category of "GFAP positive astrocytes distribution" between clinical and AIDS pathology groups.

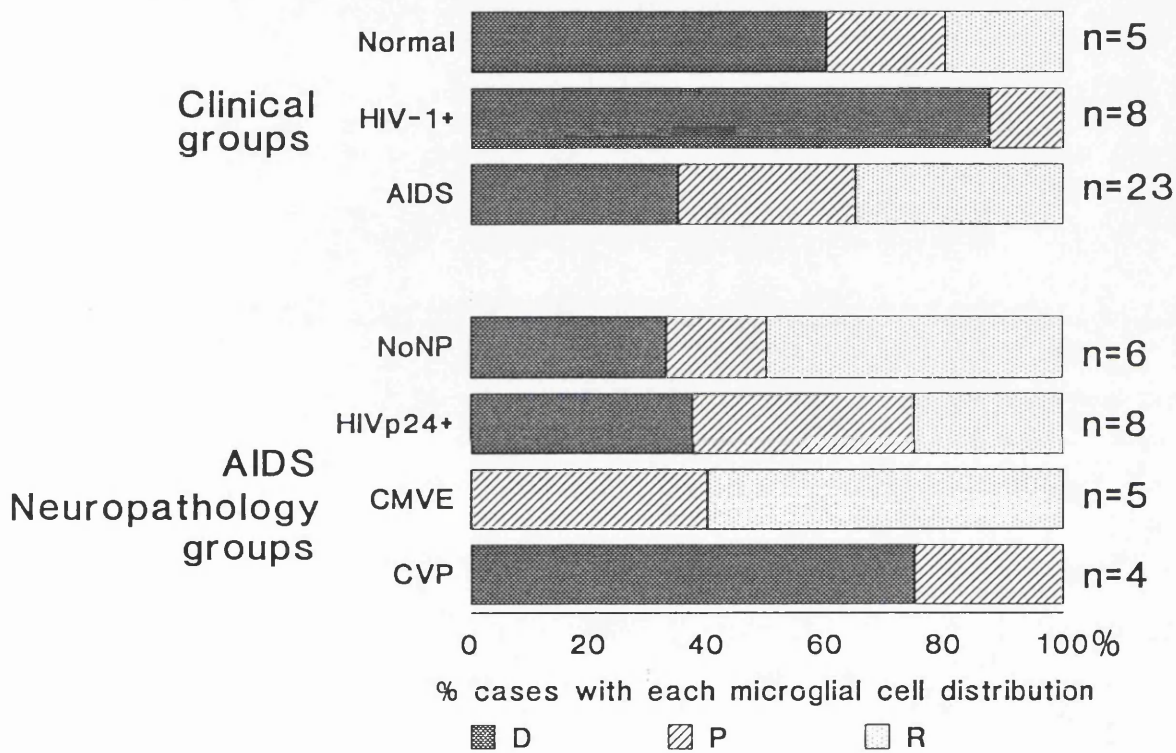


Figure 2.27 Comparison of the percentage of cases in each category of "RCA-120-positive microglial cell distribution" between clinical and AIDS pathology groups.

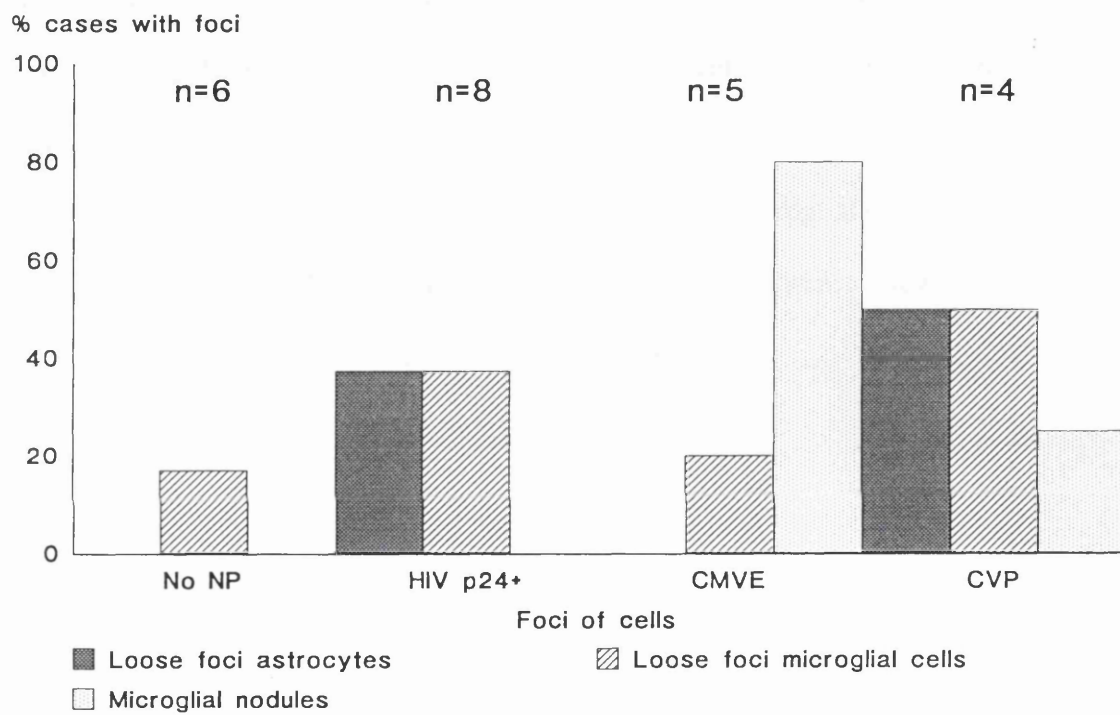


Figure 2.28 Percentage of cases in each AIDS pathology group with foci of astrocytes and microglial cells.

observed microglial cell distribution between the different groups (**fig 2.27**). However, a rare distribution was observed in most of the CMVE cases, whilst a diffuse distribution was observed in most of the cerebrovascular cases in each group. A fairly even proportion of cases from both the no neuropathology and HIV p24 antigen-positive groups were present in each category. MGNs were found in four out of five of the CMVE cases and in one of the cerebrovascular cases but not in any of the other groups (**fig 2.28**), whilst loose foci of microglial cells were observed in three cases from the HIV antigen positive group and two from the cerebrovascular group that also contained loose foci of astrocytes. Loose foci of microglial cells were also present in one case of CMVE, and one of cerebrovascular disease, however astrocytes were not observed in association with these lesions.

2.3.10 Summary

A number of changes in the appearance of astrocytes and microglial cells were observed in the frontal cortex of AIDS and HIV-1-seropositive non-AIDS cases, as compared with the normal controls. The density of GFAP positive astrocytes was increased in most AIDS cases, and there was an increased density of RCA-120-positive microglial cells in some. The latter type of cells were increased in the HIV-1-seropositive non-AIDS cases, while the density of astrocytes was variable in this group. In some of the non-AIDS cases there was a reduced density of astrocytes, in others it was increased, whilst the remainder showed no change. An increase in the nuclear volume of astrocytes was observed in the majority of AIDS cases and in some of the HIV-1-seropositive non-AIDS cases, in addition reactive astrocytes were

present in many of the AIDS cases. There was also an increase in GFAP immunoreactivity in many of the AIDS cases and some of the HIV-1-seropositive non-AIDS cases, although in some of the latter group a reduction in GFAP immunoreactivity was observed. In addition there was a loss of perivascular GFAP immunoreactivity in some of the AIDS and HIV-1-seropositive non-AIDS cases. Reactive microglial cells were present in many of the HIV-1-seropositive and AIDS cases and in some of the latter group the distribution of these cells was not diffuse, as in the normal controls but patchy. Foci of cells were observed only in the AIDS cases, MGNs as well as loose foci of microglial cells and astrocytes were observed.

None of the observed changes, in astrocyte and microglial cells, in the cortex of AIDS brains occurred exclusively in cases in which HIV-1 antigens were identified by immunohistochemistry, though differences between groups were observed. In the HIV antigen positive group the density of both astrocytes and microglial cells was high, whilst in the CMVE group the former was high and the latter low. In the cerebrovascular group astrocyte density was similar to the normal controls, whilst the density and reactivity of microglial cells was similar to the HIV-1 antigen positive cases. Perivascular GFAP immunoreactivity was reduced in the no pathology group, and there was no increase in GFAP immunoreactivity of parenchymal astrocytes. In the other three AIDS groups parenchymal GFAP immunoreactivity was increased in most cases and an obvious perivascular accumulation of astrocytes was often absent.

CHAPTER THREE:

**DETECTION OF HIV-1 PROVIRAL DNA WITH THE
POLYMERASE CHAIN REACTION, AND
THE RELATIONSHIP WITH
PATHOLOGY OF THE FRONTAL CORTEX**

3.1 Materials and Methods

3.1.1 *Subjects and Tissues*

PCR was used to detect HIV-1 proviral DNA in a total of eighteen AIDS cases from which fresh frozen brain tissue was available. All cases were from the Middlesex-University College Hospital, AIDS series and included ten of the cases studied in chapter 2. PCR was also carried out on eight additional cases that were not included in chapter 2. **Table 3.1** lists the post mortem diagnosis and neurological findings in these additional cases. They consisted of: two cases each of lymphoma (33,34), PML (35,36) and cryptococcosis (37,38), one of the latter also had a single HIVE lesion in the basal ganglia (38); one case of HIVE (39) and one of non-specific gliosis (32). In six of these (the lymphoma, PML and cryptococcosis cases) the cortex was affected by these lesions. The cortical reaction was assessed in these cases, as in chapter 2; cell counting, assessment of astrocyte nuclear changes and microglial morphological changes being performed on regions of the cortex not involved by these lesions. However, it is likely that the cortical reaction, even in areas not involved in these lesions was to some extent affected by them, and this was taken into consideration when assessing the results.

Frozen tissue samples from the HIV-1-seropositive non-AIDS cases, provided by Professor Gray and Dr Bell, were also analysed by PCR. Due to difficulty in extracting sufficient quantities of DNA from small samples of white matter, in some of these cases, PCR was only performed on cortical samples.

Table 3.1 Age, sex, post-mortem diagnosis and salient neurological details in additional AIDS cases selected for PCR.

Case	Age	Sex	PM diagnosis	Salient Neurological details
32	30	♂	Kaposi's sarcoma	None recorded
33	37	♂	Pneumonia Raised intracranial pressure	Right sided hemiplegia
34	42	♂	Pulmonary lymphoma	Dementia Limb rigidity
35	39	♂	Pneumonia	Dementia
36	56	♂	? PCP	Dysplasia Ataxia Right hemiplegia
37	48	♂	Cryptococcosis	Recurrent cryptococcal meningitis
38	25	♂	Fungal pneumonia Cryptococcal meningitis	Cerebral symptoms
39	53	♂	Pulmonary oedema Kaposi's sarcoma	Withdrawal Mania

3.1.2 Statistical analysis

3.1.2.1 Detection of HIV-1 by PCR and immunohistochemistry in the cortex and white matter.

Multiple PCR assays were carried out on DNA samples from the cortex and white matter of all cases analysed by PCR. To test whether virus is equally likely to be detected in both regions, the agreement between the frequency of positive results in these two regions was tested by calculating the confidence interval.

In order to compare the detection of HIV-1 by PCR and immunohistochemistry in

cortical and white matter regions, the percentage of cases positive with each method was compared, using McNemar's test for paired samples.

3.1.2.2 Relationship between HIV-1 detection and the occurrence of cortical pathology

Tables 3.2 and 3.3 show the morphometric results, obtained for GFAP-positive astrocytes and RCA-120-positive microglial cells respectively, in the eight additional cases on which PCR was performed (32-39). These results, together with the morphometric results already obtained for cases that were included in chapter 2, were used to compare astrocytic and microglial cell changes between the following two groups:

HIVC+ Cases in which HIV-1 proviral DNA was detected in the cortex by PCR, plus two cases in which HIV-1 antigens were visualised in the cortex, in part two, (but which were not tested by PCR) formed an HIV-1 cortex positive group.

HIVC±/- Cases that were negative or indeterminate for HIV-1 by PCR formed an HIV-1 cortex indeterminate/negative group.

A Mann-Whitney U test was used to test for differences in astrocyte and microglial cell densities between these two groups. The astrocyte MFNS, GFAP immunoreactivity, microglial cell MFMT, GFAP-positive astrocyte and RCA-120-positive microglial cell distributions were also compared. Since the data from morphometric measurements, other than cell densities, were discrete rather than

continuous measurements a test of frequency was used to examine differences between the HIVC+ and HIVC \pm /- groups. The number of cases that fell into each of the categories of: astrocyte MFNS, GFAP immunoreactivity, microglial cell MFMT, GFAP-positive astrocyte and RCA-120-positive microglial cell distributions, were compared using the chi-squared test for trends in proportions between the HIVC+ and HIVC \pm /- groups.

The astrocyte and microglial cell reactions of the two HIV-1-seropositive non-AIDS cases in which HIV-1 proviral DNA was detected by PCR, were compared with cases in this group that were negative or indeterminate. There was not sufficient data for any statistical analysis to be carried out in this group.

In five of the additional eight cases that were not included in chapter 2 (32,33,34,38 and 39), the formalin fixation time was prolonged (longer than four months) and this may have reduced immunoreactivity or lectin reactivity. Therefore comparisons were made between cases fixed for longer than or less than four months, prior to testing for differences between the HIVC+ and HIVC-/ \pm groups. A Mann-Whitney U test was used to test for differences in cell astrocyte and microglial cell density between these two groups. Data from the other measurements were insufficient for statistical analysis; however, in order for visual comparisons to be made it was illustrated in graph form.

Table 3.2 Density, reactivity, and distribution of GFAP-positive astrocytes in the frontal cortex of cases 33-40.

Case	Astrocyte Density Cells/mm ² (Mean of 10 counts)	Astrocyte MFNS	GFAP immuno- reactivity	Diffuse RA	Predominant distribution of GFAP- positive astrocytes	Loose foci of GFAP- positive astrocytes
32	7.23	2	++	-	diffuse	loose foci reactive astrocytes
33	4.45	1	+	-	rare	
34	18.63	2	++	-	diffuse/PV	
35	9.73	2	+	+	diffuse	large foci reactive astrocytes
36	18.90	1	+	-	diffuse/PV	small foci reactive astrocytes (PML)
37	28.63	1	+++	-	patchy/PV	large foci, fibrous astrocytes
38	16.68	2	+	-	patchy/PV	large foci, fibrous astrocytes
5039	10.29	2	+	-	diffuse/PV	small loose foci reactive astrocytes

MFNS- most frequently observed nuclear score

RA - reactive astrocytes

Table 3.7 Astrocyte nuclear reactivity, and distribution of RCA-120-positive microglia in the frontal cortex of cases 33-40.

Case	Microglial Density Cells/mm ² (Mean of 10 counts)	Microglial morphology MFMT	Predominant distribution of RCA-120- positive cells	Foci of RCA-120- positive cells
32	2.5	ramified +	rare	numerous small foci of ram and rod cells
33	2.78	ramified	diffuse	
34	3.34	ramified +	rare	
35	2.78	ram-ram +	rare	large loose foci (PML)
36	0.56	ramified	rare	loose foci (PML)
37	3.06	ramified +	patchy	large foci (crypto)
38	3.61	ram-ram +	patchy	large foci (crypto)
39	8.06	ramified +	diffuse	small foci ram and rod

MFMT - most frequently observed morphological type

crypto - cryptococcosis

3.1.3 Preparation of DNA

The frontal poles of brains removed within 48 hours of death were frozen at -20°C prior to separation of cortex and white matter samples. After stripping the leptomeninges from the semi-frozen specimens, thin slices of grey matter were taken from the surface of the cortex, and checked to be free of white matter. Using separate disposable scalpels, cubes of deep white matter were removed, and diced into small pieces to ensure they did not contain grey matter. Multiple samples of around 0.5g of both tissues from each brain were snap frozen by immersion in liquid nitrogen, and stored below -70°C . Tissues were digested, and DNA was purified by standard methods, described in appendix A. The concentration of DNA extracted from each sample was estimated using a Beckman spectrophotometer.

3.1.4 PCR Methodology

The methodology used to amplify HIV-1 sequences in this study was adopted following participation in a Medical Research Council (MRC), PCR collaborative study organised by the PCR reference laboratory, the National Institute for Biological Standards and controls (NIBSC) (Bootman and Kitchin, 1992). A set of reagents already tested for their efficiency was sent to each participant together with 10 coded samples that contained different amounts of HIV-1 plasmid DNA, HIV-1 negative carrier DNA, or water; the contents of each tube were unknown to participants. The purpose of this collaborative study was to test the accuracy with which different laboratories could assay samples using PCR, and to determine how frequently false

positive and false negative results occurred.

3.1.4.1 HIV-1 reference reagents (National Institute of Biological Standard and control)

HIV-1 positive samples contained the molecular clone pBH10 which has an insert from lambda BH10, sub-cloned into the *Sac I* site of the SP64 vector. This plasmid has deletions in the LTRs, which renders it non-infectious. Ten-fold serial dilutions of the plasmid from 10,000 molecules down to 0.1 molecules per 2.5 μ l were used to spike 100ng of carrier DNA; in addition samples containing carrier DNA only, and no DNA water controls were included. Three sets of primer pairs, that had been designed by Simmonds et al. (1990) to amplify conserved regions of the *gag*, *pol* and *env* genes, were provided; sequences are given in table 3.4. In addition, oligonucleotide probes, internal to the amplified sequences, were provided for Southern blotting; their sequences are given in table 3.5.

Table 3.4 Oligonucleotide primer sequences

Name (NIBSC)	Sequence	Target size (bp) within gene
HG 1214 N	5' - GGT ACA TCA GGC CAT ATC ACC	473 <i>gag</i>
HG 1686 C	5' - ACC GGT CTA CAT AGT CTC	
HP 4149 N	5' - CAT GGG TAC CAG CAC ACA AAG G	244 <i>pol</i>
HP 4392 C	5' - TCT ACT TGT CCA TGC AGT GCT TC	
HE 6539 N	5' - GAG GAT ATA ATC AGT TTA TGG	438 <i>env</i>
HE 6976 C	5' - AAT TCC ATG TGT ACA TTG TCA TG	

Table 3.5 Oligonucleotide probe sequences

Name	length	sequence
HG 1400 N	30mer	5'- CAT CAA TGA GGA AGC TGC AGA ATG GGA TAG
HG 1426 C	10mer	5'-TCC CAT TCT G
HP 4316 N	30mer	5'-CCT GTA GTA GCA AAA GAA ATA GTA GCC AGC
HP 4342 C	10mer	5'-GGC TAC TAT T
HE 6830 N	30mer	5'-CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA
HE 6856 C	10mer	5'-CTC AAA GGA T

Key to tables 3.2 and 3.3: H - HIV-1; G - *gag*; P - *pol*; E - *env*; N - normal strand; C - complimentary strand; numbers indicate position of 5' termini of primer in HIV-1 genome (HXB2)

3.1.4.2 Amplification

The reaction was carried out in a buffer containing 50mM KCL, 10mM Tris-HCL pH 8.3, 200 μ M each of dATP, dCTP, dGTP and dTTP, 1.25 units of Taq Polymerase, 0.2 μ M of each primer. The MgCl₂ concentration was titrated by the NIBSC to determine the optimal concentration for each set of primers (*gag* 1.2mM; *pol* 1.0mM; *env* 1.4mM). Forty rounds of thermal cycling were carried out on a Perkin Elmer Cetus thermal cycler; the profile used is described in appendix B.

3.1.4.3 Post-PCR analysis

Reaction products were analysed on a 1.75% agarose gel, and visualised by ethidium bromide staining. Forty μ l of each reaction product were mixed with 12 μ l of gel loading buffer, and 13 μ l of this mixture loaded onto the gel alongside a 123 base pair molecular weight marker (Gibco). PCR products were transferred onto a nylon membrane by Southern blot (appendix C) and hybridized with non-isotopically

labelled probes to confirm the specificity of the reaction products. Probes were labelled with a modification of the Saluz and Jost (1986) "filling in" method for short oligonucleotides, which was modified for labelling with digoxigenin-11-dUTP (DIG, Boehringer, Mannheim). The probe labelling procedure is described in full in appendix D. Hybridisation and detection of labelled probe was carried out according to Boehringer, Mannheim's protocols for DIG labelled probes. (detailed in appendix E).

3.1.3.4 Sensitivity of PCR for HIV-1 with NIBSC reference reagents

Following PCR and Southern blot hybridisation this PCR method was sufficiently sensitive to detect a single molecule of HIV-1 DNA in 100ng of carrier DNA (**table 3.6, fig 3.1**). There were no false negative results, indicating that the methodology used was reliable and results reproducible. Most importantly, there were no false positive results indicating that cross over contamination between samples had been prevented and that positive results were genuine.

When amplifying human DNA, 1 μ g is normally used as a template for PCR; this fairly large amount of DNA may reduce the efficiency of the reaction, due to the increased proportion of non-appropriate DNA sequences to appropriate ones. Therefore 1 μ g of DNA extracted from samples of either cortex or sub-cortical white matter was added to the above samples, and the method was repeated. In the presence of 1 μ g of human genomic DNA, the sensitivity of the reaction was reduced, 1 molecule was rarely detected, 10 molecules were detected in 80% of tests and 100 molecules were always detected. The sensitivity of the PCR test was therefore between 10 and 100 copies.



Figure 3.1 PCR amplification and Southern blot, of HIV-1 plasmid dilutions and negative control samples, provided by the NIBSC. 0.1 (lane C); 1 (lane D); 10 (lane E); 100 (lane F); 1000 (lanes B and H); 10,000 (lane I) molecules of HIV-1 plasmid DNA. Carrier DNA (Lane A); H₂O controls (lanes G and J). All samples containing 1 molecule or more of HIV-1 DNA are positive in both the gel and following Southern blotting; whilst the H₂O controls, and the 0.1 molecule dilution of HIV-1 DNA are negative. The carrier DNA sample (lane A) appears positive in the gel, but following Southern blotting is negative, demonstrating that this band is non-specific.

Table 3.6 PCR results obtained with reagents provided by NIBSC collaborative study following Southern blotting

Sample	Template: Number of HIV-1 DNA molecules per sample.	<i>gag</i>		<i>pol</i>		<i>env</i>	
		Gel	SB	Gel	SB	Gel	SB
A	Carrier DNA, No HIV	-	-	-	-	-	-
B	1000	+	+	+	+	+	+
C	0.1	-	-	-	-	-	-
D	1.0	+	+	-	+	+	+
E	10	+	+	+	+	+	+
F	100	+	+	+	+	+	+
G	H ₂ O control	-	-	-	-	-	-
H	1000	+	+	+	+	+	+
I	10,000	+	+	+	+	+	+
J	H ₂ O control	-	-	-	-	-	-

3.1.5 Measures adopted to prevent contamination

Because of the efficiency with which the polymerase chain reaction amplifies DNA, false positive results are a common problem, usually due to contamination of the reaction mixture with the specific sequence that is being amplified. Both purified template DNA and reaction products from previous PCR assays can potentially contaminate PCR mixes, by a number of routes. Aerosols of DNA molecules may enter the barrels of pipettes while templates or products are being handled and subsequently be transferred to reagents used for setting up PCR (Kwok and Higuchi, 1989). Spillage of solutions containing these molecules can persistently contaminate a laboratory area and may enter the PCR mixture. Small DNA molecules may adhere

to the skin of PCR operators and shed epidermal cells may contaminate subsequent reactions (NIBSC, personal communication). In addition, traces of DNA molecules can accidentally be transferred to the outside of tubes while pipetting. However, it is not difficult to set up this technique in a manner which greatly reduces the likelihood of contamination, and providing sufficient negative controls are included to monitor the effectiveness of these measures, the results of this method can be relied upon. The measures adopted in this study to prevent contamination are described below.

3.1.5.1 Preparation of PCR reagents

Three separate laboratory areas were used for PCR. In laboratory 1, an area where no DNA work takes place, the reaction was set up within a laminar flow cabinet which further reduces the chances of contamination. All PCR reagents were stored in a freezer located in this laboratory and a set of "clean pipettes" dedicated to setting up PCR were used. A clean coat was kept in laboratory 1 and coats worn in other laboratories kept out of this laboratory; in addition a face mask was worn when PCR reagents were being prepared.

3.1.5.2 Addition of template DNA

After preparing and aliquoting a master mix of PCR reaction buffer, reaction tubes were taken to a second laboratory. Here DNA templates were added to the reaction buffer within a microbiological safety cabinet. All DNA templates were mixed then briefly spun down to ensure that liquid was collected at the bottom of the tube, before opening. All samples containing DNA were handled with solid displacement pipettes

(Finpipettes), which prevented cross over contamination between samples. DNA templates were stored separately from PCR reagents.

3.1.5.3 Post-PCR analysis

Following addition of the template DNA, reaction tubes were transferred to laboratory 3, where the thermal cycler was situated and all post-PCR analysis took place. A third set of pipettes dedicated to handling PCR reaction products only and PCR products were stored in laboratory 3. Protective clothing (a disposable lab coat, face mask, cap and gloves) was worn in laboratory 3 while PCR products were being handled to prevent attachment of aerosols of products to skin or clothes.

3.1.5.4 Additional precautions to prevent contamination

Separate ice buckets were used in each area or designated for use in transporting samples from one area to another. Gloves were worn at all stages and changed frequently. Care was taken in the disposal of waste produced in laboratories 2 and 3; all waste was bagged and sealed separately from general laboratory waste to prevent accidental spread of DNA to other areas.

3.1.6 PCR controls

3.1.6.1 Sample control

All samples were initially amplified with human β -globin primers (Perkins, Cetus, Elmer) to ensure the suitability of the template for PCR. Only samples that were clearly positive with these primers were used for HIV-1 PCR (**figure 3.2**).

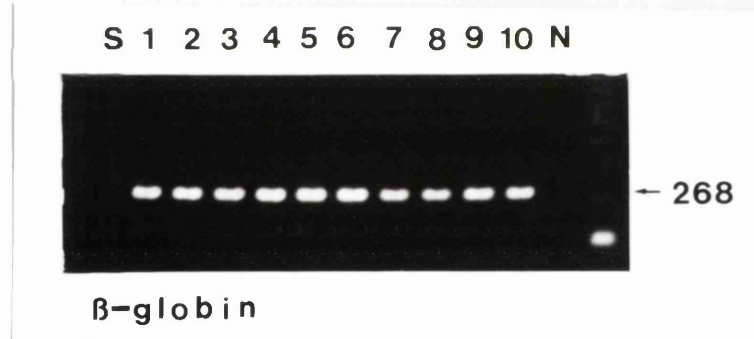


Figure 3.2 PCR amplification of 1 μ g of DNA from the cerebral cortex of AIDS and HIV-negative patients to amplify a 268 base pair fragment of human β -globin sequence. Cortical samples from HIV-1-infected (lanes 1-7) and HIV-negative (lanes 8-10) patients are positive, whereas ssDNA (S) and no-DNA (N) samples are negative.

3.1.6.2 Positive control

1 μg of DNA from HIV-1 seronegative cortical and white matter samples were spiked with 10 molecules of non-infectious plasmid containing complete re-arranged HIV-1 sequence (provided by MRC ADP repository, NIBSC) and used as positive control.

3.1.6.3 Negative controls

DNA from brains of patients not in any of the HIV-1 risk groups, salmon sperm DNA (ssDNA) and no DNA controls were taken through each experiment to ensure that there was no carry over from positive samples. HIV-negative controls consisted of 3 cases used in part 1 (c2,c4 and c5), one case of epilepsy and one of right temporal lobe intracerebral haemorrhage.

3.1.7 PCR Pilot Study

The inherent sensitivity of the PCR reaction can be problematic when tissues are used as a source of nucleic acids, as all tissue samples will be contaminated by blood cells, which may contain the target sequences being investigated. In addition, post mortem brain samples may also be contaminated by cells from the cerebrospinal fluid. Positive results may therefore occur due to contamination of tissues with infected cells from blood or CSF. In most PCR assays around 1 μg of human DNA is used as a template; since a single diploid cell in the G1 phase of cell division contains 6.6pg of DNA, 1 μg of DNA corresponds to approximately 150,000 cells (Simmonds et al., 1990). Simmonds et al. (1990) found that when amplifying DNA from purified peripheral blood mononuclear cells, between 700 and 3,300 cells were required to

detect one HIV-1 provirus molecule; depending on the stage of infection (in purified CD4 positive cells, there may be one molecule of HIV-1 DNA per 100 CD4 cells; Schnittman et al., 1989). Therefore, in order to detect contaminating infected blood cells from a brain sample, at least 700 out of the 150,000 cells present in 1 μ g of DNA would have to be peripheral blood mononuclear cells. However, since the assay used in this study has a detection limit of around 10 molecules of HIV-1 DNA per 1 μ g DNA, 7000 peripheral blood cells (or 1000 CD4 positive cells) would have to be present in the sample in order to produce a positive result. It seems unlikely that every 21st cell (150,000/7000) in a brain sample will be a peripheral blood mononuclear cell, or every 150th cell a CD4 positive mononuclear cell. Nevertheless, if only a single PCR assay were to be carried out, it is theoretically possible that the presence of infected PBMCs in that sample could yield a positive result. However, if multiple tests are performed this possibility should easily be ruled out.

In order to determine the number of tests necessary to achieve a reproducible result, and to compare the sensitivity of different primers, we carried out a pilot study in which all samples were amplified three times with each primer pair, i.e. a total of 9 PCR assays were carried out on each sample in order to assess the usefulness of the NIBSC PCR method for amplifying DNA from brain samples. All seven cases in this preliminary study were AIDS patients with and without HIV-1 related neuropathology, cases 16, 17 and 28 were described in chapter 2 while 33, 34 35 and 37 are described in section 3.1.1. DNA extraction, PCR and Southern blotting were all carried out as described above.

3.2 Results

3.2.1 Results of PCR pilot study

The results of nine PCR assays on each sample, confirmed by Southern blotting, are shown in **table 3.7** and an example of these results is illustrated in **figure 3.3**. In each case, cortical and white matter samples were tested three times with each set of primers, so that nine PCR tests were performed on each cortical and each white matter sample. The results fell into three categories. 1) Ten samples were positive in at least four (but usually more) out of nine tests and were therefore considered positive. 2) Two samples, positive in only one or two out of nine tests, were designated indeterminate. 3) The remaining two samples were consistently negative. Consistently negative results, obtained in both cortex and white matter samples of case 28, indicated that even in full-blown AIDS, this method was not sensitive enough to pick up blood or CSF cells that may have contaminated this case. Cortical and white matter samples of case 33 were positive once or twice; therefore the possibility that this is a false positive result due to contamination of the brain parenchyma by infected cells from the blood or CSF, cannot be ruled out. These samples were therefore designated as indeterminate. Alternatively, indeterminate samples may have contained less than 10 molecules of HIV-1 DNA per μg of genomic DNA, and therefore only give occasional positive results. The variability of results in some of these cases emphasises the need to perform multiple PCR tests on each sample. However, from these results it does not seem necessary to carry out nine PCR test on each sample. Before deciding how many assays were necessary to obtain a

Table 3.7 PCR pilot study: frequency of HIV-1 proviral DNA detection in cortical and white matter samples after PCR and Southern blotting.

primers	Cortex				White Matter			
	<i>gag</i>	<i>pol</i>	<i>env</i>	T	<i>gag</i>	<i>pol</i>	<i>env</i>	T
case no								
16	+++	+++	+++	9	+++	- ++	+++	8
17	+++	+++	+++	9	+++	+++	+ - +	8
28	- - -	- - -	- - -	0	- - -	- - -	- - -	0
33	- - +	- - -	- - +	2	- - -	- - +	- - -	1
34	+++	+++	- - +	7	+ - -	+++	- + -	5
35	+++	+++	+++	9	+++	+++	+ - +	8
37	- + -	+ + -	+ - -	4	- + -	+++	+ - -	5

Each symbol represents the score, either positive, + or negative, - of one test.

A total of nine tests, three with each set of primers, was performed on each sample.

T - total number of positive results after 9 PCR assays.

Samples with 4 or more positive tests out of 9 were considered positive, samples that were positive in only 1 or 2 tests were considered indeterminate.

Primers: Simmonds et al. (1990) - *gag* (881,882), *pol* (001,004) and *env* (401,404)
 NIBSC - *gag* (HG1214N, HG1686C), *pol* (HP4149N, HP4392C), *env* (HE6539N, HE6976C).

reliable result, the performance of each set of primer pairs was compared.

The three different sets of primers gave very similar results; the *gag* primers gave a positive result in 25 out of 42 assays, the *pol* in 29 and the *env* in 21. A one way analysis of variance did not detect a significant difference between the number of positive results obtained using these three sets of primer pairs ($p=0.374$). The *gag*

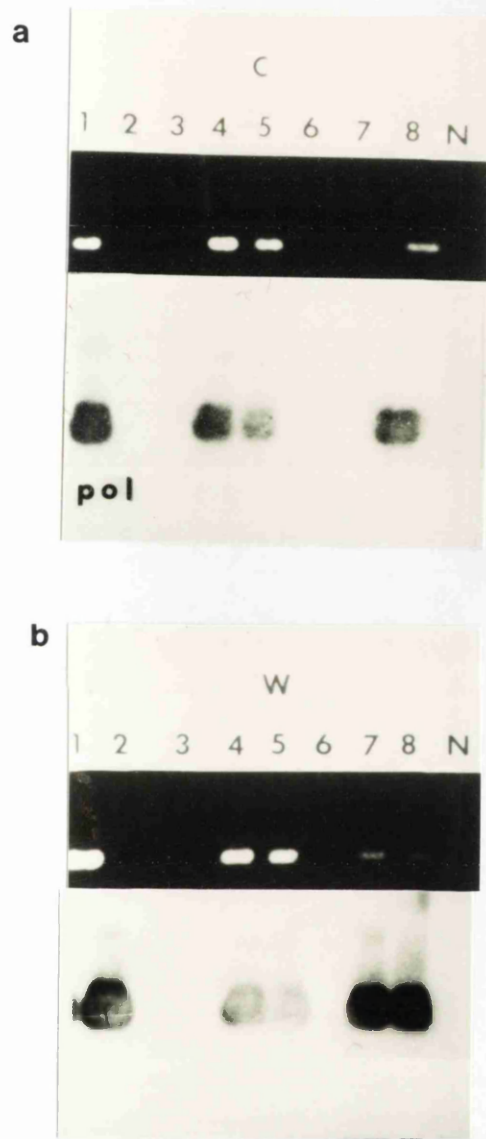


Figure 3.3. Detection of HIV-1 *pol* sequence in cortical (a) and white matter (b) samples of seven AIDS cases and one HIV-1-seronegative control, following DNA extraction, amplification by PCR and Southern blotting. Lanes 1 to 8: cases 17, c3,33,35,34,28,37 and 16. Lane N:No DNA control. Both cortical and white matter samples were positive in cases 16,17,34 and 35; the cortical sample of case 37 was positive while the white matter sample was negative. Both samples of cases 28,33 and c3 were negative.

primers, which were the most readily available set, were therefore selected to carry out initial amplifications of all samples. Following three PCR assays with this set of primers those that were positive in all three tests were considered positive. Samples negative in one or more of these assays were amplified twice with the *pol*, and once with the *env* sets of primers. Thus either 3 or 6 PCR assays were carried out on the remaining AIDS cases. Sufficient DNA was extracted from cortical samples of the HIV-1-seropositive non-AIDS cases to perform five PCR assays, three with the *gag* primers, and one each with the *env* and *pol* primers.

3.2.2 PCR results: controls

At least six PCR assays were performed on all control cases and these were consistently negative. Each PCR run included ssDNA and water controls, as well as a positive control consisting of 10 molecules of HIV-1 DNA in 1 μ g of human genomic DNA. The negative controls were negative on all PCR runs, while the positive control was positive in 24 out of 30 PCR assays.

3.2.3 PCR results: HIV-1 seropositive non-AIDS.

The results of all PCR assays performed on DNA from cortical samples from the eight HIV-1-seropositive non-AIDS cases are shown in **table 3.8** and an example of these results is illustrated in **figure 3.4**. Two cases (2 and 5) were positive in four out of five assays and therefore considered positive, in four cases (1,3,6 and 7) only one or two assays were positive, these cases were designated as indeterminate and the remaining two cases (4 and 8) were negative in all five PCR tests.

Table 3.8 Frequency of positive, negative and indeterminate results following PCR for HIV-1, with primers to *gag*, *pol* and *env* primers and Southern blotting; on DNA extracted from cortex of frontal lobe tissue from HIV-1-seropositive non-AIDS cases.

case	Neuropathology	Number of + PCR assays	Score
1	Discrete meningeal reaction Myelin pallor	1/5	±
2	Cortical and WM - gliosis	4/5	+
3	Meningitis PV - infiltrate	1/5	±
4	oedema WM - gliosis PV - mac	0/5	-
5	Discrete LM - infiltrate PV - mac cortex - foci of ischemia	4/5	+
6	PV - mac mineralisation slight IV haemorrhage	1/5	±
7	oedema discrete PV and LM - infiltrate slight subarachnoid haemorrhage	2/5	±
8	oedema LM and PV - infiltrate PV - mac	0/5	-

WM - white matter, LM - leptomeningeal, PV - perivascular, mac - macrophage

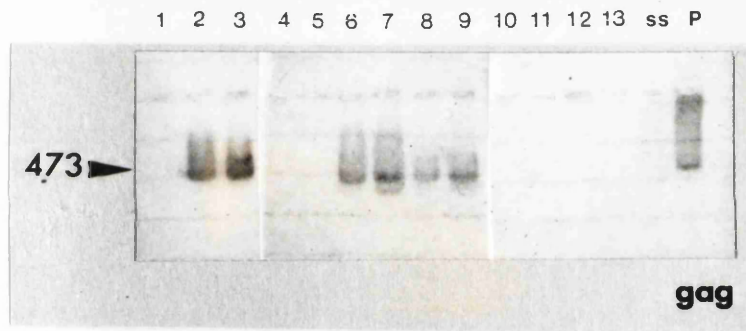


Figure 3.4 Detection of HIV *gag* sequences in cortical samples of three AIDS cases, eight HIV-1-seropositive non-AIDS controls and two HIV-1-seronegative drug users; following DNA extraction, amplification by PCR and Southern blotting. Lanes 1-13: Cases 30,27,22,6,3,2,5,1,7,4,8,c3,c4; lane SS:salmon sperm DNA; lane P:positive control. Of the AIDS cases both 27 and 22 are positive (lanes 2 and 3) while case 31 is negative (lane 1). A positive result was obtained in four of the HIV-1-seropositive cases (2,5,1 and 7; lanes 6,7,8 and 9), whilst cases 6,3,4 and 8 (lanes 4,5,10 and 11), the two HIV-1-seronegative drug users (cases c4 and c5; lanes 12 and 13), and the ssDNA control are negative.

3.2.4 PCR results: AIDS.

The results of all PCR assays performed on the AIDS cases are shown in **table 3.9**. In addition to the seven cases described in the pilot study, PCR was carried out on a further eleven cases (eleven cortical samples and eleven white matter samples). An example of these results is illustrated in **fig 3.4** and **3.5**. Of these additional twenty-two samples, ten were positive in all three tests with the *gag* primers. Three more PCR assays were performed on the remaining twelve samples; in two of these samples four out of six PCR assays were positive and these samples were therefore considered positive. Five samples were consistently negative in all PCR assays and of the remaining five samples only one or two out of six assays were positive; these samples were therefore considered indeterminate.

