SOME ASPECTS OF HTLV-I INFECTION IN NATAL, SOUTH AFRICA

BY

AHMED IQBAL BHIGJEE

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF MEDICINE

in the

Sub-Department of Neurology

University of Natal

Durban

1992

ABSTRACT

In recent years an association between Tropical Spastic Paraparesis (TSP) and HTLV-I has been demonstrated. Based on this observation a clinical, laboratory and epidemiological study of this virus and TSP was undertaken in Natal. Antibody positive patients were investigated according to a myelopathy protocol. The associated disorders of peripheral neuropathy and polymyositis, and co-infection with the human immunodeficiency virus (HIV 1/2) were also investigated. Spinal cord pathology was evaluated in one patient. The epidemiological arm of this study was a survey of HTLV-I seroprevalence and the risk factors for exposure in the Ngwelezane district, north of Durban.

Ninety Black patients with HTLV-I associated myelopathy (HAM /TSP) were identified, 31 men and 59 women. Their mean age was 43.5 ($\pm$ 12.5)years. The duration of symptoms prior to presentation was 6 months or less in 62% of the patients. Apart from symptoms of weakness (96%) and stiffness (100%), bladder disturbance (81%) and backache (67%) were common. A large proportion (55%) were wheelchair bound and sensory deficits were noted in 64% of the patients. Ten patients were treated with corticosteroids without benefit.

Common laboratory abnormalities included anaemia (59%), raised ESR (71%), polyclonal gammopathy (80%) and CSF pleocytosis (66%). The peripheral blood lymphocytes of 10 patients were subjected to culture. HTLV-I was isolated from 6 samples. HLA typing in 56 patients did not show any definitive trends.

Muscle biopsies from three patients with disproportionate proximal limb weakness showed an inflammatory myopathy. Sural nerve biopsies of another six patients showed features of axonal degeneration and regeneration, demyelination and remyelination, and globule formation. No viral particles, protein or proviral DNA was detected in the biopsied muscles and nerves.

The spinal cord from a transfusion related HAM/TSP case demonstrated perivascular infiltrates, myelin pallor and axonal degeneration. No viral particle or protein was detected but the HTLV-I "gag" gene was amplified from the spinal cord.

Co-infection with HIV-I was noted in six patients in whom myelopathy was the sole neurological manifestation.

HTLV-I seroprevalence amongst healthy individuals in the Ngwelezane district was 2.6%. Seropositivity increased with age. No significant association was demonstrated between infection and the risk factors of gender, history of blood transfusion, scarifications and number of sexual partners. This thesis confirms that HTLV-I is endemic in Natal and is an important cause of neurological disability. Future research will be directed towards (1) a detailed study of the pathogenesis of HAM/TSP (2) evaluating antibody negative cases of TSP and (3) determining intra-familial spread of the virus.

PART OF THIS STUDY HAS BEEN DONE IN CONSULTATION WITH THE INSTITUTE FOR BIOSTATISTICS OF THE MEDICAL RESEARCH COUNCIL

PREFACE

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Neurology, under the supervision of Professor P L A Bill.

A I BHIGJEE

September 1992

While this study was in progress, the following thesis related papers were submitted for publication:

1. Bhigjee AI, Harvey MM, Windsor I, Bill PLA. Blood transfusion and HTLV-I associated myelopathy. *S Afr Med J* 1989; 76(12): 700

2. Bhigjee AI, Kelbe C, Haribhai HC et al. Myelopathy associated with human T cell lymphotropic virus type I (HTLV-I) in Natal, South Africa. *Brain* 1990; 113: 1307 - 1320.

3. Bhigjee AI. There are other human retroviruses too - a note on myelopathy associated with HTLV-I. *S Afr Med J* 1991; 79: 524 - 525.

4. Bhigjee AI, Wiley CA, Wachsmann W, Amenomori T, Pirie D, Bill PLA, Windsor IM. HTLV-I associated myelopathy: Clinico-pathologic correlation with localization of provirus to spinal cord. *Neurology* 1991; 41: 1990 - 1992.

5. Bhigjee AI, Bill PLA, Hammond MG, Windsor IM. HLA profile and HTLV-I associated myelopathy (HAM/TSP) in Natal, South Africa. *J Neurol Neurosurg Psychiatr* 1992; 55: 329 - 330.

6. Bhigjee AI, Bill PLA, Wiley CA et al. Peripheral nerve lesions in HTLV-I associated myelopathy (HAM/TSP). *Muscle & Nerve* (in press).

7. Bhigjee AI, Bill PLA, Tait D. Dual infection with HTLV-I and HIV-1: A clinico-laboratory study of six cases. *S Afr Med J* (submitted).

8. Bhigjee AI, Vinsen C, Windsor IM, Tait D, Bill PLA, Gouws E. Prevalence and transmission of HTLV-I infection in Natal/Kwa Zulu, South Africa. *S Afr Med J* (submitted).

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to the following individuals:

1) The many patients who participated in this study.

2) Professor P L A Bill, supervisor and head of the Neurology Department, for his support and encouragement.

3) The many registrars who helped in the management of the patients.

4) Dr Dwarkapersad, Superintendent of Wentworth Hospital for use of facilities and his cooperation.

5) Dr Haselau, Superintendent of Ngwelezane Hospital for use of facilities during the field study.

6) Ms J Nkomakhazi of RIDTE for assistance with the field study.

7) Dr R Cooppan, formerly of RIDTE for providing paediatric serum samples.

8) Dr S Khan and Mrs C Steele for proof reading the manuscript.

9) Miss P Enstrom for secretarial assistance.

10) Mr P Govender from the Computer Services, University of Natal for his assistance.

11) My parents Mohammed and Hazera who, barely educated themselves, saw the importance of educating their children.

12) My wife Rokaya, and children Shoaib, Mohammed and Suraiya for their patience.

The role of the following collaborators is gratefully acknowledged:

1. Drs I Windsor and D Tait of the Department of Virology, University of Natal, for the serological testing.

2. Dr M Hammond of the Institute of Immunology for the HLA study.

3. Drs Wiley, Wachsman and Amenomori from the San Diego School of Medicine, University of California, for assistance with the in situ hybridization, immunocytochemistry and PCR studies on skeletal muscle, peripheral nerve and spinal cord.

4. Professor W Becker, Department of Medical Virology, University of Stellenbosch, for viral cultures.

This thesis was supported in part by the MRC of South Africa and the K.M. Browse Research Scholarship.

Last but not least thanks are due to the Almighty for His Guidance.