HIV-1 proviral DNA was detected in a total of twelve out of eighteen cases (66%). Eleven cortical samples (61%) and eleven white matter samples (61%) gave a positive result. An indeterminate result was obtained in three cortical samples (17%) and four white matter samples (22%). Four cortical samples (22%) and three white matter samples (17%) were consistently negative. The correlation between the number of positive assays in the cortex and white matter samples from each case was very close. However in case 24 the white matter was positive and the cortex indeterminate, while in case 27 the opposite result was obtained. To test for agreement between the frequency of positive results in these two regions the confidence interval was calculated. The mean difference between the frequency of positive results in the cortex and white matter was 0.83 with a 95% confidence interval of 0.32 to 1.34

Table 3.9 Frequency of positive, negative and indeterminate results following PCR for HIV-1, with primers to *gag*, *pol* and *env* primers and Southern blotting; on DNA extracted from cortex and white matter of frontal lobe tissue from AIDS cases.

case	Neuropathology	cortex		white matter	
		No. of + assays	Score	No. of + assays	Score
14	No abnormalities	0/6	-	0/6	-
16	Diffuse gliosis	9/9	+	7/9	+
17	HIVE	9/9	+	8/9	+
19	HIVE	3/3	+	3/3	+
22	HIVE	3/3	+	3/3	+
24	CMVE	1/6	±	4/6	+
27	CMVE	4/6	+	1/6	±
28	Ischaemia	0/9	-	0/9	-
30	Ischaemia	0/6	-	0/6	-
31	Haemorrhage	0/6	-	1/6	±
32	Diffuse gliosis	6/6	+	6/6	+
33	Lymphoma	2/9	±	1/9	±
34	lymphoma	7/9	+	5/9	+
35	PML	9/9	+	9/9	+
36	PML	1/6	±	2/6	±
37	Cryptococcosis	4/9	+	5/9	+
38	HIVE/Cryptococcosis	3/3	+	3/3	+
39	HIVE	3/3	+	3/3	+

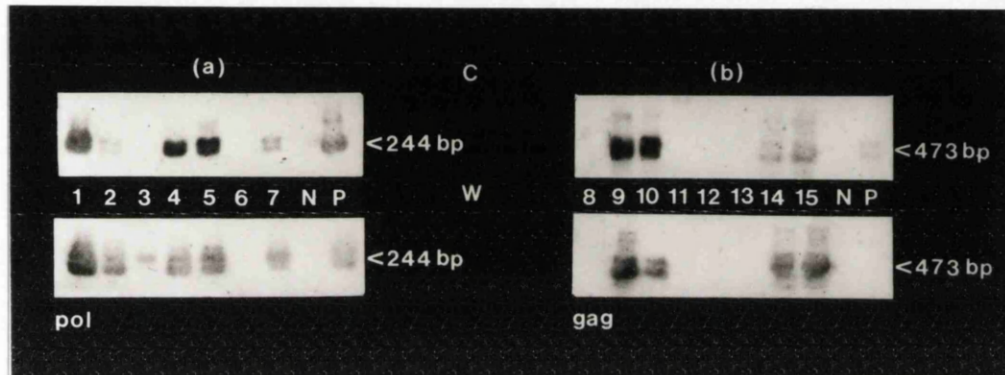


Figure 3.5 Detection of HIV-1 *pol* (a) and *gag* (b) sequences in cortical (C) and white matter (W) samples of fifteen AIDS cases, following DNA extraction, amplification by PCR and Southern blotting. Lanes 1 to 15: cases 17,16,33,35,34,28,37,14,39,38,24,36,31,32 and 19. Lane N: no DNA control; lane P: 10 molecules of HIV-1 plasmid DNA and 1 μ g of HIV-1-seronegative genomic DNA. Samples from both cortex and white matter are positive following detection of a region of the *pol* (a) or *gag* (b) gene in cases 17,16,35,34,37,39,38,34 and 19 and negative in cases 28,14,24,36,31 whilst in case 33 the cortical sample is negative and the white matter sample positive. The no DNA controls (N) are negative while the plasmid control (P) was positive with the *pol* primers, and faintly positive and negative in the cortical and white matter samples amplified with the *gag* primers. The sensitivity of the PCR test appears to be between 10 to 100 HIV proviral DNA copies.

The t value (3.46) was significant at the 5% level.

3.2.5 Comparison of immunohistochemical and PCR detection of HIV

HIV-1 antigens were detected in eight of the eighteen cases (44%) tested by PCR for HIV-1 proviral DNA; in the white matter of eight cases (44%) and the cortex of three (17%). Cases 16, 17, 19 and 22 were described in chapter 2; the remaining four cases consisted of one each of HIVE (case 39), diffuse gliosis (case 32), lymphoma (case 34) and PML (case 35). In two of these cases (lymphoma and PML), HIV-1 antigens were detected in both the cortex and white matter, although, as described in other cases in which p24 antigens were detected in the cortex, the amount of immunoreactive product was considerably less abundant in the cortex of both cases.

HIV-1 proviral DNA was detected by PCR from frozen tissue of all eight cases, in which HIV-1 antigens were detected in paraffin sections. In four cases, proviral DNA was detected by PCR while immunohistochemistry was negative. In one of these four with both cryptococcosis and HIVE, while no antigen was detected in the frontal lobe, HIV-1 antigen was detected in the basal ganglia, the only region where lesions of HIVE were found. The remaining three cases, consisted of cryptococcosis (1 case) and CMVE (2 cases). Of the two CMVE cases, HIV-1 proviral DNA was detected in the white matter but not in the cortex of one case, and in the cortex but not the white matter of the other. Cases in which both cortical and white matter samples were negative, or indeterminate by both techniques, consisted of one case with no abnormalities, three with cerebrovascular disorders, one with PML and one

with lymphoma.

HIV-1 antigen was visualized in the white matter of eight of the eleven cases in which HIV-1 proviral DNA was detected. In contrast HIV-1 antigens were observed in the cortex of only three of the eleven cases in which HIV-1 proviral DNA was detected. Using McNemar's test for paired samples the difference between the proportion of positive cases in the white matter detected by immunohistochemistry versus PCR, was 17% ($p=0.25$) which is not significant with a 95% confidence interval. However the difference in the proportion of cases positive with each method in the cortex was 41% which is significant at the same level ($p=0.016$).

3.2.6 Detection of HIV-1 proviral DNA and antigens in the cortex of different pathological cases.

The results of both immunohistochemical and PCR detection of HIV-1 in the cortex are shown in table 3.9, according to the type of associated neuropathology. HIV-1 proviral DNA was detected in the cortex of all five cases with HIVe/lep and both cases of diffuse gliosis; however HIV-1 antigen was observed in the cortex of only one of these cases (case 19; cases 18 and 21 were not included in this part of the study). HIV-1 proviral DNA was also detected in both cases with cryptococcosis and in one case each of PML, lymphoma and CMVE. HIV-1 antigens were also visualized in the cortex of these PML and lymphoma cases. PCR was negative or indeterminate for HIV-1 proviral DNA and HIV-1 antigen was not detected in the other cases of PML, lymphoma and CMVE or in any of the three cases of

cerebrovascular disease or the case with no abnormalities.

Table 3.9 Number of cases with each neuropathological lesion in which HIV-1 antigens and proviral DNA were detected HIV-1 in the frontal cortex.

Neuropathological lesions	IHC		PCR	
	+	-	+	±/-
HIVE/HIV-1ep *	1	4	5	0
Non-specific gliosis	0	2	2	0
CMVE	0	2	1	1
PML	1	1	1	1
Cryptococcosis *	0	2	2	0
Cerebrovascular	0	3	0	3
Lymphoma	1	1	1	1
No abnormalities	0	1	0	1

* case 38 is included in both categories

3.2.7 Cortical reaction and the detection of HIV-1 in the cortex.

3.2.7.1 Effect of fixation

The medians and range of GFAP-positive astrocyte and RCA-120-positive cell densities of cases fixed for longer than or less than four months are shown in **table 3.10**.

While there was no significant difference in microglial cells density between these

two groups ($\alpha = 0.554$); a significant difference was detected in astrocyte density ($\alpha = 0.0384$). These results are in agreement with those in chapter 2, where it was found that, while GFAP immunoreactivity may or may not be affected by formalin fixation, RCA-120 lectin reactivity is resistant to prolonged fixation.

Comparisons of astrocyte density were therefore carried out on this smaller group of cases, while all cases on which PCR was carried out were used to compare RCA-120 cell density which seems resistant to prolonged fixation. In addition, comparisons of GFAP density, numbers of reactive astrocytes and distribution of astrocytes, which may also have been affected by reduced GFAP immunoreactivity were carried out on the smaller group of cases.

Table 3.10 Median astrocyte and microglial cell density of cases fixed for less than and longer than four months.

MEASURE	Cases fixed < 4 months (n = 13)	Cases fixed > 4 months (n = 5)	Mann-Whitney $\alpha =$
Astrocyte density	26.60 (8.90-43.93)	10.29 (4.45-18.63)	0.0384
Microglial density	3.06 (0.56-9.18)	3.34 (2.78-8.06)	0.5542

The number of cases with each astrocyte MFNS, microglial cell MFMT and microglial cell distribution, in cases fixed for longer than four months and cases fixed for less than four months are illustrated as percentages in **figure 3.6**.

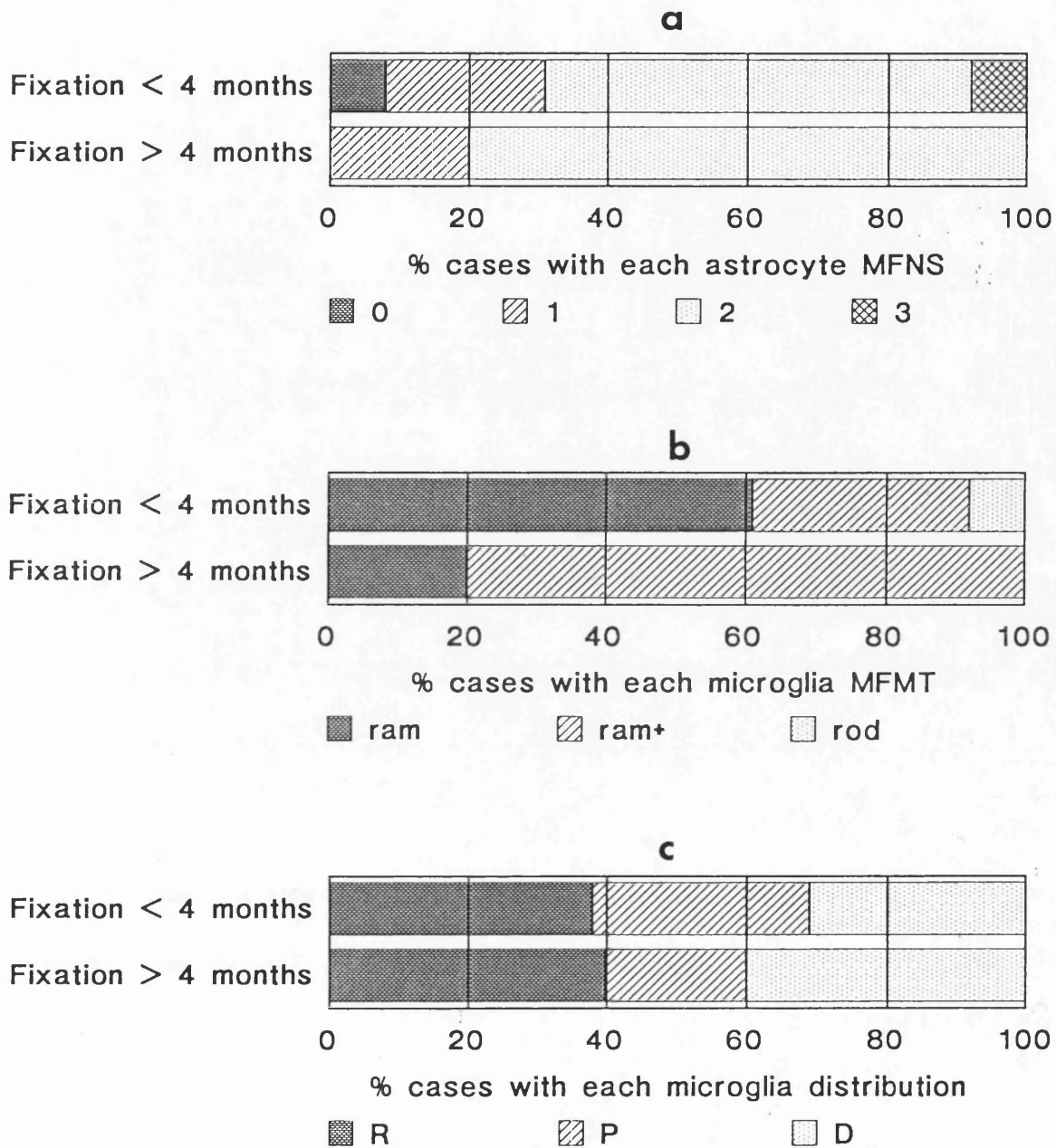


Figure 3.6 Comparison of percentage of cases in each category of: "astrocyte MFNS (a); microglia MFMT (b) microglia distribution (c), between cases fixed for longer than and less than 4 months.

The percentage of cases in each category, of astrocyte MFNS score, did not appear to differ significantly between cases fixed for less than or longer than 4 months (**fig 3.6a**). Although there was more variability in cases fixed for less than four months, this is may be due to the fact that there were more cases in this group. In the group fixed for longer than four months, a higher percentage of cases have enlarged ramified cells, as the MFMT of microglial cell, than in the group fixed for less than four months (**fig 3.6b**). This indicates that fixation does not reduce the staining intensity of RCA-120-positive cells. The number of cases with each distribution of microglia seemed very similar in cases fixed for longer than, and less than four months (**fig 3.6c**). Thus, comparisons between HIVC+ and HIVC \pm /- groups, of astrocyte MFNS, microglial cell MFMT, and distribution of microglial cells, were carried out using all cases on which PCR was performed.

3.2.7.2 GFAP-positive astrocyte and RCA-120-positive cell density

AIDS

Median astrocyte and microglial cell densities and reactivities were calculated for both the HIVC+ and HIVC \pm /- groups (**table 3.13**) and the Mann-Whitney test was used to calculate the significance of differences in cell densities between these groups. Median astrocyte density was higher in the HIVC+ group than in the HIVC \pm /- group, and a Mann-Whitney U test detected a difference in astrocyte density between these two groups, which marginally missed significance ($\alpha=0.054$). Microglial density was not significantly different ($\alpha=0.428$).

HIV-1-seropositive non-AIDS

The astrocyte density of the two cases that were positive for HIV-1 proviral DNA by PCR was low in case 2, and within the range of the normal controls in case 5. The density was highest in case 8, however a negative result was obtained in all PCR assays on cortical DNA from this case. The density of microglial cells was increased in most cases in the HIV-1-seropositive non-AIDS group including case 5. However, in case 2 there was the lowest density of microglial cells in the group.

Table 3.13 Cortical astrocyte and microglial cell density in HIVC+ and HIVC±/- cases; Medians and Mann-Whitney.

MEASURE	HIVC +	HIVC ±/-	$\alpha =$
Astrocyte density	27.75 (n=9)*	17.85 (n=6)*	0.054
Microglial density	3.34 (n=13)	3.05 (n=7)	0.428

* cases fixated for longer than 4 months excluded (i.e. cases 32 33 34, 38 and 39)

3.2.7.3 Astrocyte MFNS

AIDS

The percentage of cases with each astrocyte MFNS, in the HIVC+ and HIVC±/- groups are compared in **figure 3.7a**. In the HIVC+ group the percentage of cases with a MFNS of 2 was twice that of the HIVC±/- group, while a higher percentage of cases in the HIVC±/- group had a MFNS of 0 or 1. However one of the cases in the HIVC±/- group had a most frequent score of 3. The chi-squared test for trend in proportions was not significant ($p=0.22$), suggesting a higher nuclear score is not related to the presence of HIV-1 proviral DNA in the cortex.

HIV-1-seropositive non-AIDS

In the HIV-1-seropositive non-AIDS cases, the two PCR positive cases had a MFNS of 0 and 1. Case 4 had the highest nuclear score of 2, but was negative in all PCR tests.

3.2.7.4 GFAP immunoreactivity

The percentage of cases with each GFAP immunoreactive density in the HIVC+ and the HIVC \pm /- groups appeared very similar (**fig 3.7b**), and the chi-squared test for trends in proportions between these two groups was not significant ($p=0.72$).

Of the two HIV-1-seropositive non-AIDS cases that were positive, case 5 had very few GFAP-positive cells (\pm) while an increase in GFAP immunoreactivity was found in case 2. Of the other cases, negative or indeterminate for HIV-1 by PCR, the GFAP immunoreactivity scores ranged from \pm to +++.

3.2.7.5 Reactive astrocytes

Reactive astrocytes were found in a greater percentage of HIVC+ cases (38%), as compared with the HIVC \pm /- cases (14%). However these cells were not observed in the majority of cases in both groups.

3.2.7.6 Microglial cell MFMT

AIDS

The percentage of cases in which enlarged ramified cells were most numerous, was much higher in the HIVC+ than the HIVC \pm /- group (**fig 3.7c**). The latter group

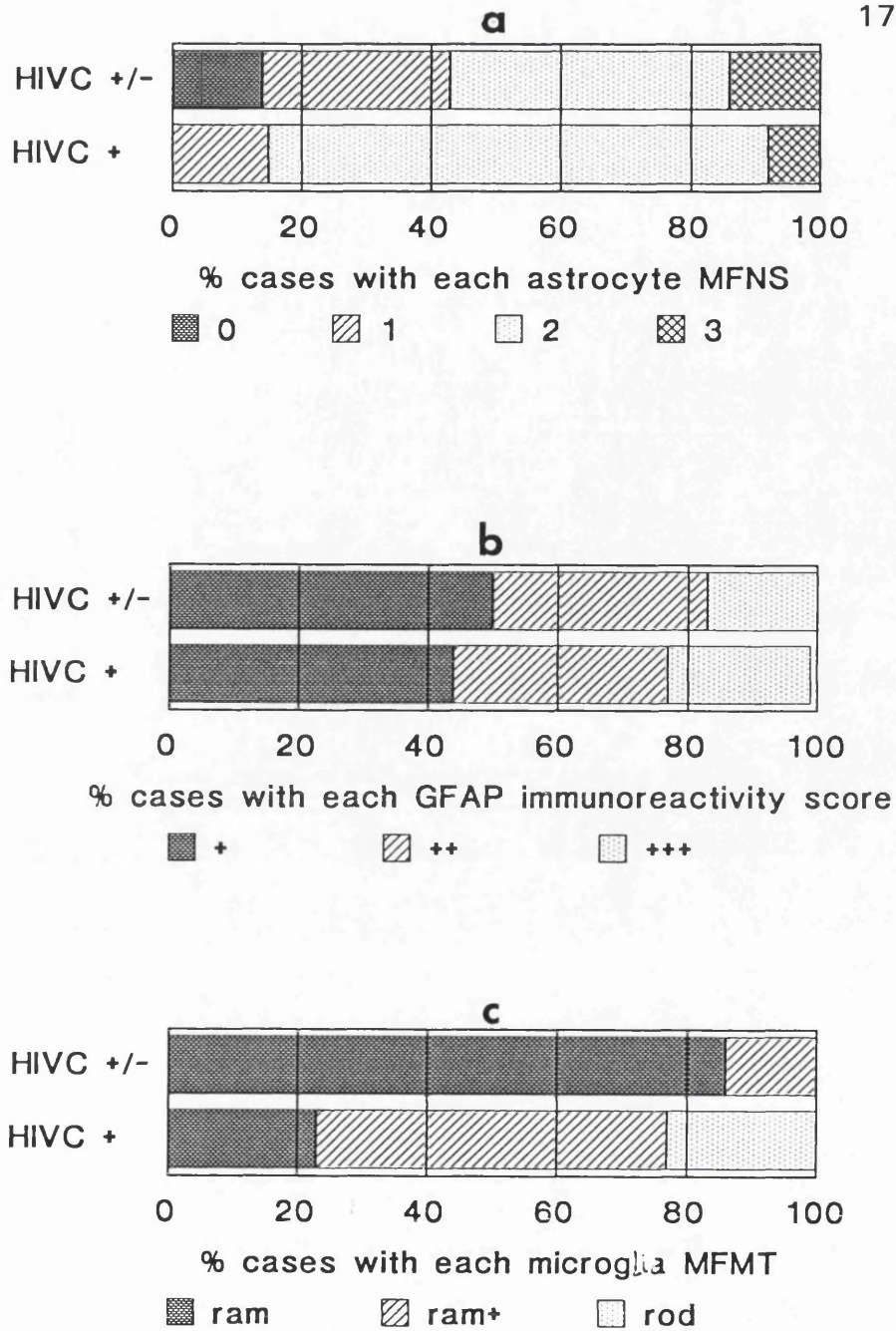


Figure 3.7 Comparison of percentage of cases in each category of: GFAP-positive astrocyte MFNS (a); GFAP immunoreactivity (b); RCA-120-positive microglia MFMT (c), between H1VC+ and H1VC \pm /- groups.

had a higher percentage of cases in which small ramified cells were most frequent. Using the chi-squared test for trends in proportions a significant difference was found between the two groups ($p=0.025$). These results suggest that HIV-1 is more likely to be detected (either by PCR or immunohistochemistry) in cases with enlarged ramified cells or rod cells.

HIV-1-seropositive non-AIDS

The most reactive microglial cells were seen in case 8 which was negative by all PCR assays, the appearance of the two positive cases was not different from other negative or indeterminate cases.

3.2.7.7 GFAP-positive astrocyte distribution

AIDS

Both groups had an identical percentage of cases with a diffuse distribution of astrocytes (**fig 3.8a**). The remaining cases had either a diffuse/PV or patchy/PV; the latter was not seen in the HIVC+ group. The chi-squared test for trends in proportions was not significant ($p=0.66$).

HIV-1-seropositive non-AIDS

A perivascular distribution of astrocytes was evident in both cases positive for PCR. A predominantly diffuse distribution of astrocytes was seen only in case 4, which was indeterminate by PCR.

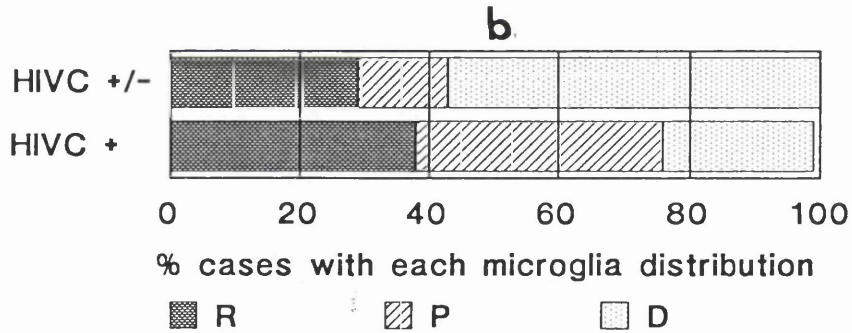
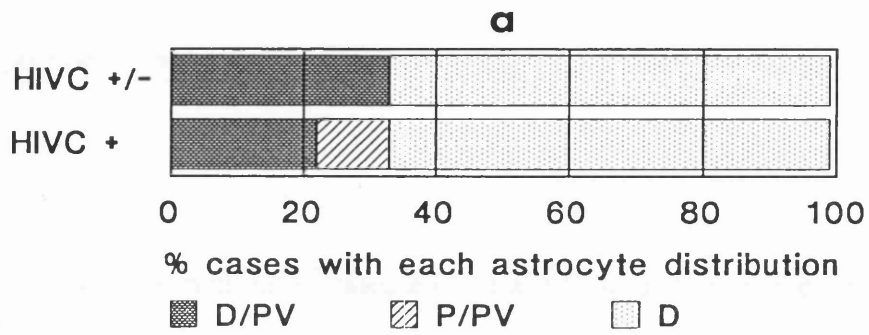


Figure 3.8 Comparison of percentage of cases in each category of: "GFAP positive astrocytes distribution" (a) and "RCA-120-positive microglia distribution" (b), between HIVC+ and HIVC \pm groups.

3.2.7.8 RCA-120-positive microglial cell distribution

AIDS

The majority of cases in the HIVC+ group had a rare or patchy distribution of microglial cells, while in the HIVC \pm group most cases had a diffuse distribution (**fig 3.8b**). However the difference in the number of cases in each category, between these two groups was not significant when compared with the chi-squared test for trends in proportions ($p=0.88$).

HIV-1-seropositive non-AIDS

In the HIV-1-seropositive non-AIDS cases all had a diffuse distribution except case 3, where there was a patchy distribution, this case was indeterminate by PCR.

3.2.7.9 Foci of astrocytes and microglial cells

Few MGNs were observed in the cases analysed by PCR, but were present in one case of CMVE and one with cerebrovascular pathology from the HIVC \pm group. Loose foci which contained both astrocytes and microglial cells were present in more of the HIVC+ cases (54%) than the HIV \pm cases (29%).

3.2.8 Effect of including cases with pathogens, other than HIV-1, in the cortex, on the analysis of differences between cases in which HIV-1 was detected or not detected in the cortex.

Eleven of the eighteen cases on which PCR was performed had lesions in the cortex

which are likely to have contributed to the pathological reaction of astrocytes and microglial cells in this region. PML, lymphoma, and CVME lesions were seen in two cases each; however HIV-1 proviral DNA was detected by PCR in one case only of these three pathologies. The effect these lesions may have produced on cellular changes is thus unlikely to have biased the results when comparisons of cases with and without HIV-1 in the cortex were made.

However, both cases of *cryptococcosis* were included in the HIVC+ group and may have affected the findings of increased GFAP-positive astrocyte density and reactive microglial cells associated with this group. In practice, only one case of *cryptococcosis* was included in the comparison of astrocyte density as the other was fixed for longer than four months. When this case was excluded from the HIVC+ group the median value of astrocyte density was higher (30.72) than the median value when this case was included (26.60); therefore, inclusion of this case did not produce an artificially higher astrocyte cell density in this group. Enlarged ramified cells were the MFMT of microglial cells in both *cryptococcosis* cases, however since there was only three out of thirteen cases in the HIVC+ group in which enlarged ramified cells or rod cells were not frequently seen, it is unlikely that these two cases unduly affected this comparison.

The cerebrovascular lesions were all in the HIVC \pm /- group. It was determined in chapter 2 that the astrocyte density of cerebrovascular lesions was lower than any of the other groups. The lower density of astrocytes may be due to the fact that HIV-1 is not present in the cortex of any of these cases or it may be related to

cerebrovascular pathology. If the latter is true, then the lower density of astrocytes in the HIVC \pm /- group may have been partly due to the presence of three cases with cerebrovascular lesions in this group. The median value of the three cases with cerebrovascular lesions (10.87) is lower than that of the median value of the other three cases (18.90). In addition, the presence of diffusely distributed small ramified cells in all three cases with cerebrovascular lesions may have biased the results of the HIVC \pm /- group.

3.2.9 Summary of cortical changes related to the presence of HIV-1 in the cortex.

Comparison of AIDS cases in which HIV-1 was detected in the cortex, either by PCR or by immunohistochemistry, suggests that detection of HIV-1 in the cortex is associated with an increased density of GFAP-positive astrocytes. However, as explained above, it is not clear if the low density of GFAP-positive astrocytes in the cerebrovascular cases (included in the HIVC \pm /- group) was due to the lack of HIV-1 in the cortex, or related to cerebrovascular pathology. The presence of HIV-1 in the cortex may also be associated with enlarged nuclear volume, although this trend was not statistically significant and increases in nuclear size clearly also occur in the absence of detectable HIV-1. HIV-1 is also more likely to be detected from the cortex of AIDS cases with enlarged ramified microglial cells or rod cells, rather than small ramified cells, although there was no relationship between microglial cell density and the detection of HIV-1. These results are contradicted to some extent by the HIV-1-seropositive cases, though since HIV-1 was detected in only two of these cases, it is difficult to assess the significance of these results.

Reactive astrocytes and foci of reactive astrocytes and microglial cells were more frequently observed in cases from which HIV-1 was detected, although some of these foci were in response to other pathogens such as PML.

The amount of GFAP immunoreactivity, the distribution of GFAP-positive cells, and the distribution of microglial cells were not related to the detection of HIV-1 in the cortex.

CHAPTER FOUR:

DISCUSSION AND CONCLUSIONS

4.1 Overview

Astrogliosis, microglial cell activation and neuronal loss may contribute to the development of dementia, which has been associated with HIV-1 infection. Indeed, their identification may help to establish whether a correlation exists between clinical signs of dementia and pathological findings in the cortex and may even help to explain the lack of correlation between dementia and HIV-related white matter pathology. However the pathogenesis of cerebral cortical changes are still poorly understood. It has been suggested that infection of the brain with HIV-1 may be involved in the development of these changes (Ciardi et al., 1990, Budka 1991, Lipton 1991). This suggestion is based on studies that have correlated neurological symptoms with the presence of HIV-1 in the CSF of patients at different stages of disease (Ho et al., 1985; Resnick et al., 1988; Shaunak et al., 1990; Goswami et al., 1991) and *in vitro* studies that have shown HIV-1 to be toxic to some neuronal cell lines (Lipton 1991,1992; Brenneman et al., 1988). However the distribution of HIV-1 antigens and nucleic acids in post-mortem AIDS brains is predominantly subcortical. While HIV-1 is often visualised within the cortex, a number of studies have commented on the rarity of HIV-1 in this region as compared with that seen in subcortical areas (Budka, 1990, Kure et al., 1990, Sinclair and Scaravilli 1991). In contrast reports on cortical abnormalities suggest that they may be widespread in AIDS brains (Ciardi et al., 1990; Everall et al.,1991).

Therefore it seemed pertinent to correlate the presence of HIV-1 in the cortex with some of these cortical changes, as detection of the virus in the cortex of affected

brains would increase the likelihood that HIV-1 and diffuse gliodystrophy are linked.

To this end, immunohistochemical detection of HIV-1 was carried out on the frontal lobes of a series of AIDS brains, in which changes in astrocyte and microglial cell populations were also examined in the cerebral cortex. In addition to immunohistochemistry, PCR was carried out on frozen tissues from the frontal lobes of ~~these~~ ^{a number of} cases to detect HIV-1 proviral DNA. The rationale for employing this additional method was twofold. Firstly, it is well known that the sensitivity of immunohistochemistry on paraffin sections is limited, antigen may lose its immunoreactivity during fixation and processing, or be present in small amounts below the resolution of this technique. In contrast, PCR is renowned for its sensitivity and it is therefore more suitable for determining the presence or absence of small quantities of virus. It has been suggested that small amounts of HIV-1 may exert toxic effects (Giulian et al., 1990), therefore, the application of PCR to AIDS brains seemed appropriate. The second reason for the use of PCR in the present study is, that HIV-1 proviral DNA can be detected with this method, whether or not viral replication was taking place. Thus, PCR is capable of detecting cells infected with HIV-1, regardless of whether they remain latently infected or whether viral replication is taking place; on the other hand immunohistochemistry can detect only the end product of viral replication, HIV-1 antigens.

The combined use of immunohistochemistry and PCR allowed another problem to be addressed; the observed predominance of viral antigen in subcortical regions of AIDS brains, as already described, has never been explained. This distribution implies that HIV-1 preferentially infects the white matter or, if both regions of the brain are

infected, viral replication is more restricted in the white matter.

The polymerase chain reaction is very subject to false positive results; in a recent NIBSC study in which this laboratory took part (Bootman and Kitchin, 1992), 50% of participating laboratories reported positive results in samples that did not contain the target DNA. This report recommended precautions, to prevent contamination and controls, to monitor their effectiveness. Strict safe-guards in this laboratory seemed effective in preventing contamination, as HIV-1 DNA was not detected in any of the negative controls that were included in each PCR run.

A previous study from this department (Ciardi et al., 1990) found that astrogliosis and microglial cell activation occur in AIDS; both in cases with and without obvious white matter pathology. In a further investigation (Sinclair and Scaravilli, 1992) the presence of HIV-1 proviral DNA, detected by PCR, was related to changes in the cerebral cortex, previously described as DPD in AIDS (Budka et al., 1991). However the results of both studies were complicated by the presence of pathological changes in a number of cases that were not related to HIV-1. Although it was clear from the latter study that factors other than HIV-1 may also produce cortical pathology, the results suggested that HIV-1 may also be involved in the development of such changes.

In the first part of this study, described in part two, various aspects of the astrogliosis and microglial cell reaction in the cerebral cortex of the frontal lobe, were examined in AIDS brains with different pathological lesions, in HIV-1-seropositive non-AIDS

brains and brains from HIV-seronegative controls. Astrogliosis and microglial cell activation are non-specific reactions to injury. However, since the reaction of these cells varies in response to different pathological stimuli, an attempt was made to determine whether there were any patterns of astrocyte or microglial cell reaction that were specific to brains infected with HIV; or if diffuse poliodystrophic changes are a general disorder in AIDS, independent of the presence of HIV-1.

The distribution, morphology, and density of GFAP-positive astrocytes and RCA-120-positive microglial cells were examined in cases with identifiable HIV-1 antigen in the brain and compared with cases in which no antigen could be detected. The antigen-negative brains consisted of cases with: no neuropathological abnormalities (on routine examination); CMV encephalitis and cerebrovascular disorders.

CMV encephalitis and cerebrovascular pathologies comprise two different types of lesion that are commonly seen in AIDS brains. CMV encephalitis is a common viral infection of AIDS patients and in most cases it produces MGNs in the cortex and white matter (Morgello et al., 1987). More severe lesions are produced by this virus, commonly around the ventricles; however in this study, the only cortical pathology present in selected cases was the appearance of microglial nodules, some of which contained astrocytes with intranuclear inclusions, typical of CMV. Cerebrovascular cases consisted of one case of haemorrhage, two of ischemia and one of hypoxia. The haemorrhage was limited to one area of the brain, which was not used for this study, in the other three ischaemic lesions were found throughout the cortex.

Three cases that were not in any of the risk groups for HIV-1 infection, and two HIV-1-seronegative drug users, provided a base line for comparison of astrocyte and microglial cell changes. It was difficult to find normal brains which matched the AIDS cases for age, sex and fixation time and therefore only five cases were selected. In one of the normal controls (c2) immunohistochemical stains revealed a mild microglial cell and astrocytic reaction. This slight reaction may have been a result of the measles like illness that lead to the patient's death.

HIV-1-seropositive non-AIDS cases were also examined to determine whether diffuse poliodystrophic changes occur at an early stage of infection, as HIV-1 is believed to enter the brain at an early stage (Ho et al., 1985; Resnick et al., 1988; Shaunak et al., 1990; Goswami et al., 1991; Sinclair et al., 1992).

Immunohistochemistry was used to identify cases with cerebral HIV-1 infection and those in which antigens were present in the cortex. Although HIV-1 antigens were detected in the cortex of only three cases in chapter 2, all of these cases comprised an HIV-1 p24 antigen positive group for the purpose of comparison with cases lacking detectable HIV-1 cerebral infection.

A diffuse astrogliosis was present in the anterior frontal cortex of most AIDS cases that we examined, and a patchy microglial cell reaction was present in the cortex of many of these, in agreement with previous studies (Budka et al., 1987; Ciardi et al., 1990). The astrocyte reaction consisted of one or more of the following changes: 1) an increase in the density of GFAP-positive astrocytes; 2) an increase in astrocyte

nuclear size and reduction in chromatin density; 3) an increase in GFAP staining intensity and 4) changes in the distribution of GFAP-positive cells. The microglial cell reaction consisted of one or more of: 1) an increase in the size and thickness of cell processes, the appearance of rod cells, macrophages and multinucleated giant cells; 2) an increase in cell density; 3) loss of the normal diffuse distribution of these cells.

Astrocyte and microglial cell changes were also identified in a number of the HIV-seropositive non-AIDS cases; however the pattern of changes was different from the AIDS groups. In some HIV-1-seropositive non-AIDS cases there was a reduction in GFAP immunoreactivity while in others it was increased. In addition, there was a much more marked increase in microglial cell density in this group as compared with the AIDS cases.

Changes in the different groups described above are compared below and the implications of these results for the pathogenesis of cortical pathology in AIDS are considered.

In the second part of this study (chapter 3), PCR was used to detect HIV-1 proviral DNA in the cortex and white matter of AIDS brains and in the cortex of HIV-1-seropositive non-AIDS cases. Although some of the changes in the cerebral cortex, mentioned above were more severe in the HIV-1 antigen positive group, they were not specific to these cases. Therefore the first part of the study (chapter 2) did not provide any firm evidence to support HIV-1 as the aetiological agent in diffuse poliodystrophy, especially since HIV-1 could only be located in the cortex of three

cases. The lack of sensitivity of immunohistochemistry has already been mentioned; although HIV-1 was not detected in the brains of the CMVE, cerebrovascular or no neuropathological changes groups with immunohistochemistry, small amounts of virus below the resolution of this technique may have been present. Indeed, in one of the CMVE cases, negative for HIV-1 antigen, HIV-1 proviral DNA was detected by PCR in the cortex. Furthermore, HIV-1 proviral DNA was detected in the cortex of all cases with HIVe/lep, tested by PCR, regardless of whether or not antigen had been detected in this region of the brain. These findings suggest that immunohistochemical detection of an infectious agent may not always be sufficient for correlations with pathological changes to be made.

Fresh frozen tissue was used as a source of DNA for this technique; unfortunately this type of material was not available from all the cases that were studied in chapter 2. Although PCR can be used to identify viral sequences in paraffin embedded tissues (Nicoll et al., 1991), in this study, since cortical and white matter tissues were examined separately, paraffin embedded material was not considered suitable for this procedure. Ten of the cases described in chapter 2 were included in chapter 3 and an additional twelve cases were also examined. The cortex of these additional cases was examined in the same way as the cases in chapter 2, however pathological lesions which impinged on or affected the cerebral cortex, were present in some and these lesions had to be taken into consideration in the discussion of the results.

The density of GFAP-positive astrocytes was higher in cases in which HIV-1 proviral DNA was detected, as compared to cases in which it was not detected, and this

difference was close to significance. Furthermore, in cases with enlarged ramified microglial cells or rod cells, HIV-1 proviral DNA was significantly more likely to be detected. These results are discussed in detail below together with the results from chapter 2 and the implications of these pathological findings for the pathogenesis of cortical pathology in AIDS is considered. Firstly the use of immunohistochemistry and PCR for the detection of HIV-1 in the brain are compared.

4.2 Detection of HIV-1 in the brain

In previous comparative studies of the various HIV-1 antibodies, LAV gp41.1 was found to be the most sensitive (Budka, 1990; Kure et al., 1990). However in the present study false positive staining with this antibody could not be eliminated. Kure noted that the antibody did give false positive results in HIV-negative tissues containing macrophages, although this could be eliminated at higher antibody dilutions. In the above mentioned studies tissues were fixed in formalin for one or two weeks, while in the present study most cases were fixed for considerably longer periods. This may explain why LAV gp 41.1 was negative when higher dilutions were used. However Yamada et al. (1991) raised an antibody to gp41 that cross reacted with astrocytes in both human and rodent CNS tissues which suggests that this HIV-1 molecule may share a common epitope with astrocytes. Thus antibodies directed against this molecule may be liable to give false positive staining.

Budka (1990) found that all HIV-1-associated lesions were labelled with LAV gp41.1, in addition to a number of cases without these specific changes. While in Kure's

study this antibody labelled 26 out of 33 cases (79%) with HIV-1 related changes. In both studies NEA p24 was positive in fewer cases suggesting that detection of HIV-1 with this antibody, as in the present study, may not be optimal. However, in initial experiments (Section 2.3.1), p24 antigen was detected in 17 out of 22 (78%) cases with HIV-specific changes in which fixation ranged from 2 weeks to 15 months. Therefore, in the present study immunohistochemistry using NEA p24 appeared to be as sensitive for the detection of HIV-1, as LAV gp41.1 was in Kure's study.

HIV-1 antigen was confined to cells of the macrophage/monocyte cell type in all HIV-1 p24 antigen positive cases, selected for study of cortical cellular changes. This finding is in agreement with previous studies, as well as experiments carried out in this laboratory, that have employed double staining, to identify the type of HIV-1-infected cell. All thirty-one HIV-seropositive and AIDS cases included in chapters 2 and 3 were tested for the presence of HIV-1 p24 antigen. Antigen was not detected in any of the eight HIV-seropositive non-AIDS cases but was found in a total of twelve out of twenty-three AIDS cases (52%). In five of these cases (22%) HIV-1 antigen was detected in the cortex as well as the white matter. All cases in which HIV-1 was visualised in the cortex, by immunohistochemistry, had fewer positive cells in this region than in the white matter.

PCR results were classified as positive, indeterminate or negative following multiple PCR assays. When only one or two out of either six or nine PCR tests were positive, these results may have been due to the presence of contaminating blood or CSF cells that are infected by HIV-1, and were therefore considered indeterminate. However

since the sensitivity level of the test was relatively high (10 molecules of HIV-1 DNA per microgram of genomic DNA), it seems unlikely that sufficient infected peripheral blood cells or CSF cells would be present in each sample since only 1 out of 700 peripheral blood mononuclear cells or 1 out of 100 CD4 lymphocytes are infected, even at late stages of infection. Considering the low frequency of HIV-1 infected peripheral blood mononuclear cells, even late in the course of AIDS, it seems unlikely that the sufficient peripheral blood cell DNA would be present in any assay to produce a positive result. Alternatively, indeterminate results may be produced by samples which are infected by HIV-1, but have a level of infection that is lower than the detection limit of the test, and are therefore usually negative. The positive control sample, which contained 10 molecules of HIV-1 proviral DNA per microgram of human genomic DNA, was positive in 80% of PCR assays. The sensitivity of the PCR assay was therefore limited to between 10 and 100 molecules of the target sequence per microgram of genomic DNA. A negative result may have been obtained in some brains that contained a lower level of infection and the HIVC+ and HIVC \pm /- groups may therefore have consisted of cases with high and low levels of HIV-1 proviral DNA. Nevertheless, there was a distinct difference in the frequency of positive results between the positive and indeterminate cases, and comparisons between these two groups were considered to be valid ones to make.

Direct quantification of the amount of HIV-1 proviral DNA in brain tissues may be useful for assessing the relationship between infection and pathological changes. Several studies have used PCR in a quantitative way, to this end (Pang et al., 1990; Simmonds et al., 1990). However, the non-radioactive detection system used in this study is not as well suited to quantification as isotopic detection systems. The amount

of radioactivity released from an isotopic probe can be directly measured or visualised; whilst non-radioactive probes are detected indirectly, by binding of an antibody followed by an enzymatic reaction to produce a coloured substrate. More stages are involved and the enzymatic production of the coloured substrate can be variable; thus detection by non-isotopic probes is a less direct measurement of the amount of DNA present.

Previous investigations utilizing PCR to detect HIV-1 proviral DNA in brain tissues from AIDS patients, suggested that there was also a close correlation between the detection of proviral DNA and HIVE/lep (Pang et al., 1990, Kleihues et al., 1991). However, in the present study, HIV-1 proviral DNA was detected in cases with no morphological evidence of HIVE/lep and in a few cases in which HIV-1 antigens could not be visualised using immunohistochemistry.

Both immunohistochemistry and PCR were carried out on eighteen AIDS cases: in the cortex and white matter of paraffin sections and fresh frozen samples respectively, both from anterior frontal lobe. The number of cases in which both techniques were positive were comparable in samples of white matter. In the cortex more than three times as many samples were positive with PCR as with immunohistochemistry, a difference which was statistically significant. This finding confirms that HIV-1 infects both cortical and subcortical regions of the brain (Sinclair and Scaravilli, 1992). There are two possible explanations for the difference in virus detection by these two methods. 1) PCR is simply a more sensitive technique; 2) PCR detects latent viral infection that cannot be identified by immunohistochemistry. The large

difference between the results of PCR and immunohistochemical detection of HIV-1 in the cortex, in contrast to the small difference in the white matter, suggest that latently infected cells may be present in the cortex, which are not be detected by immunohistochemistry. In addition, the level of replication of HIV-1 may be lower in the cortex than the white matter and be below the resolution of the immunohistochemical technique.

4.3 Morphometric assessment of astrocytes and microglial cells

4.3.1 Methodological considerations

The density of both types of cells was assessed by standard quantitative methods. The result of this type of cell count on fixed post-mortem tissues must be interpreted with caution as several variables may have affected these results. During fixation, processing and sectioning both swelling and shrinkage of tissue components occur. Subjecting all tissues to an identical regime, as described in the methods section, can reduce variations between samples; however factors affecting alteration in tissue volume and mass are not well understood, and may change in an unpredictable way. For example, following fixation of a brain in 10% formalin its weight may either increase or decrease compared with the fresh brain weight. Most shrinkage occurs during tissue processing, and this variable can be quantified by measuring blocks before, and after processing. However this study used material that had already been processed to paraffin wax, as remaining unprocessed tissues from many of the cases, selected for investigation, had already been fixed for more than 12 months.

Formalin fixation is also presumed to affect antigen integrity and, if prolonged, immunoreactivity may be reduced. The effect of formalin fixation on immunoreactivity of GFAP and HIV-1 p24 and reactivity of the lectin RCA-120 was tested. The density of GFAP-positive astrocytes declined with increasing fixation time in one of the three cases that was tested, suggesting that in this case formalin fixation may have reduced GFAP immunoreactivity. However, no relationship between fixation time and GFAP-positive astrocyte cell density was found in the other two cases; therefore fixation may affect some tissue antigens in an unpredictable manner. In order to reduce the variability produced by prolonged fixation, cases selected for study in part one had all been fixed for periods of less than four months. This period of time was chosen as all three fixation controls appeared to have a relatively stable GFAP-positive cell count up till this time. In contrast to GFAP, HIV-1 p24 immunoreactivity and RCA-120 lectin reactivity seemed to be resistant to prolonged fixation.

Previous studies examining particular neuropathological lesions that occur in AIDS have often depended on comparing cases that have more than one type of lesion. The aim of this study was to eliminate such confounding variables as far as possible in order to compare cellular changes of cases with a pathological entity produced by a single aetiological factor. This reduced the number of suitable cases, especially in the CMVE and cerebrovascular groups which contained only 5 and 4 cases each. Unfortunately, in the study of AIDS, obtaining cases with a single type of neuropathology is extremely difficult due to the frequency with which neuropathological lesions due to more than one agent occur in these patients.

In addition to methodological problems there are other problems that must be considered when comparing pathological and non-pathological samples. The increased density of GFAP-positive cells may be the result of loss of another cell type rather than, or in addition to, an increase in GFAP-positive cells. Neuronal cell loss for example may produce cortical shrinkage (Wiley et al., 1991) which in turn, may have the effect of increasing astrocyte density. Morphometric studies in Huntington's chorea have shown that increases in astrocyte density are due to neuronal loss (Vacca et al., 1984, cited by Björklund et al., 1986). However, cortical atrophy was not commonly seen in the majority of cases in this study during macroscopic brain examinations and the brain weights were within normal limits.

The criteria used to assess morphological changes and changes in astrocyte and microglial cell distributions, were based on preliminary observations described in section 2.1, on a number of AIDS cases and normal controls. Changes in astrocyte nuclear size as well as perivascular distribution were noticed. RCA-120-positive microglial cells were much more obvious, due to increased size and staining intensity of their processes; rod cells were also present and in a few cases macrophages and MGCs were seen. These changes in the appearance of GFAP-positive astrocytes and RCA-120-positive microglial cells may occur independently of changes in cell density. Thus, measurements of cell density alone may not reveal the true extent of cortical injury. In an attempt to measure these changes, a scoring system was devised as described in section 2.24.

Astrocyte nuclei were graded according to increased size and staining of chromatin

and the density of GFAP-positive processes surrounding astrocyte nuclei was also assessed. Microglial cells were classified as small ramified cells (ram) enlarged ramified cells (ram+), rod cells (rod) and macrophages (mac). This scoring system may have represented a continuum from least to most reactive; however in some cases abundant small ramified cells together with rod cells were seen, while enlarged ramified cells were not present. The difference in the thickness of microglial cell processes is not likely to be due to fixation, as comparison of short and long fixation time cases in Part 3, revealed no significant difference between the two. In addition, enlargement of cell processes is usually accompanied by nuclear enlargement. Development of ramified cells into larger cells or into rod cells may represent two different pathways of activation.

4.3.2 Astrocyte and microglial cell reaction in HIV-negative pathological brains

The distribution of microglial cells, macrophages and astrocytes varies in different pathological processes as observed in section 2.1. Whilst in SSPE there is a diffuse increase in astrocytes and macrophage/microglial cells throughout the cortex, the cortical reaction in PML when widespread, is uneven. In the latter, large areas of cortex contained aggregates of densely stained astrocytes and macrophage/microglial cells, while other areas appear unaffected. This may be due to the fact that when the cortex is involved in PML, it involves spread of JC virus from the white matter to this region. In JBE, focal lesions were present in the cortex, and consisted mainly of microglial cells. Although a few astrocytes surrounded these lesions, there was no notable increase in GFAP staining throughout the cortex. The appearance of

MGNs, such as those seen in JBE, may reflect an appropriate immune response within the CNS, where the invading organism is contained within a focal aggregate of microglial cells. In the Hypoxia case, large patches of reactive astrocytes were confined to regions of the cortex that are most vulnerable to reduced oxygen. In AIDS, the diffuse distribution of astrocytes throughout all layers of the cortex was similar to that observed in the SSPE case, and unlike that seen in the PML, and hypoxia cases. Esiri and Morris (1991), suggested that the astrocyte response in SSPE may be maintained by IL-1 secreted from macrophages; however, the response of macrophages and microglial cells in AIDS is very limited in comparison to SSPE.

4.3.3 *GFAP-positive astrocyte density*

Astrocyte density was significantly increased in AIDS cases as compared with normal controls, while there was no difference between the density of cells observed in the HIV-1-seropositive non-AIDS and controls. Among the AIDS groups, GFAP-positive astrocyte density was highest in cases with HIVE or HIV-1ep, although the difference between this group and cases with no pathological changes, or with CMVE did not reach significance. However, there was a significant difference between the astrocyte density of the cerebrovascular and HIV antigen positive groups; the density of cells in the former being within the range of the normal controls. All cerebrovascular cases were negative for HIV-1 by immunohistochemistry, and the three that were tested were negative or indeterminate with PCR. The absence of HIV-1 in these cases with a low astrocytes density, suggests that the increased astrocyte density in the other groups may be related to the presence of HIV-1. Comparison of astrocyte

density between all cases in which HIV-1 was detected by immunohistochemistry or PCR (HIVC+) and those that were negative or indeterminate by PCR (HIVC \pm /-), revealed that astrocyte density was higher in the former group. However, the lower density of astrocytes in the cerebrovascular cases as compared to other AIDS cases, is not necessarily due to the absence of cerebral HIV-1 infection. For example, astrocyte density may appear reduced as a consequence of swelling within the cortical ribbon, as a result of oedema; which may be more likely to occur in cases with cerebrovascular insults. However, the GFAP-positive density was relatively low in the three other cases in the HIVC \pm /- group, which supports the suggestion that the presence of HIV-1 in the cerebral cortex is related to an increase in GFAP astrocyte density.

This interpretation also implies that the increased astrocyte density in the groups with no neuropathological abnormalities and CMVE may also result from HIV-1 infection of the cerebral cortex. These cases were all negative for HIV-1 using immunohistochemistry, though proviral DNA, may have been detected using PCR. Unfortunately, frozen tissue was available from only three of these cases. In one with no neuropathological abnormalities, PCR was negative, and this case had the lowest GFAP-positive astrocyte density of the group. One of the two CMVE cases had detectable HIV-1 provirus in the cortex, while the result in the other case was indeterminate, and astrocyte density was similar in these two cases. The examination of more cases, by PCR, would help to clarify this issue.

The HIV-1-seropositive non-AIDS cases were considered separately from those with

AIDS in the analysis of the relationship between morphometric features and the presence of HIV-1 in the cortex. Since non-AIDS cases were at a different clinical stage of disease, the changes induced by HIV-1 in the brain may appear different. HIV-1 proviral DNA was detected in the cortex of only two of the eight cases tested, and there was no relationship between astrocyte density and HIV-1 proviral DNA in the cortex in this group.

Overall, these results suggest that HIV-1 may be related to an increased density of GFAP-positive astrocytes in the cortex of patients with AIDS; though it is clear that HIV-1 is not the only factor responsible for this gliotic reaction, as the density of these cells may also be increased in cases from which HIV-1 cannot be detected by either immunohistochemistry or PCR.

The increased density of GFAP-positive astrocytes in the cortex may result from either an increase in the number of astrocytes in the cortex, or from an increase in the number of astrocytes that are expressing GFAP, or of both. Protoplasmic astrocytes express little, or no GFAP in the normal cortex but may switch on GFAP expression under certain pathological conditions. Budka (1992) found that while the number of cells expressing GFAP is increased in AIDS brains, the number of cells expressing S-100, a neuroectodermal marker that stains both protoplasmic and fibrous cells, remains the same. He interpreted these findings as suggesting that whilst the overall number of astrocytes in the brain is consistent, there is an increase in the number of cells expressing GFAP, during AIDS. In addition, Miyake et al., (1988) using tritiated thymidine to label dividing cells, found that although some GFAP-

positive astrocytes were labelled with tritiated thymidine in the cerebral cortex of mice following a stab wound, the percentage of labelled cells is very low. Although astrocytes may have a limited ability to proliferate, it seems likely that the increased numbers of GFAP-positive cells in injured CNS is mainly due to hypertrophy of astrocytes, with increased expression of GFAP, rather than to hyperplasia.

4.3.4 Changes in morphology and distribution of GFAP-positive astrocytes

In normal cortex, GFAP labelled cells showed two distinct appearances: In one, nuclei appeared to be surrounded by abundant GFAP immunoreactive processes and corresponded to fibrous astrocytes; in the other, nuclei were surrounded by a small amount of ill-defined GFAP immunoreactivity and corresponded to protoplasmic astrocytes. The fibrous appearance was seen mainly in the subpial region or was closely associated with blood vessels, although some parenchymal cells with this appearance were also seen. The protoplasmic type was seen in occasional cells throughout the parenchyma. Increased expression of GFAP is a common reaction of astrocytes to injury; reactive astrocytes are generally recognised to have intensely GFAP-positive hypertrophied cytoplasm (Duchen, 1992). The nuclear volume of these cells also increases and the nucleus may appear eccentrically placed in the cell. In the cortex of AIDS cases, reactive astrocytes were seen in four cases with HIVE or HIV-lep, one case with no obvious changes, one case with CMVE and two cases with cerebrovascular changes. The appearance of these cells in the brains of cases with cerebral HIV-1 infection may be in response to the presence of the virus; indeed reactive astrocytes were observed in a higher percentage of cases from the HIVE+

group, as compared with the HIVC \pm /- cases. However, these cells are also commonly seen in response to other pathological conditions in AIDS.

Alterations in the nuclear appearance of GFAP-positive astrocytes was the most noticeable feature in many of the AIDS cases. Nuclei were enlarged and pale or watery with marginalised chromatin (nuclear score 2 or 3) in the majority of AIDS cases (19/23) and normal appearing nuclei were observed in only one of the AIDS cases, with CMVE. In contrast, enlarged pale nuclei were seen in only one of the HIV-1-seropositive non-AIDS cases, although mild nuclear enlargement was present in another four.

Although these nuclear changes were not specific to cases with detectable HIV-1 proviral DNA in the cortex, and there was no statistically significant difference between the nuclear scores of the HIVC+ and HIVC \pm /- groups; HIV-1 proviral DNA was detected in most of the cases with a high nuclear score. Since nuclear scores are also increased in cases where HIV-1 proviral DNA cannot be detected, HIV-1 cannot be the only factor responsible for this change; although these results suggest that the virus may be involved.

Nuclear enlargement was not confined to cells with increased GFAP-positive cytoplasm. In five cases from the group, with no neuropathological changes, in one case with diffuse gliosis, in one case with HIVE, and one of those with cerebrovascular changes, large pale or watery nuclei were seen in the absence of typically reactive cytoplasmic changes. In addition, this lack of GFAP

immunoreactivity was also observed in the single HIV-1-seropositive non-AIDS case with enlarged pale nuclei. These nuclei were surrounded by a few GFAP-positive branching processes, whilst in some cases the nuclei were surrounded only by a thin rim of GFAP-positive staining. Although GFAP expression may be reduced by prolonged fixation, it is unlikely that the lack of GFAP positivity of these cells was due to fixation as, following prolonged fixation cells with plentiful GFAP-positive cytoplasm were still obvious. Furthermore astrocytes with enlarged pale nuclei and little GFAP staining were found in cases that had been fixed for some of the shortest periods of time.

These large watery nuclei were reminiscent of Alzheimer type II astrocytes that occur in the cortex, in conditions such as Wilson's disease and hepatic encephalopathies (Kimura and Budka, 1986). In addition, biochemical and immunohistochemical analysis of GFAP have revealed that this protein is reduced in the cortex in these conditions. Alzheimer type II astrocytes are described as having little or no GFAP-positive cytoplasm, a swollen, vesicular nucleus with margination of chromatin and may also have an intranuclear inclusion (Kimura and Budka, 1986; Dolman, 1985; Duchen, 1992). Although nuclei observed in some of the AIDS cases in the present study have a similar appearance, most had at least some GFAP-positive processes; moreover no nuclear inclusions were seen. However, with AIDS, it is possible that patients die from systemic infectious disorders, before the effects of a metabolic disturbance on the brain become full-blown. Metabolic encephalopathies may result from a longer period of severe metabolic insult prior to the patients death than in AIDS. It is therefore possible that the enlarged, watery, nuclei with little GFAP-

positive processes seen in AIDS brains, are a response to hepatic dysfunction, that has not become severe enough to produce the typical appearance of metabolic encephalopathy before death. Indeed, hepatic dysfunction occurs frequently in both AIDS patients and HIV-1-seropositive patients that have not developed AIDS (reviewed by Harcourt-Webster, 1992). Pathological examination of livers from AIDS patients reveals a variety of changes including granulomatous hepatitis (associated with mycobacterial and fungal infections); inflammatory reactions associated with CMV and herpes simplex infection; involvement by Kaposi's sarcoma or malignant lymphoma and in most patients who are HIV-1-seropositive there is serological evidence of Hepatitis B, which in many cases may be associated with a history of hepatitis or jaundice. The possibility of a relationship between hepatic and brain pathology in AIDS has however, not been investigated. It is unlikely that a metabolic insult alone is responsible for the cortical reaction in AIDS, since increased GFAP expression, which occurs commonly in these cases, is the opposite of what is observed in cases with metabolic encephalopathy.

In addition to the predominantly nuclear changes, in a few cases (one case of HIVE and one with CMVE) nuclear changes were mild, and extensive GFAP-positive branching processes were seen throughout the cortex. This appearance was similar to the gliofibrillary reaction seen as response to invading organisms such as *Cryptococcus neoforms* and often occurs in AIDS patients. However these organisms were not detected in any of the cases in chapter 2, in which this reaction was observed. Rather there was a diffuse gliofibrillary reaction throughout the cortex.

In the AIDS brains with no obvious pathological changes, and in some of the HIV-1-seropositive non-AIDS cases, a dramatic reduction in perivascular GFAP staining was observed. In cases with no neuropathological abnormalities, there was an increase in GFAP expression by parenchymal cells and a decrease by perivascular astrocytes. However in some of the non-AIDS cases, there appeared to be a reduction in GFAP staining of both. The loss of perivascular staining seemed to be a result of reduced GFAP expression rather than loss of GFAP-positive perivascular astrocytes, as GFAP-positive cells with a small amount of GFAP-positive cytoplasm or a few GFAP-positive processes were still present in this location. Pulliam (1992) observed a loss of GFAP cytoplasmic fibrils in human fetal brain aggregates, to which HIV-1 gp120 had been applied. In addition, astrocyte cultures treated with gp120, showed a loss of GFAP immunoreactivity. Pulliam concluded that gp120 directly damages astrocytes which, in turn may damage neurons. This loss of GFAP in cultured astrocytes treated with GFAP, may correlate with the reduction in GFAP immunoreactive processes that was observed in some cases, notably those with no obvious neuropathological abnormalities, in this study. However, HIV-1 was not detected by immunocytochemistry in the cortex of cases with reduced GFAP immunoreactivity, and only one case from the group with no neuropathological abnormalities was tested by PCR. Since this case did not contain detectable amounts of HIV-1 proviral DNA it cannot be concluded that the gp120 induced loss of astrocyte processes in cell culture, also occurs in the cerebral cortex of AIDS cases. A loss of GFAP immunoreactivity was also observed in the cortex of three of the HIV-1-seropositive non-AIDS cases, HIV-1 proviral DNA was detected in the cortex of one of these cases but not in the other two.

A more severe reduction in GFAP immunoreactivity than was observed in any of the AIDS cases in this study, was seen in the HSVE case observed in the initial observations (section 2.1). Here very few perivascular GFAP-positive cells were observed whilst infiltration of lymphocytes and macrophages was observed around many vessels. In addition, large quantities of HSV antigen were detectable in the cortex of this case. In contrast, antigens were not detected in any of the cases in the no neuropathology group, or among the HIV-1-seropositive non-AIDS cases, whilst perivascular infiltrates of macrophages and lymphocytes were sometimes present in the latter but not in the former. The loss of GFAP immunoreactive processes may result from the secretory products of infiltrating inflammatory cells. In AIDS, the microglial cell/macrophage reaction in the cortex was unremarkable in most of the cases with reduced perivascular GFAP immunoreactivity, whilst in the HIV-1-seropositive non-AIDS cases there was no relationship between the presence of an inflammatory infiltrate and a reduction GFAP immunoreactivity.

These findings are difficult to interpret; loss of normal appearing perivascular fibrous astrocytes could result from a metabolic insult to these cells, as discussed above or from an immunological insult, derived from the circulatory system. Astrocytes are essential for maintenance of the blood-brain barrier (Abbott et al., 1992) and loss of these cells may contribute to its breakdown, morphological evidence of which has been reported in AIDS (Smith et al., 1990). The absence of perivascular insulation by astrocytes seemed to occur predominantly in cases that lacked any obvious neuropathological abnormalities. In the HIV-1-seropositive non-AIDS cases, although a few perivascular macrophages containing haemosiderin were present in the latter

group, no morphological evidence of blood-brain barrier breakdown was evident in the former. However damage to the blood-brain barrier may facilitate the entry of HIV-1 infected monocytes or blood-borne viral particles into the CNS.

GFAP immunoreactive staining around vessels was more abundant in HIV antigen positive, CMVE and cerebrovascular cases; astrocytes with enlarged cytoplasmic volume and thickened processes were present throughout the parenchyma, and although no obvious perivascular distribution was observed in many cases, astrocytes did appear to be in contact with vessels. The increase in GFAP immunoreactivity seen in these cases may reflect a secondary response to an initial insult that induced a reduction in GFAP expression; or may represent a reaction of astrocytes to another pathological insult, such as HIV-1 induced toxicity.

A temporary loss of GFAP immunoreactivity was demonstrated in rats by Björklund et al. (1986) in response to intracerebral injection of QUIN, a known excitotoxin. A reduction in GFAP staining around the site of QUIN injection, was not observed in animals injected with non-toxic amino acids. GFAP staining around the injection site recovered within three days, and within six a gliotic reaction was seen. Furthermore, within six hours of administration of QUIN, an increased density of GFAP-positive astrocytes was observed some distance from the site of injection. Björklund's study demonstrates that astrocytes have the ability to recover from some types of insult that result in GFAP loss. Furthermore, it has been demonstrated that QUIN is present in increased levels in the brain and CSF of AIDS patients and that the level of QUIN seems to correlate with the presence of neurological abnormalities (Heyes et al.,

1989, 1991). QUIN has been demonstrated to produce neuronal damage (Schwartz et al., 1983) as well as astrocytic damage. This kind of excitotoxic damage induced by QUIN, has also been demonstrated to occur in response to HIV-1 gp120 (Brenneman, 1988; Lipton, 1991,1992). Although, unlike QUIN, gp120 is not an antagonist of the NMDA receptor (Lipton 1992), it may indirectly facilitate neurotoxic damage by interfering with the neurotrophic functions of astrocytes, or by inducing the release of neurotoxins from microglial cells (Brenneman, 1988; Lipton 1992). Excitotoxic damage may therefore occur in the brains of AIDS patients in response to increased levels of QUIN, and in cases where HIV-1 is also present, release of gp120 by infected cells may accentuate the neurotoxic processes (Lipton 1992). The observations in the present study of increased astrocyte density and nuclear enlargement in cases with cortical HIV-1 infection, and in some without, may be relevant to the toxic effects of gp120 and QUIN. Thus, the astrocyte reaction in the cortex may be due to increased levels of QUIN, or the presence of HIV-1; whilst the combined effects of QUIN and gp120 may result in more severe damage. The synergistic interaction between these two neurotoxins could be responsible for some of the highly reactive changes, observed in cases with detectable HIV-1 in the brain. The detection of QUIN and HIV-1 gp120 from the cortex of AIDS cases with and without cortical abnormalities would help to resolve this issue.

4.3.5 Changes in RCA-120 microglial cell density

The density of microglial cells was variable in AIDS cases and was not significantly different from normal controls. Surprisingly, there was a significant increase in

microglial density in the HIV-1-seropositive non-AIDS cases; however since the non-AIDS cases were all drug users and the AIDS cases all homosexuals the difference in microglial cell density between these two groups may have been related to difference in their risk factors.

The significant increase in microglial density in the HIV-1-seropositive non-AIDS cases, as compared with normal controls, demonstrates that a microglial cell reaction occurs at an early stage of the disease, at least in HIV-1 infected drug users. The significantly higher density of microglial cells in the non-AIDS cases compared with the AIDS cases suggests that this increase may be transient. Interestingly, Esiri et al. (1991) identified an increase in the density of oligodendrocytes at an early stage of HIV-1 infection, which was not present in cases with AIDS. Examination of the cortex of drug-users with AIDS is necessary to establish whether the increased density in microglial cells is a transient process occurring early in AIDS, or if there is a difference between the microglial cell reaction in HIV-1 infected drug users and homosexuals.

Perivascular cuffing of lymphocytes, a common reaction to viral infection of the brain (Esiri and Kennedy 1992), and subacute meningitis are commonly observed in the brains of HIV-1-seropositive non-AIDS cases (Gray et al., 1992) and were observed in some of the cases in this study. Infiltration of the brain and meninges by inflammatory cells and the increase in microglial cell density, may both be part of the same pathological response. However, the results of this study do not support HIV-1 as being responsible for inducing these changes as HIV-1 proviral DNA was detected

in only two out of the eight non-AIDS cases tested, and not in cases with the highest density of microglial cells. Nevertheless, this early microglial cell reaction may facilitate the entry of HIV-1 into the brain by acting as receptive host cells.

Although the density of microglial cells did not appear to be increased in the cortex of the AIDS cases in the present study, many of these cases had abundant microglial cells and macrophages in the white matter, as described in section 2.1. Although the reason for this difference is not clear, the low density of microglial cells in the cortex, as compared to the white matter is interesting in terms of HIV-1 antigen distribution in the brain. Since microglia and macrophages are the main reservoir of HIV-1 in the CNS, the lower amount of antigen found in the cortex may be related to the lower density of appropriate host cells in this region. In addition, studies on rat brain have demonstrated that CD4 antigen expression is lower in the cortex than the white matter (Perry and Gordon, 1987). Although the expression of CD4 by human microglial cells seems to be very low (Peudenier et al., 1991; Jordan et al., 1991) or in some studies undetectable (Williams et al., 1992), CD4 expression by microglial cells, isolated from adult human brain, has been demonstrated and infection of these cells, by macrophage adapted strains of HIV-1, can be blocked by anti-CD4 antibodies or soluble CD4 (Jordan et al., 1991). There was no difference in detection of HIV-1 proviral DNA from cortical and white matter samples in this study, while there was considerably less viral antigen in the cortex; these findings may reflect the reduced availability of active host cells in the cortex, as compared with the white matter, in which virus replication is facilitated.

There seemed to be no correlation between the density of microglia and the density of astrocytes. Amongst the AIDS cases, both were highest in the HIVE/lep group. However, in the CMVE group, although the astrocyte density was similar to the HIVE/lep group the microglial density was significantly lower. In contrast the microglial reaction in cases with cerebrovascular disease was similar to the HIVE/lep group while the density of astrocytes was significantly lower.

4.2.6 Changes in microglial cell morphology and distribution

Although microglial cell density was not significantly different from normal controls, reactive cells, of either morphological type, were a frequent finding in twelve of the twenty one AIDS cases. Although the density of microglial cells was higher in HIV-1-seropositive non-AIDS cases than the AIDS cases, rod cells were more frequently observed in the cortex of more of the latter cases. Macrophages and MGCs were observed in small numbers in a few of the HIVE/lep cases, and were present in all three of the cases in this group in which HIV-1 antigen was detected in the cortex. These findings indicate that although a microglial reaction is present in the cortex, it would not be revealed by cell counting alone. However, the extent of the microglial cell reaction in the frontal cortex of AIDS cases is undoubtedly mild in comparison to that seen in some other viral infections of the brain, such as the SSPE, PML and HSV cases observed in the preliminary observations (section 2.1).

Reactive microglial cells (enlarged ramified, rod and macrophage cell types) were more likely to be found in the cortex of AIDS cases from which HIV-1 provirus was

detected by PCR, than in cases that were negative or indeterminate. In addition, rod cells were the most frequent cell type in more cases with HIV-1 antigen in the cortex, compared to cases in which only proviral DNA was found. This suggests that HIV-1 is more likely to be present in cases with reactive microglial cells in the cortex than in cases where these cells are not found, when other pathological lesions are not obvious.

Foci of cells observed in some cases were of two distinct appearances: MGNs which consisted of tightly packed aggregates of microglial cells, lacking GFAP-positive astrocytes or, 'loose foci' of microglial cells, less densely packed than in MGNs. GFAP-positive astrocytes were frequently associated with these loose foci of microglial cells. CMV inclusions were sometimes observed in the former type of foci, but never in the latter. The latter type of focal lesion was observed in cases with HIV-1 and in those with cerebrovascular disease. MGNs may represent an appropriate reaction to the presence of CMV in the brain; these foci often contain lymphocytes, which are usually absent from the loose foci. The loose foci of microglial cells and astrocytes are presumed to form in response to HIV-1 infection (Budka et al., 1987; Budka 1991), and HIV-1 antigens can be detected within them. The reason why these foci should differ from those produced in response to CMV is not clear, although the lack of lymphocytes in loose foci may be involved.

Microglial cells and infiltrating macrophages are the major cell types in the nervous system identified as being infected with HIV-1. Gp120 and other potentially toxic

HIV-1 proteins are presumed to be released from these cells. The low levels of detectable antigen in the cortex, suggests that HIV-1 may not be present in sufficient quantities in this region of the brain to induce toxic reaction. However the amount of antigen present may be considerably more than can be detected by immunohistochemistry on paraffin sections; in addition, it has been suggested that relatively low levels of viral products may be sufficient to induce neuronal damage (Giulian et al., 1990). Furthermore, the present study detected the HIV-1 p24 antigen, which does not necessarily reflect the amount of gp120 present in this region.

Microglial cells and macrophages release a range of potentially toxic substances including QUIN and cytokines (Dickson, 1991). Elevated levels of a variety of cytokines have been detected in the CSF and serum of AIDS patients (Grimaldi et al., 1991; Matsuyama et al., 1991) and it has been demonstrated that some of these substances, for example tumour necrosis factor (TNF), can cause CNS damage (Selmaj and Raine, 1988). The possibility that astrocytes may also release cytokines must also be considered. Some astrocyte cell lines can be induced to secrete IL-1, IL-3 and prostaglandins (Frei et al, 1987), although evidence for secretion of these substances by astrocytes *in vivo* is lacking.

Although the reason for over-production of cytokines in the CNS is not clear, it suggests that the immune function within the brain occurring in response to HIV-1 or opportunistic infections is disturbed. Achim et al. (1991a) have demonstrated increased expression of β_2 -Microglobulin (a fragment of the MHC I receptor) by endothelial cells, oligodendrocytes and macrophages in the CNS of AIDS cases with

a viral encephalitis. In addition, increased expression of MHC II by macrophages and microglial cells was observed, by Achim et al.(1991a), but only in cases with HIV. Similar findings were reported by Kennedy and Gairns (1992). Expression of the MHC I receptor, in conjunction with antigen, is necessary for recognition of infected cells by cytotoxic T lymphocytes whilst expression of MHC II is essential for antigen presentation to T-helper (CD4) lymphocytes (Rook, 1989). However, as already mentioned there is a distinct lack of lymphocytes, in AIDS brains as compared with other viral infections of the CNS. Achim et al (1991a&b) proposed that this may be due to the peripheral depletion of CD4 cells and indeed at an early stage of disease, prior to the peripheral depletion of CD4 cells, perivascular cuffing of lymphocytes is observed in the CNS (Gray et al., 1992). In order to induce proliferation of T cells, antigen presenting cells secrete cytokines, including IL-1 and IFN- γ , as they present antigen (Rook, 1989). In AIDS, the presentation of antigen may fail to induce an immune reaction, and in the absence of appropriate feedback, inappropriate release of cytokines by macrophage/microglial cells may result.

Since some astrocyte cell lines have been shown to present antigen and release a range of cytokines, astrogliosis in AIDS may represent inappropriately stimulated astrocytes, which also may release toxic cytokines. However although occasional reports of MHC II expression by astrocytes *in vivo* can be found (Hickey et al., 1985; Frank et al., 1986) numerous recent studies employing immunoelectron microscopy (Sasaki and Nakazato, 1992; Graeber et al., 1992) and double labelling immunohistochemistry (McGeer, 1988) have identified MHC II only on microglial cells and perivascular cells in normal brains and in various pathological conditions

including AIDS encephalopathy. However astrocytes may express MHC I *in vivo* (Sobel and Ames, 1988) and release IL-1 and IFN in response to injury or infection (Frei et al., 1987). Although the increased density of astrocytes in the AIDS cases would suggest that these cells may be responsible for the elevated levels of cytokines that are associated with HIV-1 infection, it seems more likely that microglial cells and macrophages are the source of these factors within the brain, although astrocyte release of some cytokines such as TGF- β (Whal et al., 1991), which may accentuate neurological damage (Matsuyama et al., 1991).

In some pathological conditions, such as EAE in rats, a microglial cell reaction is seen as an initial response, before an astroglial reaction occurs (Matsumoto et al., 1992). The astrocyte response occurs later in EAE and serves to isolate microglial cells from infiltrating inflammatory cell aggregates. This temporal difference in the reaction of microglia and astrocytes was also observed in section 2.1, where the reaction of these cells to the invasion of the brain by *Cryptococcus neoforms* was described. Matsumoto et al. (1992) suggested that while microglia augment the response of infiltrating T cells, astrocytes may suppress the proliferation of these cells and thus limit the inflammatory response and consequent damage to the brain parenchyma. In the present study, the microglial cell reaction was most notable in the cortex of HIV-1-seropositive non-AIDS cases, in which the astrocytic response was not marked. In AIDS cases where a huge increase in the density of astrocytes was observed in some cases, the microglial cell response was not remarkable. If the microglial cell reaction is transient, the reduced density of these cells in cases with full-blown AIDS may be a result of down-regulation of these cells by astrocytes. The

presence of fewer microglial cells but with a more reactive morphology in the cortex of AIDS brains, may be an end result of this process. Although HIV-1 proviral DNA and antigens were not detected in the cortex of most of the non-AIDS cases, the microglial cell reaction in these cases may facilitate the infection of the brain by HIV-1, rather than occurring in response to the virus, as already suggested. The down regulation of an immune response by astrocytes may therefore limit to some extent the proliferation of HIV-1 in this region.

4.2.7 Conclusions on the relationship between HIV-1 detection and the presence of cortical abnormalities.

The detection of HIV-1 was associated with an increased density of GFAP-positive astrocytes, and the presence of reactive microglial cells in the cortex of AIDS cases. In addition, HIV-1 may affect the changes observed in astrocyte nuclear appearance. However, none of these changes were found exclusively in cases with cerebral HIV-1 infection or in cases from which HIV-1 proviral DNA was detected. It is therefore likely that pathological appearances previously described as DPD in AIDS, have a multifactorial aetiology. It has recently been suggested (Sharer, 1992) that the presence of HIV-1 in the brain is merely a "red herring"; thus, infected monocytes that enter the CNS in response to a stimuli, other than the presence of HIV-1, allow the replication of the virus upon becoming activated. HIV-1 expression in the brain according to this scenario, is merely a side effect of other inflammatory stimuli. However, our results suggest that activation of astrocytes and microglial cells is more severe in the cortex of cases where HIV-1 can be detected. Therefore, while the cortical reaction may at first be a response to other opportunistic infections, metabolic dysfunction, or immune factors, the reaction seems exacerbated by the presence of HIV-1, in the cortex.

It is important for the treatment of neurological conditions in AIDS, that the underlying cause of the pathological observations described in this study is determined. Initial observations on the effect of Zidovudine suggest that treatment with this drug may reduce the incidence of AIDS dementia (Portegies et al., 1989)

whilst the identification of HIV-1 p24 antigen in the brains of patients that had been treated with Zidovudine was reduced (Achim et al., 1991a). Together, these findings suggest that HIV-1 is involved in the development of dementia; however clinico-pathological correlations of cortical pathology and neurological symptoms are now urgently required.

This study has also confirmed that HIV-1 can infect the brain parenchyma at an early stage of disease. Previous reports have detected HIV-1 in the CSF of HIV-1 seropositive non-AIDS patients (Ho et al., 1985; Resnick et al., 1988; Shaunak et al., 1990; Goswami et al., 1991; Sinclair et al., 1992).but apart from a report on a single case, from this department (Sinclair et al., 1992) it has not been established if HIV-1 is also present within the brain itself. The results suggest however, that a relatively small proportion of HIV-1-seropositive non-AIDS patients have cerebral HIV-1 infection, although the presence of an indeterminate result in a number of these cases, raises the possibility that a low level of HIV-1 infection is frequently present. Further study of HIV-1-seropositive cases, to determine if HIV-1 is present in the white matter as well as the cortex, is now required.

It has not yet been established if the astrocyte and microglial cell changes in AIDS brains are related to the neuronal loss that occurs in AIDS. However Everall et al. (1991) found that neuronal loss was present in both cases with HIV and those they described as having minimal changes, while there was no neuronal loss in HIV-seropositive non-AIDS cases. These results bear similarities to those obtained in this study for astrocyte density; increased in both HIV/lep and no pathology cases, while

there was no increase in the HIV-1-seropositive non-AIDS cases. At present, it has not been established if there is a correlation between the presence of HIV-1 in the cortex and neuronal loss. Further study of astrocyte, microglial cell and neuronal changes are necessary to establish associations with: expression of HIV-1 proteins; expression of QUIN; cytokine expression and hepatic dysfunction. In addition, clinico-pathological correlations of neurological dysfunction and cortical damage are urgently required.

REFERENCES

Abbott NJ, Revest PA, Romero IA (1992) Astrocyte-endothelial interaction: physiology and pathology. *Neuropathology and Applied Neurobiology* **18**:424-433.

Achim CL, Morey MK, Wiley CA (1991a) Expression of major histocompatibility complex and HIV antigens within the brains of AIDS patients. *AIDS* **5**:535-541.

Achim CL, Schrier RD and Wiley CA (1991b) Immunopathogenesis of HIV encephalitis. *Brain Pathology* **1**:177-184.

AIDS Information, International Literature on Acquired Immunodeficiency Syndrome and related retroviruses, Leeds University Press. August 1992; Vol 8:pp32.

American Academy of Neurology AIDS Task Force (1989) Human immunodeficiency virus (HIV) infection and the nervous system. *Neurology* **83**:119-122.

American Academy of Neurology AIDS Task Force (1991) Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. *Neurology* **41**:778-785.

Anderson JM, Milner RDG, Strich SJ (1967) Effects of neonatal hypoglycaemia on the nervous system: a pathological study. *Journal of Neurology Neurosurgery and Psychiatry* **30**:295-310.

Artigas J, Niedobitek F, Grosse G, Heise W, Gosztonyi G (1989) Spongiform encephalopathy in AIDS dementia complex: report of five cases. *Journal of Acquired Immune Deficiency Syndrome* **2**: 374-381.

Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**:868-871.

Battula N, Temin HM (1987) Sites of integration of infectious DNA of avian reticuloendothelial viruses in different avian cellular DNAs. *Cell* **13**:387.

Beal MF (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Annals of Neurology* **31**:119-130.

Bélec L, Martin PMV, Vohito MD, Gresenguet G, Tabo A, Georges AG (1989) Low prevalence of neuro-psychiatric clinical manifestations in central African patients with acquired immune deficiency syndrome. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **83**:844-846.

Belman AL, Ultmann MH, Horoupian D, Novick B, Spiro AJ, Rubinstein A, Kurtzberg D, Cone-Wesson B (1985) Neurological complications in infants and children with acquired immune deficiency syndrome. *Annals of Neurology* **18**:560-566.