CONTENTS

ABSTRACT i

PREFACE iv

ACKNOWLEDGEMENTS vii

CONTENTS x

LIST OF TABLES xviii

LIST OF FIGURES xx

LIST OF PLATES xxi

ABBREVIATIONS xxii

CHAPTER 1 **1 - 5**

Introduction 2

1.1 Historical Background 2

1.2 HTLV-I Infection and Disease 3

CHAPTER 2 **6 - 16**

HAM/TSP: Clinical Aspects 7

2.1 Demographic Features 7

2.2 Symptoms 7

2.3 Signs 8

2.4 Discussion 8

2.4.1. Area of Residence 8

2.4.2. Race 8

2.4.3. Age 9

2.4.4. Sex 9

2.4.5. Familial Cases 10

2.4.6. Disease Duration, Severity & Pattern 10

2.4.7. Therapy 11

CHAPTER 3 **17 - 31**

HAM/TSP: Laboratory Studies 18

3.1 Methods 18

3.1.1. Routine Blood Investigations 18

3.1.2. Cerebrospinal Fluid Tests 18

3.1.3. Virological Studies 19

3.1.4. Radiological Studies 20

3.1.5. Electrodiagnostic Studies 20

3.2 Results 21

3.2.1. Routine Blood Tests 21

3.2.2. Cerebrospinal Fluid Tests 21

3.2.3. Virological Studies 22

3.2.4. Radiological Studies 23

3.2.5. Electrodiagnostic Studies 23

3.3 Discussion 24

3.3.1. Routine Laboratory Studies 24

3.3.2. Cerebrospinal Fluid Tests 25

3.3.3. Virological Studies 26

3.3.4. Radiological Studies 26

3.3.5. Electrodiagnostic Studies 27

3.4 Conclusion 27

CHAPTER 4 **32 - 41**

HAM/TSP: HLA Studies 33

4.1 Introduction 33

4.2 Subjects & Methods 33

4.3 Results 34

4.4 Discussion 35

CHAPTER 5 **42 - 56**

HAM/TSP: Spinal Cord Pathology 43

5.1 Introduction 43

5.2 Case History 43

5.3 Materials and Methods 44

5.3.1. Light Microscopy Studies 45

5.3.2. Electron Microscopy Studies 45

5.3.3. Lymphocyte Marker Studies 45

5.3.4. Viral Protein Detection Studies 45

5.3.5. Polymerase Chain Reaction Studies 45

5.3.6. In-situ Hybridization Studies 46

5.4 Results 46

5.4.1. Light Microscopy Studies 46

5.4.2. Lymphocyte Marker Studies 47

5.4.3. Electron Microscopy Studies 47

5.4.4. Viral Protein Detection Studies 48

5.4.5. Polymerase Chain Reaction Studies 48

5.4.6. In-situ Hybridization Studies 48

5.5 Discussion 49

5.6 Conclusion 52

CHAPTER 6 **57 - 67**

HTLV-I and Peripheral Neuropathy: A Clinicopathological Study 58

6.1 Introduction 59

6.2 Methods 59

6.2.1. Light Microscopy Studies 59

6.2.2. Electron Microscopy Studies 59

6.2.3. Viral Protein Detection Studies 59

6.2.4. In-situ Hybridization Tests to detect Proviral Nucleic Acids 59

6.2.5. Polymerase Chain Reaction Assays 59

6.2.6. Morphometric Analysis 59

6.3 Results 60

6.3.1. Light Microscopy Studies 60

6.3.2. Immunofluorescent Studies 60

6.3.3. Electron Microscopy Studies 60

6.3.4. Teased Fibre Preparations 61

6.3.5. Morphometric Studies 61

6.3.6. Viral Protein Detection Studies 61

6.3.7. In-situ Hybridization Tests 61

6.3.8. Polymerase Chain Reaction Assay 61

6.4 Discussion 62

CHAPTER 7 **68 - 75**

HTLV-I and Inflammatory Myopathy: A Clinico-pathological Study 69

7.1. Introduction 69

7.2 Materials & Methods 69

7.3 Case Histories 70

7.3.1. Case 1 70

7.3.2. Case 2 70

7.3.3. Case 3 71

7.4 Results 71

7.5 Discussion 72

7.6 Conclusion 73

CHAPTER 8 **76 - 88**

Dual HTLV-I and HIV Infection: A Clinicolaboratory Study of Six Patients . 77

8.1 Introduction 77

8.2 Methods 77

8.3 Case Histories 78

8.3.1. Case 1 78

8.3.2. Case 2 79

8.3.3. Case 3 80

8.3.4. Case 4 80

8.3.5. Case 5 81

8.3.6. Case 6 82

8.4 Discussion 82

8.5 Conclusion 85

CHAPTER 9 **89 - 99**

HTLV-I : Sero-epidemiological Study in the Natal/Kwa Zulu Region 90

9.1 Introduction 90

9.2 Methods 91

9.3 Results 92

9.4 Discussion 93

9.5 Conclusion 94

CHAPTER 10 **100 - 103**

Conclusion and Future HTLV-I Protocol 101

10.1 Pathogenesis of HAM/TSP 101

10.2 Clinical Aspects of HAM/TSP 101

10.2.1. Diagnosis 101

10.2.2. Management 102

10.3 Epidemiology 102

10.4 HTLV-I and Other Neurological Diseases 103

10.5 Future HTLV-I Research in South Africa 103

BIBLIOGRAPHY **104 - 126**

APPENDIX A

Myelopathy Protocol 127

APPENDIX B

MRC Power Grading System 129

APPENDIX C

Methodology for the Detection of Oligoclonal Bands in the CSF ... 130

APPENDIX D

Results of Routine CSF Studies 132

APPENDIX E

Results of IgG Indices and CSF IgG Oligoclonal Bands 136

APPENDIX F

Results of Serum and CSF Beta-2- Microglobulin Levels 139

APPENDIX G

Results of Sural Nerve Conduction Studies 140

APPENDIX H

Results of Common Peroneal Nerve Conduction Studies 143

APPENDIX I

Results of Visual Evoked Response Studies 146

APPENDIX J

Results of Brainstem Auditory Evoked Response Studies 148

APPENDIX K

Methodology for PCR from paraffin embedded tissue sections 156

APPENDIX L

HTLV-I Seroepidemiology Questionnaire 158

LIST OF TABLES

2.1 Areas of patient residence 12

2.2 Summary of symptoms at time of presentation 15

2.3 Summary of neurological findings 16

4.1 Frequencies of HLA-A locus antigens in patients and controls 37

4.2 Frequencies of HLA-B locus antigens in patients and controls 38

4.3 Frequencies of HLA-C locus antigens in patients and controls 39

4.4 Frequencies of HLA-DR locus antigens in patients and controls 40

4.5 Frequencies of HLA-DQ locus antigens in patients and controls 41

6.1 Results of nerve fibre teasing 64

6.2 Myelinated fibre density studies 65

7.1 Laboratory data of myopathy patients 75

8.1 Results of relevant blood tests in combined HTLV-I/HIV-1 infection 86

8.2 Results of CSF tests in combined HTLV-I/HIV-1 infection 87

8.3 Lymphocyte marker studies in combined HTLV-I/HIV-1 infection 88

9.1 Selected worldwide HTLV-I seroprevalence rates 95

9.2 Selected HTLV-I seroprevalence rates in Africa 96

9.3 Selected HTLV-I seroprevalence rates in South Africa 97

9.4 Age adjusted HTLV-I seroprevalence in different age groups 98

LIST OF FIGURES

2.1 Map of Natal and Transkei illustrating areas of residence of HAM/TSP cases 13

2.2 Age distribution of HAM/TSP cases 14

3.1 Example of positive IgG oligoclonal bands in the CSF 28

3.2 Example of positive Western Blot results in the serum 29

3.3 Example of positive Western Blot results in CSF 30

3.4 CT myelogram demonstrating spinal arachnoiditis 31

3.5 CT myelogram demonstrating thoracic cord atrophy 31

5.1 Results of PCR assays demonstrating HTLV-I proviral DNA 55

9.1 Age related rise in HTLV-I seropositivity 99

LIST OF PLATES

5.1 Section of thoracic cord showing myelin pallor of the lateral columns 53

5.2 Section of thoracic cord showing perivascular inflammatory infiltrate 53

5.3 Section of thoracic cord showing reactive changes 54

6.1 Sections of sural nerves showing varying degrees of degeneration, fibre loss and remyelination 66

7.1 Sections of muscle from patients with associated inflammatory myopathy 74

ABBREVIATIONS

ANF	Antinuclear factor
AOP	Adult-onset polymyositis
ATLL	Adult T-cell leukaemia/lymphoma
β2M	Beta-2-microglobulin
BAER	Brainstem auditory evoked response
CSF	Cerebrospinal fluid
CT	Computed tomography
DNA	Deoxyribose nucleic acid
EBV	Ebstein-Barr virus
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
FTA	Fluorescent treponemal antibody
GGT	Gamma glutamyl transpeptidase
HAM	HTLV-I associated myelopathy
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HTLV-I	Human T-cell lymphotropic virus Type I
HTLV-II	Human T-cell lymphotropic virus Type II
Ig	Immunoglobulin
LTR	Long terminal repeats
MHC	Major histocompatibility complex
PBL	Peripheral blood lymphocyte

PCR	Polymerase chain reaction
PND	Peripheral nerve dysfunction
RPR	Rapid plasma reagin
TAN	Tropical ataxic neuropathy
TPHI	Treponema pallidum haemagglutination inhibition
TSP	Tropical spastic paraparesis
VER	Visual evoked response
WB	Western blot

CHAPTER 1

INTRODUCTION

1.1 HISTORICAL BACKGROUND

Spastic paraparesis without evidence of spinal cord compression has been described from a number of tropical and sub-tropical areas including Jamaica (Cruickshank, 1956; Montgomery et al. 1964), South India (Mani et al. 1969), Seychelles (Kelly & De Mol 1982) and Colombia (Roman et al. 1985). It is an endemic disorder and was first referred to as Tropical Spastic Paraparesis (TSP) by Mani et al (1969). TSP must be distinguished from epidemics of spinal disorders such as the cassava-related myelopathy in Mozambique (Casadei et al. 1984) and lathyrism in India (Prasad & Sharan 1979) as well as tropical ataxic neuropathy (TAN) (Montgomery et al. 1964; Osuntokun 1968). The clinical features of TSP include a subacute to chronic progressive paraparesis, frequent sphincter disturbance and insignificant sensory disturbance. Some studies showed equal frequency of disease in males and females whereas others observed a male preponderance. Most studies indicated that the disease tended to stabilise and the disability to be seldom severe. TSP represents a major cause of neurological disability in the indigenous people of the countries mentioned earlier. For example, Kelly & De Mol (1982) found a prevalence ratio of 127 per 100,000 of the population in the Seychelles.

The Natal/Kwa Zulu region (henceforth referred to as simply Natal) constitutes the smallest province of the Republic of South Africa and lies between the latitudes 27-31°S and longitudes 29 - 33°E. It is bordered on the east by the Indian Ocean while the Drakensberg mountains form the western boundary. TSP was first reported from this region by Cosnett (1965) who described 41 cases comprising mainly young adult males. In a later series of 330 black paraplegic patients in Natal, Wallace & Cosnett (1983) noted that TSP was the most common diagnostic category after trauma and vertebral tuberculosis, again emphasising its importance as a cause of neurological disability amongst the indigenous people of the tropics and sub-tropics.

When the Neurology Unit was established in 1985 at Wentworth Hospital in Durban as a referral centre for Natal, a myelopathy protocol (Appendix A) was drawn up to study TSP in greater detail. After a clinical assessment extensive investigations were undertaken. The battery of tests were reviewed at regular intervals. Where a test yielded no useful information it was deleted from the protocol except in special circumstances. Despite the in-depth study it was noted that up to one third of the myelopathy cases still remained undiagnosed (unpublished data).

1.2. HTLV-I INFECTION AND DISEASE

The aetiology of TSP remained elusive. Treponemal infections, toxins and nutritional aberrations were postulated but were never proven.

The human T-cell lymphotropic virus type I (HTLV-I), the first human retrovirus to be identified, was isolated from a patient with cutaneous T-cell lymphoma (Poeisz et al. 1980). HTLV-I was then linked to other cases of adult T-cell leukaemia/lymphoma (ATLL) (Yoshida et al. 1982; Hinuma et al. 1982). In 1985 serendipity led Gessain et al (1985) to demonstrate antibodies to HTLV-I in two patients with TSP. This association between TSP and HTLV-I was rapidly confirmed by others. In the Seychelles, Roman et al (1987) found antibodies in 17 (85%) of 20 serum samples from TSP patients, whilst Rodgers-Johnson et al (1988) detected HTLV-I antibodies in 82% of 47 Jamaican TSP patients. The Colombian experience was similar with 52 of 55 (94%) patients testing positive (Zaninovic et al. 1988).

Osame et al (1987) described similar cases from Japan and called it HTLV-I associated myelopathy (HAM). Since HAM and TSP are the same condition, the disorder is now commonly referred to as HAM/TSP.

Following the initial studies, testing for antibodies to HTLV-I was included in the Wentworth Hospital myelopathy protocol. This thesis represents the clinical and sero-epidemiological experience with HTLV-I infection and disease in Natal. A total of 90 HAM/TSP were collected up to December 1991. Chapters 2 to 4 describe the clinical, laboratory, virological, neurophysiological and HLA findings in these patients. Chapter 5 describes in detail the spinal cord pathology of another patient. Chapters 6 and 7 discuss other HTLV-I associated disorders viz peripheral neuropathy and inflammatory myopathy. The interesting occurrence of co-infection

with HTLV-I and the human immunodeficiency virus (HIV) forms the basis of chapter 8. Chapter 9 discusses the sero-epidemiological study of over 1000 apparently healthy blacks. The thesis ends with a look into the future.

ATLL has been seen in Natal (Jogessar et al. 1992), but is rare in comparison to HAM/TSP and is beyond the scope of this thesis.

CHAPTER 2

HAM/TSP: CLINICAL ASPECTS

2.1. DEMOGRAPHIC FEATURES

From August 1988 to December 1991 a total of 90 patients were seen at Wentworth Hospital. They were referred from all parts of Natal and included 6 patients from the Transkei which is an adjacent independent Black homeland (Fig.2.1; Table 2.1). All patients were Black.

There were 31 men and 59 women. The mean age at the onset of symptoms was 43.5 ($\pm$ 12.5) years. The youngest was 18 years and the oldest 70 years (Fig. 2.2). The duration of symptoms prior to referral to Wentworth Hospital ranged from 1 to 144 months with a mean of 14 months. In 56 (62%) patients the duration of symptoms was 6 months or less. A further 9 (10%) had an illness duration between 7 - 12 months. Of the 47 patients questioned, 10 (21%) had received blood transfusion in the past. Thirty four patients were asked about the number of sexual partners engaged. One denied sexual activity, 19 had a single partner, 8 between 2 - 5 partners and 6 more than 5 partners. No patient had a family history of a similar problem.

2.2. SYMPTOMS

The main neurological symptoms are summarised in Table 2.2. Apart from complaints of weakness and stiffness, bladder disturbance (81%) and backache (67%) were common. No patient complained of headache, visual, auditory or bulbar dysfunction.

2.3. SIGNS

The main neurological signs are summarised in Table 2.3. Weakness and spasticity were present in all (MRC grading used for power testing - Appendix B). Sensory abnormalities were common (64%) and a large proportion were wheelchair bound.

2.4 DISCUSSION

2.4.1. Area of Residence

The patients came from all parts of Natal but especially from the coastal and northern areas. The relatively fewer cases from the South Coast and inland areas may be related to poorer health facilities and non referral (personal observation).

2.4.2. Race

All patients were Black (84 Zulus and 6 Xhosas). Apart from a specific situation of transfusion related HAM/TSP in a White patient (discussed separately - chapter 5), no case has been seen in Whites and Asians. The occurrence of HAM/TSP almost exclusively in people of Black African ancestry is a world wide observation (Roman 1988). Gallo et al (1983) postulated that the chronic viral infection originated in Africa and spread to other endemic areas via the slave trade dating back to the sixteenth century. This suggestion does not explain the high endemicity of HTLV-I amongst the South-western Islands of Kyushu and Shikoku in Japan.

More attractive is the hypothesis that the virus existed in man since prehistoric times but died out in all except a few groups of people (Ishida and Hinuma 1986).

2.4.3. **Age**

The mean age at onset of symptoms was 43.5 ($\pm$ 12.5) years. The youngest patient was 18 years and the oldest 70 years. Symptoms began after the age of 30 in 83% of the patients. These findings are similar to those reported from other parts of the world (Montgomery et al. 1964; Vernant et al. 1987; Roman et al. 1985; Roman et al. 1987). While the findings on age distribution confirm that HAM/TSP is a disease of adults, it provides no information concerning the incubation period. The data obtained regarding blood transfusion, age at first sexual contact and number of sexual partners are inadequate and no reliable conclusions can be made.

2.4.4. **Sex**

While both men and women can be infected, earlier studies showed a preponderance of men. For example 53% of the Jamaican cases (Montgomery et al. 1964) were men and, in Natal Cosnett (1965) found that 66% of his patients were male. More recent studies, however, show a reverse trend with a predominance of women (Vernant et al. 1987; Roman et al. 1987). The present study found a female dominance of 2:1. Possible explanations for the present gender pattern could include case ascertainment bias e.g. women may seek medical attention more often than men or may

have more severe disease (Roman 1988). However, it could be argued that in Natal the observed male preponderance by Cosnett (1965) could have reflected easier access to hospitals for males because of the previous practice of migrant labour. It is also noteworthy that the virus is more effectively transmitted from male to female than vice versa (Kajiyama et al. 1986).

2.4.5. **Familial Cases**

The 90 HAM/TSP cases were unrelated and none had a history of a similar illness amongst family members. It was not possible to personally interview family members as many patients were from far flung areas and had lost contact with spouses, partners, parents and siblings. Thus an in depth family study was beyond the scope of this thesis.

2.4.6. **Disease Duration, Severity and Pattern**

Most of the patients had a short duration of illness (72% less than one year) and approximately half the patients were wheelchair bound or bedridden at the time of presentation. These findings differ from other studies which showed that most patients remain ambulant with or without physical aids (Rodgers-Johnson et al. 1988; Zaninovic et al. 1988). The course of the disease is therefore more rapid and aggressive in this study population. The reason for this is unclear but may be due to a high viral load (chapter 3).

Unlike other studies, 64% of the patients had some form of sensory disturbance on examination. A pinprick level could be obtained in 52% of the cases and patchy or peripheral sensory impairment was noted in a further 12%. This may again reflect a more aggressive disease in our patients.

2.4.7. **Therapy**

Japanese studies have demonstrated that the myelopathy responds to steroids, other immunosuppressive agents and plasmapheresis (Osame et al. 1987; Matsuo et al. 1988). The occasional case report from the West has also suggested steroid responsiveness of the myelopathy (McArthur et al. 1990).

In an open study, 10 patients of the present series were given prednisone (1mg/kg body weight) for periods varying from 3 to 9 months. Two were ambulant with aid and the rest wheelchair bound. No improvement was noted in any of the patients. It is probable that this group had too far advanced and irreversible disease to show any response to steroids.

TABLE 2.1: AREAS OF RESIDENCE OF PATIENTS

Area	Number
Bizana	2
Durban & surrounding townships	10
Edendale	1
Empangeni	10
Eshowe	3
Flagstaff	2
Gingindlovu	2
Hammarsdale	1
Hluhluwe	1
Ingwavuma	3
Inyoni	1
Jozini	1
Kranskop	2
Lusikisiki	1
Mahlabatini	1
Manguzi	1
Mandini	3
Maphumulo	3
Mbonambi	2
Mlalati	1
Mondlo	2
Mpolweni	1
Mseleni	2
Mthuzwini	2
Mtubatuba	4
Ndwedwe	4
Ngwanase	2
Nkandla	1
Nongoma	3
Nqutu	1
Ntumeni	1
Pleisslaer	1
Polamont	1
Pongola	3
Scottburgh	3
Stanger	1
Sweetwater	1
Thabenkhulu	1
Tongaat	2
Umbombo	2
Ulundi	1
Umtata	1
Verulam	1

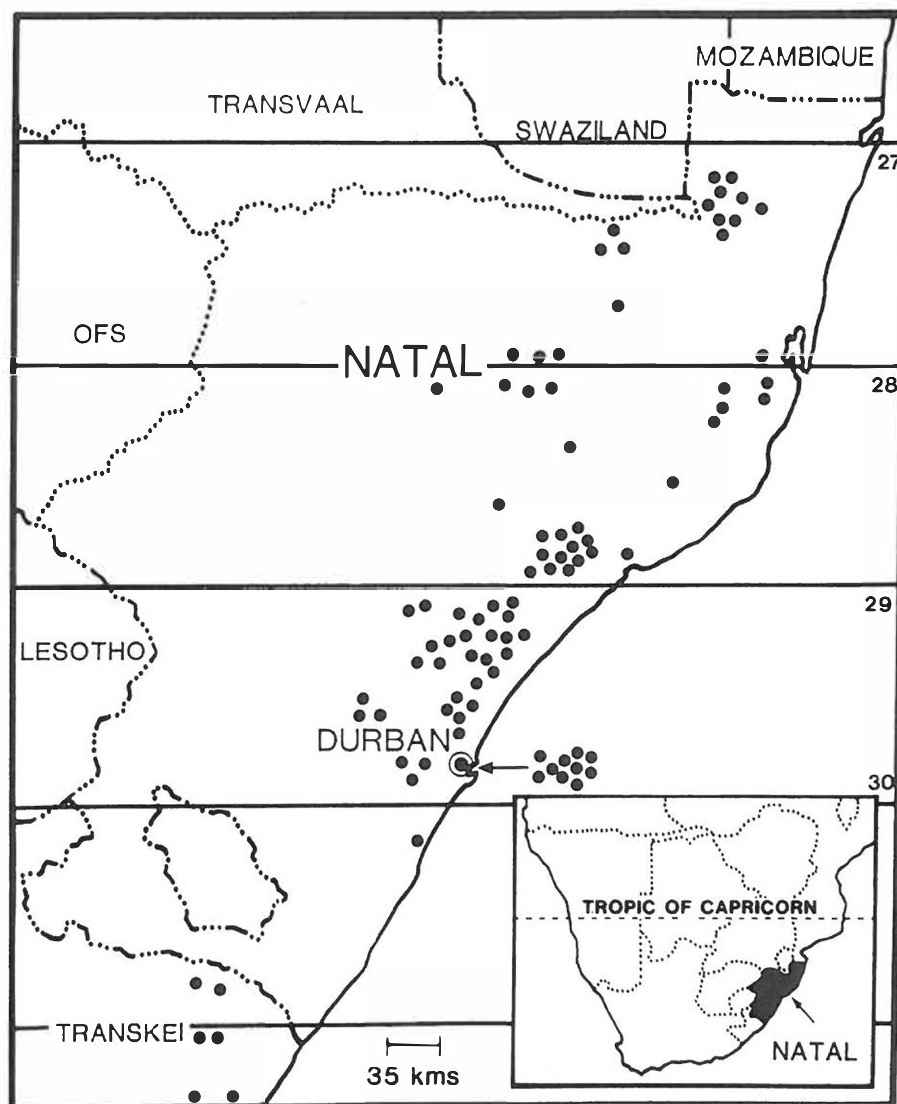


Fig 1.2. Map of Natal and Transkei showing areas of residence of HAM/TSP cases. Note patients come from as far north as Ingwavuma and as far south as the Transkei.

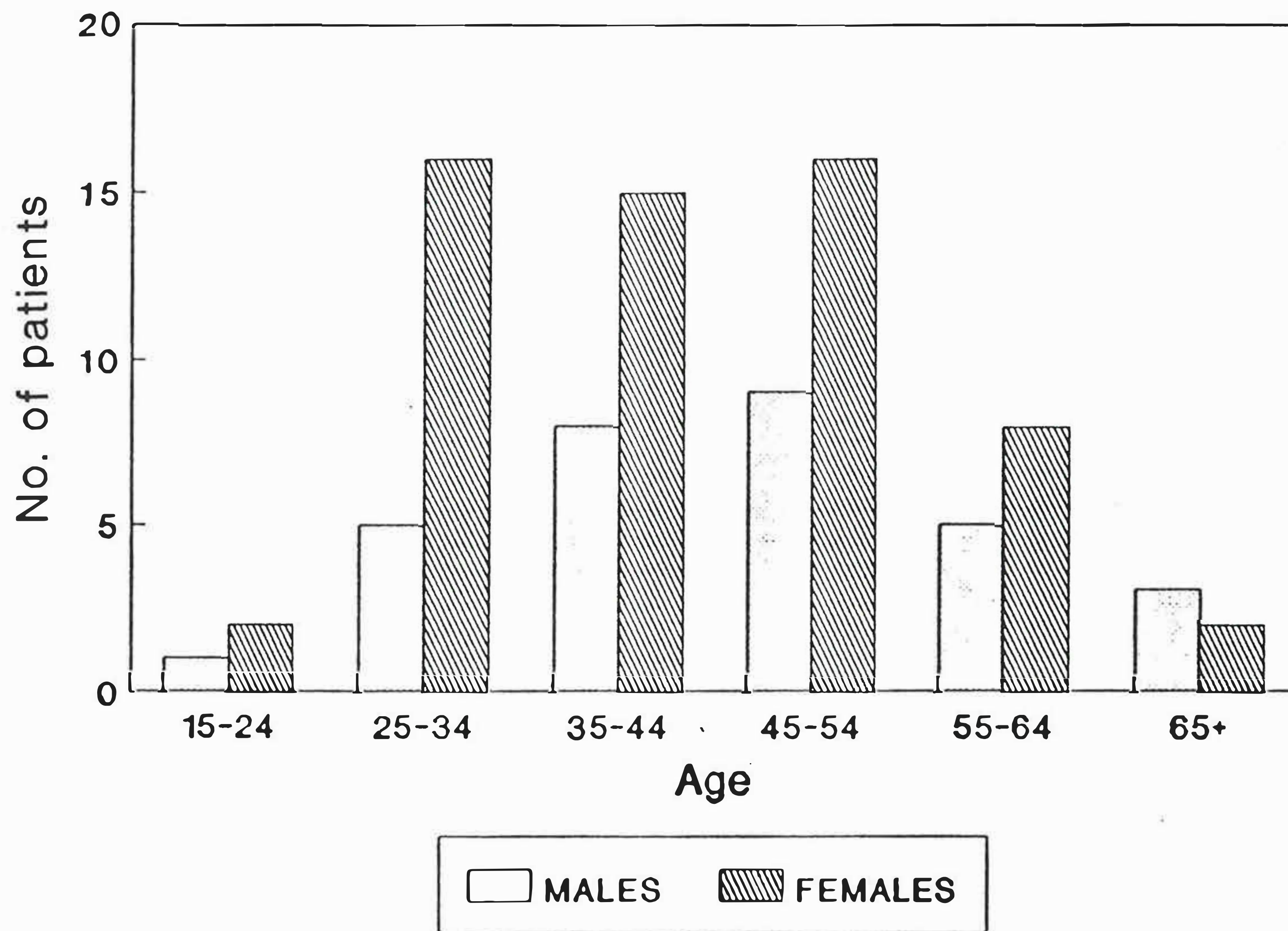


Figure 2.2. Distribution of patients according to age at onset of symptoms.

TABLE 2.2: SUMMARY OF SYMPTOMS AT THE TIME PRESENTATION

Symptom	Number	Percentage
Stiffness of legs	90	100
Weakness of legs	86	96
Urinary bladder dysfunction	73	81
Thoracic or lumbar backache	60	67
Numbness	59	66
Paraesthesia	57	63
Bowel dysfunction	41	47

TABLE 2.3: SUMMARY OF NEUROLOGICAL FINDINGS

Neurological Finding	Number	Percentage
Lower Limbs		
Spasticity	90	100
Weakness	90	100
Brisk knee tendon reflexes	80	89
Brisk ankle tendon reflexes	65	73
Upper Limbs		
Weakness	31	34
Increased tendon reflexes	70	78
Abdominal Reflexes (recorded in 80 cases)		
Absent	76	95
Plantar Response (recorded in 87 cases)		
Extensor	70	80
Equivocal	10	12
Flexor	7	8
Degree of Motor Disability		
Ambulatory without assistance	19	21
Ambulatory with walker or stick	22	24
Wheelchair bound/bedridden	49	55
Sensory Disturbance, Lower Limbs		
Impaired pinprick & touch	55	61
Impaired proprioception	27	30
Impaired vibration sense	28	31
Approximate Sensory Level (recorded in 89 cases)		
None	32	36
Lumbosacral	11	12
Lower Thoracic (T9-T12)	18	20
Mid Thoracic (T5-T8)	11	12
Upper Thoracic (T1-T4)	6	8
Peripheral/Patchy loss	11	12

CHAPTER 3

HAM/TSP: LABORATORY DATA

3.1. METHODS

3.1.1. Routine Blood Investigations

Each patient had the following blood tests in the routine service laboratory: full blood count (FBC), erthyrocyte sedimentation rate (ESR), plasma urea, electrolytes and glucose, liver function tests (serum albumin, globulin, and glutamyl transpeptidase (Alpha and GGT), aspartate aminotransferase (ALT), alanine aminotransferase (AST), and alkaline phosphatase), serum protein electrophoresis, serum immunoglobulins (IgG, IgA, IgM), antinuclear factor (ANF), serum rapid plasma reagin (RPR) and treponema pallidum haemagglutination inhibition (TPHI) tests for syphilis, serum folate and vitamin B12 levels. Other serological tests done were as per myelopathy protocol (Appendix A).

3.1.2. Cerebrospinal fluid (CSF) tests

CSF was sampled for cell count, protein, globulin, glucose, bacterial culture and serological tests for syphilis. Selected cases were tested for cryptococcal antigen and cysticercus antibodies. To assess immune response and de novo antibody production within the central nervous system compartment the following tests were done: (1) concentrations of CSF and serum β2-microglobulin (β2M) were assayed using a commercially available solid phase radio-immunoassay (Phadebas β2-microtests, Pharmacia,

Uppsala,Sweden) (2) calculation of the IgG index according to the method of Lefvert and Link (1985) and (3) detection of CSF IgG oligoclonal bands using the method of Kier et al (1990; Appendix C).

3.1.3. **Virological Studies**

Specimens of blood and CSF were tested for the presence of antibodies to HTLV-I using an enzyme linked immunosorbent assay. All positive specimens were subsequently confirmed by Western blot (WB) (Dupont HTLV-I ELISA and Dupont HTLV-I Western blot, Biotech Research Laboratory, Rockville, MD, USA). All blood specimens were also screened for the presence of antibodies to the human immunodeficiency virus (HIV) by ELISA and any positive result confirmed by WB (Abbot Recombinant HIV 1/2 EIA, Abbot Diagnostic Products, Weisbaden-Delkenheim, Germany, and HIV 1 Western blot IgG Assay, Diagnostic Biotechnology, Singapore).