Bignami A, Railston HJ (1969) The cellular reaction to Wallerian degeneration in the central nervous system of the cat. *Brain Research* 13:444-61.

Björklund H, Olson L, Dahl D, Schwarcz R (1986) Short- and long-term consequences of intracranial injections of the excitotoxin, quinolinic acid, as evidenced by GFA immunohistochemistry of astrocytes. *Brain Research* 371:267-277.

Bootman JS, Kitchin PA (1992) An international collaborative study to assess a set of reference reagents for HIV-1 PCR. *Journal of Virological Methods* 37:23-42.

Bredesen DE, Messing R (1983) Neurological syndromes heralding the acquired immune deficiency syndrome. *Annals of Neurology* 14:141 (abstract).

Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, Pert CB (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 335:639-642.

Brew BJ, Bhalla RB, Paul M, Sidtis JJ, Keilp JJ, Sadler AE, Gallardo H, M^cArthur MK, Schwartz MK, Price RW (1992) Cerebrospinal fluid β_2 -microglobulin in patients with AIDS dementia complex: an expanded series including response to zidovudine treatment. *AIDS* 6:461-465.

Budka H (1986) Multinucleated giant cells in brain: A hallmark of the acquired immune deficiency syndrome (AIDS). *Acta Neuropathologica (berl)* 69:253-258.

Budka H, Costanzi G, Cristina S, Lechi A, Parravivini C, Trabattoni R, Vago L (1987) Brain pathology induced by infection with the human immunodeficiency virus (HIV). *Acta Neuropathologica (berl)* 75:186-198.

Budka H (1988) Human immunodeficiency virus in glial cells. *Journal of Infectious Disease* 157:203.

Budka H (1990) Human immunodeficiency virus (HIV) envelope and core proteins in CNS tissues of patients with the acquired immune deficiency syndrome (AIDS). *Acta Neuropathologica* 79:611-619

Budka H (1991) Neuropathology of human immunodeficiency virus infection. *Brain Pathology* 1:163-175.

Budka H, Wiley CA, Kleihues P, Artigas J, Asbury AK, Cho E-S, Cornblath DR, Dal Canto MC, DeGirolami U, Dickson D, Epstein LG, Esiri MM, Giangaspero F, Gosztanyi G, Gray F, Griffin JW, Henin D, Iwasaki Y, Janssen RS, Johnson RT, Lantos PL, Lyman WD, M^cArthur JC, Nagashima K, Peress N, Petito CK, Price RW, Rhodes RH, Rosenblum M, Said G, Scaravilli F, Sharer LR, Vinters HV (1991) HIV-associated disease of the nervous system: review of nomenclature and proposal for neuropathology-based terminology *Brain Pathology* 1:143-152.

Budka H (1992) Neuropathology of HIV infection: Update. *Neuroscience of HIV infection: Satellite conference, VIIIth International Conference on AIDS*, Amsterdam, July 14-17th.

Burgess A, Riccio M (1992) Cognitive impairment and dementia in HIV-1 infection. In: Rudge (ed) *Bailliere's Clinical Neurology: Neurological Aspects of the Human Retroviruses*, Baillière Tindall, London; pp155-174.

Center for Disease Control (1981a) Pneumocystis pneumonia - Los Angeles, *Morbidity and Mortality Weekly Report June 5th*, **30**:250-252.

Center for Disease Control (1981b) Kaposi's sarcoma and pneumonia among homosexual men - New York and California. *Morbidity and Mortality Weekly report July 3rd*, **30**:305-308.

Cheng-Mayer C, Levy JA (1988) Distinct biological and serological properties of human immunodeficiency viruses from the brain. *Annals of Neurology* **23**:S58-S61.

Cheng-Mayer C (1990) Biological and molecular features of HIV-1 related to tissue tropism. *AIDS* **4**:S49-S56.

Choi BH (1981) Hematogenous cells in the central nervous system of developing human embryos and fetuses. *Journal of Comparative Neurology* **196**:683-694.

Ciardi A, Sinclair E, Scaravilli F, Harcourt-Webster NJ, Lucas S (1990) The involvement of the cerebral cortex in human immunodeficiency virus encephalopathy: a morphological and immunohistochemical study. *Acta Neuropathologica (berl)* **81**:51-59.

Clague CPT, Ostrowski MA, Deck JHN, Harnish DG, Colley EA, Stead RH (1988) Severe diffuse necrotizing cortical encephalopathy in acquired immune deficiency syndrome (AIDS): an immunocytochemical and ultrastructural study. *Journal of Neuropathology and Experimental Neurology* **47**: 346 (Abstr).

Collewijn H and Schadé JP (1965) Changes in the size of astrocytes and oligodendrocytes during anoxia, hypothermia and spreading depression. In: EDP De Robertis and R Carrea (eds) *Progress in Brain Research*, vol. 15 Elsevier, Amsterdam; pp 184-95.

Crooks DA, Scholtz CL, Vowles G, Greenwald S, Evans S (1991) The glial reaction in closed head injuries. *Neuropathology and Applied Neurobiology* **17**:407-414.

Cullen B, Greene W (1989) Regulatory pathways governing HIV-1 replication. *Cell*:**58**:423-426.

Dal canto MC (1989) AIDS and the nervous system: Current status and future perspectives. *Human pathology* **20**:410-418.

Dal canto MC, Rabinowitz SG (1982) Experimental models of virus-induced demyelination of the central nervous system. *Annals of Neurology* 11:109-127.

Dalgleish AG, Beverley PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA (1984) The CD4(T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* 312:763-766.

Davies JF, Zuana H, Hostomsky Z, Jordan SR, Matthews DA (1991) Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase. *Science* 252:88-95.

Deen KC, McDougal JS, Inacker R, Folena-Wasserman G, Arthos J, Rosenberg J, Maddon PJ, Axel R, Sweet RW (1988) A soluble form of CD4 (T4) selectively inhibits AIDS virus infection. *Nature* 313:82-84.

Dickson DW, Mattiace LA, Kure K, Hutchins K, Lyman WD, Brosnan CF (1991) Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. *Laboratory Investigation* 64:135-156.

Dolman CL (1985) Microglia. In: Davis R & Robertson DM (Eds), *Text book of Neuropathology*. Williams & Wilkins; pp117-137.

Dorfman LJ (1973) Cytomegalovirus encephalitis in adults. *Neurology* 23:136-143. Dreyer EB, Kaiser PK, Offermann JT, Lipton SA (1990) HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science* 248:364-367.

Duchen LW (1992) General Pathology of neurons and glia. In: H Adams LW & Duchen (Eds), *Greenfield's Neuropathology, 5th edition*, Edward Arnold: pp1-68.

Eilbott DJ, Peress N, Burger H, LaNeve D, Orenstein J, Gendelman HE, Seidman R, Weiser B (1989) Human immunodeficiency virus type 1 in spinal cords of acquired immunodeficiency syndrome patients with myelopathy: Expression and replication in macrophages. *Proceedings of the National Academy of Science (USA)* 86:3337-3341.

Eng LF, Vanderhaegen JJ, Bignami A, Gerstl B (1971) An acidic protein isolated from fibrous astrocytes. *Brain Research* 28:351-354.

Epstein LG, Sharer LR, Cho E-S, Meyenhofer MF (1984). HLTV-II/LAV-like retrovirus particles in the brains of patients with AIDS encephalopathy. *AIDS Research and Human Retroviruses* 5:447-454.

Erlich HA (1989) Basic methodology. In: HA Erlich (ed) *PCR Technology*, Stockton Press:pp1-5.

Esiri MM, Oppenheimer DR, Brownell B, Haire M (1982) Distribution of measles antigen and immunoglobulin-containing cells in the CNS in subacute sclerosing

panencephalitis and atypical measles encephalitis. *Journal of Neurological Sciences* 53:29-43.

Esiri MM, Moris CS (1991) Immunocytochemical study of macrophages and microglial cells and extracellular matrix components in human CNS disease. 2. Non-neoplastic disease. *Journal of the Neurological Sciences* 101:59-72.

Esiri MM, Moris CS, Millard PR (1991) Fate of oligodendrocytes in HIV-1 infection. *AIDS* 5:1081-1088.

Esiri MM (1992) Role of the Macrophage in HIV encephalitis. In: F scaravilli (ed), *The Neuropathology of HIV infection*, Springer-Verlag pp 235-254.

Esiri MM, Kennedy PGE (1992) Virus disease. In: JH Adams & LW Duchon (eds) *Greenfield's Neuropathology, 5th edition*. Edward Arnold: pp335-399.

Everall IP, Luthert PJ, Lantos PL (1991) Neuronal loss in the frontal cortex in HIV infection. *Lancet* 337:1119-1121.

Feinberg MB, Greene WC (1992) Molecular insights into human immunodeficiency virus type 1 pathogenesis. *Current Opinion in Immunology* 4:466-474.

Fenyö EM, Albert J, Åsjö B (1989) Replicative capacity, cytopathic effect and cell tropism of HIV. *AIDS* 3(suppl 1):S5-S12.

Festoff BW, Rao JS, Brenneman DE (1990) Vasoactive intestinal peptide (VIP) is a secretagogue for protease nexin 1 (PN1) release from astrocytes. *Society of Neuroscience Abstracts* 16:909.

Fisher RA, Bertonis JM, Meier W, Johnson VA, Costopoulos DS, Liu T, Tizard R, Walker BD, Hirsch MS, Schooley RT, Flavell RA (1988) HIV infection is blocked *in vitro* by recombinant soluble CD4. *Nature* 331:76-78.

Fontana A, Frei K, Bodmer S, Hoffer E (1987) Immune-mediated encephalitis: on the role of antigen presenting cells in brain tissue. *Immunological Reviews* 100:185-201.

Frank E, Pulve M, de Tribolet N (1986) Expression of class II major histocompatibility antigens on reactive astrocytes and endothelial cells within the gliosis surrounding metastases and abscesses. *Journal of Neuroimmunology* 12:29-36.

Frei K, Siepl C, Bodmer S, Fontana A (1987) Astrocytes and microglial cells as immune regulatory cells of the brain. In: MD Norenberg, L Hertz, A Schousboe (Eds) *The biochemical Pathology of astrocytes, Neurology and Neurobiology*. 39:239-246.

Gabuzda DH, Ho DD, de la Monte S, Hirsch MS, Rota TR, Sobel RA (1986)

Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. *Annals of Neurology* **20**:289-295.

Gajdusek DC, Amyx HL, Gibbs CJ, Ashier DM, Rodgers-Johnson P, Epstein LG, Sarin PS, Gallo RC, Maluish A, Arthur LO, Montagnier C, Mildvan D (1985) Infection of chimpanzees by human T-lymphotropic retroviruses in brain and other tissues from AIDS patients. *Lancet* **1**:55-56.

Gallo RC, Salahudin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B, White G, Foster P, Markham PD (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS or at risk for AIDS. *Science* **224**:500-503.

Gartner S, Markovitz P, Markovitz DM, Betts RF, Popovic M (1986) Virus isolation from and identification of HTLV-III/LAV-producing cells in brain tissue from a patient with AIDS. *Journal of the American Medical Association* **256**:2365-2371.

Gilboa E, Mitra SW, Goff S, Baltimore D (1979) A detailed model of reverse transcription and tests of crucial aspects. *Cell* **18**:93-98.

Giulian D, Vaca K, Noonan CA. (1990) Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* **250**:1593-1596.

Giulian D (1992) Production of neurotoxins by HIV-1 infected mononuclear cells. *Neuroscience of HIV infection: Satellite conference, VIIIth International Conference on AIDS*, Amsterdam, July 14-17th.

Goff S (1990) Retroviral reverse transcriptase: synthesis, structure, function. *Journal of the Acquired Immune Deficiency Syndrome* **3**:817-825.

Goswami KK, Miller RF, Harrison MJ, Hamel DJ, Daniels RS, Tedder RS (1991) Expression of HIV-1 in the cerebrospinal fluid detected by the polymerase chain reaction and its correlation with central nervous system disease. *AIDS* **5**:797-803.

Gosztanyi G, Lamperth L, Webster H de F (1988) *In situ*-hybridization study of HIV DNA and RNA distribution in AIDS encephalopathy lesions. *Journal of Neuropathology and Experimental Neurology* **47**: 346 (Abstr).

Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A (1981) *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *New England Journal of Medicine* **305**:1425-1431.

Goudsmit J, Wolters EC, Bakker M, Smit I, Van der Noordaa J, Hische EAH, Tutuarima JA, Van der Helm HJ (1986) Intrathecal synthesis of antibodies to HTLV-III in patients without AIDS or AIDS related complex. *British Medical Journal* **292**:1231-1234.

Graeber MB, Streit WJ (1990) Microglia: Immune network in the CNS. *Brain Pathology* 1:2-5.

Graeber MB, Streit WJ, Buringer D, Sparks DL, Kreutzberg (1992) Ultrastructural location of major histocompatibility complex (MHC) Class II positive perivascular cells in histologically normal human brain. *Journal of Neuropathology and Experimental Neurology* 51:303-311.

Gray F, Gherardi R, Keohane C, Favolini M, Sobels A, Poirier (1988a) Pathology of the central nervous system in 40 cases of acquired immune deficiency syndrome (AIDS). *Neuropathology and Applied Neurobiology* 14:365-380.

Gray F, Gherardi R, Scaravilli F. (1988b) The neuropathology of the acquired immune deficiency syndrome (AIDS): A review. *Brain* 111:245-266.

Gray F, Gaulard P, le Bezu M, Sinclair E, Gherardi R, Scaravilli F and Poirier J (1990) HIV encephalitis-like multinucleated giant cells in a nodal lymphoma in AIDS. *Histopathology* 16:402-405.

Gray F, Haug H, Chimelli L, Geny C, Gaston A, Scaravilli F, Budka H (1991) Prominent cortical atrophy with neuronal loss as a possible correlate of Human Immunodeficiency virus encephalopathy. *Acta Neuropathologica (berl)* 82:229-233.

Gray F, Lescs M-C, Keohane C, Paraire F, Marc B, Durigon M, Gherardi R (1992) Early brain changes in HIV infection: neuropathological study of 11 HIV-seropositive non AIDS cases. *Journal of Neuropathology and Experimental Neurology* 51:177-185.

Grimaldi LME, Martino GV, Franciotta DM, Brustia R, Castagna A, Pristera R Lazzarin A (1991) Elevated alpha-tumour necrosis factor levels in spinal fluid from HIV-1-infected patients with central nervous system involvement. *Annals of Neurology* 29:21-25.

Gyorkey F, Melnick JL, Gyorkey P. (1987) Human immunodeficiency virus in brain biopsies of patients with AIDS and progressive encephalopathy. *Journal of Infectious Disease* 155:870-876.

Haase AT. (1986) Pathogenesis of lentivirus infections. *Nature* 322:130-136.

Hahn BH, Shaw GM, Taylor ME, Redfield RR, Markham PD, Salahuddin SZ, Wong-Staal F, Gallo RC, Parks ES, Parks WP (1986) Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. *Science* 232:1584-1553.

Harcourt-Webster JN (1992) General Pathology. In: F Scaravilli (ed) *The Neuropathology of HIV Infection*. Springer-Verlag:pp 53-97.

Harrison MJG (1992) Neurological complications of HIV infection: clinical aspects. In: F Scaravilli (ed) *The Neuropathology of HIV Infection*. Springer-Verlag:pp 21-33.

Hayes GM, Woodrooffe MN, Cuzner ML (1987) Microglia are the major cell type expressing MHC Class II in human white matter. *Journal of Neurological Science* 80:25-37.

Hénin D, Duyckaerts C, Chaunu M P, Vazeux R, Brousse N, Rozenbaum W, Haun JJ (1987) Etude neuropathologique de 31 cas de syndrome d'immuno-dépression acquise. *Revue Neurologique (Paris)* 143:631-642.

Hertz L (1978) An intense potassium uptake into astrocytes, its further enhancement by high concentrations of potassium and its possible involvement in potassium homeostasis at the cellular level. *Brain Research* 145:202-208.

Heyes MP, Rubinow D, Lane C, Markey SP (1989) Cerebrospinal fluid quinolinic acid concentrations are increased in acquired immune deficiency syndrome. *Annals of Neurology* 26:275-277.

Heyes MP, Brew BJ, Martin A, Price RW, Salazar AM, Sidtis JJ, Yergey JA, Mourdain MM, Sadler AE, Kelip J, Rubinow D, Markey SP (1991) Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: Relationship to clinical and neurological status. *Annals of Neurology* 29:202-209.

Hickey WF, Osborn JP, Kirby WM (1985) Expression of Ia molecules by astrocytes during experimental allergic encephalomyelitis in the Lewis rat. *Cellular Immunology* 91:528-35.

Hickey WF and Kimura H (1988) Perivascular microglia cells of the CNS are bone marrow-derived and present antigen *in vivo*. *Science* 239:290-292.

Ho DD, Rota TR, Schooley RT, Kaplin JC, Allen JD, Groopman JE, Resnick L, Felstein D, Andrews CA, Hirsch MS (1985) Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *New England Journal of Medicine* 313:1493-1497.

Ho DD, Kaplin JC, Rackauskas IE, Gurney ME (1988) Second conserved domain of gp120 is important for infectivity and antibody neutralization. *Science* 239:1021-1023.

Hoover EA, Mullins JI, Quackenbush SL, Gasper P (1987) Experimental Transmission and pathogenesis of immunodeficiency syndrome in cats. *Blood* 70:1880-1892.

Howlett WP, Nkya WM, Mmuni KA, Missalek WR (1989) Neurological disorders in AIDS and HIV disease in the northern zone of Tanzania. *AIDS* 3:289-296.

Hussey RE, Richardson NE, Kowalski M, Brown NR, Chang H-C, Siliciano RF, Dorfman T, Walker B, Sodroski J, Reinhertz EL (1988) A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. *Nature* 331:78-82.

Jordan FL and Thomas WE (1988) Brain macrophages: questions of origin and interrelationship. *Brain Research Reviews* 13:165-178.

Jordan CA, Watkins BA, Kufta C, Dubois-Dalcq M (1991) Infection of brain microglial cells by human immunodeficiency virus type 1 is CD4 dependent. *Journal of Virology* 65:736-742.

Juhler M, Neuwelt EA (1989) The blood-brain barrier and the immune system. In: Neuwelt EA (ed) *Implications of the blood-brain barrier and its manipulation; volume 1 Basic Science Aspects*. Plenum Publishing Corporation, NY; pp261-285.

Kauss J, Zurlinden B, Schlote W (1991) Axonal volume and myelin sheath thickness of CNS myelinated fibers are reduced in HIV encephalopathy. *Clinical Neuropathology* 10:38 (Abstract).

Keating JN, Trimble KC, Mulcahy F, Scott JM, Weir DG (1991) Evidence of brain methyltransferase inhibition and early brain involvement in HIV-positive patients. *Lancet* 337:935-938.

Kennedy PGE, Gairns J (1992) Major histocompatibility complex (MHC) antigen expression in HIV encephalitis. *Neuropathology and Applied Neurobiology* 18:478-522.

Ketzler S, Weis S, Haug A, Budka H. (1990) Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropathologica (Berl)* 80:92-94.

Kimura T, Budka H (1986) Glial fibrillary acidic protein and S-100 protein in human hepatic encephalopathy: Immunocytochemical demonstration of dissociation of two glial-associated proteins. *Acta Neuropathologica (Berl)* 70:17-21.

Klatzmann D, Champagne E, Chamaret S, Gruest S, Guetard D, Hercend T, Gluckman J-C, Montagnier L (1984) T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* 312:767-771.

Kleihues P, Lang W, Burger PC, Budka H, Vogt M, Maurer R, Luthy R, Siegenthaler W (1985) Progressive diffuse leukoencephalopathy in patients with acquired immune deficiency syndrome (AIDS). *Acta Neuropathologica (berl)* 68:333-339.

Kleihues P, Leib SL, Strittmatter C, Wiestler OD, Lang W (1991) HIV encephalopathy: incidence, definition and pathogenesis. Results of a Swiss collaborative study. *Acta Pathologica (Japan)* 43:197-205.

Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS (1986) Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 233:1089-93.

Kowalski M, Potz J, Basiripour L, Dorfman T, Goh WC, Terwilliger E, Dayton A, Rosen C, Haseltine W, Sodroski J (1987) Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. *Science* **237**:1351-1355.

Kure K, Weidenheim KM, Lyman WD, Dickson DW. (1990) Morphology and distribution of HIV-1 gp41-positive microglia in subacute AIDS encephalitis. *Acta Neuropathologica (berl)* **80**:393-400

Kwok S, Higuchi R (1989) Avoiding false positives with PCR. *Nature* **339**:237-238.

Lahdevirta J, Maury CPJ, Teppo A-M, Repo H (1988) Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immune deficiency syndrome. *American Journal of Medicine* **85**:289-291.

Lang W, Miklossy J, Deruaz JP, Pizzalato GP, Probst A, Schaffner T, Gessaga E (1989) Neuropathology of the acquired immune deficiency syndrome (AIDS): a report of 135 consecutive autopsy cases from Switzerland. *Acta Neuropathologica (berl)* **77**:379-390.

Lasky LA, Groopman JE, Fennie CW, Benz PM, Capon DJ, Dowbenko DJ, Nakamura GR, Nunes WM, Renz ME, Berman PW (1986) Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. *Science* **233**:209-212.

Levy RM, Bredeisen DE, Rosenblum ML (1985a) Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): experience at UCSF and review of the literature. *Journal of Neurosurgery* **62**:475-495.

Levy JA, Shimabukuro J, Hollander H, Mills J, Kaminsky L (1985b) Isolation of AIDS-associated retroviruses from cerebrospinal fluid and brain of patients with neurological symptoms. *Lancet* **II**:586-588.

Lipton SA (1991) HIV-related neurotoxicity. *Brain Pathology* **1**:193-199.

Lipton SA (1992) Models of neuronal injury in AIDS: Another role for the NMDA receptor? *Trends in Neuroscience* **15**:75-79.

Ma KC, Chang ZH, Shih H, Zhu JH, Wu JY (1991) The compensatory 'rebound' of reactive astrogliosis: glial fibrillary acidic protein immunohistochemical analysis of reactive astrogliosis after a puncture wound to the brain of rats with portocaval anastomosis. *Acta Neuropathologica* **82**:72-77.

Maddon PJ, Daglish AG, McDougal JS, Clapham PR, Weiss RA, Axel R (1986) The T₄ gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**:333-384.

Mandybur TI, Ormsby I, Zemlan FP (1989) Cerebral aging: a quantitative study of

gliosis in old nude mice. *Acta Neuropathologica (berl)* **77**:507-513.

Martinez-Hernandez A, Bell KP, Norenberg MD (1977) Glutamine synthetase: glial localization in brain. *Science* **195**:1356-1358.

Matsumoto Y, Ohmori K, Fujiwara M (1992) Microglial and astroglial reactions to inflammatory lesions of experimental autoimmune encephalomyelitis in the rat central nervous system. *Journal of Neuroimmunology* **37**:23-33.

Matsuyama T, Kobayashi N, Yamamoto N. (1991) Cytokines and HIV infection: is AIDS a tumor necrosis factor disease? *AIDS* **5**:1405-1417.

Matthijs T, Miedema F (1990) Interactions between HIV and the host immune system in the pathogenesis of AIDS. *AIDS* **4**:S57-S66.

M^cArthur JC (1987) Neurologic manifestations of AIDS. *Medicine* **66**:407-437.

M^cDougal JS, Kennedy MS, Sligh JM, Cort SP, Malwe A, Nicholson JKA (1986) Binding of HLT^v-III/LAV to T⁴⁺ cells by a complex of the 110K viral protein and the T4 molecule. *Science* **331**:382

M^cGeer PL, Itagaki S, McGeer EG (1988) Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathologica (berl)* **76**:550-557.

M^cKeating JA, Griffiths PD & Weiss RA (1990) HIV susceptibility conferred to human fibroblasts by cytomegalovirus-induced Fc receptor *Nature* **343**:659-661.

Meltzer MS, Skillman DR, Gomatos PJ, Kalter DC, Gendelman HE (1990) Role of mononuclear phagocytes in the pathogenesis of human immunodeficiency virus infection. *Annual Review of Immunology* **8**:169-194.

Meyenhofer MF, Epstein LG, Cho E-S, Sharer LR (1987) Ultrastructural morphology and intracellular production of human immunodeficiency virus (HIV) in brain. *Journal of Neuropathology and Experimental Neurology* **46**:474-484.

Michaels J, Sharer LR, Epstein LG (1988) Human immunodeficiency virus type 1 (HIV-1) infection of the nervous system: A review. *Immunodeficiency Reviews* **1**:71-104.

Mims CA, White DO (1984) Infection and the spread of viruses through the body. In: *Viral Pathogenesis and Immunology*. Blackwell; pp68.

Mirra SS, del Rio C (1989) The fine structure of the acquired immunodeficiency syndrome encephalopathy. *Archives of Pathology and Laboratory Medicine* **113**:858-865.

Mitrani E, Coffin J, Boedtker H, Doty P (1987) Rous sarcoma virus is integrated but not expressed in chicken early embryonic cells. *Proceedings of the National Academy of Science (USA)* **84**:2781-2784.

Miyake T, Hattori T, Fukuda M, Kiramura T, Fujita S (1988) Quantitative studies on proliferative changes of reactive astrocytes in mouse cerebral cortex. *Brain Research* **451**:133-138.

Mizrachi Y, Zeira M, Shahabuddin M, Sinangil, GL Volsky IF (1991) Efficient binding, fusion and entry of HIV-1 into CD4-negative neural cells: a mechanism of neuropathogenesis in AIDS. *Bulletin of the Institute Pasteur* **89**:81-96.

Monte SM de la, Ho DD, Schooley RT, Hirsch MS, Richardson EP (1987) Subacute encephalomyelitis of AIDS and its relation to HTLV-III infection. *Neurology* **37**:562-569.

Morgello S, Cho E-S, Nielson S, Devinsky O, Petit CK (1987) Cytomegalovirus in the nervous system of patients with the acquired immunodeficiency syndrome: An autopsy study of 30 cases and review of the literature. *Human Pathology* **18**:289-297.

Moskowitz LB, Hensley GT, Chan JC, Gregorios J, Conley FK (1984) The neuropathology of acquired immune deficiency syndrome. *Archives of Pathological and Laboratory Medicine* **108**:867-872.

Murabe Y, Sano Y (1982) Morphological studies on neuroglia. V Microglial cells in the cerebral cortex of the rat with special reference to their possible involvement in synaptic function. *Cell and Tissue Research* **223**: 493-506.

Narayan O, Clements JE, Strandberg JD, Cork LC, Griffin DE (1980) Biological characterization of the virus causing leukoencephalitis and arthritis in goats. *Journal of General Virology* **50**:69-79.

Nathanson N, Georgsson G, Palsson PA, Najjar JA, Lutley R, Petursson G (1985) Experimental visna in Icelandic sheep: The prototype lentiviral infection. *Reviews of Infectious Disease* **7**:75-82.

Navia BA, Jordan BD, Cho E-S, Petit CK, Price RW (1985) AIDS dementia: Clinical-pathological analysis. *Neurology* **35**(suppl 1):145(Abstract).

Navia BA, Cho E-S, Petit CK, Price RW (1986b) the AIDS dementia complex: II Neuropathology. *Annals of Neurology* **19**:525-535

Navia BA, Jordan BD, Price RW, (1986a) The AIDS dementia complex: I. Clinical features. *Annals of Neurology* **19**:517-524.

Nelson JA, Reynolds-Kohler C, Oldstone MBA, Wiley CA (1988) HIV and HCMV coinfect brain cells in patients with AIDS. *Virology* **165**:286-290.

Nelson JA, Ghazal P, Wiley CA (1990) Role of opportunistic viral infections in AIDS. *AIDS* 4:1-10.

Nicoll JAR, Maitland NJ, Love S (1991) Use of the polymerase chain reaction to detect herpes simplex virus DNA in paraffin section of human brain at necropsy. *Journal of Neurology, Neurosurgery, and Psychiatry* 54:167-168.

O'Brien WA, Koyanagi Y, Chen ISY (1990a) Virological aspects of the nervous system. In: Vinters HV and Anders KH (eds) *Neuropathology of AIDS*, CRC Press, Florida; 189-207.

O'Brien WA, Zack JA, Chen ISY (1990b) Molecular pathogenesis of HIV-1. *AIDS* 4:S41-S48.

Olney JW (1989) Excitatory amino acids and neuropsychiatric disorders. *Biological Psychiatry* 26:505-525.

Pang S, Koyanagi Y, Miles S, Wiley C, Vinters HV, Chen ISY (1990) High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients. *Nature* 343:85-89.

Peluso R, Haase A, Stowring L, Edwards M, Ventura P (1985) A Trojan Horse mechanism for the spread of visna virus in monocytes. *Virology* 147:231-236.

Perentes E, Rubinstein LJ (1987) Recent applications of immunoperoxidase histochemistry in human neuro-oncology. *Archives of Pathology and Laboratory Medicine* 111:796-812.

Perry VH, Gordon S (1987) Modulation of CD4 antigen on macrophages and microglia in rat brain. *Journal of Experimental Medicine* 166:1138-1143.

Perry VH, Gordon S (1988) Macrophages and microglia in the nervous system. *Trends in Neuroscience* 6:273-277.

Pert CB, Mervis RF, Hill JM, Breneman DE, Ruff MR, Switzer RC, Warren B, Glowa JR (1989) Morphological and behavioural response to acute and subacute i.c.v. gp120 administration in the rat. *Society of Neuroscience Abstracts* 15:1387

Peters A, Palay SL, Webster H deF (1976) *The fine structure of the nervous system*, 2nd edn, Saunders, Philadelphia.

Petito CK, Cho E-S, Lemann W, Navia BA, Price RW. (1986) Neuropathology of the acquired immune deficiency syndrome (AIDS): An autopsy review. *Journal of Neuropathology and Experimental Neurology* 45:635-646.

Peudenier S, Hery C, Montagnier L, Tardieu M (1991) Human microglial cells: Characterization in cerebral tissue and in primary culture, and study of their

susceptibility to HIV-1 infection. *Annals of Neurology* **29**:152-161.

Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**:497-500

Portegies P, de Gans J, Lange JM, Derix MMA, Speelman H, Bakker M, Danner SA (1989) Declining incidence of AIDS dementia complex after introduction of Zidovudine treatment *British Medical Journal* **299**:819-821

Porwit A, Parravicini C, Petren A-L, Barkhem T, Costanzi G, Josephs S, Biberfeld T (1989) Cell association of HIV in AIDS-related encephalopathy and dementia *APIMS* **97**:79-90.

Price RW, Brew B (1988) AIDS commentary: the AIDS dementia complex. *The Journal of Infectious Diseases* **158**:1079-1083.

Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988) The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239** :586-592.

Pulliam L, Herndier BG, Tang NM, M^cGrath S (1991) Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *Journal of Clinical Investigation* **87**:503-512.

Pulliam L (1992) Cellular mechanisms of HIV-encephalopathy. *Neuroscience of HIV infection: Satellite conference, VIIIth International Conference on AIDS*, Amsterdam, July 14-17th.

Pumarola-Sune T, Navia BA, Cordon-Cardo C, Cho E-S, Price RW (1987) HIV antigen in the brains of patients with the AIDS dementia complex *Annals of Neurology* **21**:490-496.

Resnick L, Berger JR, Shapshak P, Tourtellotte W (1988) Early penetration of the blood-brain barrier by HIV. *Neurology* **38**:9-14.

Rook G (1989) Cell-mediated immune responses. In: I Roitt, J Brostoff, D Male *Immunology, 2nd edition*. Gower Medical Publishing; pp 9.2-9.13.

Rosenblum MK (1990) Infection of the central nervous system by the human immunodeficiency virus type 1: Morphology and relation to syndromes of progressive encephalopathy and myelopathy in patients with AIDS. *Pathology Annual* **1**:117-69.

Rusche JR, Javaherian K, M^cDanal CM, Petro J, Lynn DL, Grimaila R, Gallo RC, Arthur LO, Fischinger PJ, Bolognesi DP, Putney SD, Matthews TJ (1988) Antibodies that inhibit fusion of human immunodeficiency virus-infected cells bind a 24-amino

acid sequence of the viral envelope gp120. *Proceedings of the National Academy of Sciences USA* **85**:3198-3202.

Sabatier J-M, Vives E, Mabrouk K, Benjouan A, Rochat, Duval A, Hue B, Bahraoui E (1991) Evidence for neurotoxic activity of tat from human immunodeficiency virus type 1. *Journal of Virology* **65**:961-967.

Saki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic Amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia *Science* **230**:1350-1354.

Saluz H, Jost JP. (1986) A simple high-resolution procedure to study DNA methylation and *in vivo* DNA-protein interactions on a single-copy gene level in higher eukaryotes. *Proceedings of the National Academy of Sciences (USA)* **86**:2602-2606.

Sasaki A, Nakazato Y (1992) The identity of cells expressing MHC class II antigens in normal and pathological human brain. *Neuropathology and Applied Neurobiology* **18**:13-26.

Sattentau QJ (1988) The role of CD4 antigen in HIV infection and immune pathogenesis. *AIDS* **2**(suppl 1):S11-S16.

Scaravilli F, Gray F, Mikol J, Sinclair E (1992) Pathology of the nervous system. In: F Scaravilli (Ed) *The Neuropathology of HIV infection*. Springer Verlag; pp99-170.

Schnittman SM, Psallidopoulos MC, Lane HC, Thompson L, Baseler M, Massari F, Fox CH, Salzman NP, Fauci AS (1989) The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* **245**:305-308.

Schüller (1990) A new strategy for the study of intrathecal immunity. In: MG Marrosu, C Cianchetti, B Tavolato (eds) *Trends in Neuroimmunology*. Plenum Press, New York; pp3-12.

Schwarcz R, Whetsell WO, Mangano RM (1983) Quinolinic acid: an endogenous metabolite that produces axon sparing lesions in rat brain. *Science* **219**:316-318.

Schwenk J, Cruz-Sanchez F, Gosztonyi G, Ceruos-Navarro J (1987) Spongiform encephalopathy in a patient with acquired immune deficiency syndrome (AIDS). *Acta Neuropathologica (berl)* **74**:389-392.

Selmaj KW, Raine CS (1988) Tumour necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. *Annals of Neurology* **23**:339-346.

Sen R, Baltimore D (1986) Inducibility of kappa immunoglobulin enhancer binding protein NF- κ B by a post transcriptional mechanism. *Cell* **47**:921-298.

Sharer LR, Cho E-S, Epstein LG (1985) Multinucleated giant cells and HTLV-III in AIDS encephalopathy. *Human Pathology* **16**:760.

Sharer LR, Epstein LG, Cho E-S, Joshi VV, Meyenhofer F, Rankin LF, Petito CK (1986) Pathologic features of AIDS encephalopathy in children. *Human Pathology* **17**:271-284.

Sharer LR (1992) Pathology of HIV-1 infection of the central nervous system. A review. *Journal of Neuropathology and Experimental Neurology* **51**:3-11.

Shaunak S, Albright RE, Klotman ME, Henry SC, Bartlett JA, Hamilton JD (1990) Amplification of HIV-1 provirus in the cerebrospinal fluid and its correlation with neurological disease. *Journal of Infectious Disease* **161**:1068-1072.

Shaunak S, Weber JN (1992) The retroviruses: classification and molecular biology. In: P Rudge (ed) *Bailliere's Clinical Neurology. Neurological aspects of human retroviruses*. Baillière Tindall, London; 1:1-21.

Shaw KGM, Harper ME, Hahn BH, Epstein LG, Gajdusek DC, Price RW, Navia BA, Petito CK, O'Hara CJ, Groopman JE, Cho E-S, Oleske JM, Wong-Staal F, Gallo RC (1985) HTLV-III infection in brains of children and adults with AIDS encephalopathy. *Science* **227**:177-182.

Shaw GM, Hahn BH, Arya SK, Groopman JE, Gallo RC, Wong-Staal F (1984) Molecular characterization of Human T-cell Leukemia (Lymphotropic) Virus Type III in the Acquired Immune Deficiency Syndrome. *Science* **226**:1165-1171.

Shimotohno K, Temin HM (1980) No apparent nucleotide sequence specificity in cellular DNA juxtaposed to retrovirus proviruses. *Proceedings of the National Academy of Sciences (USA)* **77**:7357-7361.

Simmonds P, Balfe P, Peutherer JF, Ludlam CA, Bishop JO, Brown AJL (1990) Human immunodeficiency virus-infected individuals contain proviruses in small numbers of peripheral mononuclear cells and at low copy numbers. *Journal of Virology* **64**:864-872.

Sinclair E and Scaravilli F (1992) Detection of HIV proviral DNA in cortex and white matter of AIDS brains by non-isotopic polymerase chain reaction: correlation with diffuse gliodystrophy. *AIDS* **6**:925-932.

Sinclair E, Gray F, Scaravilli F (1992) PCR detection of HIV proviral DNA in the brain of an asymptomatic HIV-positive patient. *Neurology* **239**:469-471.

Smith TW, DeGirolami U, Hénin D, Bolgert F, Hauw J-J (1990) Human immunodeficiency virus (HIV) leukoencephalopathy and the microcirculation. *Journal of Neuropathology and Experimental Neurology* **49**:357-370.

Smith I, Howells DW, Kendall B, Levinsky R, Hyland K (1987) Folate deficiency and demyelination in AIDS. *Lancet* **2**:215.

Snider WD, Simpson DM, Nielson S, Gold JWM, Metroka CE, Posner JB (1983) Neurological complications of acquired immune deficiency syndrome: analysis of 50 patients. *Annals of Neurology* **14**:403-418.

Sobel RA, DeArmond SJ, Forno LS, Eng LF (1981) Glial fibrillary acidic protein in hepatic encephalopathy: an immunohistochemical study. *Journal of Neuropathology and Experimental Neurology* **40**:625-632.

Sobel RA, Blanchette BW, Bhan AK, Colvin RB (1984) The immunopathology of experimental allergic encephalomyelitis. I. Quantitative analysis of inflammatory cells *in situ*. *Journal of Immunology* **132**:2393-2401.

Sobel RA, Ames MB (1988) Major histocompatibility complex molecule expression in the human central nervous system: immunohistochemical analysis of 40 patients. *Journal of Neuropathology and Experimental Neurology* **47**:19-28.

Steffy K, Wong-Staal F (1991) Genetic regulation of human immunodeficiency virus. *Microbiological Reviews* **55**:193-205.

Stoler MH, Thomas AE, Benn S, Angerer RC, Angerer (1986) Human T-cell lymphotropic virus type III infection of the central nervous system. *Journal of the American Medical Association* **256**:2360-2364.

Streit WJ, Graeber MB, Kreutzberg GW (1988) Functional plasticity of microglia a review. *Glia* **1**:310-307.

Surtees R, Hyland K, Smith I (1990) Central-nervous-system methyl-group metabolism in children with neurological complications of HIV infection. *Lancet* **335**:619-612.

Takeda A, Tuazon CU, Ennis FA, (1988) Antibody-enhanced infection by HIV-1 via Fc receptor mediated entry. *Science* **242**:580-583.

Tersmette M, Miedema F (1990) Interactions between HIV and the host immune system in the pathogenesis of AIDS. *AIDS* **4**:S57-S66.

Trauneker A, Luke W, Karjalainen K (1988) Soluble CD4 molecules neutralize human immunodeficiency virus type 1. *Nature* **331**:84-86.

Vazeux R, Brousse N, Jarry A, Henin D, Marche C, Vedrenne C, Mikol J, Wolff M, Michon C, Rozenbaum W, Bureau J-F, Montagnier L, Brahic M (1987) AIDS subacute encephalitis: identification of HIV-infected cells. *American Journal of Pathology* **126**:403-410.

Vitkovic L, Wood GP, Major EO, Fauci AS (1991) Human astrocytes stimulate HIV-1 expression in a chronically infected promonocyte clone via interleukin-6. *AIDS Research and Human Retroviruses* 7:723-727.

Wain-Hobson S (1989) HIV-1 genome variability in vivo. *AIDS* 3:S13-S18.

Watkins BA, Dorn HH, Kelly WB, Armstrong RC, Potts BJ, Michaels F, Kufta CV, Dubois-Dalcq M (1990) Specific tropism of HIV-1 for microglial cells in primary human brain cultures. *Science* 249:549-553.