Virus isolation was attempted from 10 of the HTLV-I seropositive patients using methods previously described (Becker et al 1988). Peripheral blood lymphocyte (PBL) cultures were established from heparinised venous blood. The criteria used to determine HTLV-I infection were (1) the establishment of a continuous long-term cell line with the morphology of lymphocytes transformed by HTLV-I infection, (2) the demonstration of characteristic morphogenesis of HTLV-I particles by electron microscopy of ultra-thin sections of these cells, and (3) specific hybridization of DNA extracted from

these cells with the reference HTLV-I DNA probe kindly provided by R. Gallo.

For comparison serum HTLV-I antibody testing was also done in a control group consisting of patients with other neurological diseases, patients admitted for plastic surgery and trauma cases admitted to orthopaedic wards.

3.1.4. **Radiological Studies**

All patients had chest and spinal radiographs and all underwent conventional myelograms. Myelo-CT and CT head scans (GE9800) were done in selected cases.

3.1.5. **Electrodiagnostic Studies**

Motor and sensory nerve conduction studies were performed using standard techniques and taking the necessary precautions to ensure consistent results. A minimum of 1 motor and 1 sensory nerve each in upper and lower limbs were evaluated. Full field pattern reversal visual evoked potentials (VEPs) and brainstem auditory evoked potentials (BAEP) were also done in some of the patients. All studies were undertaken with a DISA Neuromatic 2000 machine.

3.2. RESULTS

3.2.1. **Routine Blood Tests**

Anaemia was present in 33/59 (56%) females (haemoglobin less than 12 g/dl) and 18/29 (62%) males (haemoglobin less than 14 g/dl). The haemoglobin was not measured in a further two males. The mean ESR (measured in 87 subjects) was 50 mm/hour with a range from 1 to 157 mm/hour. Sixty two subjects (71%) had an ESR of greater than 20 mm/hour. Leucopenia (less than 4.8 x 10^9/dl) was present in 9 (10%) of 88 subjects tested.

A polyclonal gammopathy was seen in 63/79 (80%) patients. The serum IgG (normal 0,7 - 3,1 g/l) was elevated in 60/71 (85%) samples, IgA (normal 0,7 - 3,1 g/l) was elevated in 60/71 (85%) samples, and IgM (normal 0,6 - 2,7 g/l) in 38/71 (54%).

The serum RPR was positive in 20/89 (22%) samples tested. In only 2 of these was the titre > 1:8. The TPHA was positive in all except 1 of 20 RPR positive cases. Antinuclear antibodies were detected in 8/78 (10%) samples. The titres were less than 1:100 in all except 1 case.

3.2.2. **Cerebrospinal fluid tests**

The routine cell count, protein, globulin and glucose levels are summarised in Appendix D. Pleocytosis (>5 cells/μl) was present in 60/90 (66%) samples and an elevated protein (>0.4 g/l) in 34/90 (38%) samples.

Bacterial culture (including M.tuberculosis) of CSF was negative in all samples. No patient had a positive CSF fluorescent treponemal antibody test. Cryptococcal antigen was not detected in any of the samples tested.

Appendix E summarises the results of IgG indices and CSF IgG oligoclonal bands, whilst Appendix F illustrates the serum and CSF β2M levels and the CSF: Serum albumin ratios. The IgG index was calculated in 59 patients and was greater than 0,7 in 43 (73%). CSF IgG oligoclonal bands were present in 23 (85%) of 27 samples tested. Figure 3.1 illustrates some of the positive results. The serum β2M was raised in 24/25 (96%) and CSF β2M in 25/25 (100%). The CSF β2M was greater than the serum β2M in 17/25 (68%) paired samples.

3.2.3. **Virological Studies**

The ELISA and WB was positive in the sera of all 90 patients. The WB showed strong specific multiple banding pattern indicative of well established infection (Figure 3.2). The 86 CSF samples tested yielded similar results (Figure 3.3). No sera from the control group tested positive. The virus was isolated from 6 of 10 PBLs samples subjected to culture. The cultures from 5 of the samples were positive within 7 weeks and the sixth sample after 3 months.

Six patients also had antibodies to HIV in their sera and CSF. This group is discussed in detail in chapter 8.

3.2.4. **Radiological Studies**

The chest radiographs were normal in all except 9 patients. Four had changes consistent with old tuberculosis whilst the remainder showed non-specific shadowing. None of the 9 patients had chest symptoms at the time of the neurological presentation. The plain spinal radiographs were abnormal in 13 patients. All of these showed degenerative changes but none had changes thought to be clinically relevant. No myelogram showed a clinically relevant compressive lesion. Myelo-CTs were done in 77 patients. Arachnoiditis was noted in 9 (Figure 3.4) and thoracic cord atrophy in 16 (Figure 3.5). CT of the head was done in 24 patients. Minor degrees of cerebral atrophy was noted in 10, basal ganglia calcification in 1 and periventricular lucencies in 1. The remainder were normal.

3.2.5. **Electrodiagnostic Studies**

Median sensory and motor nerve conduction studies were performed in 64 patients. Median sensory latency was prolonged in 9 patients (greater than 2.99 msec; stimulus to onset), velocity decreased in 3 patients (less than 36.3 m/sec) and amplitude abnormal in 1 patient (normal : greater than 8.58 μV). The median distal motor latency was prolonged in 13 patients (greater than 4.38 msec) but the forearm velocity was normal in all. The evoked motor amplitude was decreased in 12 patients (less than 7.0 μV) and 'F' latency prolonged in 4 (greater than 32.7 msec). Sural and common peroneal nerve conduction studies are summarised in Appendices H and I. The sural studies were abnormal in 33/62 (53%) nerves studied, the main

abnormalities being either low amplitude or lack of stimulability. The distal motor latency of the common peroneal nerve studies was abnormal in 12 (23%), the velocity in 16 (30%), the amplitude in 29 (55%) and the 'F' wave latency in 4 (8%) of 53 nerves tested. A further 5 nerves were unstimulable.

Pattern reversal VERs (Appendix I) showed prolonged latencies in 3 (6%) of 69 right eyes tested and in 5 (10%) of 48 left eyes tested. The results of the BAER studies are summarised in Appendix J. One or more inter wave latency abnormalities were seen in 13 (25%) of 54 right ears tested and in 14 (27%) of 51 left ears tested.

3.3 DISCUSSION

3.3.1. Routine Laboratory Studies

The routine laboratory studies did not demonstrate specific trends. Anaemia, elevated ESR and hypergammaglobulinaemia were common, all features consistent with a chronic infective illness.

The serum RPR test for syphilis was positive in 22% of the patients but no patient had a positive CSF FTA-ABS test. These findings differ from Jamaican patients who have demonstrated positivity rates of 46% and 4% in serum and CSF respectively (Rodgers-Johnson et al. 1988). Similar high positivity rates were obtained in Colombia (Zaninovic et al. 1988). Vernant et al (1987), however, found figures comparable to ours in Martinique.

Osame et al (1987) did not comment on syphilitic serology in their Japanese patients. The high frequency of treponemal antibodies in some of the studies remain unexplained but they are probably not relevant to the pathogenesis of HAM/TSP.

3.3.2. **Cerebrospinal Fluid Tests**

CSF pleocytosis (greater than 5 cells/μl) and raised protein levels were common. These findings differ from the typical TSP cases but resemble those noted in the Japanese patients (Osame et al. 1987). A possible explanation may be that many of our patients presented early and therefore in the active stage of their disease. The IgG index was elevated in 73% of the samples tested and CSF IgG oligoclonal bands were detected in 85% (23/27) samples. The results of these tests indicate de novo intrathecal antibody production.

The β2 microglobulin protein is a 11,500 dalton molecule bound to the class I antigens of the MHC. Raised levels are seen when there is induction of MHC expression on surfaces of activated lymphocytes. In this study the CSF β2M was raised in all 25 samples tested and was greater than the serum β2M in 17 cases. The CSF/serum albumin ratio (used as an index of blood-brain barrier integrity) was measured in 21 and shown to be normal in 13. In 10 of these 13 cases the CSF β2M was greater than serum β2M. These results provide further evidence of immune activation within the CNS.

3.3.3. Virological Studies

The strong multiple specific bands seen on WB confirmed exposure to HTLV-I. The question of cross reacting HTLV-II antibodies was handled by the isolation of the HTLV-I virus from the peripheral blood lymphocytes of 6 patients. The CSF demonstrated bands similar to that seen in the serum. The tests for intra-thecal antibody production discussed above would suggest that there was specific anti HTLV-I antibody synthesis in the CNS compartment.

3.3.4. Radiological Studies

No myelogram or myelo-CT showed clinically relevant spinal cord compression. However, myelo-CT showed evidence of arachnoiditis and atrophy in some of the cases, reflecting features noted pathologically. Spinal cord MRI was not done because of lack of facilities but others (Kermode et al. 1990) have confirmed cord atrophy with this technique.

CT brain showed some abnormality in half of the cases studied (12/24), the main feature being atrophy. The finding of periventricular lucencies in only one patient reflects the relative insensitivity of CT in detecting white matter changes when compared to MRI. Both Newton et al (1987) and Kira et al (1988) found white matter high intensity signals on brain MRI. These changes were similar but less extensive to those seen in Multiple Sclerosis.

3.3.5. **Electrodiagnostic Studies**

Electrophysiological evidence of peripheral nerve dysfunction was common. For example, some abnormality of the common peroneal nerve was noted in 58.6% of the nerves studied. The peripheral nerve dysfunction is discussed in detail in chapter 7.

The VER and BAER studies confirmed the presence of subclinical abnormalities in other parts of the central nervous system.

3.4. CONCLUSION

The extensive investigations discussed in this chapter:

1) confirmed the association between HTLV-I and HAM/TSP
2) excluded other recognisable causes of myelopathy and
3) demonstrated that, while the thoracic cord bears the brunt of the disease, the pathological process affects other parts of the neuraxis as well.

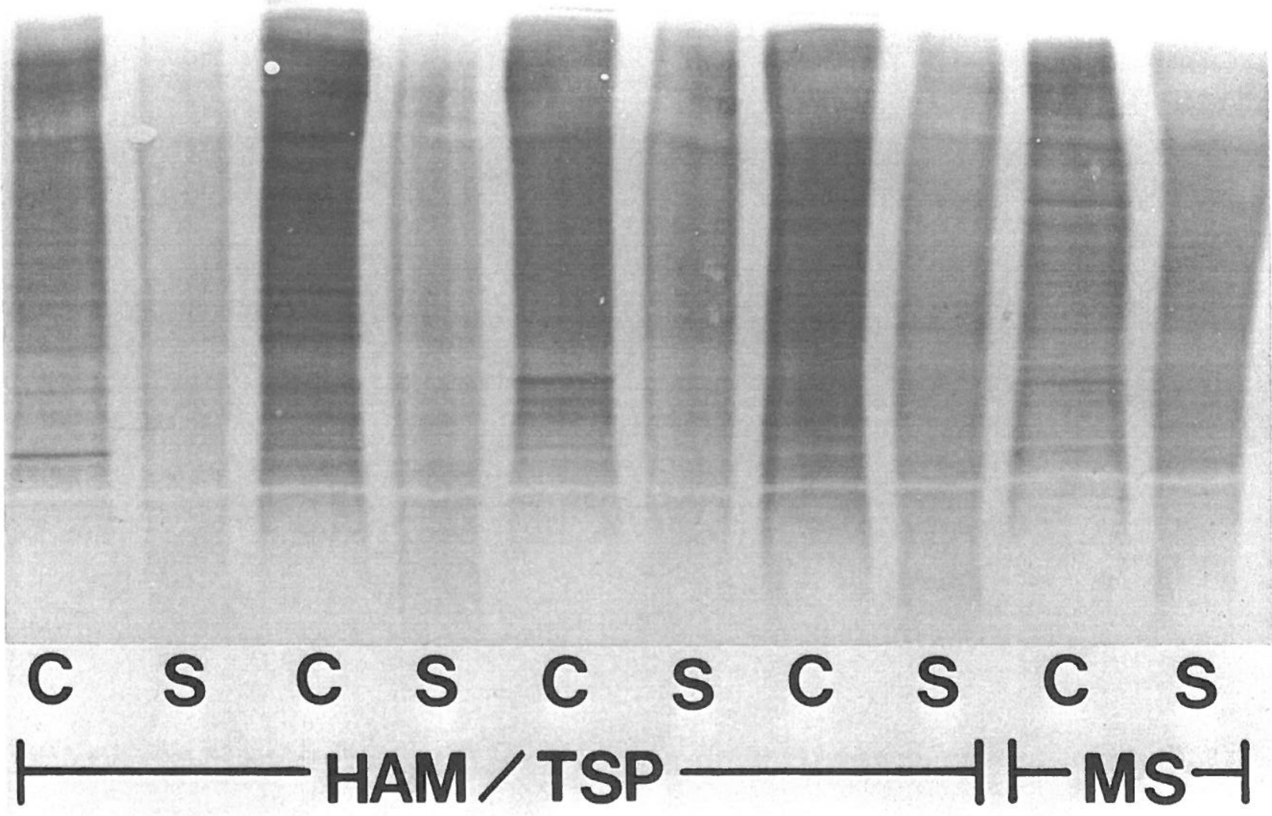


FIG 3.1: Examples of IgG Oligoclonal bands in the CSF
C = CSF, S = Serum , MS = Multiple Sclerosis

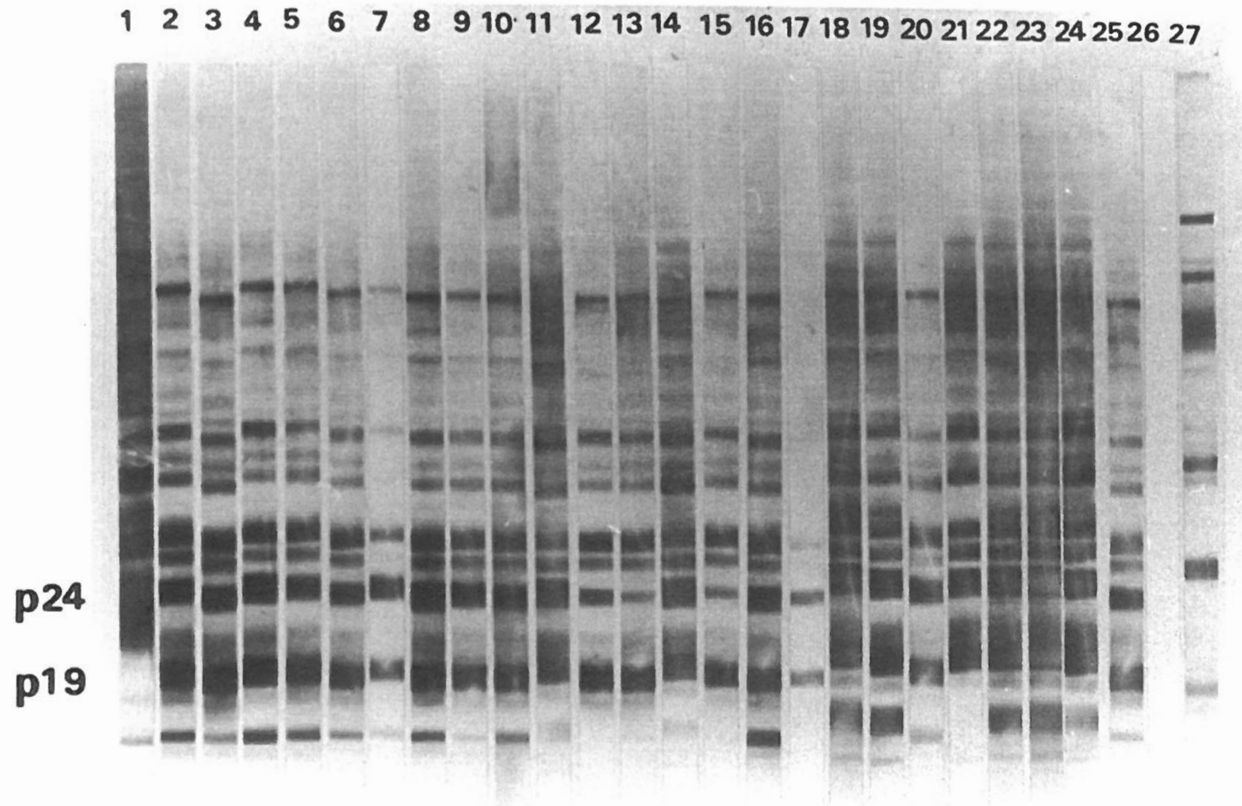


FIG 3.2: POSITIVE SERUM WESTERN BLOT RESULTS OF THE FIRST 24 PATIENTS.
Lanes 1-24 : HAM/TSP patients; Lane 25 : HTLV-I positive control serum ; Lane 26 : HIV blot using HTLV-I positive serum ; Lane 27 : HIV-1 blot using HIV-1 positive serum.

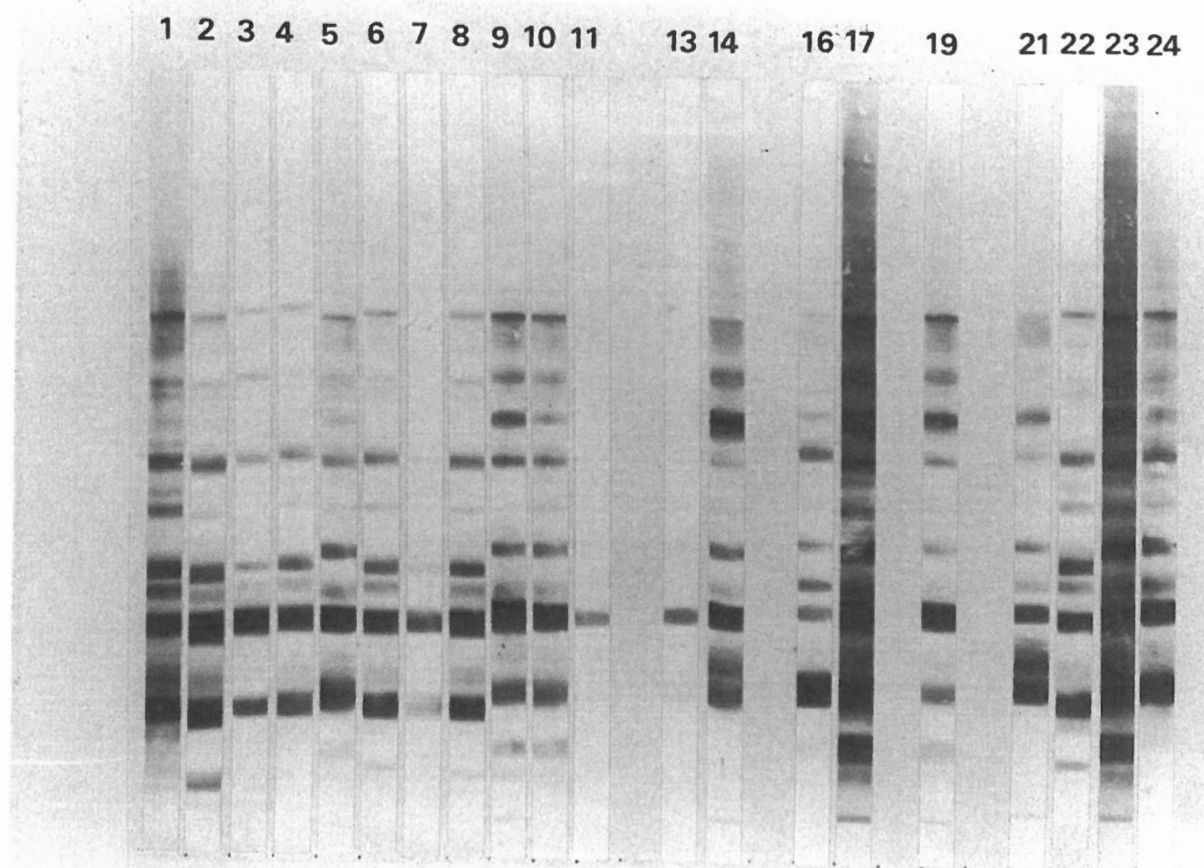


FIG.3.3: Positive CSF Western Blot Results. Samples 12,15,18 & 20 are missing.

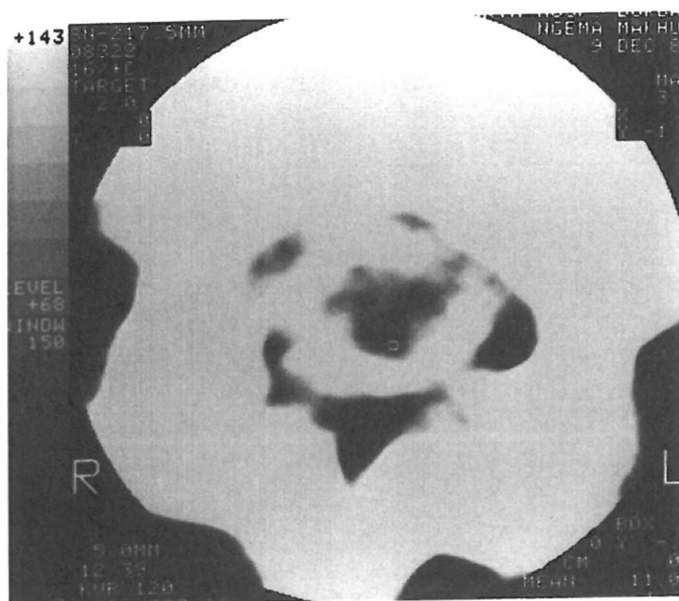


FIG 3.4: CT Myelogram demonstrating arachnoiditis.

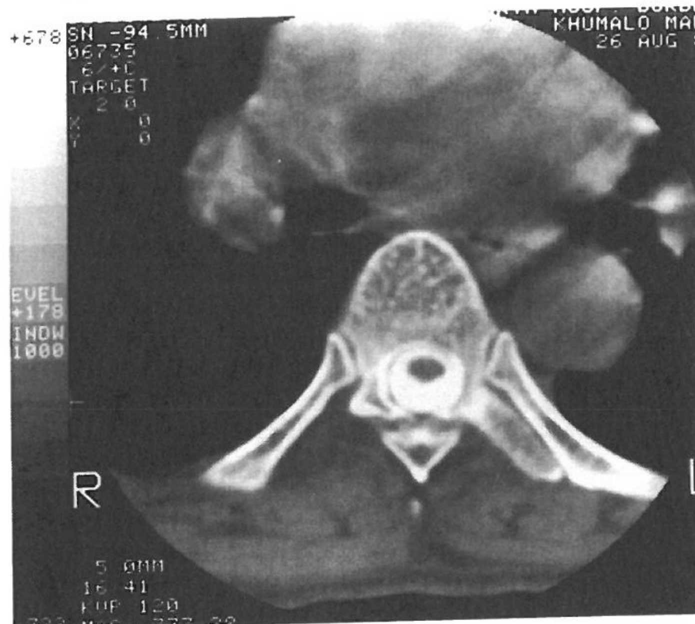


FIG. 3.5: CT Myelogram demonstrating thoracic cord atrophy.

CHAPTER 4

HAM/TSP: HLA STUDIES

4.1 INTRODUCTION

Although the association between HTLV-I and myelopathy is well established, only a small proportion of antibody positive individuals develop HAM/TSP eg Osame et al (1990) estimated a prevalence rate of HAM/TSP of 1 per 2000 to 3600 antibody positive persons in Japan. Further, the same virus (Yoshida et al. 1987) causes a different disorder in other antibody positive individuals viz ATLL.

These observations raise the possibility of host factors determining the development of HAM/TSP. To explore this, the Human Leucocyte Antigen (HLA) profile was determined in 56 of the 90 HAM/TSP patients.

4.2 SUBJECTS AND METHODS

HLA studies were undertaken in 56 HAM/TSP patients (18 men and 36 women). The control group consisted of 1848 normal black adults who were either staff or randomly selected blood donors. HLA class I antigens were determined in all patients and control subjects by a two stage microlympho-cytotoxicity test (Terasaki & McClelland 1964) with 180 antisera. These consisted of local sera that have been requested for use in International Histocompatibility Workshops, local sera that have been verified with International Workshop sera and sera that have been exchanged with other laboratories world-wide. Similarly, 120 sera were used to define the class II antigens on B-lymphocyte enriched suspensions prepared with the aid of straws packed with nylon wool (Danilovs et al. 1980). The class II

antigens were determined in 43 patients and 556 control subjects except that there were only 340 control subjects tested for HLA DQ antigens.

Difference in HLA frequencies were tested for significance with the Chi square test (without Yate's correction) and the probability was corrected by multiplying the p-value by the number of comparisons made i.e. the number of antigens tested (Svejgaard et al. 1974). Relative risks were calculated according to the formula recommended by Woolf (1955). The difficulties of establishing negative correlations which may indicate a "protective" antigen have been discussed by Svejgaard et al (1975). Haplotype frequencies were estimated by the method of Mattiuz et al (1970).