Weber JN (1992) HIV infection: A cellular approach. In: F Scaravilli (ed) *The Neuropathology of HIV infection*. Springer Verlag; pp1-8.

Weiser B, Peress N, La Neve D, Eilbott DJ, Seidman R, Burger H (1990) Human immunodeficiency virus type 1 expression in the central nervous system correlates directly with extent of disease. *Proceedings of National Academy of Sciences (USA)* 87:3997-4001.

Weiss RA (1987) Retroviruses and human disease. *Journal of Clinical Pathology* 40:1064-1069.

Weller SK, Joy AE, Temin HM (1980) Correlation between cell killing and massive second round superinfection by members of some subgroups of Avian Leukosis virus. *Journal of Virology* 33:494-506.

Werner T, Ferroni S, Saermark T, Brack-Werner R, Banati RB, Mager R, Steinaa L, Kreutzberg GW, Erfle V (1991) HIV-1 nef protein exhibits structural and functional similarity to scorpion peptides interacting with K⁺ channels. *AIDS* 5:1300-1307.

Whal SM, Allen JB, M^cCartney-Francis N, Morganti-Kossmann MC, Kossmann T, Ellingsworth L, (1991) Macrophage- and astrocyte-derived transforming growth factor β as a mediator of central nervous system dysfunction in acquired immune deficiency syndrome. *Journal of Experimental Medicine* 173:981-991.

Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone (1986) Cellular localization of human immunodeficiency virus infection within the brain of acquired immune deficiency syndrome patients. *Proceedings of the National Academy of Sciences (USA)* 83:7089-7093.

Wiley CA, Masliah E, Morey M, Lemere C, DeTeresa R, Grafe M, Hansen L, Terry R (1991) Neocortical damage during HIV infection. *Annals of Neurology* 29:651-657.

Wiley CA (1992) Immunohistochemical and in situ hybridization study of HIV encephalitis. In: F Scaravilli (ed) *The Neuropathology of HIV infection*. Springer Verlag; pp251-258.

Wiley RL, Smith DH, Lasky LA, Theodore TS, Earl PL, Moss B, Capon DJ, Martin MA (1988) *In vitro* mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *Journal of Virology* **62**:139-147.

Williams K, Bar-Or A, Ulvestad E, Oliver A, Antel JP, Yong VW (1992) Biology of adult human microglia in culture: comparisons with peripheral blood monocytes and astrocytes. *Journal of Neuropathology and Experimental Neurology* **51**:538-549.

Wisniewski HM, Lossinsky AS (1991) Structural and functional aspects of the interaction of inflammatory cells with the Blood-brain barrier in experimental brain inflammation. *Brain Pathology* **1**:89-96.

Yamada M, Zurbriggen A, Oldstone MB, Fujinami RS (1991) Common immunological determinant between human immunodeficiency virus type 1 gp41 and astrocytes. *Journal of Virology* **65**:1370-1376.

Zimatore GB, Simone IL, Carbonara S, Casalino C, Rosato A, Coluccia B, Panico N, Angarano G (1989) Intrathecal synthesis of IgG in HIV infections. *Acta Neurologica (Napoli)* **11**:127-135.

APPENDICES

APPENDIX A

Extraction of DNA from HIV-positive frozen brain specimens

Reagents

HIRT Buffer

0.5% SDS

10mM TRIS-HCL pH7.4

1mM EDTA

Proteinase K Stock solution 25 mg/ml distilled water

Preparation of Phenol (500g Molecular biology grade, Sigma.)

1. Remove from freezer and warm to room temperature
2. Melt at 68°C in water bath
3. Weigh out 0.05g of 8-Hydroxyquinoline in to 10, 100 ml plastic beakers with lid.
4. Add 50 mls of melted phenol, mix briefly store at -20°C in PCR reagent freezer.

Equilibration

1. Melt an aliquot of phenol at 68°C in water bath.
2. Add 50 mls 0.5 M TRIS-HCL pH 8, stir 15 mins then aspirate the upper phase.
3. Add 50 mls 0.1 M TRIS-HCL pH 8 and repeat above 3 times.
4. Add 10 mls 0.1 M TRIS-HCL pH 8 with 2 μ l of β -metacarptoethanol

Preparation of chloroform:isoamyl alcohol (C:I)

Add 10 mls isoamyl alcohol per 250 mls isoamyl alcohol

Working Solution Phenol:Chloroform:Isoamyl alcohol (P:C:I)

1. Mix equal volumes of phenol and C:I in large glass Bijou (autoclaved).
2. Spin down to remove contaminating buffer.

Method

1. Defrost tissue.
2. Add HIRT buffer with 0.5% proteinase k - 10 μ l of stock solution in 1 ml of HIRT buffer to give a final volume of 250 μ g/ml. (use about 1 ml/ 0.3g of tissue).
3. Add a few glass beads, incubate at 56°C, vortex every 5 mins until tissue begins to break down.
4. Digest overnight at 56°C in waterbath, then vortex.
5. Prepare P:C:I and add 0.5 mls to a screw top tube, for each sample, cap and label tubes.
6. Add 0.5 mls of sample to P:C:I using disposable pastures and taking care not to create any aerosols (do not leave more than one tube open at a time to prevent possible cross contamination of samples).
7. Spin down, 3000 rpm, 30 min.
8. Transfer upper aqueous phase to fresh eppendorf.
9. Transfer lower phase to organic waste.
10. Add 0.5mls P:C:I and repeat 7-9 until there is no visible protein at interface (2 or 3 times).

11. Add equal volume of C:1 and repeat 7-10.
12. Repeat 12, transfer aqueous phase to fresh eppendorf.
13. Add 5M NaCL to aqueous phase to give a final concentration of 1M NaCL.
14. Add twice the volume of ice cold ethanol, mix well.

Leave -70°C from 30 mins to overnight.

15. Spin 15 min at 3000 rpm, discard supernatant.

Stand tubes upright to drain.

16. Wash in ice cold 70% ethanol, spin down 15 mins, 3 times.
17. Allow most ethanol to evaporate, add between 500 and 1000 μl of sterile distilled water. Dissolve overnight at 37°C and 1 hr 56°C .
18. Estimate the concentration of DNA on spectrophotometer.

APPENDIX B**Thermal cycling protocol****Cycle 1**

94°C for 1.5 mins

55°C for 2.0 mins

72°C for 3.0 mins

Cycles 2-39

94°C for 1.0 mins

55°C for 2.0 mins

72°C for 3.0 mins

Cycle 40

94°C for 1.0 mins

55°C for 2.0 mins

72°C for 10.0 mins

4°C hold

APPENDIX C

Transfer of DNA from agarose gels to nylon membrane

1. Photograph the gel, and cut out molecular weight marker of interest to mark position, trim away excess gel and transfer to prepared staining dish.
2. Wrap a piece of Whatman 3MM paper around a stack of glass plates. Place the wrapped support inside a large staining dish. Fill the dish with 1.5 M NaCl with 0.5 M NaOH almost to the top of the support and smooth out all air bubbles in the 3MM Whatman paper with a glass rod.
3. Invert the gel so that its underside is uppermost. Place the gel on the damp 3MM Whatman paper. Make sure there are no air bubbles between the 3MM paper and the gel.
4. Using a fresh scalpel cut a piece of nylon membrane about 1-2 mm larger than the gel in both dimensions. Use gloves and forceps to handle the membrane.
5. Float the membrane on the surface of a solution of 2 x SSC until it completely wets from beneath. Then immerse the filter in 2 x SSC FOR 2-3 min
6. Place the membrane on top of the gel so that one edge extends just over the line of slots at the top of the gel. Be careful to remove all air bubbles trapped between the gel and the filter.
7. Wet two pieces of Whatman 3MM paper, cut to exactly the same size as the gel, in 2 x SSC and place them on top of the membrane. Again remove all air bubbles.
8. Cut a stack of paper towels (5-8 cm high) just smaller than the 3 MM paper.

Put a glass plate on top of the stack and weigh it down with a 500g weight, and leave overnight.

9. Remove the towels and the 3MM filters above the gel. Turn over gel and filter and lay them, gel side up on a dry sheet of 3MM paper. Mark the positions of the gel slots on the filter with a very soft pencil or a ball point pen.
14. Peel off and discard the gel. Soak the filter in 6 x SSC at RT for 5 min.
15. Allow excess fluid to drain from the filter and set the filter to dry at RT on a sheet of 3MM paper
16. Place the dried filter between two pieces of 3MM paper. Bake at 120°C if filter is not to be used immediately store at RT between two sheets of 3MM paper.

APPENDIX D

Labelling oligonucleotides with DIG-UTP for Southern blotting (modified from Saluz and Jost, 1986)

N strand primer = 30mer $\sim 0.05 \mu\text{g}/\mu\text{l}$

C strand primer = 10mer $\sim 0.04 \mu\text{g}/\mu\text{l}$

Oligonucleotide buffer

500 mM sodium chloride

100 mM Tris-HCL pH 7.5

100 mM Magnesium chloride

10mM dithiothreitol

1. Annealing mix

10 μl 30mer

4 μl 10mer

2.5 μl oligo buffer

1. Incubate at 75°C , 2 min
2. Incubate at RT, 20 min
3. Incubate on ice, 10 min
4. Add 1 μl each of 10 mM dGTP, dATP, dCTP

Store at -20°C

2. Reaction Mix

2 μl Annealing mix

0.5 μl 11-DIG-dUTP

1 μl Klenow

3. Removing Unincorporated nucleotides

1. Add 1 μl glycogen and 200 μl 2M ammonium acetate in 70% ethanol
2. Mix well
3. Incubate overnight at -70°C

4. Spin down, discard supernatant and repeat
5. Wash twice in ice cold 70% ethanol
6. Dissolve in 50 μ l of buffer 10mM TRIS-HCL pH8.0, 1mM EDTA

APPENDIX E

Hybridization with and detection of DIG labelled probes (modified from Boehringer Mannheim)

1. Hybridization

Solutions

Hybridization solution	100 ml
5 x SSC	25 ml 20 x SSC
20% formamide	20 ml deionised
0.1% N-lauroylarcosine, Na salt (Sigma)	0.1 g
0.02% SDS	200 μ l 10 x SDS

Add 1% blocking reagent (Boehringer Mannheim) to freshly prepared solution.

Prepare 1 hr in advance by dissolving at 50-70°C (will not become clear)

Wash solution	1L
0.2 x SCC	10 ml 20 x SSC
0.1% SDS	10 ml 10 x SSC

Method

1. Prehybridize in a sealed box with at least 20mls hybridization solution per 100 cm² of membrane at 42°C for at least 1 hr. Redistribute the solution from time to time
2. Denature 10 μ l of DIG labelled probe in 100 μ l of hybridisation buffer at 95°C for 10 min, then quench on ice for 10 min.
3. Make plastic bags for overnight hybridisation, seal on two sides only. Insert the membrane into plastic bags, seal third side and add 5mls of hybridisation buffer and removing bubbles, seal fourth side.
4. Add diluted probes to bags and reseal, incubate overnight at 42°C.

5. Rinse filters in wash solution then wash 4 x 15 min at RT in 250 mls.
6. Membranes can then be used directly for detection of hybridized DNA or air-dried and stored for later detection.

2. Detection

Solutions

Buffer 1	(1L)	100 mM Tris-HCL pH 7.5 150 mM Nacl
Buffer 2	(300 ml)	0.5% blocking reagent (Boehringer Mannheim) in buffer 1 Dissolve 1 hr in advance at 50-70°C.
Buffer 3	(1L)	100mM Tris-HCL pH 9.5 100 mM Nacl 50 mM MgCl ₂
Buffer 4	(1L)	10 mM Tris-HCL pH 8.0 1 mM EDTA
Colour Solution (prepare fresh)		45 µl NBT solution (Boehringer Mannheim) 35 µl X-phosphate-solution (Boehringer Mannheim) 10 ml buffer 3

Method

All stages except the colour reaction require shaking or mixing.

1. Wash filters in buffer 1 for 1 min
2. Incubate for 30 min with 100mls of buffer 2
3. Wash again briefly with buffer 1
4. Dilute antibody conjugate (Boehringer Mannheim) to 1:5000 in buffer 1 (stable for 12 hrs at 4oC)
5. Incubate membranes for 30 mins with 20 mls of diluted antibody conjugate solution

6. Remove unbound antibody by washing 2 x 15 min with 100 ml of buffer 1
7. Equilibrate membrane with 20 ml of buffer 3 for 2 mins
8. Incubate membrane in dark with 10 mls freshly prepared colour solution sealed in a plastic bag. The colour precipitate starts to form within a few minutes and the reaction is usually complete after 1 day. Do not shake or mix while colour is developing
9. When desired spots are detected, stop the reaction by washing the membrane for 5 mins with 50 mls of buffer 4

List of Supplementary Material Submitted for the degree of Doctor of Philosophy**1. Published Papers**

Gray F, Gaulard P, le Beuz M, **Sinclair E**, Gherardi R, Scaravilli F and Poirier J (1990) HIV encephalitis-like multinucleated giant cell in a nodal lymphoma in AIDS. *Histopathology* **16**:402-405.

Ciardi A, **Sinclair E**, Scaravilli F, Harcourt-Webster J N and Lucas S (1990) The involvement of the cerebral cortex in human immunodeficiency virus encephalopathy: a morphological and immunohistochemical study. *Acta Neuropathologica (Berl.)* **81**:51-59.

McGowan I, Potter M, George R J D, Michaels L, **Sinclair E**, Scaravilli F and Miller R (1991) HIV encephalopathy presenting as hypomania. *Genitourinary Medicine* **67**:420-424.

Sinclair E and Scaravilli F (1992) Detection of proviral DNA in cortex and white matter of AIDS brains by non-isotopic PCR: Correlation with diffuse poliosystrophy. *AIDS* **6**:925-932.

Sinclair E, Gray F, Scaravilli F (1992) PCR detection of HIV proviral DNA in the brain of an asymptomatic HIV-positive patient. *Neurology* **239**:469-471.

2. Abstracts

Sinclair E, Ciardi A, Harcourt-Webster JN, Lucas S, Scaravilli F (1991) Morphological and immunohistochemical changes in HIV encephalopathy. *Neuropathology and Applied Neurobiology* 17:246.

Sinclair E, Scaravilli F (1991) Human immunodeficiency virus (HIV) proviral sequences in the cortex and white matter of AIDS patients with and without HIV encephalopathy *Neuroscience of HIV infection: Satellite conference, VIIIth International Conference on AIDS*, Padova, Italy, June 12-15th.

Sinclair E, Scaravilli F (1992) HIV proviral DNA is present in the cortex and white matter of AIDS brains with and without HIV encephalitis. *Neuropathology and Applied Neurobiology* 18:292.

Sinclair E, Bell J, Gray F, Scaravilli F (1992) Detection of HIV proviral DNA in HIV-positive non-AIDS drug users and its relationship to pathology of the cerebral cortex. *Neuroscience of HIV infection: Satellite conference, VIIIth International Conference on AIDS*, Amsterdam, Holland, July 14-17th.

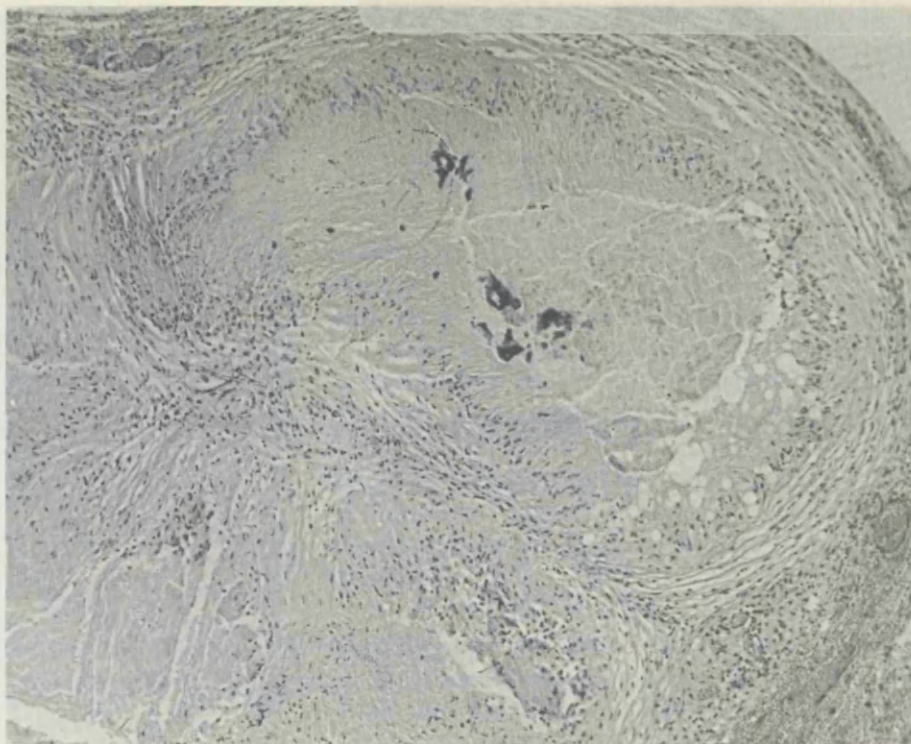


Figure 1. Necrotizing granulomas of rheumatoid type containing diathermy pigment. H & E. $\times 120$.

Case history

A 48-year-old woman (gravida 1, para 1) with a history of endometriosis was admitted for treatment of abdominal pain. A hysterectomy, bilateral oophorectomy, pelvic neurectomy and excision of a hard right ovarian nodule were performed. A bilateral salpingectomy had been performed for bilateral hydrosalpinx three years previously and diathermy ablation of extensive pelvic endometriosis was undertaken 18 months later.

PATHOLOGICAL FINDINGS

The uterus ($8 \times 4 \times 3$ cm) showed adenomyosis, left cornual endometriosis, endometrial atrophy and a normal cervix. Each ovary showed marked capsular fibrosis containing occasional fragments of sequestered omentum and multiple cortical epithelial inclusion cysts. The right ovarian nodule ($3 \times 2 \times 1$ cm) was composed of multiple granulomas of rheumatoid type (Figure 1) and an occasional extant focus of endometriosis. Each granuloma consisted of a central core of amorphous, eosinophilic, necrotic material containing flecks of brown, non-ferric (Perls' negative), diathermy pigment which was surrounded by a rim of palisaded histiocytes and an occasional multinucleate foreign body type giant cell. A thick band of hyalinized collagen encircled each granuloma. Haemosiderin-laden, lipofuscin-laden and xanthomatous histiocytes were not present. Ziehl-Neelsen

and Grocott's silver methenamine stains were performed and did not show either mycobacteria or fungi respectively. Also, parasites, ova or birefringent foreign material were not found.

Discussion

The necrotizing granulomas found at repeat prostatectomy are considered to be due to tissue necrosis caused by diathermy during the initial transurethral resection (Haboubi, Khan & Ali 1984). Rheumatoid-type, necrotizing granulomas have been described following tubal diathermy for sterilization (Roberts *et al.* 1977) and after wedge biopsy of kidney where thermocautery was used to control bleeding (Balogh 1986). Unlike the necrotic pseudoxanthomatous nodules of ovary and peritoneum associated with endometriosis (Clement, Young & Scully 1988), the ovarian nodule in this case did not contain foamy histiocytes, lipofuscin-rich histiocytes or siderophages. The formation of necrotizing granulomas following diathermy necrosis is a phenomenon which may become increasingly recognized in sites other than the prostate.

References

- Balogh K. Palisading granuloma in the kidney after open biopsy. *Am. Surg. Pathol.* 1986; 10; 441-442.
- Clement PB, Young RH, Scully RE. Necrotic pseudoxanthomatous

nodules of ovary and peritoneum in endometriosis. *Am. J. Surg. Pathol.* 1988; 12; 390-397.

Evans CS, Klein HZ, Goldman RL, Kohout ND. Necrobiotic granulomas of the uterine cervix: a probable postoperative reaction. *Am. J. Surg. Pathol.* 1984; 8; 841-844.

Haboubi NY, Khan MK, Ali HH. Necrotising granulomatous prostatitis after transurethral resection. *J. Clin. Pathol.* 1984; 37; 103-104.

Lee G, Shepherd N. Necrotising granulomata in prostatic resection specimens—a sequel to previous operation. *J. Clin. Pathol.* 1983; 36; 1067-1070.

Roberts JT, Roberts GT, Maudsley RF. Indolent granulomatous necrosis in patients with previous tubal diathermy. *Am. J. Obstet. Gynecol.* 1977; 129; 112-113.

BRIEF REPORT

HIV encephalitis-like multinucleated giant cells in a nodal lymphoma in AIDS

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A case of acquired immune deficiency syndrome (AIDS), in which multinucleated giant cells, characteristic of the human immunodeficiency virus (HIV)-encephalitis, were found in a mediastinal nodal deposit of lymphoma, is reported. Immunocytochemical studies confirmed the macrophage/histiocytic origin of these cells and the presence of HIV antigen in their cytoplasm. The occurrence of such multinucleated giant cells, representing the hallmark of HIV infection, has not been previously reported outside the central nervous system.

Keywords: acquired immune deficiency syndrome (AIDS), human immunodeficiency virus (HIV), encephalitis, multinucleated giant cell, non-Hodgkins' lymphoma

Introduction

Multinucleated giant cells were described by Sharer, Cho & Epstein (1985) in the brains of AIDS patients where the human immunodeficiency virus (HIV) had been demonstrated by hybridization. Immunohistochemistry has confirmed their histiocytic origin and ultrastructural studies have shown HIV particles in their cytoplasm

(Gray, Gherardi & Scaravilli 1988). Koenig *et al.* (1986) have demonstrated that macrophages and macrophage-derived multinucleated giant cells represent the main cells capable of synthesizing viral RNA in the central nervous system. Therefore, they may be both the main reservoir and the vehicle of spread for the virus. The majority of authors agree that these multinucleated giant cells represent the hallmark of HIV encephalitis (Gray *et al.* 1988). In sharp contrast, to our knowledge, such multinucleated giant cells have not been documented outside the central nervous system.

Case report

A 40-year-old man with AIDS developed a primary anorectal malignant large cell lymphoma, extensive herpes zoster skin eruption and terminal confusional state. At post mortem examination, one mediastinal node involved by a malignant large cell lymphoma with a stronger plasmacytic differentiation than in the anorectal and cerebral localizations, showed numerous typical multinucleated giant cells. These were similar to those described in HIV-encephalitis. Their histological appearance was characteristic: the cytoplasm was eosinophilic, granular with peripheral vacuoles and was more densely stained in the centre of the cell than at the

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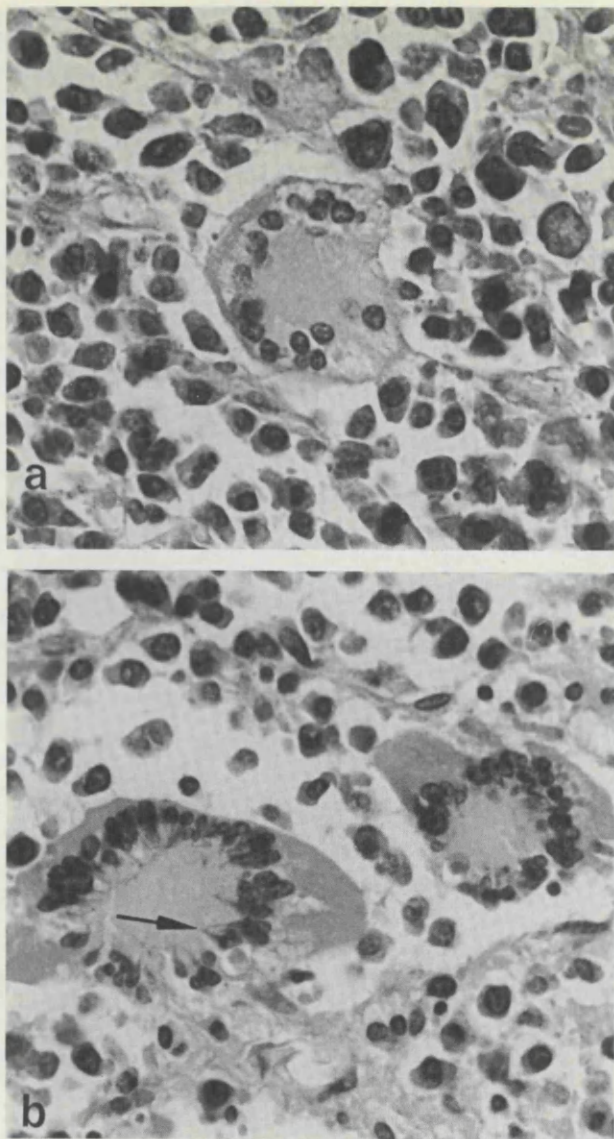


Figure 1. Mediastinal lymph node: a Multinucleated giant cell with eosinophilic, granular cytoplasm more densely stained in the centre of the cell, peripheral vacuoles and multiple basophilic nuclei arrayed about the periphery; b some nuclei showed nuclear bridges or filaments (arrow) radiating toward the centre of the cell. H & E. $\times 600$.

periphery; multiple basophilic nuclei were usually arrayed about the periphery and some of them showed nuclear bridges or filaments radiating toward the centre of the cell (Figure 1). HIV antigen was demonstrated in the cytoplasm of these cells using immunoperoxidase staining with a modified avidin-biotin method and gp 41 antiserum (Figure 2a & b). These cells were negative for CD 45 (using monoclonal antibody to leucocyte common antigen) but stained strongly positive for CD 68 (using KP1 or Y1/82A monoclonal antibody) (Figure 2c)

and CD 15 (using leu M1 monoclonal antibody) (Figure 2d); these reactions are consistent with a macrophage/histiocytic origin. Microorganisms were not detected in sections stained for bacteria, acid-fast bacilli, fungi or parasites. Viral inclusions were not observed.

Post mortem examination of the brain revealed three types of lesions: (1) diffuse white matter lesions with multinucleated giant cells characteristic of HIV encephalitis, (2) numerous cortico-subcortical necrotic lesions of varicella-zoster encephalitis with prominent Cowdry-type intranuclear inclusion bodies and (3) a deposit of malignant large cell lymphoma with plasmacytic differentiation in the white matter of the left cerebral hemisphere. In both the latter lesions, numerous multinucleated giant cells were also present (Figure 3). These multinucleated giant cells had the same immunohistochemical properties as those found in the nodal lymphoma.

Discussion

Multinucleated giant cells, similar to those seen in the CNS in AIDS patients, have not previously been reported in extra-cranial sites. These cells are morphologically different from other types of multinucleated cells which may be observed in lymph nodes in AIDS. In particular, the multinucleated cells observed in lymph nodes of male AIDS patients with lymphadenopathy and in lymph nodes and lungs of children with AIDS as first quoted by Sharer *et al.* (1985), are Warthin Finkeldey giant cells which are not specific and may occasionally be found in lymphoid structures in AIDS (Diebold *et al.* 1985).

In contrast, multinucleated syncytial cells similar to multinucleated giant cells have been observed in liver, gastrointestinal tract, lymph nodes, spleen and brain of monkeys infected by the simian immunodeficiency virus (Sharer *et al.* 1987).

Multinucleated giant cells are thought to be the result of changes induced by HIV in infected macrophages (Lifson *et al.* 1986). Since HIV has been demonstrated in the cytoplasm of histiocytes in lymph nodes, the exceptional occurrence of multinucleated giant cells in lymphoid organs is puzzling. In the present case, the occurrence of multinucleated giant cells within lymphomatous tissue both within the brain and the lymph node is noteworthy.

Acknowledgements

The authors wish to thank Dr David Mason for kindly providing the KP1 (Y1/82A) monoclonal antibody, Martine Favolini and Gilles Cochet for histological preparations and Marie-Claude Lesc for photography.

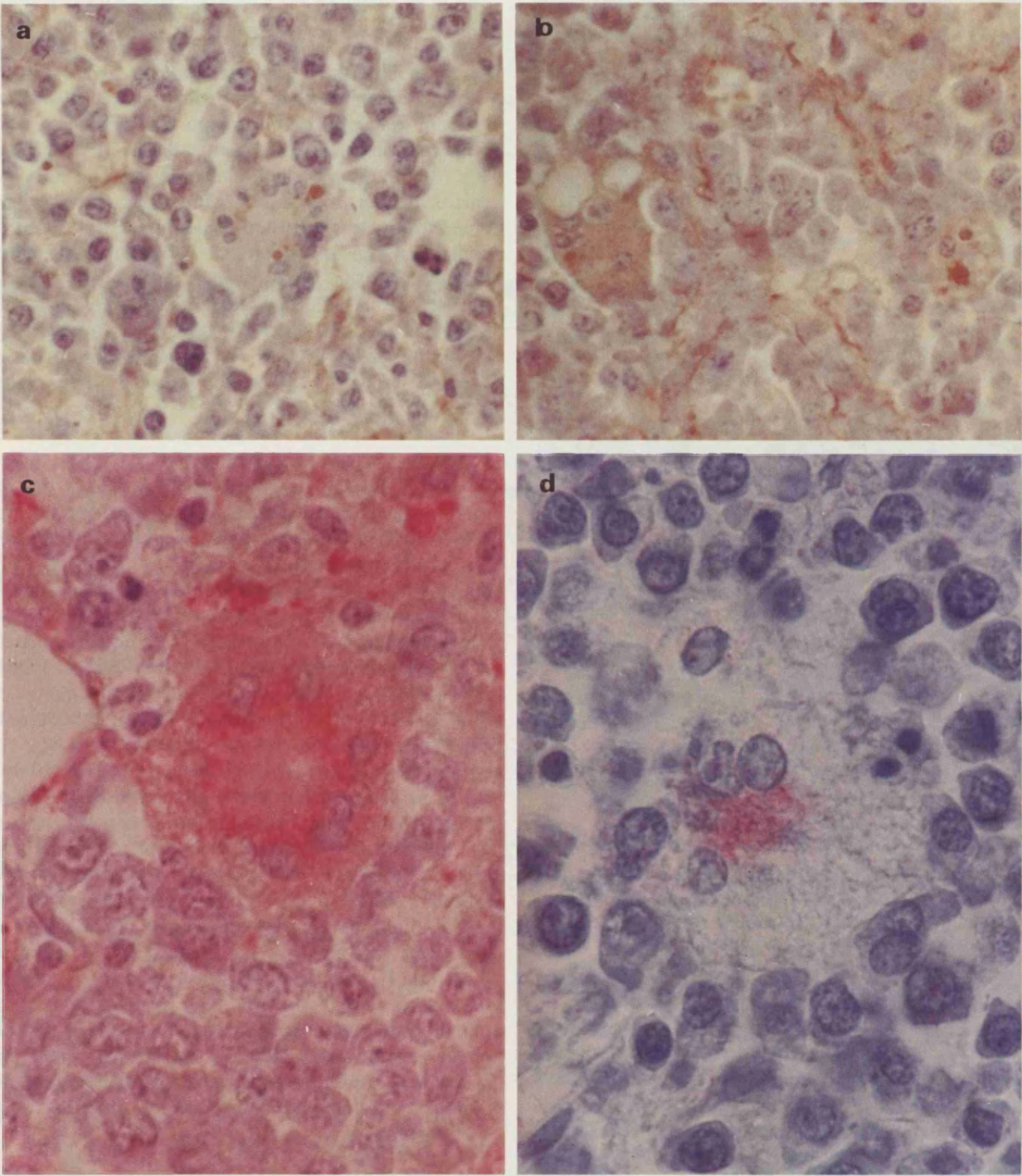


Figure 2. Mediastinal lymph node: **a & b** positive staining of the cytoplasm of the giant cells for HIV antigen using gp 41 antiserum. Peroxidase and modified avidin-biotin. **c & d** Cytoplasmic staining of a multinucleated giant cell **c** with KP1 (CD 68) and **d** with Leu MI (CD 15) antibodies using an indirect immunoalkaline phosphatase method. $\times 600$.

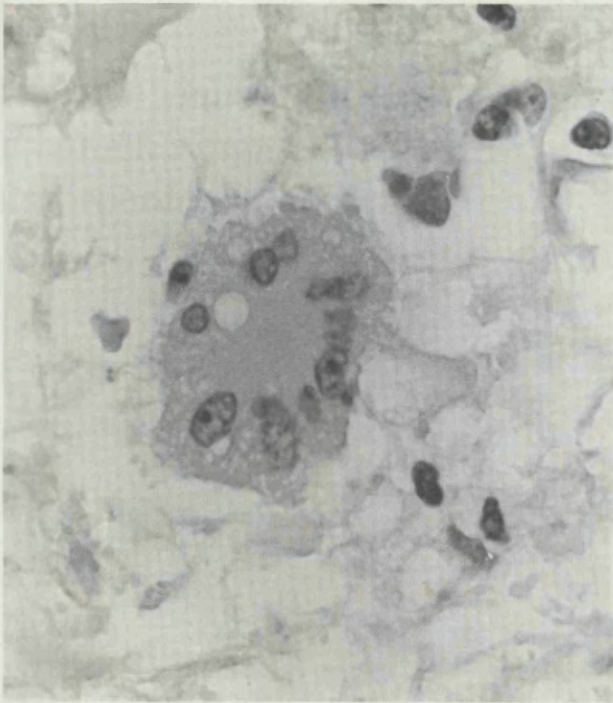


Figure 3. Brain: lymphoma deposit showing a typical multinucleated giant cell. H & E. $\times 600$.

References

- Diebold J, Marche CL, Audouin J, Aubert JP, Le Tourneau A, Bouton C, Reynes M, Wizniak J, Capron F, Tricottet V. Lymph node modifications in patients with the acquired immunodeficiency syndrome (AIDS) or with AIDS related complex (ARC). A histological, immuno-histopathological and ultrastructural study of 45 Cases. *Path. Res. Pract.* 1985; **180**: 590-611.
- Gray F, Gherardi R, Scaravilli F. The neuropathology of the acquired immune deficiency syndrome (AIDS): a review. *Brain* 1988; **111**: 245-266.
- Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkapour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 1986; **233**: 1089-1093.
- Lifson JD, Reyes GR, McGrath MS, Stein BS, Engelman EG. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. *Science* 1986; **232**: 1123-1127.
- Sharer LR, Cho E-S, Epstein LG. Multinucleated giant cells and HTLV-III in AIDS encephalopathy. *Hum. Pathol.* 1985; **16**: 760.
- Sharer LR, Epstein LG, Cho E-S, Murphey-Corb M, Baskin GB. Comparative pathology of HIV and SIV encephalitis. (Abstract) *J. Neuropathol. Exp. Neurol.* 1987; **46**: 349.

BRIEF REPORT

Pre-menopausal cytomegalovirus oophoritis

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Keywords: cytomegalovirus, ovary

Introduction

Cytomegalovirus oophoritis is easily misdiagnosed as malignant disease (Subietas, Deppisch & Astarloa 1977). These authors and others (Livolsi & Merino 1979) have pointed out that cytomegalovirus oophoritis occurs only in post-menopausal women. We report the first case of pre-menopausal cytomegalovirus oophoritis; morphological differences from the post-menopausal form are described.

Case report

A 40-year-old female died six weeks after liver transplantation for cholangiocarcinoma. Post mortem examination established that death was due to extensive infarction of the transplant liver caused by dissection of the hepatic artery. Cholangiocarcinoma was found within the wall of the portal vein.

The ovaries were virtually spherical, 32 mm greatest dimension. Blunt, white, papillary, cortical projections, ranging in size from 2 to 4 mm diameter, were noted on the haemorrhagic surface of each ovary and section revealed a normal medulla but a blotchy, haemorrhagic expanded cortex (Figure 1a).

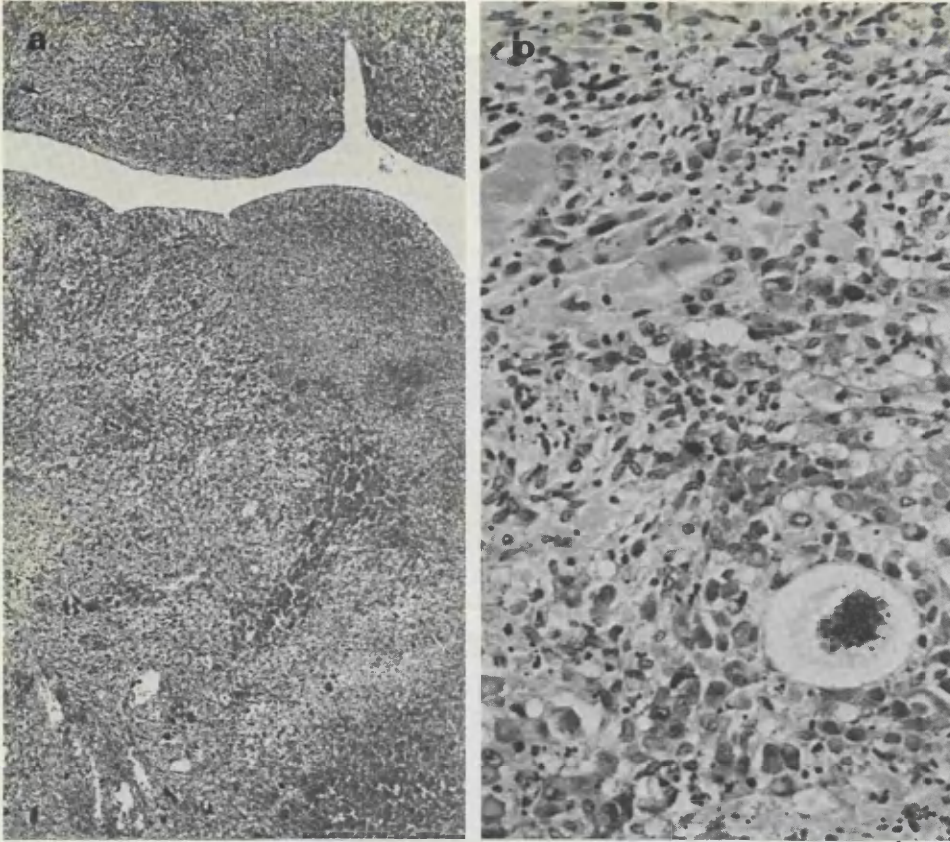


Figure 1: a: Papillary appearance to the ovarian cortex. b: An oocyte among numerous cytomegalovirus infected cells. H & E. a $\times 70$. b $\times 250$.

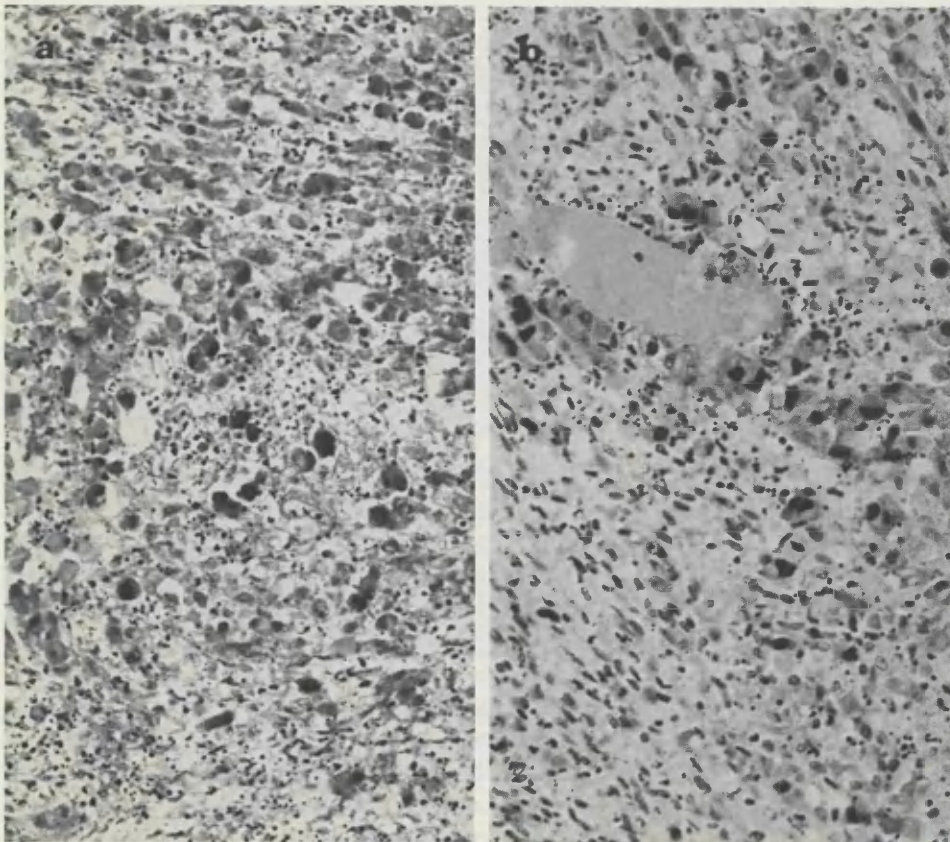


Figure 2: a: Cellular staining using anti-cytomegalovirus antibody. There is staining of both nuclei and cytoplasm. b: DNA probe for cytomegalovirus. Note the intense nuclear positivity. $\times 250$.