4.3 RESULTS

The frequencies of HLA A, B and C locus antigens in patients and controls are show in Table 4.1, 4.2 and 4.3 respectively. Table 4.4 and 4.5 list the frequencies of the HLA DR and DQ antigens. The X^2 and relative risk (R-R) for each antigen are shown in the tables.

HLA Bw57 was detected in 10 of 54 patients (18.5%) but only in 4.8% of controls. The X^2 is 20.3 and the R-R 4.5. Even correcting for the total number of class I antigens tested (47), the p value for Bw57 still remained significant at the 1% level. The increased frequencies of A2 (35.2% vs 24.3%), B7 (29.6% vs 23.3%) and DR1 (13.9% vs 5.2%) were of borderline significance. There were no significant

differences in the frequencies of HLA-C and HLA DQ antigens. Interestingly A26 was less frequent in patients when compared to controls.

4.4 DISCUSSION

In contrast to our largely negative findings, Usuku et al (1988) found specific HLA haplotypes in 70% of their HAM/TSP patients. Furthermore none of the HAM/TSP associated HLA haplotypes were seen in ATLL. However, Usuku et al (1988) did not correct for the number of antigens tested. Another Japanese group (Nishimura et al. 1991) found increased frequencies of HLA-A31, B7 and DR1 in HAM/TSP patients but these associations were not statistically significant when their p values were corrected for the number of alleles tested. The antigens A11, Bw54 and Bw52 associated with Japanese HAM/TSP (Usuku et al. 1988), are not found in the Zulus.

There is evidence suggesting that much of the neurological injury in HAM/TSP is immune mediated (Dalgleish et al. 1988; see also chapter 5). When this is viewed against (1) greater immune responsiveness of HAM/TSP patients compared to ATLL and asymptomatic HTLV-I carriers (Usuku et al. 1988) (2) the existence of class II associated auto-immunity (Todd et al. 1988) and (3) the binding of viral peptides by class I molecules to activate CD8 and cytotoxic T cells (Todd et al. 1988), it is possible that a more refined examination of the HLA system may yet prove fruitful in Zulu HAM/TSP cases. The recent molecular genetic study by Usuku et al (1990) showed a relationship between a particular amino acid sequence of the HLA DR beta 1 chain and susceptibility to HAM/TSP. Control frequencies

in Zulus for HLA polymorphism using PCR amplified DNA, dot-blots and probes have been already established. A project to determine if any of the DNA markers are relevant to HAM/TSP is being planned.

TABLE 4.1: FREQUENCIES OF HLA-A LOCUS ANTIGENS IN PATIENTS AND CONTROLS

	Controls		HAM/TSP			
	Number	%	Number	%	CHI-SQ	R-R
A1	152	8.23	5	9.26	0.01	1.1
Aw36	18	0.97	1	1.85	0.41	1.9
A2	449	24.30	19	35.19	3.35	1.7
A3	205	11.09	5	9.26	0.18	0.8
A11	0	0.00	0	0.00	-	-
A23	338	18.29	8	14.81	0.43	0.8
A24	110	5.95	5	9.26	1.01	1.6
A25	61	3.30	0	0.00	1.84	0.0
A26	215	11.63	2	3.70	3.26	0.3
Aw34	158	8.55	6	11.11	0.44	1.3
A26	394	21.32	16	29.63	2.14	1.6
A29	270	14.61	5	9.26	1.21	0.6
Aw74	23	1.24	0	0.00	0.68	0.0
A30	583	31.55	13	24.07	1.36	0.7
A31	62	3.35	1	1.85	0.37	0.5
A32	37	2.00	2	3.70	0.76	1.9
A33	66	3.57	2	3.70	0.00	1.0
Aw43	6	0.32	0	0.00	0.18	0.0
Aw66	3	0.16	0	0.00	0.09	0.0
TOTAL	1848	100.00	54	100.00		

R-R = relative risk

TABLE 4.2: FREQUENCIES OF HLA-B LOCUS ANTIGENS IN PATIENTS AND CONTROLS

	Controls		HAM/TSP			
	Number	%	Number	%	CHI-SQ	R-R
B7	432	23.38	16	29.63	1.14	1.4
B8	235	12.72	7	12.96	0.00	1.0
B13	62	3.35	2	3.70	0.02	1.1
B14	112	6.06	4	7.41	0.17	1.2
B18	95	5.14	3	5.56	0.02	1.1
B21	35	1.89	0	0.00	1.04	0.0
Bw22	1	0.05	0	0.00	0.03	0.0
B27	8	0.43	0	0.00	0.23	0.0
B35	135	7.31	5	9.26	0.29	1.3
B37	2	0.11	0	0.00	0.06	0.0
B38	32	1.73	1	1.85	0.00	1.1
B39	29	1.57	0	0.00	0.86	0.0
Bw41	33	1.79	0	0.00	0.98	0.0
Bw42	368	19.91	8	14.81	0.86	0.7
B44	303	16.40	11	20.37	0.60	1.3
B45	174	9.42	2	3.70	2.04	0.4
Bw47	2	0.11	0	0.00	0.06	0.0
Bw48	1	0.05	0	0.00	0.03	0.0
B51	20	1.08	1	1.85	0.28	1.7
Bw52	1	0.05	0	0.00	0.03	0.0
Bw53	29	1.57	2	3.70	1.49	2.4
Bw5I	0	0.00	0	0.00	-	-
Bw57	88	4.76	10	18.52	20.32	4.5
Bw58	585	31.66	12	22.22	2.17	0.6
Bw60	1	0.05	0	0.00	0.03	0.0
Bw61	0	0.00	0	0.00	-	-
Bw62	12	0.65	0	0.00	0.35	0.0
Bw63	43	2.33	0	0.00	1.29	0.0
Bw70	512	27.71	7	12.96	5.75	0.4
TOTAL	1848	100.00	54	100.00		

TABLE 4.3: FREQUENCIES OF HLA-C LOCUS ANTIGENS IN PATIENTS AND CONTROLS

	Controls		HAM/TSP			
	Number	%	Number	%	CHI-SQ	R-R
Cw1	1	0.23	1	1.85	3.18	8.4
Cw2	99	22.30	8	14.81	1.60	0.6
Cw3	51	11.49	11	20.37	3.49	2.0
Cw4	73	16.44	9	16.67	0.00	1.0
Cw5	5	1.13	1	1.85	0.21	1.7
Cw6	36	8.11	3	5.56	0.43	0.7
Cw7	151	34.01	21	38.89	0.51	1.2
Cw8	8	1.80	2	3.70	0.89	2.1
TOTAL	444	100.00	54	100.00		

TABLE 4.4: FREQUENCIES OF HLA-DR LOCUS ANTIGENS IN PATIENTS AND CONTROLS

	Controls		**HAM/TSP**			
	Number	%	Number	%	CHI-SQ	R-R
DR1	29	5.22	6	13.95	5.54	2.9
DR2	133	23.92	15	34.88	2.58	1.7
DR3	195	35.07	14	32.56	0.11	0.9
DR4	50	8.99	4	9.30	0.00	1.0
DR5	175	31.47	15	34.88	0.21	1.2
DRw6	111	19.96	4	9.30	2.92	0.4
DR7	94	16.91	5	11.63	0.81	0.6
DRw8	23	4.14	0	0.00	1.85	0.0
DR9	5	0.90	1	2.33	0.82	2.6
DRw10	14	2.52	3	6.98	2.88	2.9
TOTAL	556	100.0	43	100.00		

TABLE 4.5: FREQUENCIES OF HLA-DQ LOCUS ANTIGENS IN PATIENTS AND CONTROLS

	Controls		**HAM/TSP**			
	Number	%	Number	%	CHI-SQ	R-R
DQw1	213	62.65	31	72.09	1.47	1.5
DQw2	76	22.35	8	18.60	0.31	0.8
DQw3	102	30.00	16	37.21	0.93	1.4
TOTAL	340	100.00	43	100.00		

CHAPTER 5

HAM/TSP: A CLINICO-PATHOLOGICAL STUDY OF INFECTION ACQUIRED BY BLOOD TRANSFUSION

5.1 INTRODUCTION

HTLV-I is transmitted through sexual intercourse, blood transfusion, intravenous drug abuse and from mother to child through breast feeding. The latency from infection to disease is shorter and the disease more aggressive when transmission is by blood transfusion (Osame et al. 1990). This probably reflects the higher viral load as well as the immunosuppressive effect of blood transfusion (Manns and Blattner 1991).

The clinical and pathological features in a white male who developed transfusion associated HAM/TSP are presented. He was not included amongst the 90 cases described in chapter 2.

5.2 CASE HISTORY

A 49 year old White South African man received 48 units of blood following a massive gastro-intestinal haemorrhage in February 1987. Fourteen months later (April 1988), he noticed progressive weakness and stiffness of his legs. By June 1989 he was wheelchair bound and incontinent of urine and faeces. Examination at this stage revealed normal mental state and cranial nerves. There was mild increase in tone in the upper limbs but the power was normal. The lower limbs were

markedly spastic with spontaneous clonus. Power in the legs was graded 2/5 (MRC grading). There was generalised hyper-reflexia and bilateral extensor plantar responses. He had patchy impairment of pinprick sensation to the T6 dermatome on the right and L2 on the left. Other modalities of sensation were intact.

A full length myelogram and magnetic resonance imaging of the head and cervical spine were normal. The CSF was acellular but had raised protein (0.75 g/l). Antibodies to HTLV-I were detected in the serum. Antibodies to HIV 1/2 were absent. No CSF was available for antibody testing.

The patient declined further investigations. A 3 month course of steroids produced no benefit. His disability gradually progressed to the extent that he became quadriplegic, developed pressure sores and recurrent respiratory and urinary tract infections. He died in April 1990. Autopsy permission was limited to examining the spinal cord which was removed approximately 24 hours after death.

5.3 MATERIALS AND METHODS

5.3.1. Light Microscopy Studies

Representative paraffin sections of the cervical, thoracic and lumbar spinal cord were stained using standard techniques. The stains used were haematoxylin and eosin, PTAH, Servier Munger and Luxol fast blue. Immunocytochemical studies using antibodies to glial fibrillary acidic protein (GFAP; DAKO, Santa Barbara, CA) and neurofilament (NF; Lab Systems,

Research Triangle Park, North Carolina, USA) were undertaken according to the technique of Wiley et al (1986).

5.3.2. **Electron Microscopy Studies**

Specimens of cervical, thoracic and lumbar spinal cord were fixed in 3% phosphate buffered gluteraldehyde and processed for electron microscopy according to standard techniques.

5.3.3. **Lymphocyte Marker Studies**

Lymphocyte marker studies (Dako-Pan B, T11, T4(CD4), T8(CD8), LC, MAC 387) were done on frozen sections of the thoracic cord according to the recommended methods (Dakopatts-Glostrup, Denmark).

5.3.4. **Viral Protein Detection Studies**

Protein A purified polyclonal rabbit antiserum to HTLV-I and a series of commercially available monoclonal antibodies (Olympus Corporation, Lake Success, NY) were used on paraffin embedded tissues. Six of the monoclonal antibodies were specific for HTLV-I core protein and two were specific for HTLV-I envelope proteins. The method used for immunostaining was that described by Wiley et al (1986).

5.3.5. **Polymerase Chain Reaction Assay**

Total cellular DNA was extracted from formalin fixed paraffin embedded blocks of the spinal cord (Wright & Manos 1990) and amplified for the viral

gag gene. Details of methodology are presented in Appendix L. For comparison, a positive control HTLV-I infected cell line (SLB-I), a negative control non-infected human T-cell line (HVT 78), liver and lymph node tissue from a seropositive case of non-Hodgkins lymphoma and muscle from a seropositive case of polymyositis were similarly tested.

5.3.6. **In-situ Hybridization Studies**

These studies were performed according to the methods of Wiley et al (1986), except that instead of labelling the probe with ^{35}S labelled nucleotides, a digoxigenin label was employed. In brief, a 1.2 Kb HTLV-I tax sequence and a comparably sized pBR plasmid sequence were labelled by random primed incorporation of digoxigenin-labelled deoxyuridine-triphosphate according to the Genius kit protocol (Boehringer Mannheim Biochemicals, Indianapolis, In, USA). The average length of the digoxigenin labelled probes is approximately 750 bases. Hybridization was performed for 18 hours at 37°C. The slides were extensively washed as per kit protocol, then developed with an immunocytochemical reaction using sheep anti-digoxigenin antisera.

5.4. **RESULTS**

5.4.1. **Light Microscopy Studies**

Sections from the cervical, thoracic and lumbar levels of the spinal cord demonstrated mild meningeal chronic inflammation. Chronic perivascular

and parenchymal inflammation was noted in all levels of the cord but was most severe within the thoracic level (plates 5.1-5.3). Inflammation was restricted mostly to the white matter tracts in the lateral and posterior columns. Demyelination and axonal loss were noted in the inflamed regions. Bodian silver stains and immunocytochemical stains for neurofilament proteins demonstrated dystrophic dilatations of axonal processes. The GFAP stain demonstrated moderate gliosis in the inflamed areas.