The involvement of the cerebral cortex in human immunodeficiency virus encephalopathy: a morphological and immunohistochemical study*

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Summary. The encephalopathy resulting from direct infection of the brain by human immunodeficiency virus (HIV), which correlates clinically with the AIDS dementia complex, has been reported as being localized to the white matter where it induces myelin loss, gliosis and perivascular infiltration by mononuclear macrophages and multinucleated giant cells. Damage to the cortical grey matter in HIV encephalopathy was investigated in nine randomly selected HIV-positive cases with or without clinical or morphological evidence of encephalopathy and in five age-matched controls, using routine histology and immunohistochemical methods [glial fibrillary acidic protein (GFAP), microglia and HIV antibodies]. Increased numbers of GFAP-expressing astrocytes and *Ricinus communis* agglutinin 1–120-expressing microglial cells were found in all the HIV-positive cases (including asymptomatic) and their severity could be correlated with the severity of the encephalopathy in the white matter; the increase in number of cells expressing GFAP was diffuse and the intensity of the staining higher than that of microglial cells. The subpial region was the most severely involved. It is suggested that involvement of the cortical grey matter is more common in HIV infection than previously suspected and that clinical evidence of a dementing process in AIDS is not necessarily due only to white matter lesions.

Key words: AIDS – HIV encephalopathy – Immunohistochemistry – Cortex

A high proportion of patients with AIDS develop neurological symptoms. These include sensory and motor dis-

turbances, impairment of memory and concentration and psychomotor retardation [28] and may progress to dementia. This syndrome, called the AIDS-dementia complex (ADC) by Navia et al. [33], has been classified as subcortical in type because of the low incidence of dyspraxia and dysphasia which are prominent signs of cortical involvement.

The main pathological abnormalities associated with the ADC were found in the white matter and subcortical grey nuclei and include myelin pallor, diffuse gliosis, scattered microglial nodules and multinucleated giant cells (MGC) [34]. The latter (MGC) are composed of fused macrophages [11], which are the cells predominantly involved in this illness [25, 37, 40, 45] and have been considered the hallmark of the infection [2].

In most large neuropathological series [26, 35, 42] changes in the cortical grey matter received relatively little attention compared with the severe lesions present in the subcortical white matter. Budka et al. [5] and de la Monte et al. [32], however, described cortical changes in adults and, in addition, Sharer et al. [39] observed nerve cell loss in brains of children with AIDS.

However, although these reports correlate the changes in the cortex with those in the subjacent white matter, they do not make any reference to the severity of the dementia. In this report we describe the changes in the grey and white matter in the frontal lobe of nine unselected brains of HIV-positive patients and correlate the findings with the clinical presentation.

Patients and methods

The brains used in this study were: HIV-negative controls, consisting of three females and two males and HIV-positive cases (six homosexuals, two drug abusers and one, male, partner of a female drug abuser). The mean age of the former was 41.2 years, that of the latter was 36.5. The brains of HIV-positive patients were classified according to the pathological findings; those with HIV encephalopathy were further subdivided according to the severity of the pathological process: (1) normal brains (cases 1 and 2); (2) non-

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Table 1. Summary of cases studied

Cases	Age (years)/ Sex	Risk factor for HIV	Clinical findings	Neurological symptoms	Brain pathology
<i>HIV negative:</i>					
1	45/F	None	Congenital myopathy	None	No abnormalities
2	38/M	None	Thoracic melanoma	None	No abnormalities
3	62/F	None	PML, follicular lymphoma	Right hemianopia, dysphasia, impairment of memory, cortical blindness, sensory and motor disturbances	Severe loss of white matter
4	17/M	None	MS (biopsy)	Raised intracranial pressure	Acute MS plaque
5	35/F	None	Japanese B encephalitis	Brain stem signs, peripheral neuropathy, intermittent headache, photophobia	Polioencephalitis, diffuse microglial nodules
<i>HIV positive:</i>					
6	32/M	Homo	Diarrhoea, cyanosis, <i>P. carinii</i> pneumonia	Terminal confusion	No abnormalities
7	41/M	Homo	Kaposi's sarcoma, anaemia, cardiomyopathy	Peripheral neuropathy	No abnormalities
8	47/M	Homo	Generalised lymphadenopathy, hepatosplenomegaly, anaemia, hypercalcaemia	None	No abnormalities
9	45/M	Homo	<i>P. carinii</i> pneumonia	None	Mild HIV encephalopathy, toxoplasmosis
10	40/M	Homo	Generalised MAI infection	None	Microglial nodules, myelin pallor and MGC
11	34/M	Partner drug abuse (F)	Fever, headache, pharyn- gitis, malaise and loss of weight	Loss of vision and memory	HIV encephalopathy with MGC, cytomegalovirus
12	24/M	Drug abuse	Kaposi's sarcoma, weight loss, fever, oral candidiasis	Confusion	Diffuse microglial nodules, MGC, myelin pallor
13	29/M	Homo	Fever	Intellectual deterioration, peripheral neuropathy	Myelin pallor, MGC in white matter and cortical ribbon
14	28/M	Drug abuse		Dysarthria, right hemiparesis, altered consciousness, became comatosed	Diffuse leucoencephalopathy with oedema and MGC, astrocytic hyperplasia, cytomegalovirus, toxoplasmosis

HIV: Human immunodeficiency virus; homo: homosexual; PML: progressive multifocal leukoencephalopathy; MS: multiple sclerosis; MAI: mycobacterium avium-intracellulare; MGC: multinucleated giant cells

HIV related pathological changes (cases 3–5); (3) HIV-positive patients (cases 6–8) without any clinical or morphological involvement of the brain; (4) HIV-positive patients (cases 9–11) with mild or moderate HIV encephalopathy, only one of whom (11) presented neurological symptoms; (5) HIV-positive patients (cases 12–14) with obvious neurological symptoms and severe pathological changes of HIV encephalopathy. Details of the salient clinical and pathological findings are summarised in Table 1. Although care was taken to select brains with and without pathological changes and, among the former, with and without clinical signs of dementia, within each group cases were chosen at random. The three pathological HIV-negative cases represent examples of primary changes of the grey (case 5) or white matter (case 4) or of white matter changes producing secondary damage of the grey (case 3).

Clinical signs suggestive of dementia were present only in cases 11–14 and referred to as memory loss, confusion, intellectual deterioration and altered consciousness. Neurological signs were not

present in cases 6–10, with the exception of peripheral neuropathy in case 7 and terminal confusion in case 6, the latter probably in relation with central cyanosis and *Pneumocystis carinii* pneumonia.

Since the pathological changes in the white matter of the HIV-positive patients were those of HIV encephalopathy with MGC [15, 16, 35], a more detailed description of their pathological features was considered beyond the scope of the present study.

The brains were fixed in 10% buffered formalin for 3 weeks to 4 months. Two blocks of frontal lobe were selected for this investigation. The biopsy specimen was immersed in the same fixative for 72 h. Paraffin sections were stained with haematoxylin and eosin, Luxol fast blue/cresyl violet and with Gleys and Marsland's silver impregnation for axons. Immunohistochemical methods were applied using antibodies to glial fibrillary acidic protein (GFAP, polyclonal; DAKO Ltd, UK) and to a core HIV protein (p24, monoclonal, Dupont, UK). Histochemistry to visualise microglia was also done using the lectin *Ricinus communis* agglutinin (RCA)

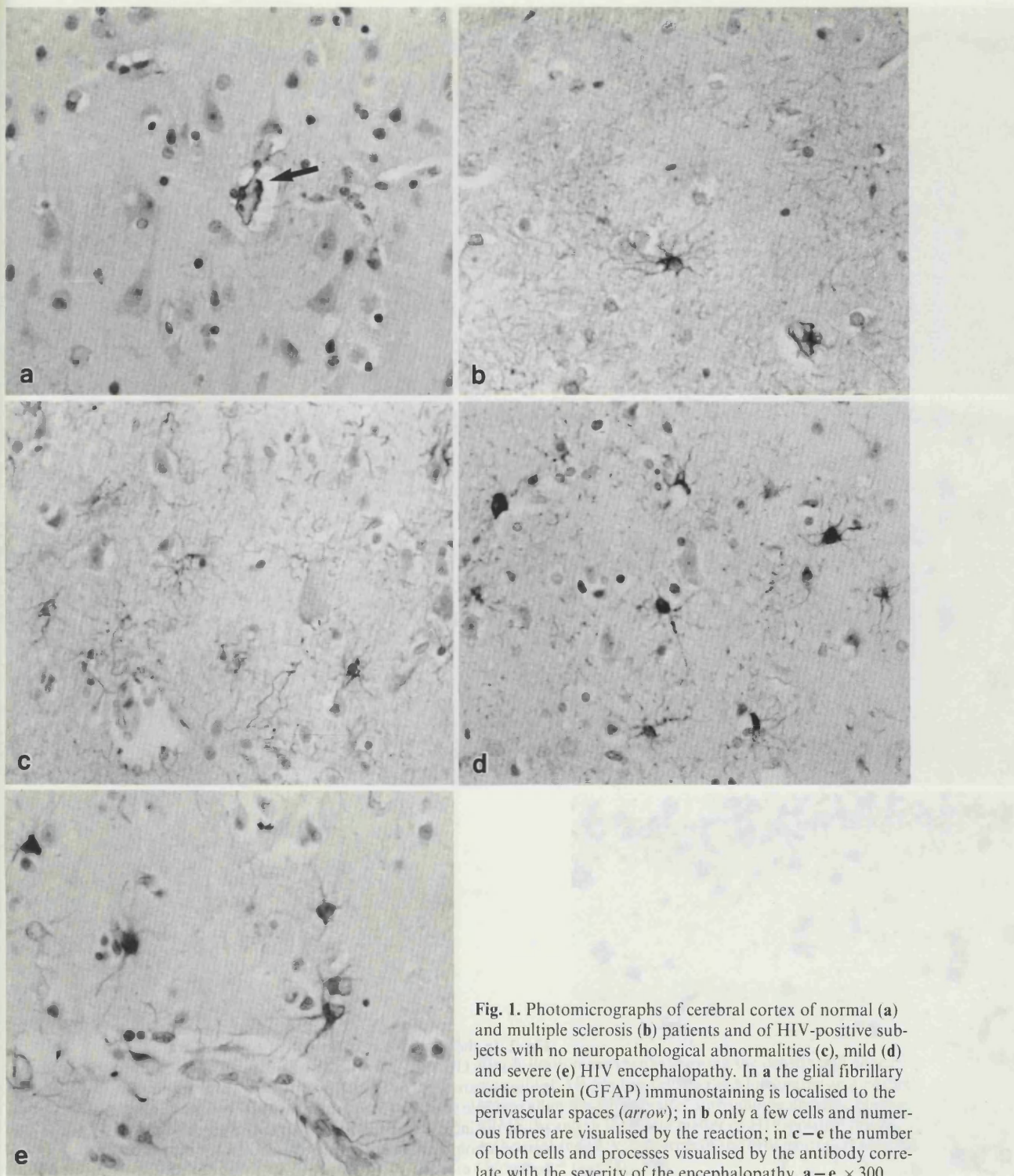


Fig. 1. Photomicrographs of cerebral cortex of normal (a) and multiple sclerosis (b) patients and of HIV-positive subjects with no neuropathological abnormalities (c), mild (d) and severe (e) HIV encephalopathy. In a the glial fibrillary acidic protein (GFAP) immunostaining is localised to the perivascular spaces (*arrow*); in b only a few cells and numerous fibres are visualised by the reaction; in c–e the number of both cells and processes visualised by the antibody correlate with the severity of the encephalopathy. a–e $\times 300$

1–120 (Vector Labs., UK). The antibodies and the lectin were visualised using an avidin-biotin peroxidase technique.

Morphometric studies

The density of GFAP- and RCA 1–120-positive cells with a recognisable nucleus was counted in ten high-power fields ($\times 400$) and the mean value was calculated in the subcortical white matter, the region of the U-fibres and the deep, intermediate and subpial cortical layers. A semiquantitative method was also applied to evaluate the

size of the astrocytes and the amount of immunoreactive material present in astrocytes and microglial cells. The following grading was used for astrocytes: +/- indicates that the scanty cytoplasm had only a thin rim of positive reaction situated beneath the cell membrane; 1+ corresponds to cells whose amount of cytoplasm was as large as the area of the nucleus and was homogeneously positive; 2+ and 3+ indicate cells whose cytoplasm had twice and three times the size of the nucleus, respectively, and was homogeneously positive. For the evaluation of microglial cells, P+, P++ and P+++ indicate the presence of less than 10, between 10 and 20 and more than 20 cell processes, respectively, per high-power field.

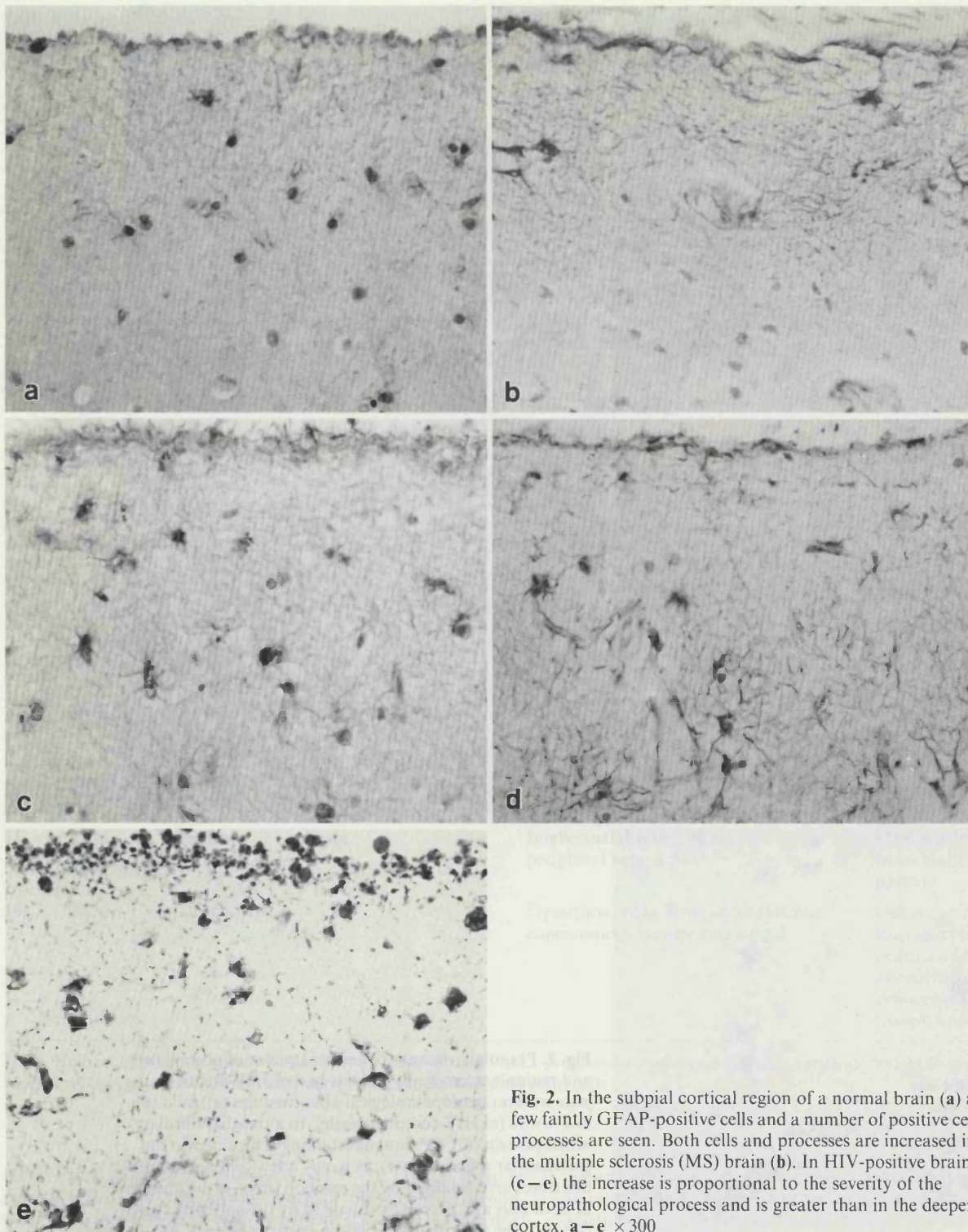


Fig. 2. In the subpial cortical region of a normal brain (a) a few faintly GFAP-positive cells and a number of positive cell processes are seen. Both cells and processes are increased in the multiple sclerosis (MS) brain (b). In HIV-positive brains (c–e) the increase is proportional to the severity of the neuropathological process and is greater than in the deeper cortex. a–e $\times 300$

Results

Glial fibrillary acidic protein

Normal controls. In the grey matter, GFAP antibodies identified only a small number of cells and cells processes; in the former, the reaction was seen beneath the cell membrane and was stronger around blood vessels (Fig. 1a). On the other hand, in the subpial region

(Fig. 2a) and in the white matter (Fig. 3a) the immunostaining was localised predominantly in numerous cell processes.

Pathological HIV-negative controls. A considerable increase in number and size of cells expressing GFAP was observed in all cortical layers of case 3. In case 4, the increase was moderate in all layers (Fig. 1b), except the

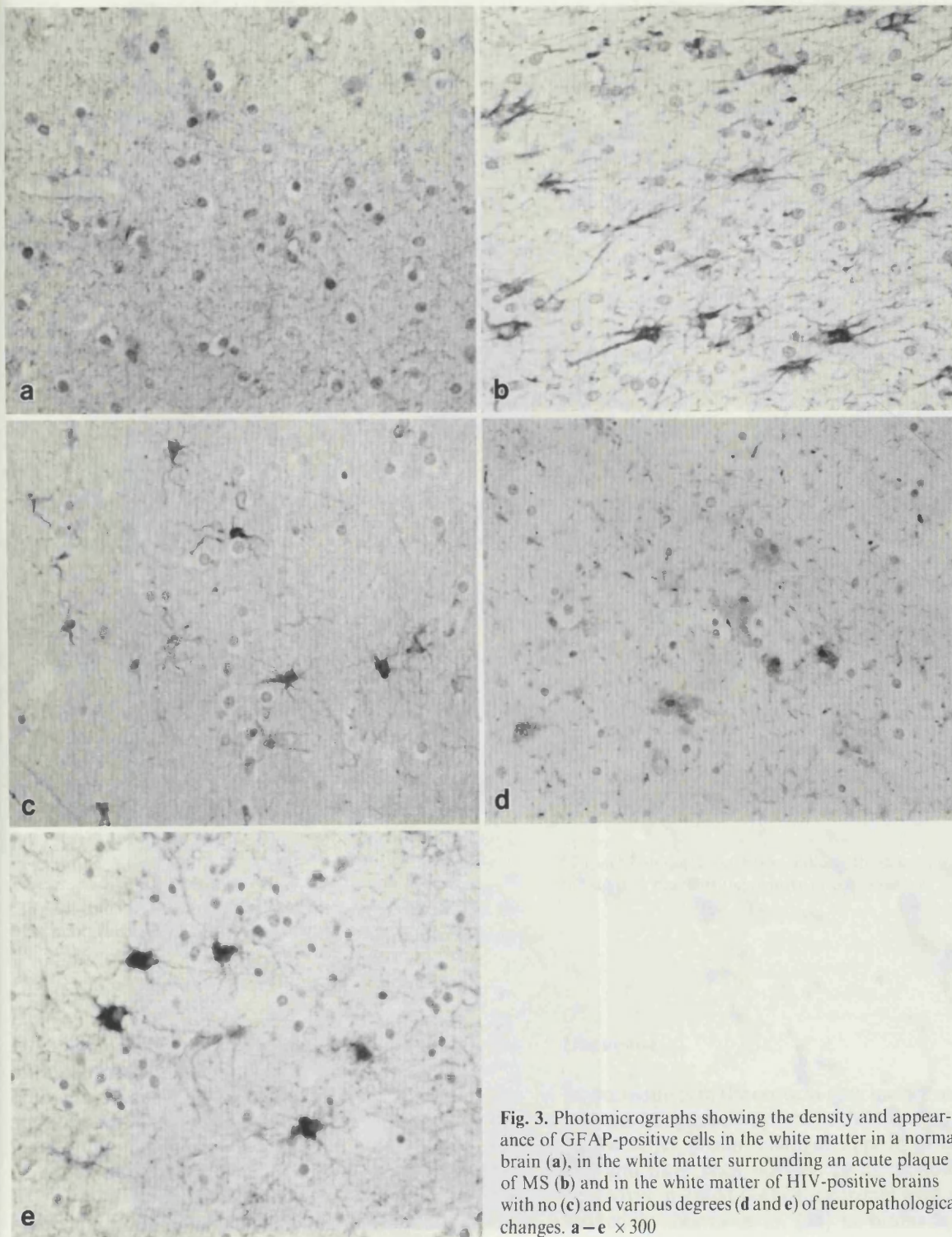


Fig. 3. Photomicrographs showing the density and appearance of GFAP-positive cells in the white matter in a normal brain (a), in the white matter surrounding an acute plaque of MS (b) and in the white matter of HIV-positive brains with no (c) and various degrees (d and e) of neuropathological changes. a–e $\times 300$

subpial, where it was severe (Fig. 2b). In addition, the area of normal myelin between the cortex and the plaque showed a considerable number of cells expressing GFAP (Fig. 3b). In the cortex of case 5, GFAP-positive cells were seen in large numbers only throughout the subpial and deep layers and in circumscribed areas in the layers II–V. On the other hand, the density of GFAP-positive cells was high at all levels of the white matter.

HIV-positive patients. In group 3, cells expressing GFAP (Fig. 1c) were increased in the subpial areas (Fig. 2c) and in the white matter (Fig. 3c). The intensity of the immunostaining increased in group 4, both in deep (Fig. 1d) and in the subpial region (Fig. 2d) and to lesser extent in the white matter (Fig. 3d). Group 5 showed the largest number of GFAP-positive cells with equal density in the grey (Figs. 1e, 2e) and white matter (Fig. 3e).

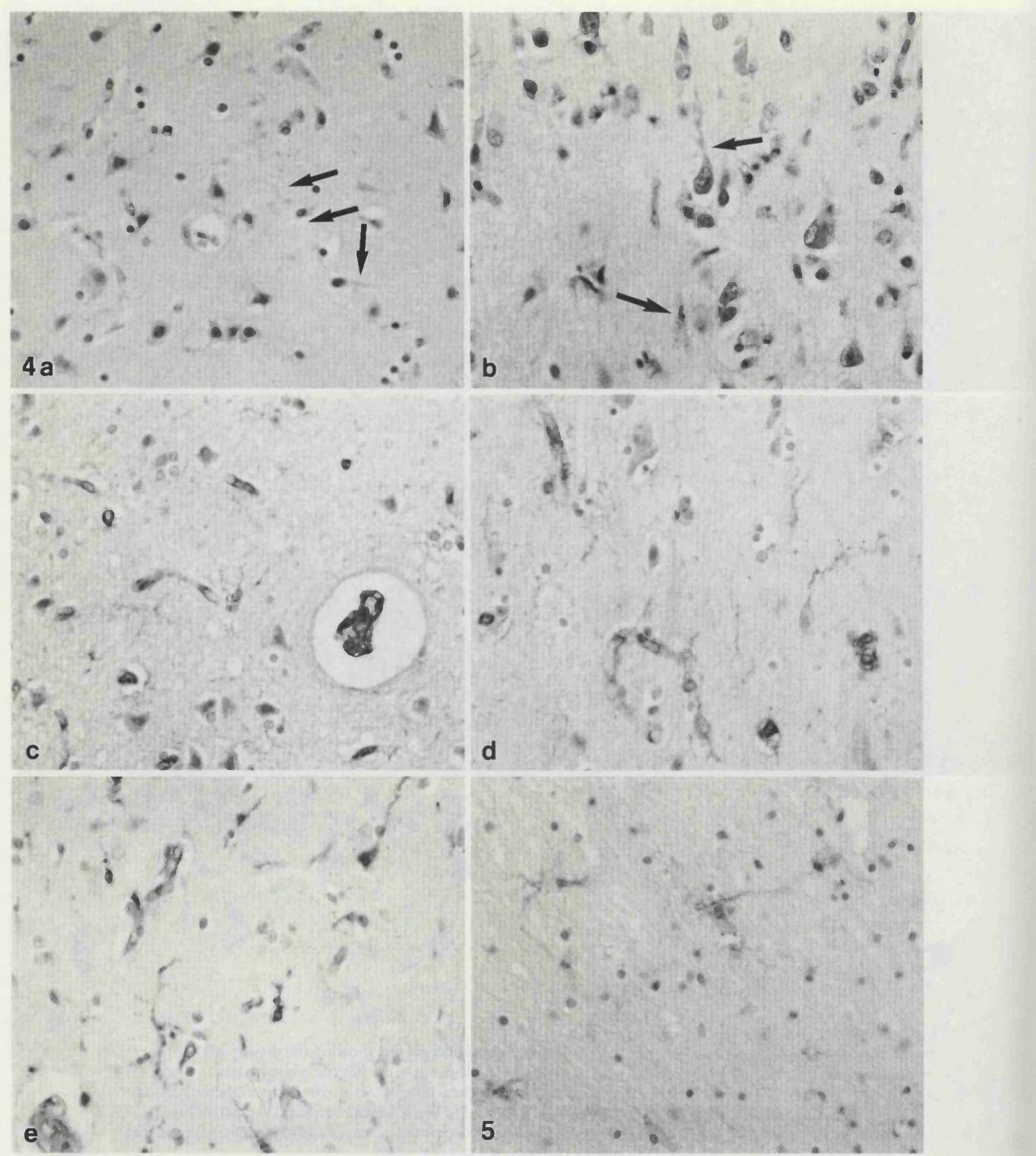


Fig. 4a–e. Photomicrographs showing cortical microglial cells and processes visualised with *Ricinus communis* agglutinin 1–120. In the normal brain (a) only a few processes are faintly stained (arrows); in the brain with PML (b) cell bodies and processes are seen (arrows); in HIV-positive brains (c–e) more cells are seen and processes are

more strongly stained and more branched with increasing severity of the illness. × 300

Fig. 5. Photomicrograph of the white matter of case 13. Several microglial cells react with the HIV antibody p24. × 300

RCA 1–120

Normal controls. Positive reaction to RCA 1–120 was faint and localised predominantly in thin cell processes (Fig. 4a).

Pathological HIV-negative controls. In case 3 the number of positive cell processes was slightly increased in the cortex (Fig. 4b), whereas in case 5 they were localised only in the nodules.

Table 2. Density and size of glial fibrillary acidic protein-positive cells

	Cortex			White matter	
	Subpial	Interm.	Deep	U-fibres	Deep
1	2–3 ^a (1+)	0.5–1 (±)	<0.5 (±)	2 (±)	2.5 (±)
2	1–2 (1+)	<1 (1+)	1 (±)	2 (1+)	2 (±)
3	20 (3+)	30 (3+)	15 (2+)	NV –	NV –
4	6 (1+)	1 (1+)	1.5 (1+)	20 (2+)	14 (2+)
5	10 (1+)	<2 (±)	7 (±)	20 (2+)	20 (2+)
6	5 (1+)	1 (1+)	1 (1+)	7 (1+)	5.5 (1+)
7	15 (1+)	5 (±)	2 (±)	15 (2+)	11 (1+)
8	10 (2+)	0.5 (1+)	3 (1+)	9 (2+)	2.5 (1+)
9	25 (3+)	6 (2+)	8 (1+)	7 (2+)	8 (2+)
10	25 (2+)	13 (2+)	13 (2+)	15 (2+)	6 (1+)
11	15 (3+)	6.5 (2+)	7.5 (2+)	13 (2+)	10 (2+)
12	20 (2+)	16 (2+)	14 (2+)	20 (3+)	14 (2+)
13	20 (3+)	10 (2+)	12 (2+)	17 (3+)	14 (3+)
14	28 (3+)	12 (2+)	10 (2+)	18 (3+)	14 (3+)

^a In each column the numbers indicate the density of glial cells, the signs in brackets the intensity of the immunostaining

HIV-positive patients. In group 3 positive staining was diffusely present in a small number of cell processes in both grey (Fig. 4c) and white matter. In group 4 the appearances of two cases with slight encephalopathy differed only moderately from those in group 3 (Fig. 4d), while in cases with moderate involvement the number of RCA 1–120-positive cells in the deep cortical layers was higher. In group 5 the numbers of RCA 1–120-positive cells was increased, particularly in the grey matter (Fig. 4e).

The density of GFAP- and RCA 1–120-positive cells, their size and the number of processes are shown in Tables 2 and 3.

Immunostaining with the antibody p24 was seen only in HIV-positive cases with neuropathological changes and predominantly in the white matter and around blood vessels. Positive cells included microglia (Fig. 5), macrophages and MGC and there was correlation between number of these cells and severity of the encephalopathy as previously described by Budka [3].

Table 3. Density of microglial cells and their processes

	Cortex		U-fibres	Deep white matter
	Interm.	Deep		
1	0.2 (P±) ^a	0.2 (P±)	0.2 (P±)	0.2 (P±)
2	0.1 (P±)	0.2 (P±)	0.1 (P±)	0.3 (P+)
3	4 (P+)	>7 (P+)	NV	NV
4	ND	ND	ND	ND
5	3.5 (P+)	5 (+)	10 (P++)	5 (P+)
6	<0.5 (P+)	<0.5 (P+)	<1 (P+)	<0.5 –
7	0.8 (P++)	0.9 (P++)	<2 (P+)	1.6 (P+)
8	1 (P++)	<1 (P+)	<2 (P+)	1.5 –
9	<2 (P++)	<2 (P++)	2.5 (P++)	2 (P++)
10	0.2 (P+)	–	0.4 (P+)	0.4 (P+)
11	0.5 (P++)	2 (P++)	10 (P++)	8 (P++)
12	6 (P++)	8 (P+)	11 (P++)	10 (P+)
13	3 (P+++)	3 (P+++)	2 (P++)	5 (P+)
14	2 (P++)	4 (P++)	15 (P++)	10 (P++)

^a In each column the numbers indicate the density of microglial cells, the signs in brackets the density of cell processes

Discussion

Abnormalities in the cortical grey matter in HIV-positive patients have been reported in children [39] and in adults. In the latter group, changes appear as cortical gliosis [32] and have been referred to as 'poliodystrophy' [5]. Moreover, marked cortical neuronal loss, previously described by Navia et al. [34] in brains with 'the most advanced white matter disease', has recently been confirmed in a morphometric study [3]. In group 5, the changes in the white matter were so diffuse and severe that they may possibly have extended to, or determined those of, the grey matter. However, the latter did not show any histological evidence of nerve cell loss. On the other hand, the findings in group 4 and, although milder, group 3, were unsuspected until examined quantitatively. They consisted of a diffuse increase in number of GFAP-positive cells in areas in which the subjacent white matter showed only subtle or moderate changes. These changes were the main pathological abnormality since the increase

in RCA 1–120-positive cells was much less apparent than the astrocytic reaction. Although the increase in cellular expression of GFAP and RCA was seen also in HIV-negative cases, changes were patchy and mainly localised to areas of obvious histological abnormality.

Discussing the pathogenesis of the diffuse poliodystrophy, Budka [4] suggested that the mechanisms producing the lesion in the white matter might also operate in the cortical grey. According to the theory of the 'Trojan horse' [20, 36], lymphocytes and macrophages bind to the endothelium [44] and can enter both into the white and the grey matter. Another mechanism suggested, that of a direct infection of endothelial cells [43, 45], also implies entry of HIV in both grey and white matter. The leptomeninges too may provide a route of entry for the virus: HIV has been recovered from the CSF of patients [6], sometimes in the absence of detectable virus in the peripheral blood [27] and CSF abnormalities are common finding in these patients [30]. An acute or subacute meningitis has been described at various times after exposure to HIV [21, 22, 24], although it has rarely been seen in patients suffering from full-blown AIDS [23]. Although at the time of death the severity of the glial changes in our material was proportional to the degree of cerebral involvement, its existence at abnormal levels in non-neurological cases suggests that in some circumstances it may represent morphological sequela of an early process and, in particular, that the subpial gliosis be secondary to an episode of meningitis.

Although the type and rate of progress of the lesions support the localisation of HIV in the cortical grey matter, the nature and extent of the possible damage produced is not clearly understood. Among the various mechanisms proposed for the production of lesions in the white matter, the most probable is a 'bystander effect' [8]. According to this theory [9] myelin is destroyed by soluble immune factors liberated by activated monocytes and lymphocytes with a mechanism similar to the demyelination in Theiler's virus infection [7]. Although no comparable damage takes place in the cortex of HIV-positive patients, at least until the terminal stages of the illness, release of soluble immune factors could be responsible for the proliferation of astrocytes and microglia seen in the present study and shown to have an immune function in the brain [10, 38]. The possibility of a direct cytopathic effect by HIV on nerve cells was supported by the demonstration that the viral envelope glycoprotein gp120 exerts toxic effect on embryonic hippocampal nerve cells of the mouse *in vitro* [1]; however, since rodents cannot be infected by this virus, it seems unlikely that such effect could take place *in vivo*. Another possibility is based on the demonstration that a segment of the gp120 molecule shows sequence homology with several neurotrophic factors, including neuroleukin [17, 18] and VIP [1]. The former has been found to support continuous survival of spinal cord and sensory neurons *in vitro* [17, 18]. The latter was found to be neurotrophic for embryonic hippocampal cell cultures [1]. Damage could derive from competition of gp120 with these factors for their specific cell surface receptors.

Our results showed that HIV antigens in the cortex were localised only in cells of the microglia-macrophage lineage as found in the white matter by previous workers [5, 12, 13, 25, 31, 41] and ourselves. No immunostaining was seen in neuroepithelial cells, thus confirming that, in spite of sporadic evidence of virus expression by astrocytes [14, 19, 45], virus has not been unequivocally demonstrated in these cells.

The presence of lesions in the grey matter, quite independent from those in the subjacent white, is important for the interpretation of the cause of dementia in HIV-positive patients. Although the severity of dementia appears to correspond to the degree of immunosuppression [29], there seems to be poor correlation between the clinical signs and pathological changes in the white matter. The findings of the present study do not allow us to make any reliable conclusion about the frequency of the cortical involvement in HIV-positive patients; however, they suggest that these abnormalities are commoner than previously suspected and may be responsible for unusual or otherwise unexplained clinical findings in patients suffering from ADC.

References

1. Breneman DE, Westbrook GL, Fitzgerald SP (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 335:639–642
2. Budka H (1986) Multinucleated giant cells in brain: a hallmark of the acquired immune deficiency syndrome (AIDS). *Acta Neuropathol (Berl)* 69:253–258
3. Budka H (1990) Neuropathology of AIDS. Proceedings of the 14th World Congress of Neurology, Int Conf Ser 883. Elsevier, Amsterdam (in press)
4. Budka H (1989) Human immunodeficiency virus (HIV)-induced disease of the central nervous system: pathology and implications for pathogenesis. *Acta Neuropathol* 77:225–236
5. Budka H, Costanzi G, Cristina S, Lechi A, Parravicini C, Trabattoni R, Vago L (1987) Brain pathology induced by infection with the human immunodeficiency virus (HIV). A histological, immunocytochemical and electron microscopical study of 100 autopsy cases. *Acta Neuropathol (Berl)* 75:185–198
6. Chiodi F, Asjo B, Fenyo E-M, Norkrans G, Hagberg L, Albert J (1986) Isolation of human immunodeficiency virus from cerebro-spinal fluid of antibody-positive virus carriers without neurological symptoms. *Lancet* II:1276–1277
7. Clatch RJ, Melvold RW, Miller SD (1985) Theiler's murine encephalomyelitis (TMEV)-induced demyelinating disease in mice is influenced by the H-2D region: correlation with TMEV-specific delayed-type hypersensitivity. *J Immunol* 135:1408–1414
8. Dal Canto MC (1989) AIDS and the nervous system: current status and future perspectives. *Hum Pathol* 20:410–418
9. Dal Canto MC, Rabinowitz SG (1982) Experimental models of virus-induced demyelination of the central nervous system. *Ann Neurol* 11:109–127
10. Frei K, Siepl C, Bodmer S, Fontana A (1988) Astrocytes and microglial cells as immune regulatory cells of the brain. In: Norenberg MD, Hertz L, Schousboe A (eds) *The biochemical pathology of astrocytes* (Neurology and Neurobiology series, vol 39). Alan R Liss, New York, pp 239–245
11. Gabuzda DH, Hirsch MS (1987) Neurologic manifestations of infection with human immunodeficiency virus. *Ann Intern Med* 107:383–391
12. Gabuzda DH, Ho DD, de la Monte SM, Hirsch MS, Rota Tr, Sobel RA (1986) Immunohistochemical identification of HTLV-

III antigen in brains of patients with AIDS. *Ann Neurol* 20:289–295

13. Gartner S, Markovitz P, Markovitz DM, Betts RF, Popovic M (1986) Virus isolation from and identification of HTLV-III/LAV-producing cells in brain tissue from a patient with AIDS. *J Am Med Assoc* 256:2365–2371
14. Gosztonyi G, Lamperth L, Webster HdeF (1989) In situ hybridization study of HIV DNA and RNA distribution in AIDS encephalopathy lesions (abstract). *J Neuropathol Exp Neurol* 47:346
15. Gray F, Gherardi R, Baudrimont M, Gaulard P, Meyrignac C, Vedrenne C, Poirier J (1987) Leucoencephalopathy with multinucleated giant cells containing human immune deficiency virus-like particles and multiple opportunistic cerebral infections in one patient with AIDS. *Acta Neuropathol (Berl)* 73:99–104
16. Gray F, Gherardi R, Scaravilli F (1988) The neuropathology of the acquired immune deficiency syndrome (AIDS). A review. *Brain* 111:245–266
17. Gurney ME, Apatoff BR, Spear GT, Baumel MJ, Antel JP, Brown Bania M, Reder AT (1986) Neuroleukin: a lymphokine product of lectin-stimulated T cells. *Science* 234:574–581
18. Gurney ME, Heinrich SP, Lee MR, Yin H-S (1986) Molecular cloning and expression of neuroleukin, a neurotrophic factor for spinal and sensory neurons. *Science* 235:566–574
19. Gyorkey F, Melnick JL, Gyorkey P (1987) Human immunodeficiency virus in brain biopsy of patients with AIDS and progressive encephalopathy. *J Infect Dis* 155:870–876
20. Haase AT (1986) Pathogenesis of lentivirus infection. *Nature* 322:130–136
21. Ho DD, Rota TR, Schooley RT, Kaplan JC, Allan JD, Groopman JE, Resnick L, Felsenstein D, Andrews CA, Hirsch MS (1985) Isolation of HTLV-III from cerebro-spinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *N Engl J Med* 313:1493–1497
22. Hollander H, Levy J (1987) Neurological abnormalities and human immunodeficiency virus recovery from cerebrospinal fluid. *Ann Intern Med* 106:692–695
23. Hollander H, Stringari S (1987) Human immunodeficiency virus-associated meningitis. *Am J Med* 83:813–816
24. Johnson RT, McArthur JC, Narayan O (1988) The neurobiology of human immunodeficiency virus infections. *FASEB J* 2:2970–2981
25. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS (1986) Detection of AIDS virus in macrophages in brain macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 233:1089–1093
26. Lang W, Miklossy J, Deruaz IP, Pizzolato GP, Probst A, Schaffner T, Gessaga E, Kleihues P (1989) Neuropathology of the acquired immune deficiency syndrome (AIDS): a report of 135 consecutive autopsy cases from Switzerland. *Acta Neuropathol* 77:379–390
27. Levy JA (1988) The biology of the human immunodeficiency virus and its role in neurological disease. In: Rosenblum ML, Levy RM, Bredesen DE (eds) *AIDS and the nervous system*. Raven Press, New York, pp 327–345
28. Levy RM, Bredesen DE, Rosenblum ML (1985) Neurological manifestations of the acquired immunodeficiency syndrome: experience at UCSF and review of the literature. *J Neurosurg* 62:475–495
29. McArthur JC (1987) Neurologic manifestations of AIDS. *Medicine* 66:407–437
30. McArthur JC, Cohen BA, Farzedgan H, Cornblath DR, Selnes OA, Ostrow D, Johnson RT, Phair J, Polk BF (1988) Cerebrospinal fluid abnormalities in homosexual men with and without neuropsychiatric findings. *Ann Neurol* 23 [Suppl]:S34–S37
31. Meyenhofer MF, Epstein LG, Cho E-S, Sharer LR (1987) Ultrastructural morphology and intracellular production of human immunodeficiency virus (HIV) in brain. *J Neuropathol Exp Neurol* 46:474–484
32. Monte SM de la, Ho DD, Schooley RT, Hirsch MS, Richardson EP Jr (1987) Subacute encephalomyelitis of AIDS and its relation to HTLV-III infection. *Neurology* 37:562–569
33. Navia BA, Jordan BD, Price RW (1986) The AIDS-dementia complex. I. Clinical features. *Ann Neurol* 19:517–524
34. Navia BA, Cho E-S, Petite CK, Price RW (1986) The AIDS-dementia complex. II. Neuropathology. *Ann Neurol* 19:525–535
35. Petite CK, Cho E-S, Lemann W, Navia BA, Price RW (1986) Neuropathology of acquired immunodeficiency syndrome (AIDS): an autopsy review. *J Neuropathol Exp Neurol* 45:635–646
36. Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988) The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* 239:586–592
37. Pumarola-Sune T, Navia BA, Cordon-Casro C., Cho E-S, Price RW (1987) HIV antigen in the brains of patients with the AIDS dementia complex. *Ann Neurol* 21:490–496
38. Salamat SM, Tallent MW, Keane RW (1988) The role of astrocytes in immune responses within the central nervous system. In: Norenberg MD, Hertz L, Schousboe A (eds) *The biochemical pathology of astrocytes (Neurology and Neurobiology series, vol 39)*. Alan R Liss, New York, pp 247–259
39. Sharer LR, Epstein LG, Cho E-S, Joshi VV, Meyenhofer MF, Rankin LF, Petite CK (1986) Pathologic features of AIDS encephalopathy in children: evidence for LAV/HTLV-III infection of brain. *Hum Pathol* 17:271–284
40. Stoler MH, Eskin TA, Benn S, Angerer RC, Angerer LM (1986) Human T cell lymphotropic virus type III infection of the central nervous system. A preliminary in situ analysis. *J Am Med Assoc* 256:2360–2364
41. Vazeux R, Brousse N, Jarry A, Henin D, Marche C, Vedrenne C, Mikol J, Wolff M, Michon C, Rozenbaum W, Bureau J-F, Montagnier L, Brahic M (1987) AIDS subacute encephalitis. Identification of HIV-infected cells. *Am J Pathol* 126:403–410
42. Vinters HV, Tomiyasu U, Anders KH (1989) Neuropathological complications of infection with the human immunodeficiency virus (HIV). *Adv AIDS Pathol* 1:101–130
43. Ward JM, O'Leary TJ, Baskin GB, Benveniste R, Harris CA, Nara PL, Rhodes RH (1987) Immunohistochemical localization of human and simian immunodeficiency viral antigens in fixed tissue sections. *Am J Pathol* 127:199–205
44. Wekerle H, Linington C, Lassmann H, Meyerman R (1986) Cellular immune reactivity within the CNS. *Trends Neurosci* 9:271–277
45. Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA (1986) Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 83:7089–7093

Clinico-pathological Conference

HIV encephalopathy presenting as hypomania

I McGowan, M Potter, R J D George, L Michaels, E Sinclair, F Scaravilli, R F Miller

Case report (Dr I McGowan)

The patient was a 40 year old male Caucasian homosexual who had worked as a nurse. In 1987 he had right thoracic single dermatomal *Herpes zoster* infection. In February 1988 he was seen at another hospital with a history of chronic diarrhoea, weight loss and oral candidiasis, and was found to have *Entamoeba histolytica* in his stool. Following counselling, he had an HIV test which was positive. He then remained well until June 1988 when he represented with cough and dyspnoea. A chest radiograph was suggestive of *Pneumocystis carinii* pneumonia. He underwent bronchoscopy which confirmed the diagnosis. He was treated with twenty-one days of nebulised pentamidine and made a rapid recovery. He then began secondary prophylaxis with daily oral co-trimoxazole, and zidovudine was commenced. Following this, he was again well until June 1989 when he presented with a short history of retrosternal discomfort. At the time he had marked oral candidiasis, and a presumptive diagnosis of pharyngeal candidiasis was made. He was treated empirically with fluconazole, and his symptoms rapidly resolved.