Immunohistochemical studies for the MHC antigen $\beta 2$ microglobulin intensely labelled endothelial cells and infiltrating inflammatory cells. Occasional glial cells and Schwann cells also showed staining. However, no neuron expressed MHC class I antigen. Comparably prepared normal spinal cord sections showed no staining for this antigen. Expression of class II antigen was restricted to inflammatory cells.

5.4.2. **Lymphocyte Marker Studies**

The inflammatory infiltrates consisted predominantly of CD8 positive cells and fewer CD4 positive cells. Scattered monocyte/macrophages and B-cells were also seen.

5.4.3. **Electron Microscopy Studies**

Thick sections of plastic embedded spinal cord tissue from the thoracic level demonstrated chronic perivascular inflammation as seen with the paraffin

sections. Cell membranes were poorly preserved and no viral particles could be identified.

5.4.4. **Viral Protein Detection Studies**

Immunocytochemical studies of paraffin embedded spinal cord sections for HTLV-I antigen were negative. Concurrently run preparations of equivalently processed paraffin embedded HTLV-I infected cell lines demonstrated abundant HTLV-I antigens.

5.4.5. **Polymerase Chain Reaction Assays**

The PCR data, shown in Fig. 5.1, indicated that HTLV-I proviral DNA was detectable in DNA extracted from all 3 levels of the spinal cord. The amount of HTLV-I DNA in the thoracic cord was approximately 3-4 fold higher than in the cervical or lumbar cord sections. The signal obtained from these latter two areas of the cord were at the virtual limits of detectability by this method. It thus appears that the thoracic cord, which exhibits the most significant pathologic alteration, also harbours the highest amount of HTLV-I DNA.

5.4.6. **In-situ Hybridization Studies**

In an attempt to localise the cellular distribution of HTLV-I proviral DNA we performed in-situ hybridization of spinal cord sections from all 3 levels examined by PCR. Using the digoxigenin label probe, no hybridization was detected in any of these sections.

5.5. **DISCUSSION**

This patient almost certainly acquired his infection from the blood transfusion as there were no other risk factors. The short latent period before symptoms is a well recognised observation when transmission is via blood and blood products (Iwasaki 1990; Gout et al. 1990).

The pathological findings in the patient were similar to those described both before (Montgomery et al. 1964; Mani et al. 1969) and after (Akizuki et al. 1988; Piccardo et al. 1988; Iwasaki 1990) the association of TSP with HTLV-I was established. It is salutary to note that Mani et al postulated a role for a slow virus as long ago as 1969. The main histological finding in HAM/TSP as seen in the patient was mononuclear infiltrates in perivascular areas especially within the lateral and posterior white matter tracts. Myelin pallor and axonal loss were noted in the same regions. All the changes were most prominent in the thoracic cord.

The pathogenesis of HAM/TSP remains unclear. Some of the problems met with in the study of the pathogenesis include paucity of autopsy material, absence of a suitable animal model and difficulty in infecting cells of neural origin in vitro (Watabe et al. 1989).

Evidence for direct viral infection is scanty. Liberski et al (1988) demonstrated viral-like particles on electron microscopy in spinal cord tissue. Due to suboptimal preservation the type of cells showing these particles was not identified. However,

neither we nor others (Wayne Moore et al. 1989; Power et al. 1991), saw particles resembling HTLV-I on electron microscopy.

Several workers (Iwasaki 1990; Piccardo et al. 1988; Cruickshank et al. 1989), including ourselves have failed to detect viral antigen in spinal cord tissue. This may in part be due to delay in performing the autopsy as well as formalin fixation. Wayne Moore et al (1989) demonstrated HTLV-I p19 core protein in cells in the perivascular regions as well as in probable glial cells in the parenchyma. This observation is complicated by the fact that the antibody used by them cross reacted with normal tissue.

The demonstration of proviral DNA in the spinal cord of the patient suggests that HTLV-I may be directly involved in the disease process. The PCR assay showed a quantitatively greater signal in the thoracic cord than in the lumbar or cervical cord. This reflected the difference in the histopathology of these regions and further suggests that the cervical and lumbar cord are directly involved in the disease process and not merely showing changes secondary to primary thoracic disease. The multilevel positivity contrasts with the finding by Power et al (1991) who could amplify the HTLV-I gag gene from the cervical cord only. Unfortunately, the in-situ hybridization studies were unsuccessful in localizing the cells of origin of the proviral DNA detected by PCR. The signal intensity by PCR was quite low and extrapolating from studies of muscle in HTLV-I seropositive patients, the signal in HAM/TSP spinal cords may be below the detectable limits of digoxigenin labelled probes.

It could be argued that the sources of the amplification product would include intraluminal monocytes and lymphocytes. However, these were scanty in the sections used for PCR and the signal was greatest in the thoracic cord which showed the maximal changes. Also Kira et al (1992) compared PCR results in HAM/TSP cords and ATLL infiltrated neural tissues. They found greater amounts of proviral DNA in HAM/TSP samples than the ATLL samples although the extent of mononuclear cell infiltration was far less in the HAM/TSP samples than the ATLL sample. This observation provides additional support for possible direct neurotoxicity of HTLV-I.

There are two further pieces of evidence suggesting direct viral infection of the spinal cord. Firstly, the persistence of HTLV-I specific IgM antibodies in the CSF (McLean et al. 1989; Jacobson et al. 1990a) indicate ongoing viral replication and the production of new viral antigens. Secondly, our lymphocyte studies showed a predominant CD8+ infiltrate. These CD8+ cells may represent a similar subset to the circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I neurological disease as noted by Jacobson et al (1990b) and may be homing in on specific viral proteins within the spinal cord.

In contrast to the difficulty of demonstrating clear cut direct viral infection there is growing evidence of immune mediated neural damage as first suggested by Dalgleish et al (1988). The histological findings have been compared to those seen in recognised situations of acute disseminated encephalomyelitis (Iwasaki 1990). The finding of activated T-cells with polyclonal gammopathy (Mori et al. 1988),

antigen specific oligoclonal bands in the CSF (Ceroni et al. 1988), increased expression of interleukin-2 receptor molecules (Jacobson et al. 1990a), increased levels of interleukin-6 in CSF (Nishimoto et al. 1987) and genetic restriction (Usuku et al 1990; Jacobson et al 1990b) provide further support for an immune based pathogenesis.

5.6 CONCLUSION

The findings in this study suggest that HTLV-I may be directly involved in the disease process and that the sites of action of the virus may be more proximal than previously believed. However, the importance of immune mediated damage should not be underplayed. Genetic susceptibility (and hence immune response) would explain why only a small proportion of seropositive individuals develop disease.

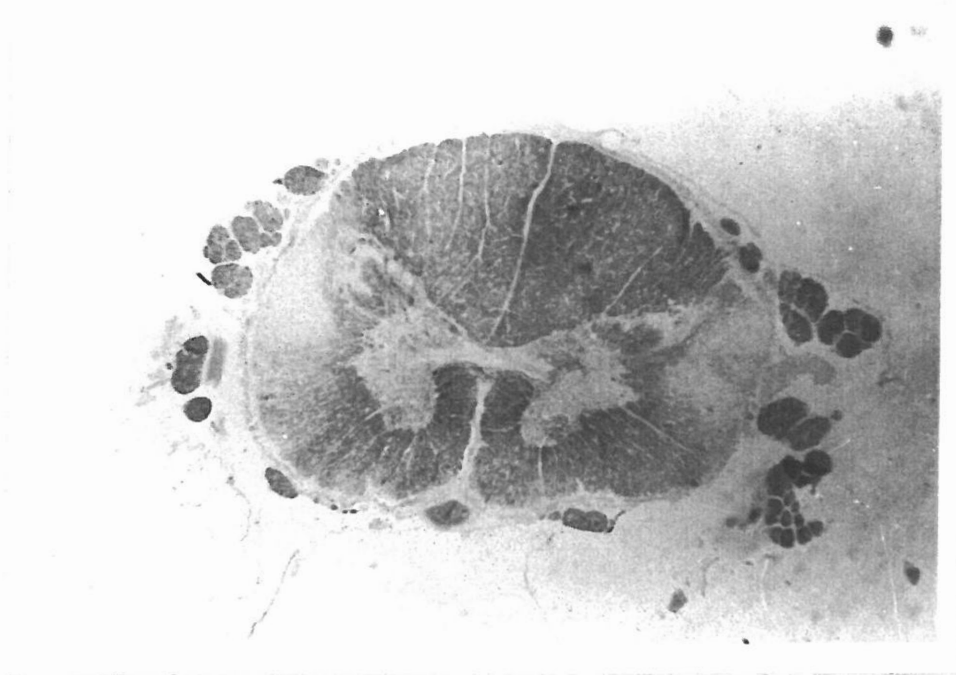


PLATE 5.1: Cross Section of the thoracic cord showing myelin pallor of lateral columns (Luxol fast blue X9)

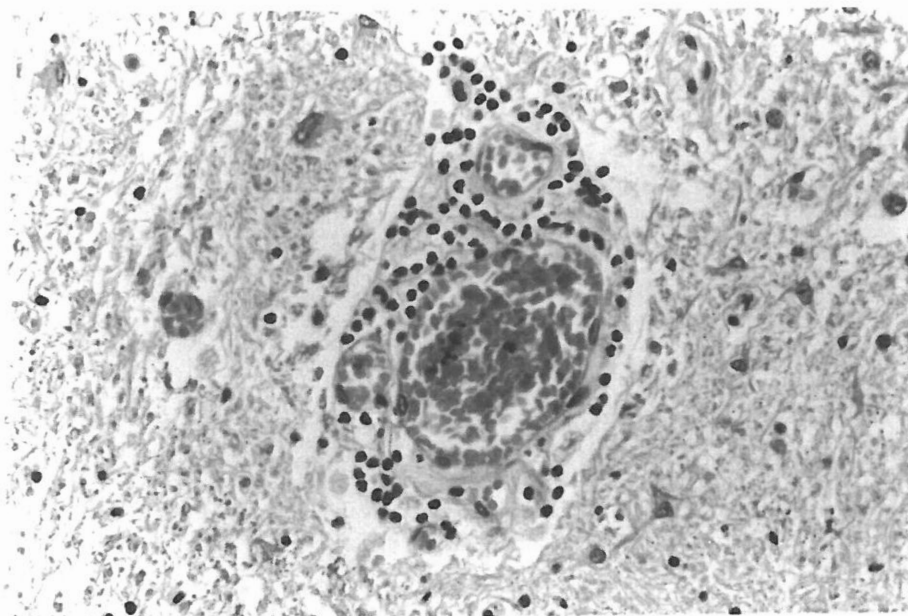


PLATE 5.2: Section of thoracic cord showing mononuclear perivascular infiltrate. (haematoxylin and eosin X 180)

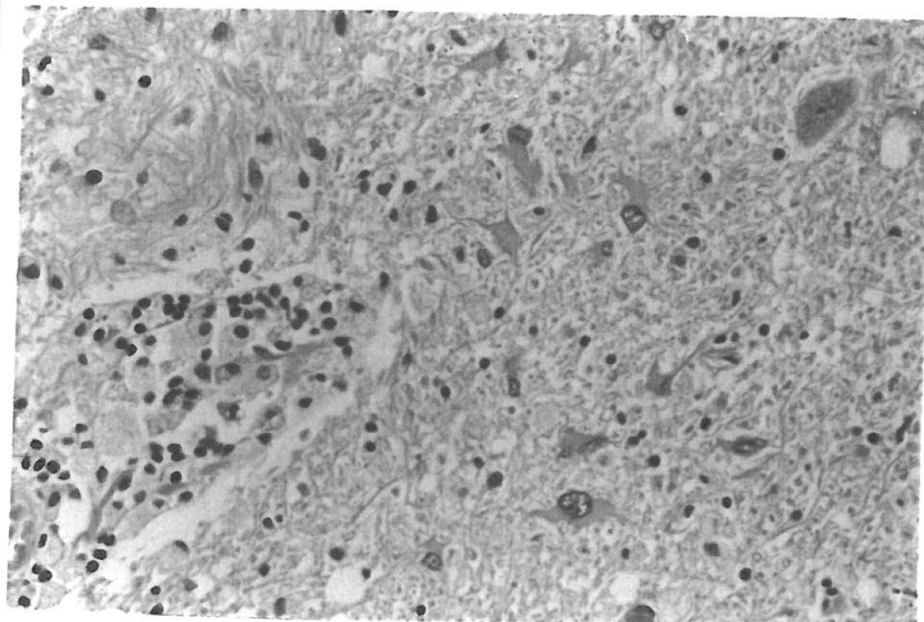


PLATE 5.3: Section of thoracic cord demonstrating perivascular and scattered parenchymal mononuclear infiltrate, foam cells and reactive astrocytes. (haematoxylin and eosin X 180)

LEGEND TO FIG. 5.1: Upper panel shows normalised HTLV-I "gag" PCR products. Lower panel contains normalised glyceraldehyde 3 phosphate dehydrogenase PCR products. Lanes 1-3: HTLV-I infected SLB-I cellular DNA at 0.1 ng, respectively. Negative controls: no template (water) (lane 4) and paraffin embedded HUT-78 T cells (lane 6). Positive controls (all DNA extracted from HTLV-I paraffin embedded cell line or patient-derived tissues): SLB-1 T cells (lane 5), muscle from HTLV-I seropositive patient with myopathy (lane 7); liver (lane 8) and lymph node (lane 9) from HTLV-I seropositive patient with non-Hodgkins lymphoma. Spinal cord specimens from HAM/TSP patient (DNA extracted from paraffin embedded tissue): cervical (lane 10), thoracic (lane 11) and lumbar (lane 12). M lanes denote markers used to define PCR products.