In November 1989 he reported mild nocturia but had no other urinary symptoms. He had a recurrence of his retrosternal discomfort, and also complained of a mucoid rectal discharge and paraesthesiae in his toes. An upper gastrointestinal endoscopy showed mild oesophagitis and also several small lesions of Kaposi's sarcoma in the pylorus. At sigmoidoscopy the rectum appeared normal but *Herpes simplex* was

cultured from a perineal swab. Treatment with acyclovir improved his rectal symptoms. Shortly after this, he became anaemic and leucopenic. His zidovudine was temporarily discontinued and then restarted at a lower dose.

In January 1990 he developed cytomegalovirus retinitis and began treatment with ganciclovir given via a Hickman line. In February he self-referred. On examination he had quite marked left-sided scrotal cellulitis. Culture from the skin revealed a heavy growth of *Staphylococcus aureus* and an ultrasound of the scrotum revealed co-existent epididymo-orchitis. He was treated with oral antibiotics, and his symptoms steadily resolved. In May that year he became non-specifically unwell with a low-grade fever and minimal dysuria. Examination at that time showed some tenderness around the Hickman insertion site. Pending the results of blood and urine cultures, broad spectrum antibiotics were begun. Subsequently a heavy growth of *Escherichia coli* was cultured from the urine. At this time the secondary prophylaxis against pneumocystis pneumonia was changed to monthly inhaled pentamidine. Later on in the summer the patient noted that the numbness and paraesthesiae had now extended up to his calves. Carbamazepine was begun with good effect. In late July he re-presented acutely with a history of night sweats, fevers and rigors. *Escherichia coli* was cultured from blood and urine and also from a skin swab taken from the Hickman entry site. Other investigations, including a prostatic ultrasound, an abdominal ultrasound and a chest radiograph were all normal. Nerve conduction studies were also performed and were consistent with an HIV peripheral neuropathy. He was treated with parenteral antibiotics for his septicaemia, and made a rapid recovery.

In September 1990 it became apparent that the patient was behaving a little oddly. He was normally an outgoing and somewhat extrovert character. His behaviour had become a little inappropriate. He had taken to visiting his neighbours dressed in only his underwear. The patient was admitted for assessment, and was clearly hypomanic. He was euphoric, disinhibited, had marked flight of ideas, insomnia and verbalised several grandiose schemes. Investigations at this stage failed to reveal a metabolic cause for his

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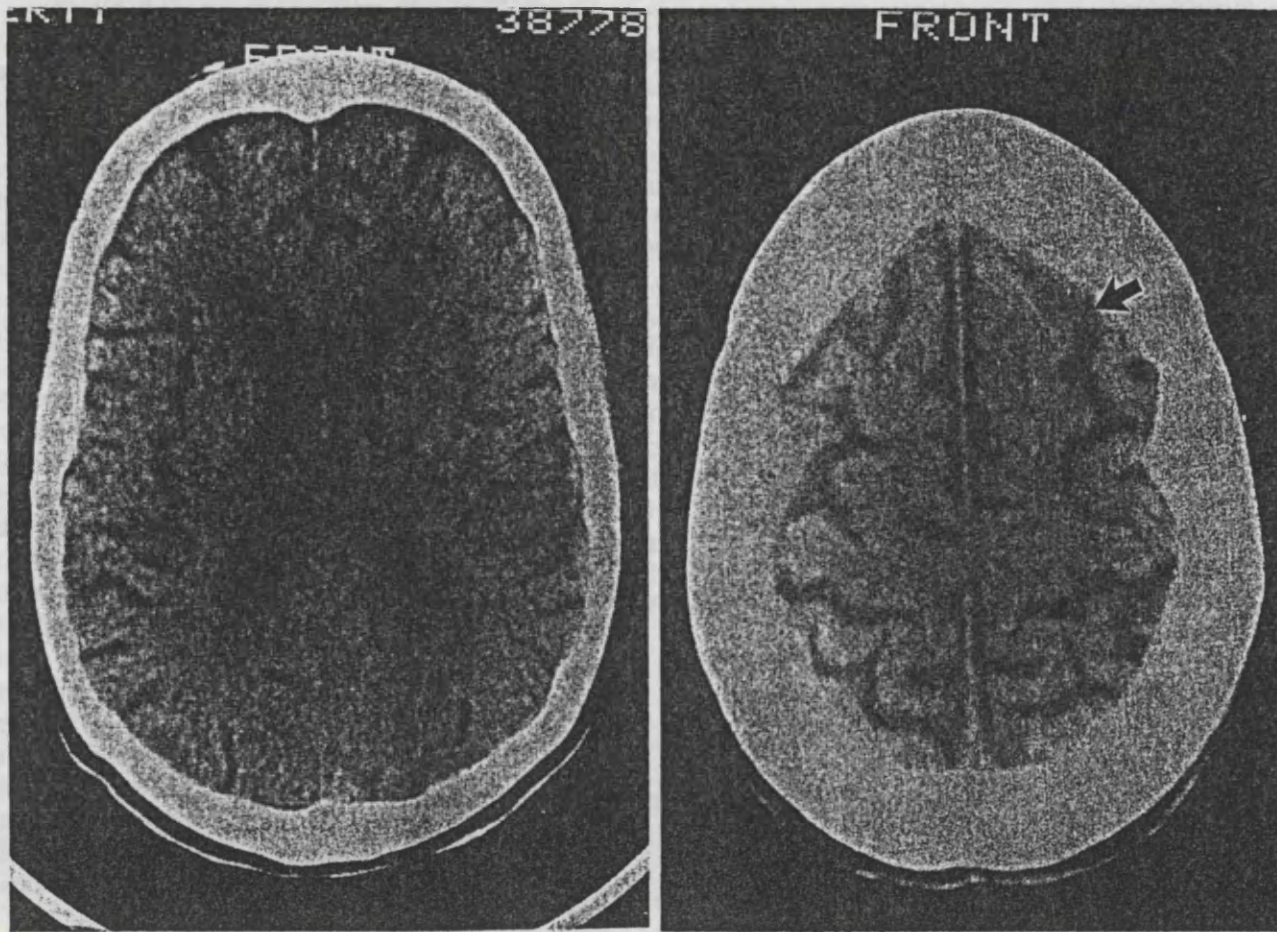


Figure 1 CT head scan taken at level of (a) the lateral ventricles (b) the hemispheres showing cortical atrophy; the sulci are widened (arrow).

problems, and a septic screen was negative. A formal psychiatric opinion was sought and the diagnosis of hypomania was confirmed. Haloperidol was commenced; this therapy was complicated by extrapyramidal side-effects which were successfully treated with procyclidine. Over the next week or so his hypomania settled. It was then planned to send him home with community psychiatric nurse support, and input from the Community Care Team. Once at home the patient discontinued his haloperidol abruptly, and rapidly became hypomanic once more. He began to spend money recklessly. He was readmitted and treated with chlorpromazine in place of haloperidol. Again there was rapid lysis of his problems. Investigations at this stage revealed that he was anaemic and he was transfused with four units of blood. CT of his head (fig 1) showed evidence of cortical atrophy with widened sulci; no focal abnormalities were evident. The patient's condition, whilst on the ward, gave continued cause for concern. He began to refuse his other medication, as he felt that to discontinue it would help him gain weight. It was felt that he was causing himself harm by his inappropriate behaviour, and after careful discussion it was decided to section him. The patient was informed of this decision, but before the section

could be activated, he left the ward and returned home. He was subsequently admitted as a voluntary patient to a psychiatric unit in another hospital, and then was discharged home once more. He was then managed in the community by the Bloomsbury Community Care Team and a community psychiatric nurse, together with a group of supportive friends and neighbours. Over this eight-week period prior to death, the patient continued to live at home. There was a steady deterioration with further weight loss and increasing weakness. Terminally he developed a bronchopneumonia and died.

Discussion (Dr M Potter)

This man was clearly hypomanic. He was restless, overactive, he had a labile mood and was disinhibited. In addition he exhibited bizarre behaviour, for example, on one occasion drawing around all his joints with a felt tip pen. We also heard that he was spending recklessly, and also had grandiose plans. He had no personal or family history of previous psychiatric problems, but he had a somewhat extrovert, premorbid personality. The patient did not drink alcohol excessively, nor did he use opiates or cocaine.

Psychosis in HIV +ve patients, unassociated with delirium or dementia, is fairly uncommon.¹² In this case the possibilities are that his hypomanic episode was unrelated to his HIV disease—the two occurring together merely as a coincidence. Alternatively this could have represented a reactive event, but against this is the long time interval between knowing he was HIV +ve and the development of the hypomania. A further possibility is that this illness was secondary to a delirium or a dementia, but there was no evidence for either of these on mental state examination. Of course, it is known that HIV +ve patients can present with psychotic features first, but it is usual for these to be followed by rapid cognitive decline or a confusional state.¹³ In such a situation it is usually obvious in retrospect that the psychotic episode was the first indication of a dementing process.⁴ This was clearly not the situation in this man's case. Very occasionally psychotic illness can occur without any evidence of an underlying organic process.¹ This has been attributed to some direct neurotropic effect of the HIV itself. This may well have been the case in this man's illness. Although of course it has to be said that trying to assess cognitive function in somebody who is hypomanic is difficult. In terms of the treatment he was given, we know that HIV +ve individuals are very sensitive to the anticholinergic and extrapyramidal side-effects of neuroleptic drugs.¹ This patient clearly showed extrapyramidal problems with haloperidol. In retrospect perhaps, thioridazine, which has fewer extrapyramidal side-effects than haloperidol, might have been a better choice of drug.¹

Grounds for detention under the Mental Health Act are quite clear. The patient must be showing evidence of causing harm to himself or to others. The patient was behaving in a reckless way and refusing his medication. However, it was still a difficult decision to make. You were faced with the options of placing this patient on a section for assessment for a month, or for treatment over 6 months knowing that the patient's overall prognosis was severely limited anyway. Another practical problem, having sectioned somebody, is attempting to contain them and this may pose significant problems particularly on an acute medical ward. It was probably a tactical error to inform the patient that he was going to be sectioned some time before the assessment was due to be carried out, since he then absconded from the ward. It might have been better for him to have been managed on a psychiatric ward, but one is faced with the ethical dilemma of restraining somebody in hospital towards the end of his life a considerable distance from his friends and neighbours and away from his own environment.

Dr R J D George

We looked after this man in his own home in another

suburb of London for several weeks before he died. The care was provided by his friends and neighbours on a day-to-day 24 hours a day basis, guided by the community psychiatric nurse, his general practitioner, an occupational therapist and ourselves. The voluntary organisation ACET (Aids Care Education and Training—National Information: 081-840 7879) visited regularly and co-ordinated and supplemented the 24 hour care network. Despite his prior psychiatric problems the patient's expressed wishes were to remain at home for as long as possible and to die there. He was fully aware of his worsening health; weight loss, progressive neuropathy affecting his mobility and deteriorating visual acuity. A month before death he decided to stop all his treatment; there was no recurrence of his psychiatric problems. In the last weeks of his life he was immobile in bed and had a urinary catheter; there was a clear but slowly progressive decline in mental function.

Clinical diagnoses:

1. Hypomania
2. Encephalopathy—HIV?, or CMV?
3. Cytomegalovirus retinitis
4. Terminal bronchopneumonia.

Pathology (*Professor L Michaels*)

The necropsy findings in this man were surprising. There was generalised lymphadenopathy. The nodes themselves were very pale. The spleen was three times its normal size, and within it there were large yellow areas. With all these changes in the reticulo-endothelial system, I thought this was going to turn out to be a lymphoma. Histologically, the lymph nodes and spleen showed massive necrosis with inflammatory cell changes (fig 2). Under high power inflammatory cells were seen to be plasma cells and some histiocytes among them. Staining of the lymph

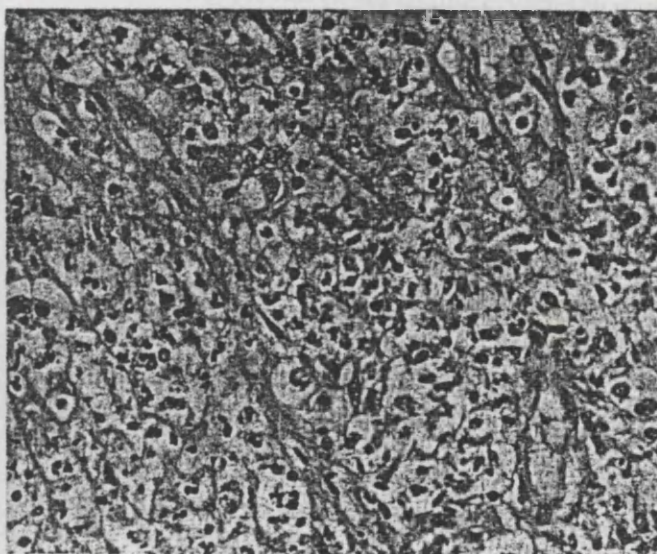


Figure 2 Para-aortic lymph node showing plasma cell and macrophage infiltration with necrosis. (×400. Haematoxylin and Eosin.)

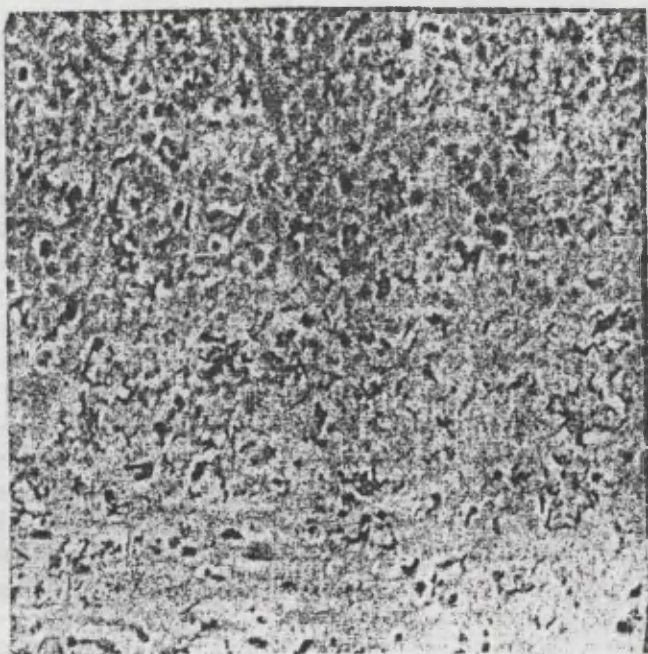


Figure 3 Para-aortic lymph node showing infiltration with vast numbers of acid fast rod shaped organisms—atypical mycobacteria. ($\times 1000$, Zeihl-Neelsen.)

nodes and spleen with Zeihl-Neelsen revealed vast numbers of acid-fast bacilli (fig 3). This is quite different from *Mycobacterium tuberculosis* in the pattern of inflammatory reaction. With the absence of granuloma formation this is likely to be an atypical mycobacterium, such as *Mycobacterium-avium intracellulare*.

In the lungs the lower lobes were consolidated. Microscopically there was evidence of bronchopneumonia. There were numerous Gram-positive cocci. *Pneumocystis carinii* were not seen, and there was no evidence of mycobacteria. In the apices, in a subpleural distribution, there was marked emphysematous change. Elsewhere in the upper



Figure 4 Left upper lobe of lung showing giant cell and macrophage reaction to numerous birefringent crystals (of pentamidine) (arrows). ($\times 400$, Haematoxylin and Eosin) photographed through partially crossed polaroids.

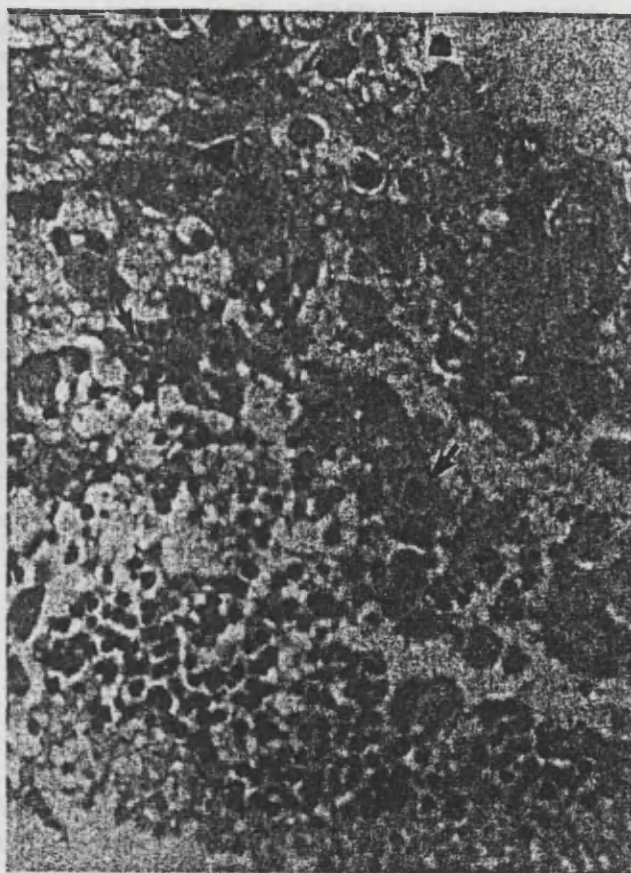


Figure 5 Ganglion cell layer of retina showing swelling and necrosis of cells, many of which show large basophilic intranuclear inclusions (arrows). The granular cell layer is not affected.

lobes, there was a granulomatous chronic inflammatory change. Within this were giant cells which had formed in reaction to foreign body material. At high power this was seen to be crystalline material (fig 4). This man had received nebulised pentamidine, and I wonder if it was pentamidine crystals that were seen deposited in the upper lobes. When nebulised pentamidine therapy is given it is at the limit of its saturation in water. It would not be surprising if some of the pentamidine were to crystallise out during inhalation and may then deposit within the lungs. A granulomatous response to pneumocystis infection has previously been reported in patients who have received inhaled pentamidine therapy.⁵ There was no evidence of Kaposi's sarcoma in the tracheobronchial tree, within the lung parenchyma or on the pleura.

In the kidneys, lying within the tubules, there were large numbers of Gram-negative rods. Although the kidneys were not cultured this was probably an *E coli* infection. In the liver there was some inflammatory change, particularly around the sinusoids, and within these were mycobacteria.

Histological examination of the left eye showed widespread retinal necrosis, foci of cells showing the inclusions of cytomegalovirus in the ganglion cell layer (fig 5).

Dr F Scaravilli

Macroscopically in the brain there was some patchy areas of discolouration but otherwise there were no gross abnormalities. At this point several possible diagnoses can be ruled out, including progressive multifocal leucoencephalopathy. Very rarely does toxoplasma encephalitis occur without macroscopic abnormalities. But macroscopically this still could be cytomegalovirus encephalitis or HIV encephalitis. On histological examination the myelin stain showed diffuse myelin pallor particularly in the hemispheres. There were no other identifying abnormalities, so this is a rather non-specific finding. The white matter did not show any abnormality other than diffuse oedema and a little perivascular cuffing with lymphocytes. In conclusion, on the basis of the histology, we were not able to make a specific diagnosis and we had to use histochemical methods.

Miss E Sinclair

Sections of the brain were stained with an anti-HIV antibody (p24, Dupont), and we detected HIV antigen, both in macrophages and microglial cells. Positive cells were detected scattered throughout the white matter, but no such cells were seen in the cortical grey matter. Using the polymerase chain reaction (PCR), proviral HIV DNA was sought. When using this technique to detect HIV, it is necessary to amplify DNA from more than one region of the genome to preclude false negative results which may occur due to sequence alterations. We routinely amplify sequences from three genes, the *gag* (which codes for HIV core protein), *pol* (which codes for reverse transcriptase) and *env* (which codes for virus cell membrane). PCR was applied separately to both cortical grey and white matter from the brain. In this case HIV sequences were detected in both grey and white matter.

Dr F Scaravilli

The presence of non-specific white matter abnormalities and the scattered inflammatory changes,

together with the evidence of HIV infection, means that this man had HIV encephalopathy. By glial fibrillary acidic protein (GFAP) staining we were able to show the extent of glial cell involvement in this process. Throughout the brain there was evidence of increased numbers of glial cells expressing GFAP, indicating that there was indeed diffuse cerebral pathology which was not detected by standard histological methods. Undoubtedly this would have accounted for the changes in higher cerebral function in this patient.

In the spinal cord at the thoracic level there was evidence of marked demyelination and vacuole formation. The patient had a vacuolar myelopathy which might explain his peripheral neurological problems.

Pathological diagnosis:

1. Disseminated mycobacterial infection
2. Terminal bronchopneumonia
3. Apical foreign body granulomatous response in the lungs
4. HIV encephalopathy
5. Vacuolar myelopathy

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- 1 Fenton TW. AIDS related psychiatric disorder. *Br J Psychiatry* 1987;151:579-88.
- 2 Halstead S, Riccio M, Harlow P, Oretti R, Thompson C. Psychosis associated with HIV infection. *Br J Psychiatry* 1988;153:618-23.
- 3 Buhrich N, Cooper DA, Freed E. HIV infection associated with symptoms indistinguishable from functional psychosis. *Br J Psychiatry* 1988;152:649-53.
- 4 Schmidt U, Miller D. Two cases of hypomania in AIDS. *Br J Psychiatry* 1988;152:839-442.
- 5 Birley HDL, Buscombe JT, Griffiths MH, Semple SJB, Miller RF. Granulomatous *Pneumocystis carinii* pneumonia in a patient with the acquired immunodeficiency syndrome. *Thorax* 1990;45:769-70.

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Detection of HIV proviral DNA in cortex and white matter of AIDS brains by non-isotopic polymerase chain reaction: correlation with diffuse poliodystrophy

Elizabeth Sinclair and Francesco Scaravilli

Objectives: (1) To determine whether detection of HIV proviral DNA sequences in the cerebral cortex correlates with the presence of pathological changes in this region, believed to contribute to the HIV-associated cognitive/motor complex. (2) To compare the frequency with which HIV infects cortical and subcortical regions of the brain.

Design: *In vitro* studies on HIV neurotoxicity suggest that HIV may be involved in the pathogenesis of cortical damage, recently defined as diffuse poliodystrophy (DPD) in AIDS. Previous detection of HIV antigen has localized HIV more frequently to subcortical than to cortical regions. It is not known whether HIV preferentially infects subcortical tissues or if viral expression varies in these two regions.

Methods: HIV antigen and proviral DNA sequences were detected in anterior frontal lobe tissues using immunohistochemistry (IHC) and the polymerase chain reaction (PCR), respectively. DPD was assessed by staining with antibodies against astrocytes (GFAP) and microglia/macrophages (HAM 56).

Results: HIV proviral DNA was detected in nine out of 15 cortical samples and in 10 out of 15 white matter samples, whilst HIV p24 antigen was localized to the cortex in three out of 15 and to the white matter in seven out of 15 cases. DPD was found in 10 cases, although in five a different aetiology may have been involved. However, DPD was present in eight out of the nine cases in which HIV proviral DNA was detected in the cortex.

Conclusions: Using a non-isotopic PCR method, HIV was detected in the brains of more cases than would be expected on the basis of IHC detection, and was present in the cortex as frequently as in the white matter. HIV, together with other factors, may contribute to the pathogenesis of DPD.

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Keywords: HIV proviral DNA, non-isotopic polymerase chain reaction, diffuse poliodystrophy, HIV-associated cognitive/motor complex.

Introduction

Pathological changes associated with HIV infection of the brain include infiltration of the white matter and subcortical grey by macrophages, presence of multinucleated giant cells (MGC) and pallor and gliosis of the white matter [1-5]. This pathological appearance is called HIV encephalitis (HIVE) if lesions are perivascular and focal, or HIV leukoencephalopathy (HIVL) if widespread throughout the deep white matter [6]. These appearances have been correlated with the HIV-associated cognitive/motor complex (previ-

ously known as the AIDS dementia complex [1,6]), although, in a number of cases, dementia is reported in patients who, at *post mortem*, do not have morphological evidence of encephalitis. Moreover, it has been suggested that pathological changes in the cerebral cortex of some of these patients may contribute to the dementia. This pathology, recently defined as diffuse poliodystrophy (DPD) in AIDS [6], has been described by several authors in HIV-positive patients with and without obvious HIVE or HIVL. Changes include astrogliosis with microglial activation [4,7,8] and neuronal loss [9-12]. MGC may also be found oc-

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asionally in the cortical grey matter of both HIVE or HIVL, but are much rarer than in the subcortical region. Similarly, HIV antigens and nucleic acids, although also found in the cortex, are predominantly located in subcortical regions [13,14] and are most often associated with changes of HIVE and HIVL [13–19].

It has been suggested that the cortical pathology seen in AIDS patients may be induced by HIV; however, since the number of cases with cortical abnormalities exceeds those in which HIV can be detected in the cortex either by immunohistochemistry (IHC) or *in situ* hybridization (ISH), it remains to be determined whether HIV exists within the cortex below the detection limit of these methods.

The recently developed polymerase chain reaction (PCR) [20] has proved to be a sensitive method for detection of HIV proviral DNA in brain tissue [21]. We used a non-isotopic PCR method to amplify HIV proviral DNA sequences from separate samples of grey and white matter. Three regions of the HIV genome were detected using primers designed to amplify conserved regions of the *gag*, *pol* and *env* genes [22]. The PCR results were related to IHC for HIV, performed on tissues from adjacent areas and to the HIV-associated neuropathological changes.

Materials and methods

Subjects and tissues

Fifteen cases were studied from a series of brains, obtained at *post mortem* from AIDS patients, treated at the University College and Middlesex Hospital, London. All subjects were homosexual men, whose age ranged from 26 to 53 years (mean, 37 years). Fixed brains weighed between 1290 and 1740 g (mean, 1463 g). At *post mortem* the frontal poles were removed and frozen and the rest of the brain was fixed in 10% formalin for 1–3 months; 15 blocks were taken from each case and routinely processed for histology.

Morphological methods

Paraffin sections of all blocks from each case were stained according to routine neuropathological methods; selected sections were also stained with special stains (PAS, Giemsa, Gram and Grocott) and immunohistochemistry for the detection of infective agents.

Immunohistochemistry

The reliability of three antibodies for the detection of HIV antigen had previously been tested on paraffin sections of blocks fixed in formalin for periods from 1 to 9 months. Cases of HIVE and HIV-negative cases (three of multiple sclerosis and one of vasculitis) were stained with the following antibodies: NEA HIV p24 (DuPont Diagnostics UK Ltd, Stevenage,

England, UK), NEA HIV p17 (DuPont), which detects the HIV core proteins p24 and p17 and LAV gp41.1 (Genetic Systems, Seattle, Washington, USA), which detects the envelope protein gp41. The two DuPont antibodies stained only HIV-positive cases, while LAV gp41.1, although reliable in other reports [13,14], stained occasional macrophages in all three multiple sclerosis lesions. Of the other two antibodies, NEA p24 was occasionally positive when NEA p17 was negative, while the reverse was never true. Therefore, NEA p24, which detected HIV in blocks fixed for up to 9 months, was used to screen paraffin sections of anterior frontal lobes from all cases in this study.

Adjacent sections were stained with antibodies to detect glial fibrillary acidic proteins (GFAP; Dako Ltd, High Wycombe, England, UK) and a macrophages/microglia marker [HAM 56; Enzo Biochem (Cambridge Bioscience, Cambridge, England, UK)]. All antibodies were visualized using the modified avidin-biotin peroxidase method [23]. GFAP and HAM 56 reactions were observed blind and independently by both investigators to assess the severity and distribution of the cellular reaction. Astrocytosis was described as mild if an increase in GFAP positive astrocyte number was the only change; moderate if enlarged, reactive nuclei were also seen; or severe when an increase in cytoplasmic volume was also present. Similarly, the microglial reaction was classified as mild when an increase in cell processes was present; moderate when numerous rod cells were present and severe only when macrophages were seen. DPD was thought to be present when astrogliosis was either moderate or severe with or without a microglial cell reaction.

Polymerase chain reaction

PCR was used to detect HIV sequences in separate specimens of grey and white matter with primer pairs from multiple regions of the HIV genome (*gag*, *pol* and *env*) provided by the UK Medical Research Council (MRC) AIDS Directed Programme (ADP) repository, National Institute of Biological Standards and Control (NIBSC). These primers were designed for use in a two-stage nested PCR procedure [22]; however, we used only the outer sets of primers. A preliminary study was performed on the first seven cases received, in which DNA was amplified from all 14 samples, three times with each primer pair in order to assess the reliability of the primers and the reproducibility of our method. DNA from a further eight cases was tested three times with the *gag* primers, which had proved reliable in our initial study. Only those samples with one or more negative results after amplification with *gag* primers were subjected to further amplifications with *pol* and *env* primers. The results of all 15 cases studied are presented here.

Preparation of DNA

The frontal poles of brains removed within 48 h of death were frozen at -20°C prior to separation of

cortex and white matter samples. After stripping the leptomeninges from the semi-frozen specimens, thin slices of grey matter were taken from the surface of the cortex and confirmed to be free of white matter. Using separate disposable scalpels, cubes of deep white matter were removed and diced into small pieces to ensure that they did not contain grey matter. Multiple samples of around 0.5 g of both tissues from each brain were snap frozen by immersion in liquid nitrogen and stored below -70°C . Tissue was digested as described previously [21], DNA purified by standard methods and its concentration estimated on a Beckman spectrophotometer.

PCR controls

All samples were initially amplified with human β -globin primers [Perkin Cetus Elmer (ILS Ltd, London, England, UK)] and only samples that were clearly positive with these primers were used for HIV PCR. DNA (1 μg) from HIV-seronegative cortical and white matter samples were spiked with 10 mol of non-infectious plasmid containing a completely rearranged HIV sequence (provided by MRC ADP repository, NIBSC) and used as positive control. DNA from brains of patients not in any of the HIV risk groups, single-stranded DNA and no DNA controls were taken through each experiment to ensure that there was no carry over from positive samples. These controls were negative by Southern blot in all cases. The different stages of PCR were physically separated as far as possible to prevent cross-contamination.

DNA amplification

Amplification was performed in 50 μl of reaction buffer (Perkin Cetus Elmer), overlaid with 50 μl of mineral oil. The reaction buffer was produced according to the manufacturer's instructions and contained 0.2 $\mu\text{mol/l}$ of each primer and 1 μg of template DNA. The MgCl_2 concentration was titrated for each primer pair. Forty cycles of a standard thermal cycling protocol were used to amplify templates on a Perkin Cetus Elmer thermal cycler. Amplified products were visualized by gel electrophoresis and their specificity was established by Southern blot hybridization using the appropriate probes.

Southern blot with non-isotopic probes

Probe labelling

The Saluz and Jost 'filling in' method [24] for labelling short oligonucleotides was used but further modified for labelling with digoxigenin-11-dUTP (DIG; Boehringer Mannheim UK Ltd, Lewes, England, UK). Probes for Southern blot hybridization were provided by the MRC PCR reference centre, NIBSC: *gag* (30 mer: 5'-CAT CAA TGA GGA AGC TGC AGA ATG GGA TAG; 10 mer: 5'-TCC CAT TCT G) *pol* (30 mer: 5'-CCT GTA GTA GCA AAA GAA ATA GTA GCC AGC; 10 mer: 5'-

GGC TAC TAT T) and *env* (30 mer: 5'-CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA; 10 mer 5'-CTC AAA GGA T). Briefly, an annealing mix containing 10 μl of the 30 mer, 10 μl of the 10 mer and 2.5 μl of oligonucleotide labelling buffer was prepared and incubated at 75°C for 2 min, at room temperature for 20 min and on ice for 10 min. This was followed by addition of 1 μl each of 10 mmol/l dGTP, dATP and dCTP. Annealing mix (2 μl) was added to 0.5 μl DIG-11-dUTP, 0.5 μl 1 mmol/l dTTP and 1 μl Klenow (Boehringer Mannheim) and incubated for 1 h at room temperature. Unincorporated nucleotides were removed by drying down the probe with 2 mol/l ammonium acetate in 70% ethanol and washing in 70% ethanol. Probes were drained then dissolved overnight at 37°C in TE buffer [10 mmol/l Tris (pH 8.0) and 1 mmol EDTA].

Hybridization

Membranes were prehybridized for 1 h at 42°C in hybridization buffer [5 \times saline-sodium citrate buffer (SSC; 20 \times 0.3 mol/l sodium citrate (pH 7.0) and 3 mol/l NaCl), 20% formamide, 0.1% lauroylarcosine, 0.02% sodium dodecyl sulphate (SDS)] and then hybridized overnight at 42°C with 10 μl of probe in hybridization buffer. They were then washed in $0.2 \times$ SSC, 0.1% SDS at room temperature.

DIG-11-dUTP detection

Detection was performed with a Boehringer Mannheim detection kit according to the manufacturer's instructions. Membranes were incubated with anti-DIG-11-dUTP antibody, labelled with alkaline phosphatase, and bound antibody was subsequently visualized with 4-Nitro blue tetrazolium chloride and X-phosphate.

Sensitivity of PCR assay

The method used in this study detected 1 mol of HIV DNA in six out of 10 PCR assays performed on a range of standardized dilutions of HIV DNA molecules (prepared by NIBSC). However, addition of 1 μg human genomic cortical or white matter DNA (from HIV-seronegative brains) reduced the sensitivity approximately 10-fold, thus 10 mol of HIV DNA were detected in five out of 10 PCR assays. The sensitivity of our method was approximately 10 mol of HIV DNA per μg of genomic DNA when multiple PCR tests were used. Although further modification (for example, nested PCR) may have resulted in a more sensitive assay, it would also have increased the likelihood of detecting HIV from infected peripheral blood mononuclear cells present in the brain sample. The low numbers of infected cell present in the circulation, even at later stages of disease [22], makes them an unlikely source of false-positive results when the detection limit of the test is relatively high.

Results

Pathological and immunohistochemical findings

The neuropathological diagnosis and HIV p24 IHC results of all 15 cases are shown in Table 1. HIVE was diagnosed in cases 1 and 10, and HIVL in cases 9 and 15. HIV p24 antigen was found in MGC, macrophages and microglial cells in the white matter in three of these cases; however, in case 10, where discrete HIVE lesions were confined to the midbrain, no p24 antigen was detected in the anterior frontal lobe. HIV antigens were detected in four other cases: in case 4, p24-positive microglia and macrophages were present in association with lesions of PML and located at the edge of foci of lymphoma in case 5, while in cases 2 and 14 positive microglial cells were scattered throughout the white matter. In cases 1, 4 and 5 HIV-infected cells were seen in both cortex and white matter although they were more numerous in the latter.

Table 1. Neuropathological diagnosis and immunohistochemical detection of HIV p24 antigen.

Case	Neuropathological diagnosis	HIV p24	
		Cortex	WM
1	HIV encephalitis	+	+
2	Non-specific reactive gliosis	-	+
3	Lymphoma	-	-
4	Progressive multifocal leukoencephalopathy	+	+
5	Lymphoma	+	+
6	Ischaemia	-	-
7	Cryptococcosis	-	-
8	No abnormalities	-	-
9	HIV leukoencephalopathy	-	+
10	HIV encephalitis, cryptococcosis	-	-
11	Cytomegalovirus encephalitis	-	-
12	Progressive multifocal leukoencephalopathy	-	-
13	Haemorrhage	-	-
14	Non-specific reactive gliosis	-	+
15	HIV leukoencephalopathy	-	+

WM, white matter.

An astrocytic reaction (highlighted by GFAP IHC) was present in the cortex of all cases and microglial cell activation (highlighted by HAM 56 IHC) in 12. However, the severity and distribution of the reaction was variable, as described in Table 2, and a diagnosis of DPD was made in 10 cases (1, 2, 5, 6, 7, 9, 10, 13, 14 and 15). The severe astrogliosis and moderate microglial cell reaction of case 1 shown in Figs 1a and b, respectively, illustrates an example of DPD. In five of these cases (5, 6, 7, 10 and 13) co-existing lesions of opportunistic infection or neoplasia may have accounted for or contributed to the cortical pathology (Table 2). In the five remaining cases with DPD, 3 (1, 9 and 15) had HIV-related lesions; in the remainder (2 and 14) the diffuse gliotic reaction, which was also present in the white matter, was the only pathological finding.

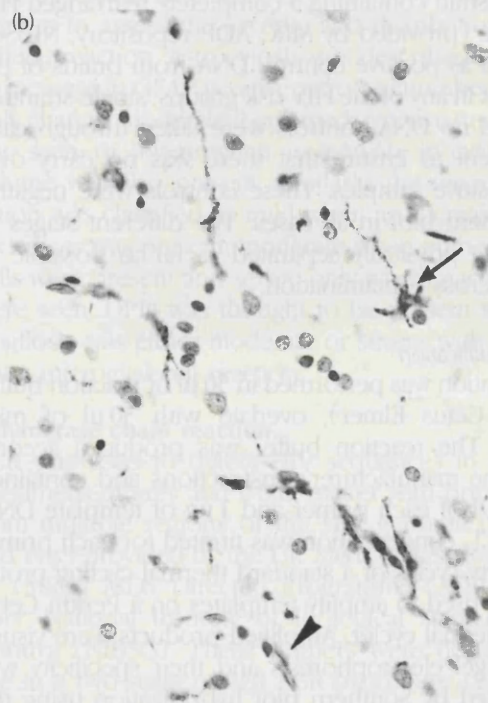
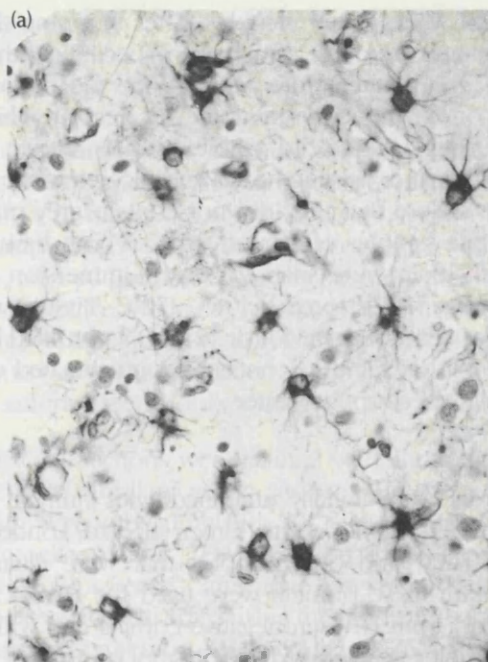


Fig. 1. Photomicrographs of anterior cerebral cortex of case 1 immunostained with (a) GFAP and (b) HAM 56. (a) Hypertrophy and hyperplasia of astrocytes. (b) Microglial cells show thickened processes (arrow); HAM 56 also stains some endothelial cells (arrowhead) ($\times 300$).

PCR results

The results of all PCR tests are shown in Table 3. Those for the first seven cases, where cortical and white matter samples were tested three times with each set of primers, fell into three categories. Ten samples were repeatedly positive after Southern blot (in at least four out of nine tests); two samples were consistently negative and the remaining samples (positive in only one or two out of nine tests) were designated indeterminate. The Southern blot from one of these tests

Table 2. Pathological changes present in the cortex immunostained by GFAP and HAM 56.

Case	Cortical reaction				Co-existing pathology
	Astrogliosis		Microglial cell activation		
1	D	Sev	D	Mod	—
2	D	Mod	U	Mod	—
3	D	Mild	—	—	Compression effect of lymphoma
4	D	Mild	—	—	Foci of PML in cortex
5	D	Mod	—	—	Compression by lymphoma
6	D	Mod	F	Sev	Many cortical nodules of ischaemia
7	D	Mod	F	Mild	Cryptococcal meningitis
8	D	Mild	—	—	—
9	D	Mod	F	Mod	—
10	D	Mod	D	Mild	—
11	D	Mild	F	Sev	Cryptococcosis involving cortex CMV microglial nodules in cortex
12	U	Mild	F	Sev	PML lesions encroaching on cortex
13	D	Mod	D	V. mild	Extensive haemorrhagic lesions
14	D	Mod	D	Mild	—
15	D	Sev	D	Mod	—

D, diffuse distribution of cells; F, focal distribution of cells; U, diffuse but uneven distribution of cells. Sev, severe; Mod, moderate; PML, progressive multifocal leukoencephalopathy; CMV, cytomegalovirus.

is shown in Fig. 2a. The remaining eight cases (16 samples), were all initially amplified three times with the *gag* primers (Fig. 2b) and eight samples were positive in all tests. The remainder were amplified twice with the *pol* and once with the *env* primers. Four of these samples were negative in all tests, three, designated indeterminate, were positive only once or twice

in the six tests and the remaining sample was positive four times in the six tests.

In 14 cases there was no significant difference between the number of times the white matter and cortical samples were positive; however, in case 11, the cortical sample was indeterminate and the white matter positive.

Correlation between DPD and proviral DNA

The results of IHC, PCR and cortical pathology are summarized in Table 4. HIV proviral DNA was detected in the cortical samples of eight out of 10 cases with diffuse poliodystrophy. Both cases 6 and 13, in which proviral DNA was not detected, had cerebrovascular abnormalities that probably account for cortical changes. In addition, of the nine cases in which proviral DNA was detected in the cortex, DPD was seen in eight. HIV p24 antigens were localized to the cortex in only three cases, in two of which DPD was present. Case 4, which lacked diffuse poliodystrophic changes, was positive with both PCR and IHC for HIV in the cortex.

Discussion

An association between HIV and the subcortical changes seen in HIVE and HIVL is well established [13]. In addition, HIV antigens can also be found in cases with no morphological evidence of HIV encephalitis (cases 4 and 5) and indeed in the absence of any specific neuropathological changes (cases 2 and

Table 3. Frequency of HIV proviral DNA detection in cortical and white matter samples after polymerase chain reaction and Southern blot.

Primers	Cortex				T	White matter				T
	<i>gag</i>		<i>pol</i>			<i>gag</i>		<i>pol</i>		
	881	882	001	004		881	882	001	004	
1	+++	+++	+++	+++	+	+++	- ++	+++	+	
2	+++	+++	+++	+++	+	+++	+++	+ - +	+	
3	-- +	---	-- +	-- +	±	---	-- +	---	±	
4	+++	+++	+++	+++	+	+++	+++	+ - +	+	
5	+++	+++	+++	+++	+	+ --	+++	- + -	+	
6	---	---	---	---	-	---	---	---	-	
7	- + -	+ + -	+ - -	+ - -	+	- + -	+++	+ - -	+	
8	---	---	---	---	-	---	---	---	-	
9	+++	+++	+++	+++	+	+++	+++	+++	+	
10	+++	+++	+++	+++	+	+++	+++	+++	+	
11	---	---	---	---	±	+ --	++	+	±	
12	- + -	---	---	---	±	+ + -	---	---	±	
13	---	---	---	---	-	---	---	---	-	
14	+++	+++	+++	+++	+	+++	+++	+++	+	
15	+++	+++	+++	+++	+	+++	+++	+++	+	

Primers numbered according to Simmonds et al. [22]. T, designated score; +, positive; ±, indeterminate; -, negative.

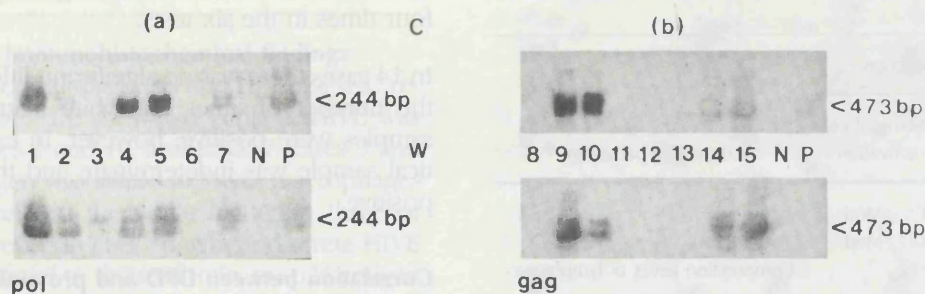


Fig. 2. Detection of HIV proviral DNA sequences in cortical (C) and white matter (W) samples following DNA extraction, amplification by polymerase chain reaction and Southern blot of cases 1-7 (a) and 8-15 (b). Samples from both cortex and white matter are positive following detection of a region of the *pol* (a) or *gag* (b) gene in cases 1, 2, 4, 5, 7, 9, 10, 14 and 15, and negative in cases 6, 8, 11, 12 and 13, while in case 3 the cortical sample is negative and the white matter sample positive. Cortical and white matter samples of a HIV-negative case (N) are negative, while 10 mol HIV plasmid DNA added to 1 µg HIV-negative DNA (P) was positive with the *pol* primers, and faintly positive and negative in the cortical and white matter samples amplified with the *gag* primers.

Table 4. Summary of HIV detection and pathological reaction in the cerebral cortex.

Case	HIV p24 antigen	Proviral DNA	DPD
1	+	+	+
2	-	+	+
3	-	-	-
4	+	+	-
5	+	+	(+)
6	-	-	(+)
7	-	+	(+)
8	-	-	-
9	-	+	+
10	-	+	(+)
11	-	-	-
12	-	-	-
13	-	-	(+)
14	-	+	+
15	-	+	+

Parentheses indicate co-existing pathology may have produced diffuse poliodystrophy (DPD).

14); this suggests that immunohistochemical detection of HIV with p24 is reasonably sensitive. We detected HIV proviral DNA in 10 out of 15 cases, seven of which were consistently positive in almost all the tests; however, more variability occurred in cases 5, 7 and 11, which gave similar results to the control sample containing 10 mol of HIV DNA per µg genomic DNA. Three cases that had only one or two positive results may have contained less than 10 mol of HIV DNA per µg genomic DNA; however, since the presence of infected peripheral blood mononuclear cells in the sample cannot be ruled out when infrequent positive results are obtained, these samples were designated indeterminate. The variability of results in some of our cases emphasizes the need to perform multiple PCR tests on each sample. Two previous PCR studies on AIDS brains [21,25] reported an association between detection of HIV DNA and the occurrence of HIVE. These results may seem at variance with ours, as only four of the 10 cases in which HIV

provirus was detected showed morphological features of HIVE or HIVL. However, morphological and/or antigenic evidence of HIV infection was present in the white matter of the majority (eight out of 10) of PCR-positive cases. HIV p24 antigen and multinucleated giant cells were never detected in cases that were negative or indeterminate by PCR. In previous PCR investigations of this kind [21,25-27], samples included both cortex and white matter. This study, using separate specimens of the two regions, has shown, for the first time, not only that HIV proviral DNA can be detected repeatedly in the frontal cortex, but also that there is a close correlation between the frequency of HIV provirus detection in the cortex and in the white matter of the same patient. In contrast, it is well known that viral antigens are more abundant in subcortical than cortical regions [13,14], which is in agreement with our localization of HIV p24 in the white matter of seven cases and in the cortex of only three; this difference in distribution remains to be explained [5]. The amount of replicating virus in cortical and subcortical tissues may be dependent on the tissue micro-environment, or host cell availability, which may be different in these two regions. Indeed, in rat brain, CD4 (the HIV binding molecule) is consistently expressed by microglial cells of the white, but only infrequently by those of the grey matter [28]. A lack of correlation between HIV antigens and proviral DNA in brain was also reported in some patients studied by Pang *et al.* [21].

Diffuse gliosis in AIDS may result from a number of causes, including co-existing opportunistic infections, neoplasia or cerebrovascular disease. However, in half of the cases with DPD in this study, none of these possible causes was present. Detection of HIV by PCR in the cortex of these cases suggests that HIV may be involved in the pathogenesis of DPD. Indeed, viral components have been found to act as toxins to neuronal cells [29]; these include the viral proteins gp120 [30] and *tat* [31] as well as unidentified factors released

from HIV-infected macrophages [32,33]. In addition, high levels of unintegrated proviral DNA, as detected in AIDS brains by Pang *et al.* [21], in avian [34–36] and feline [37,38] retroviral infections, are associated with cytotoxicity. The above observations have led to suggestions that HIV may be neurotoxic *in vivo*, the astrocytic and microglial cell reaction, as observed in this study, may be a secondary consequence of neurotoxicity or an independent reaction to the presence of HIV. However, in case 4 in this study, both HIV provirus and antigen were detected in the cortex in the absence of DPD: this suggests that, if HIV is involved, additional factors are likely to be required for the development of cortical pathology. Direct and/or indirect HIV-induced toxicity may combine with local or systemically released cytokines, some of which are increased in AIDS patients [39].

The release of proposed neurotoxins from HIV-infected cells is compatible with low levels of viral replication, or production of regulatory proteins only [32,40], suggesting that HIV may be able to induce pathology in the cerebral cortex while existing at a level below the limits of IHC detection. Alternatively, the presence of proviral DNA itself may be toxic [21]. Therefore the small amount of detectable antigen in the cortex of cases with DPD does not rule out HIV as an important factor in the development of this disorder. However, localization of proviral DNA to infected cells would help to determine the role of low-level HIV infection.

Using PCR, we have shown that infection of the brain is more widespread than was thought previously, thus demonstrating that the use of such sensitive detection techniques may be necessary to elucidate the pathogenesis of lesions underlying the HIV-associated cognitive/motor complex.

Acknowledgements

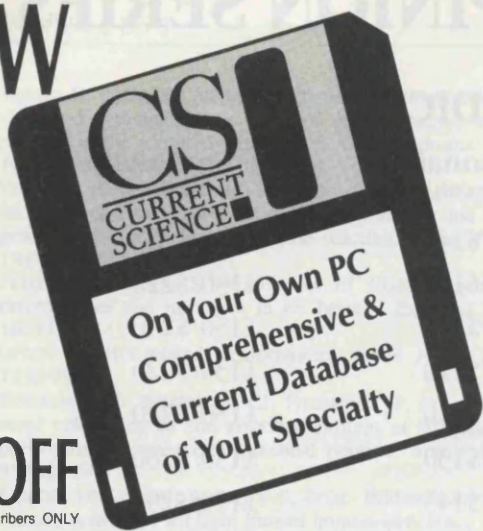
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References

- NAVIA BA, CHO E-S, PETTIO CK, PRICE RW: The AIDS dementia complex: II. Neuropathology. *Ann Neurol* 1986, 19:525–535.
- PETTIO CK, CHO E-S, LEMANN W, NAVIA BA, PRICE RW: Neuropathology of the acquired immune deficiency syndrome (AIDS): an autopsy review. *J Neuropathol Exp Neurol* 1986, 45:635–646.
- GRAY F, GHERARDI R, SCARAVILLI F: The neuropathology of the acquired immune deficiency syndrome (AIDS): a review. *Brain* 1988, 111:245–266.
- BUDKA H, COSTANZI G, CRISTINA S, *ET AL*: Brain pathology induced by infection with the human immunodeficiency virus (HIV). A histological, immunohistochemical and electron microscopical study of 100 autopsy cases. *Acta Neuropathol (Berl)* 1987, 75:185–198.
- BUDKA H: Neuropathology of human immunodeficiency virus infection. *Brain Pathol* 1991, 1:163–175.
- BUDKA H, WILEY CA, KLEIHUES P, *ET AL*: HIV-associated disease of the nervous system: review of nomenclature and proposal for neuropathology-based terminology. *Brain Pathol* 1991, 1:143–152.
- MONTE DE LA SM, HO DD, SCHOOLEY RT, *ET AL*: Subacute encephalomyelitis of AIDS and its relation to HTLV-III infection. *Neurology* 1987, 37:562–569.
- CIARDI A, SINCLAIR E, SCARAVILLI F, *ET AL*: The involvement of the cerebral cortex in human immunodeficiency virus encephalopathy: a morphological and immunohistochemical study. *Acta Neuropathol (Berl)* 1990, 81:51–59.
- KETZLER S, WEIS S, HAUG H, BUDKA H: Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropathol (Berl)* 1990, 80:92–94.
- EVERALL IP, LUTHERT PJ, LANTOS PL: Neuronal loss in the frontal cortex in HIV infection. *Lancet* 1991, 337:1119–1121.
- WILEY CA, MASLIAH E, MOREY M, *ET AL*: Neocortical damage during HIV infection. *Ann Neurol* 1991, 29:651–657.
- GRAY F, HAUG H, CHIMELLI L, *ET AL*: Prominent cortical atrophy with neuronal loss as a possible correlate of human immunodeficiency virus encephalopathy. *Acta Neuropathol (Berl)* 1991, 82:229–233.
- BUDKA H: Human immunodeficiency virus (HIV) envelope and core proteins in CNS tissues of patients with the acquired immune deficiency syndrome (AIDS). *Acta Neuropathol (Berl)* 1990, 79:611–619.
- KURE K, WEIDENHEIM KM, LYMAN WD, DICKSON DW: Morphology and distribution of HIV-1 gp41-positive microglia in subacute AIDS encephalitis. *Acta Neuropathol (Berl)* 1990, 80:393–400.
- SHAW KGM, HARPER ME, HANN BH, *ET AL*: HTLV-III infection in brains of children and adults with AIDS encephalopathy. *Science* 1985, 227:177–182.
- KOENIG S, GENDELMAN HE, ORENSTEIN JM, *ET AL*: Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 1986, 233:1089–1093.
- WILEY CA, SCHRIER RD, NELSON JA, *ET AL*: Cellular localization of human immunodeficiency virus infection within the brain of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 1986, 83:7089–7093.
- MIRRA SS, DEL RIO C: The fine structure of the acquired immunodeficiency syndrome encephalopathy. *Arch Pathol Lab Med* 1989, 113:858–865.
- PORWIT A, PARRAVICINI C, PETREN A, *ET AL*: Cell association of HIV in AIDS-related encephalopathy and dementia. *APMIS* 1989, 97:79–90.
- SAKI RK, SCHARF S, FALOONA F, *ET AL*: Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 1985, 230:1350–1355.
- PANG S, KOYANAGI Y, MILES S, *ET AL*: High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients. *Nature* 1990, 343:85–89.
- SIMMONDS P, BALFE P, PEUTHERER JF, *ET AL*: Human immunodeficiency virus-infected individuals contain proviruses in small numbers of peripheral mononuclear cells and at low copy numbers. *J Virol* 1990, 64:864–872.
- SOBEL RA, BLANCHETTE BW, BHAN AK, *ET AL*: The immunopathology of experimental allergic encephalomyelitis. I. Quantitative analysis of inflammatory cells *in situ*. *J Immunol* 1984, 132:2393–2401.
- SALUZ H, JOST JP: A simple high-resolution procedure to study DNA methylation and *in vivo* DNA-protein interactions on a single-copy gene level in higher eukaryotes. *Proc Natl Acad Sci USA* 1986, 86:2602–2606.
- KLEIHUES P, LEIB SL, STRITTMATTER C, *ET AL*: HIV-encephalopathy: incidence, definition and pathogenesis. *Acta Pathol Jpn* 1991, 41:197–205.

26. SHIBATA D, KLATT EC: Analysis of human immunodeficiency virus and cytomegalovirus infection by polymerase chain reaction in the acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 1989, 113:1239-1244.
27. VARMA VA, HUNTER S, TICKMAN RN: Acute fatal HIV encephalitis with negative serological assays for antibody and antigen: diagnosis by polymerase chain reaction. *N Engl J Med* 1989, 320:1494.
28. PERRY VH, GORDON S: Modulation of CD4 antigen on macrophages and microglia in rat brain. *J Exp Med* 1987, 166:1138-1143.
29. LIPTON SA: HIV-related neurotoxicity. *Brain Pathol* 1991, 1:193-199.
30. BRENNEMAN DE, WEATBROOK GL, FITZGERALD SP, ET AL: Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 1988, 335:639-642.
31. SABATIER J-M, VIVES E, MABROUK K, ET AL: Evidence for neurotoxic activity of *tat* from human immunodeficiency virus type I. *J Virol* 1991, 65:961-967.
32. GIULIAN D, VACA K, NOONAN CA: Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* 1990, 250:1593-1596.
33. PULLIAM L, HERNDIER BG, TANG NM, McGRATH S: Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *J Clin Invest* 1991, 87:503-512.
34. ROBINSON HL, MILES BD: Avian leukosis virus-induced osteoporosis in association with persistent synthesis of viral DNA. *Virology* 1985, 141:130-143.
35. WELLER SK, JOY AE, TEMIN HM: Correlation between cell killing and massive second round superinfection by members of some subgroups of avian leukosis virus. *J Virol* 1980, 33:494-506.
36. WELLER SK, TEMIN HM: Cell killing by avian leukosis viruses. *J Virol* 1981, 39:713-721.
37. HOOVER EA, MULLINS JI, QUACKENBUSH SL, GASPER P: Experimental transmission and pathogenesis of immunodeficiency syndrome in cats. *Blood* 1987, 70:1880-1892.
38. MULLINS JI, CHEN CS, HOOVER EA: Disease-specific and tissue-specific production of unintegrated feline leukaemia virus variant DNA in feline AIDS. *Nature* 1986, 23:333-335.
39. MATSUYAMA T, KOBAYASHI N, YAMAMOTO N: Cytokines and HIV infection: is AIDS a tumor necrosis factor disease? *AIDS* 1991, 5:1405-1417.
40. BEDNARIK DP, FOLKS TM: Mechanisms of HIV-1 latency. *AIDS* 1992, 6:3-16.

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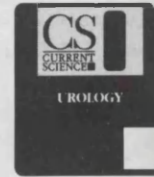
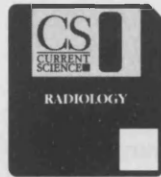
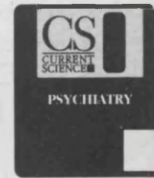
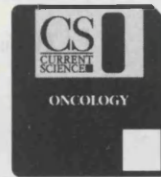
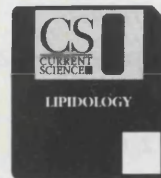
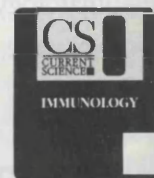
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PCR detection of HIV proviral DNA in the brain of an asymptomatic HIV-positive patient

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Sirs: Whilst it is well known that HIV is responsible for the AIDS-dementia complex (ADC), both the stage of disease at which the nervous system is infected and the pathogenetic mechanism of this disorder remain poorly understood. Examination by one of us (F.G.) of brains from HIV-positive patients who died prior to the development of AIDS often shows lymphocytic meningitis, diffuse mild to moderate myelin pallor, reactive astrogliosis, and microglial proliferation. To date, these changes have been considered "non-specific" in view of the absence of multinucleated giant cells and HIV antigens. In contrast, HIV has been frequently detected in the CSF of HIV-positive asymptomatic patients [4]; indeed, Ho et al. [5] retrieved the virus from one of their patients at the time of seroconversion, during an episode of subacute meningitis. However, there have been no reports so far of HIV detection in brains of these patients.

The polymerase chain reaction (PCR), which detects low copy numbers of nucleic acids [14], has been used to detect HIV DNA [13] and RNA [3] in the CSF of AIDS patients. In both studies the presence of HIV in the CSF correlated with the presence of neurological symptoms, although HIV was also detected in a small number of asymptomatic HIV-positive patients. PCR has also been applied to human post-mortem brain tissues, and although false-positive results might be expected, due to the presence of HIV-infected blood or CSF cells contaminating the brain sample, in practice there have been good correlations between PCR detection of HIV and HIV-related pathology [6, 9]. In addition, in our own experience post-mortem brain tissues from patients with full-

blown AIDS that lack specific neuropathological changes are consistently negative (Fig. 1, lane 2) in many cases when tested by PCR. This lack of false-positive results may be due to the low numbers of circulating peripheral blood mononuclear cells that are infected by HIV [14]. This method appears to be reliable for the detection of low levels of infection and we therefore applied PCR to post-mortem brain samples of an HIV-positive asymptomatic patient. The patient, a 31-year-old female drug user, had previously been found to be HIV-positive. By the time she died from a heroin overdose, she had not developed any symptoms of AIDS (according to CDC criteria). Neuropathology showed discrete lymphocytic meningitis and diffuse myelin pallor of the cerebral hemispheres (features which are not seen in HIV-negative drug users), but no features suggestive of HIV encephalitis were present; immunostaining with p24 antibody (Dupont, UK) for HIV was consistently negative. DNA for PCR was extracted from snap-frozen post-mortem tissue from the frontal, temporal and occipital lobes and from the deep grey nuclei. Control tissue from HIV-negative subjects and AIDS patients was also processed. Following amplification of a region of the *gag* gene, PCR products were visualized by gel electrophoresis and their specificity established using Southern blot hybridization. HIV DNA was detected in all four samples of the HIV-positive patient (Fig. 1, lanes 3–6) and in the patient with ADC (Fig. 1, lane 1) but not in HIV-negative samples (Fig. 1, lane 7) or in an AIDS patient with no neuropathological abnormalities (Fig. 1, lane 2).

The demonstration of HIV in the CSF of HIV-positive asymptomatic cases has previously been used as evidence to suggest that HIV infects the CNS at an early stage. However, it does not necessarily follow that the

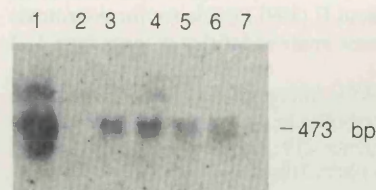


Fig. 1. Southern blot following amplification of a region of the HIV *gag* gene by polymerase chain reaction (primers 881 and 882 [14]). Samples of DNA from: the frontal lobes of AIDS patients with AIDS dementia complex (lane 1) and no neuropathological abnormalities (lane 2); the frontal (lane 3), temporal (lane 4) and occipital lobes (lane 5) and deep grey nuclei (lane 6) of the asymptomatic HIV-positive patient and frontal lobe (lane 7) of an HIV-negative patient

brain parenchyma is also infected. We have confirmed for the first time that HIV can be detected in the brain parenchyma of HIV-positive asymptomatic patients. Recent *in vitro* studies (see [8]) have shown that HIV is directly neurotoxic [1, 10]; in addition this virus may stimulate the production of neurotoxic substances such as tumour necrosis factor [7, 12], transforming growth factor- β [15] or quinolinic acid [2, 11]. We suggest that such neurotoxic mechanisms may have contributed to the pathological changes seen in this case. Further studies of similar patients may help to elucidate the role of these mechanisms in the pathogenesis of ADC.

Acknowledgement. This research was supported by a grant from the Medical Research Council (MRC).

References

- Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, Pert CB (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 335:639-642
- Cotton P (1990) Aids dementia may be linked to metabolite of tryptophan. *JAMA* 263:305-306
- Goswami KK, Miller RF, Harrison MJ, Hamel DJ, Daniels RS, Tedder RS (1991) Expression of HIV-1 in the cerebrospinal fluid detected by the polymerase chain reaction and its correlation with central nervous system disease. *AIDS* 5:797-803
- Goudsmit J, Wolters EC, Bakker M, Smit L, Van der Noordaa J, Hische EA, Tutuarima JA, Van der Helm HJ (1986) Intrathecal synthesis of antibodies to HTLV-III in patients without AIDS or AIDS-related complex. *BMJ* 292:1231-1234
- Ho DD, Rota TR, Schooley RT, Kaplan JC, Allan JD, Groopman JE, Resnick L, Felsenstein D, Andrews CA, Hirsch MS (1985) Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurological syndromes related to the acquired immunodeficiency syndrome. *N Engl J Med* 313:1493-1497
- Kleihues P, Leib SL, Strittmatter C, Wiestler OD, Lang W (1991) HIV encephalopathy: incidence, definition and pathogenesis. *Acta Pathol Jpn* 41:197-205
- Lahdevirta J, Maury CPJ, Teppo AM, Repo H (1988) Elevated levels of circulating cachectin/tumour necrosis factor in patients with acquired immunodeficiency syndrome. *Am J Med* 85:289-291
- Lipton SA (1991) HIV-related neurotoxicity. *Brain Pathol* 1:193-199
- Pang S, Koyanagi Y, Miles S, Wiley C, Vinters HV, Chen ISY (1990) High levels of unintegrated HIV-1 DNA in brain tissues of AIDS dementia patients. *Nature* 343:85-89
- Sabatier J-M, Vives E, Mabrouk K, Benjouad A, Rochat H, Duval A, Hue B, Bahraoui E (1991) Evidence for neurotoxic activity of tat from human immunodeficiency virus type 1. *J Virol* 65:961-967
- Schwarcz R, Whetsell WO, Mangano RM (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in the rat brain. *Science* 219:316-318
- Selmaj KW, Raine CS (1988) Tumour necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. *Ann Neurol* 23:339-346
- Shaunak S, Albright RE, Klotman ME, Henry SC, Bartlett JA, Hamilton JD (1990) Amplification of HIV-1 provirus from cerebrospinal fluid and its correlation with neurological disease. *J Infect Dis* 161:1068-1072
- Simmonds P, Balfe P, Peutherer JF, Ludlam CA, Bishop JO, Leigh Brown AJ (1990) Human immunodeficiency virus-infected individuals contain provirus in small numbers of periph-

eral mononuclear cells and at low copy numbers. *J Virol* 64:864-872

- Wahl SM, Allen JB, McCartney-Francis N, Morganti-Kossmann MC, Kossmann T, Ellingsworth L, Mai UE, Mergenhagen SE, Orenstein JM (1991) Macrophage- and astrocyte-derived transforming growth factor- β as a mediator of central nervous system dysfunction in acquired immunodeficiency syndrome. *J Exp Med* 173:981-991

Suppression of experimental autoimmune encephalomyelitis by a new specific leukotriene biosynthesis inhibitor

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Sirs: Leukotrienes (LT), 5-lipoxygenase products of arachidonic acid, are important mediators of inflammation, and may also be involved in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). LTB₄ acts chemotactically while LTC₄ increases vascular permeability [5]. In both MS and EAE there is an increase in the permeability of the post-capillary venules in the brain [1, 2]. An inhibitory effect on EAE is already provided by the dual cyclo-oxygenase and 5-lipoxygenase inhibitors BW 755C [3] and sulphasalazine [4].

In the present study the effect of a new specific leukotriene biosynthesis inhibitor, the compound L-663, 536 (3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2 dimethylpropanoic acid) on EAE has been investigated. Two experiments were performed on 76 young guinea pigs using low and high doses of the encephalitogenic mixture (EM). The low dose consisted of 0.2 ml 10% bovine brain emulsion mixed 1:1 with a complete Freund's adjuvant. The high-dose regimen comprised 0.3 ml 50% bovine brain emulsion. In addition, 1 mg heat-killed *Mycobacterium tuberculosis* (H₃₇-Rv) was added to the adjuvant. The mixture was injected into the dorsum of both hind pads and into the backs of the animals, which were divided into low-dose and high-dose experimental groups of 36 and 40 animals respectively.

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Half of the animals from each experimental group were treated with 5 mg/kg of the compound L-663, 536. The treatment in the first and second experimental groups began on the 3rd and 1st day respectively, following sensitization with EM. Daily intraperitoneal application was performed until day 21. The controls (18 and 20 animals from each group) were inoculated in the same way and treated intraperitoneally with the solvent alone. The animals were killed on day 22. Histological and immunohistological examinations were carried out on all the animals and the release of LTB₄ and LTC₄ by polymorphonuclear cells was measured. In the low dose experiment 5 treated animals and one control showed no signs of EAE, and complete suppression of histological lesions was observed in 5 out of 18 animals. In the high-dose experiment the sum of the lesions in the treated animals was significantly lower than in the control group. Twenty-five percent of the treated animals failed to develop clinical signs, whereas all the control animals exhibited the typical picture of EAE. Correspondingly, the release of LT was significantly reduced in the treated groups in both experiments ($P = 0.05$). In conclusion, LT inhibitors

appear to modulate or even block the inflammatory response. LT biosynthesis inhibitors such as L-663, 536 may contribute to the future treatment of EAE.

References

1. Adams CWM (1983) The general pathology of MS, permeability and relation of lesions to ventricles and veins. In: Hallpike JF, Adams CWM, Tourtellote WW (eds) Multiple sclerosis. Chapman and Hall, London, pp 223–225
2. Black KL, Hoff JJ (1985) Leukotrienes increase blood brain barrier permeability following intraparenchymal injections in rat. *Ann Neurol* 18:349–351
3. Prosiegel M, Neu I, Wildfeuer A, Ruhstroth-Bauer G (1989) Suppression of experimental autoimmune encephalomyelitis by dual cyclo-oxygenase and 5-lipoxygenase inhibition. *Acta Neurol Scand* 79:223–226
4. Prosiegel M, Neu I, Ruhstroth-Bauer G (1989) Suppressing effect of sulfasalazine on experimental autoimmune encephalomyelitis. *N Engl J Med* 321:545–546
5. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN (1987) Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 237:1171–1176

J Neurol (1992) 239:472

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The Role of Insulin-like Growth Factors in the Nervous System, Arlington, USA. *For further information, please contact:* Conference Department, New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA; tel.: 001 (212) 838-0230; FAX: 001 (212) 888-2894.

18-20 February 1993

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5-6 March 1993

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11-14 March 1993

4th International Bethel-Cleveland Clinic Epilepsy Symposium: Seizures and Syndromes, Bielefeld, FRG. *For further information, please contact:* Professor Dr. Peter Wolf, Epilepsie-Zentrum Bethel, Klinik Mara I, Maraweg 21, W-4800 Bielefeld 13, FRG.

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9th International Symposium on Brain Edema, Tokyo, Japan. *For further information, please contact:* Dr. Umeo Ito, Department of

Neurosurgery, Musashino Red-Cross Hospital, 1-26-1, Kyonanchō, Musashino-shi, Tokyo, 180 Japan; tel.: (81) 422-32-3111; FAX: (81) 422-32-3525.

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XVth World Congress of Neurology, Vancouver, Canada. *For further information, please contact:* Information Secretariat, 645-375 Water Street, Vancouver, B.C., V6B 5C6 Canada; tel.: (001) 604681-5226; FAX: (001) 604681-2503.

9 September 1993

XVth International Symposium of the Fulton Society on the Nerve Growth Factor, Vancouver, Canada. *For further information, please contact:* Professor Dr. Victor Soriano, Calle Buenos Aires 363, 11000 Montevideo, Uruguay; FAX: (5982) 956107 or (5982) 488148.

25 September-1 October 1994

XV Symposium Neuroradiologicum, Kumamoto, Japan. *For further information, please contact:* Professor Mutsumasa Takahashi, Department of Radiology, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto, 860 Japan; tel.: (096) 344-2111; FAX (096) 362-4330.

**9.15 E. SINCLAIR*, A. CIARDI*, J. N. HARCOURT-WEBSTER*, S. LUCAS*,
F. SCARAVILLI. Morphological and immunohistochemical study of the
cortical changes in HIV encephalopathy.**

The encephalopathy resulting from direct infection of the brain by HIV, which correlates clinically with the AIDS dementia complex, has been reported as localized to the white matter. Damage to the cortex has been occasionally reported in severely affected patients. We have examined the brains of 12 randomly selected HIV-positive patients with routine histology and immunohistochemical methods. Increased numbers of cells, expressing GFAP and the macrophage marker HAM 56, were found in the hemispheres of all the HIV-positive cases (including asymptomatic) and the severity of the changes correlated with that of the encephalopathy in the white matter. The changes were particularly severe in the sub-pial areas and the density of the glial staining was higher than those of microglial cells. It is suggested that involvement of the grey matter in HIV infection is more common than previously suspected and that clinical evidence of a dementing process in AIDS may not be due only to lesion of the white matter.

(Institute of Neurology, London)

Human immunodeficiency virus (HIV) proviral sequences in the cortex and white matter of AIDS patients with and without HIV encephalopathy.

Elizabeth Sinclair and Francesco Scaravilli

(Institute of Neurology, The National Hospital for Neurology and Neurosurgery, Queen Square, London, UK).

The aim of this study was to detect HIV proviral sequences in the cortex and subjacent white matter of AIDS brains and to correlate these findings with clinical and pathological changes.

Methods Eight randomly selected cases from a series of AIDS brains with various neurological disorders were examined. There were three cases of HIV encephalopathy, one of them with concurrent progressive multifocal leucoencephalopathy (PML); three cases of primary B cell lymphoma; one case of cryptococcosis infection and one with non-specific reactive gliosis. The polymerase chain reaction (PCR) was used to detect HIV sequences in the frontal lobes, grey and white matter being tested separately with primer pairs from multiple regions of the HIV genome (gag, pol and env).

Histological and immunohistochemical investigations were also carried out. Results HIV proviral sequences were detected in 5 out of 8 brains in both the grey and white matter. Two of the three lymphomas and the reactive gliosis were consistently negative as were 4 control brains from HIV negative patients. The HIV p24 antigen was detected, by immunohistochemistry, in the three cases of HIV encephalopathy but a positive reaction was seen only in the white matter.

Conclusions 1) HIV infection of the brain as detected by PCR is present in more cases and is more widespread than previously suspected on the basis of morphological, immunohistochemical and in situ hybridization studies. 2) While HIV antigens are usually restricted to the white matter and deep grey nuclei, we detected HIV proviral sequences in samples of cortex. 3) Although there is no proven relationship between the HIV provirus and neurological dysfunction, its presence in the cortex as well as in the white matter, indicates that both are infected and this result may be of significance in the pathogenesis of the AIDS dementia complex.

9.30 E. SINCLAIR*, F. SCARAVILLI. HIV proviral DNA is present in the cortex and white matter of AIDS brains with and without HIV encephalitis.

The polymerase chain reaction (PCR) was applied to detect HIV in separate specimens of hemispheric grey and white matter from unselected AIDS patients using primer pairs from multiple regions of the HIV genome. The findings were correlated with morphological and immunohistochemical changes using antibodies to detect microglia (Ham 56) astrocytes (GFAP) and HIV (p24). HIV proviral DNA as detected by PCR was found in cases that showed no morphological or immunohistochemical evidence of HIV encephalitis. In addition while HIV antigens were found more frequently in the white matter than the cortex, proviral DNA was in both regions at the same frequency. HIV infection of the brain as detected by PCR is present in more cases and is more widespread than previously suspected. (Institute of Neurology, London).

DETECTION OF HIV PROVIRAL DNA IN HIV-POSITIVE NON-AIDS DRUG USERS AND ITS RELATIONSHIP TO PATHOLOGY OF THE CEREBRAL CORTEX. Elizabeth Sinclair*, Jeanne E Bell, Françoise Gray and Francesco Scaravilli*, *Department of Neuropathology, Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG.

Diffuse poliodystrophy in AIDS may be related to clinical signs of dementia that occur in AIDS patients, however as yet the pathogenesis of these changes remains obscure. We have previously correlated the occurrence of diffuse poliodystrophy with the detection of HIV proviral DNA in the cortex of fully blown AIDS cases and found HIV DNA to be present in the cortex of the majority of cases with these cortical changes. However it is not yet known at which stage in the development of AIDS HIV enters the brain. We therefore investigated the brains of HIV positive drug users who died suddenly without developing clinical signs of AIDS. Fresh frozen postmortem frontal lobe samples from these cases were investigated for HIV proviral DNA. Samples of cortex and white matter were separated prior to the extraction of DNA and regions of the HIV *gag* and *pol* genes were amplified using the polymerase chain reaction and non-isotopic Southern blotting. Paraffin sections from frontal lobe tissues were investigated by immunohistochemistry and lectin histochemistry with markers to detect: HIV antigen (Dupont NEA p24) and to assess astrocytic (Dako GFAP) and microglial cell (Vector RCA-120) reaction in the cortex. Proviral DNA was detected in cortical and white matter samples of a small number of HIV positive drug users while p24 antigen was never present. These results confirm that HIV can infect the brain early in the course of disease. PCR results were correlated with the presence of cortical pathology and compared with fully blown AIDS cases.