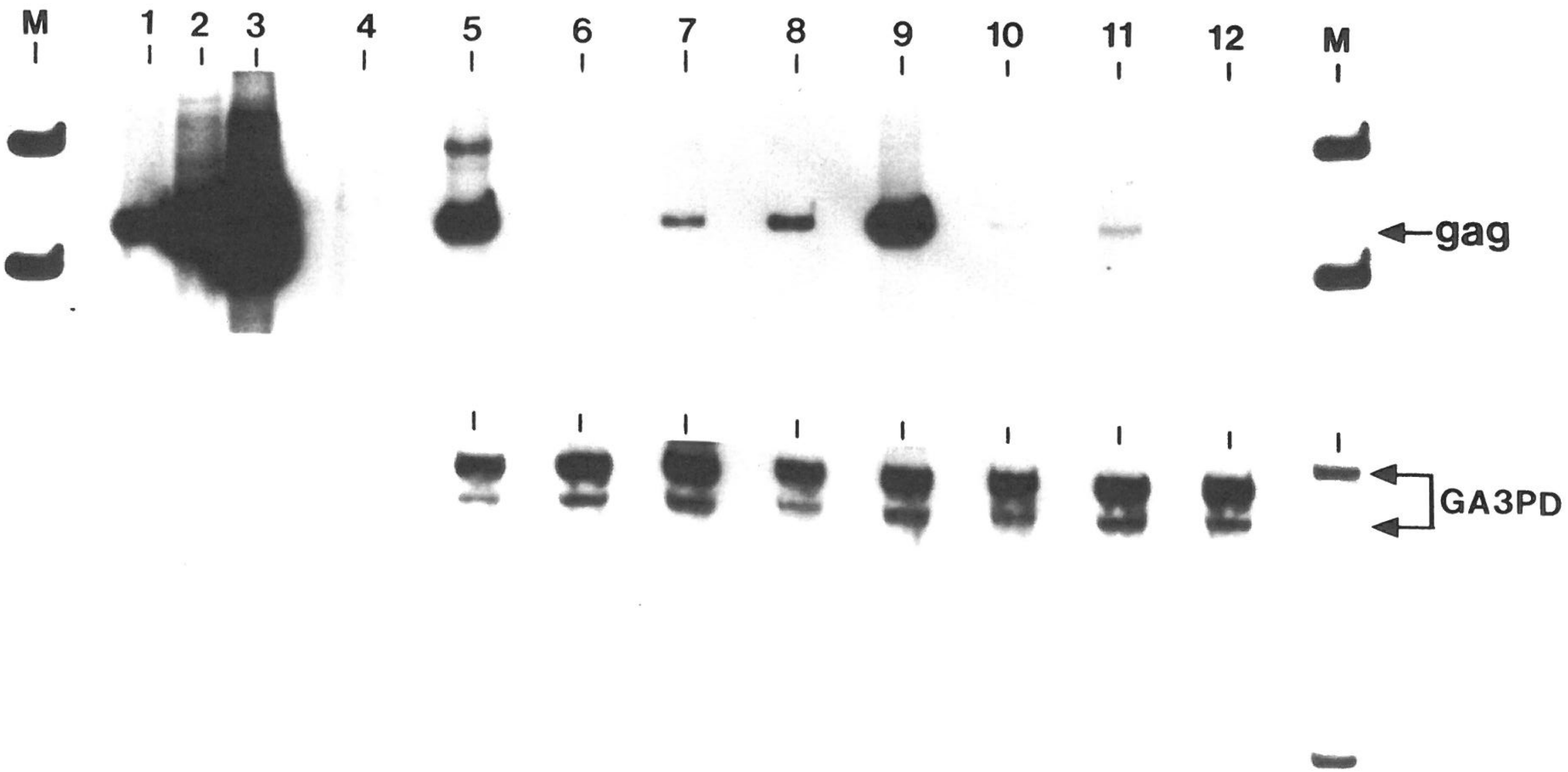


FIG 5.1: PCR analysis of DNA extracted from patient's spinal cord, tissue and cell controls. See legend on opposite page.

CHAPTER 6

HTLV-I AND PERIPHERAL NEUROPATHY:

A CLINICO-PATHOLOGIC STUDY

6.1 INTRODUCTION

Although TSP is primarily a spinal cord disorder, peripheral nerve dysfunction (PND) has been noted in various studies. The frequency of PND reported has varied from negligible to 32% (Montgomery et al. 1964; Barkhaus & Morgan 1988; Ludolph et al. 1988). The changes observed include distal wasting, stocking type peripheral sensory loss and absent ankle jerks.

Since the demonstration of antibodies to HTLV-I in patients with TSP, most reports have concentrated on multimodality evoked response studies and have shown abnormalities in the central pathways (Kakigi et al. 1988; Cruickshank et al. 1989). However, Cruickshank et al (1989) noted absent ankle jerks in 3 of their 21 patients. Arimura et al (1987) did undertake peripheral nerve conduction studies in 6 patients but found few abnormalities. Bhagavati et al (1988) noted peripheral nerve electrophysiological abnormalities in 6 of 12 patients.

We have found clinical evidence of PND in 23% of our patients and electrophysiological evidence in approximately 58% (Chapter 3). Of the patients with evidence of PND, 6 underwent sural nerve biopsies. The histopathological findings are presented.

6.2 METHODS

6.2.1. Light Microscopy Studies

6.2.2. Electron Microscopy Studies

6.2.3. Viral Protein Detection Studies

6.2.4. In-situ Hybridization tests to detect proviral Nucleic Acids.

6.2.5. Polymerase Chain Reaction Assays

The above tests were done as described in Chapter 5.

In addition nerve fibre teasing and immunofluorescent studies for IgG, IgM, IgA were done.

6.2.6. Morphometric Analysis

One micrometre thick plastic sections were stained with p-phenylenediamine for standard morphometric analysis (Forcier et al. 1991). The fascicle image was projected onto a grid scored in 0.5 cm squares using a camera Lucida attachment. The area of each fascicle was determined by digitizing prints (242 x final magnification) using a Jandel digitization pad with Sigma scan software. Mean axonal diameters of each nerve were calculated by first multiplying the percent volume fraction of axons by the total fascicular area and dividing by the total number of myelinated fibres. The calculated mean axonal area was then used to calculate mean axonal diameters by assuming a circular axonal profile and calculating the equivalent diameters. To compare these data to published standards, similar values were derived from data published by Behse (1991).

6.3 RESULTS

6.3.1. Light Microscopy Studies

No inflammatory infiltrate was seen.

6.3.2. Immunofluorescent Studies

No deposit of IgG, IgM, IgA or complement was detected.

6.3.3. Electron Microscopy

The semi-thin sections of the six sural nerves are shown in Plate 1 (a-f). The ultrastructural findings were as follows:

1. The nerves of patients 1,2,3 and 5 showed mild to moderate loss of myelinated fibres. Several fibres demonstrated disproportionately thin myelin sheaths suggestive of remyelination. Patients 1 and 2 showed occasional clusters of regenerating axons as well as proliferated Schwann cell processes without axons. The unmyelinated fibres appeared normal.
2. The biopsy from patient 4 showed severe loss of myelinated fibres, axonal atrophy, occasional degenerating axons and groups of small myelinated fibres probably representing regenerating clusters. There was a normal complement of unmyelinated fibres.
3. The sural nerve of patient 6 showed severe loss of both myelinated and unmyelinated fibres. Numerous degenerating axons were present. Bungner bands were prominent.
4. Endoneurial fibrosis was mild in nerves from patients 1,2,3 and 5 but moderate to severe in nerves from patients 4 and 6.

No virus or virus-like particles were seen in any of the specimens.

6.3.4. **Teased Fibre Preparations**

The findings of the teased fibre studies are summarised in Table 6.1. They range from predominantly normal features e.g. case 2, to severe axonal changes e.g. case 6. Several fibres showed multiple features e.g. combinations of demyelination, remyelination and "globules".

6.3.5. **Morphometric Studies**

The derived average axonal diameters of the patients' nerves were similar (4.58 $\pm$ 0.47: mean $\pm$ SD). Myelinated fibre number, number of fascicles, total fascicular area and myelinated fibre density are shown in Table 6.2. Myelinated fibre densities in all patients were below the normal range.

6.3.6. **Viral Protein Detection Studies**

No viral products were detected in the tissues examined.

6.3.7. **In Situ Hybridization Tests**

Viral nucleic acids were not demonstrated.

6.3.8. **Polymerase Chain Reaction Assays**

All samples tested negative for proviral DNA.

6.4 DISCUSSION

One of the earliest detailed reports of histological changes in the peripheral nervous system was that by Said et al (1988), who found perineurial and perivascular inflammatory infiltrates, moderate axon loss, areas of demyelination and Wallerian degeneration in a biopsy of the superficial branch of the peroneal nerve. The changes were thus similar to those in the spinal cord and roots. Makazato et al (1989) found a decrease in the number of larger myelinated fibres, frequent denervated Schwann cell clusters but no inflammatory infiltrate in a small nerve biopsy. Sigumura et al (1990) reported on sural nerve pathology in three patients. Their biopsies showed segmental demyelination, remyelination and frequent globules. No inflammatory infiltrate was observed. They considered demyelination with globule formation to be characteristic of peripheral neuropathy in HAM/TSP.

The sural nerve changes noted in the six patients of this study were similar to those described by Sigumura et al (1990). The morphometric studies gave results consistent with a normal reparative process, a decrease in myelinated fibres as well as a decrease in axonal diameters. The decreased axonal diameters may be explained by either preferential loss of larger myelinated fibres or axonal atrophy. Unlike Said et al (1988), others (Nakazato et al. 1989; Sigumura et al. 1990) including ourselves, have not been able to show any inflammatory infiltrates. A possible explanation may be the choice of nerve biopsied as Said et al (1988) biopsied a larger and more proximal nerve.

Other incidental causes of peripheral neuropathy were considered in our patients. However, all patients had symptoms of short duration; none were bedridden or critically ill and all had satisfactory nutritional status. None abused alcohol, nor had hepatic, respiratory or renal disease, underlying malignancy or diabetes mellitus. Apart from hereditary tendency to pressure palsy (Bradley et al. 1975), "globule formation" has not been properly documented in other neuropathies. We concur with Sigumura et al (1990) that the PND is part of HTLV-I associated disease and not an unrelated abnormality.

The pathogenesis of PND in HAM/TSP remains unclear. Said et al (1988) point out that the lesions they noted in the peroneal nerve were similar to those in the central nervous system. This raises the possibility of similar pathogenic mechanisms. They also cite comparable changes in the inflammatory neuropathy associated with the human immunodeficiency virus (HIV), and other retroviruses. However, many cases of this type of inflammatory neuropathy may be due to another infection viz cytomegalovirus (Grafe & Wiley 1989).

At the level of the biopsied site there was no evidence of direct HTLV-I infection. A consideration is that changes seen in the distal end of the PNS (in this study, the sural nerve) are secondary to more proximal disease, e.g. in the roots which may show the same inflammatory process as in the cord (Montgomery et al. 1964). Multi-level studies of the peripheral nerves at necropsy may shed light on the pathogenesis of PND in HAM/TSP.

TABLE 6.1: RESULTS OF NERVE FIBRE TEASING

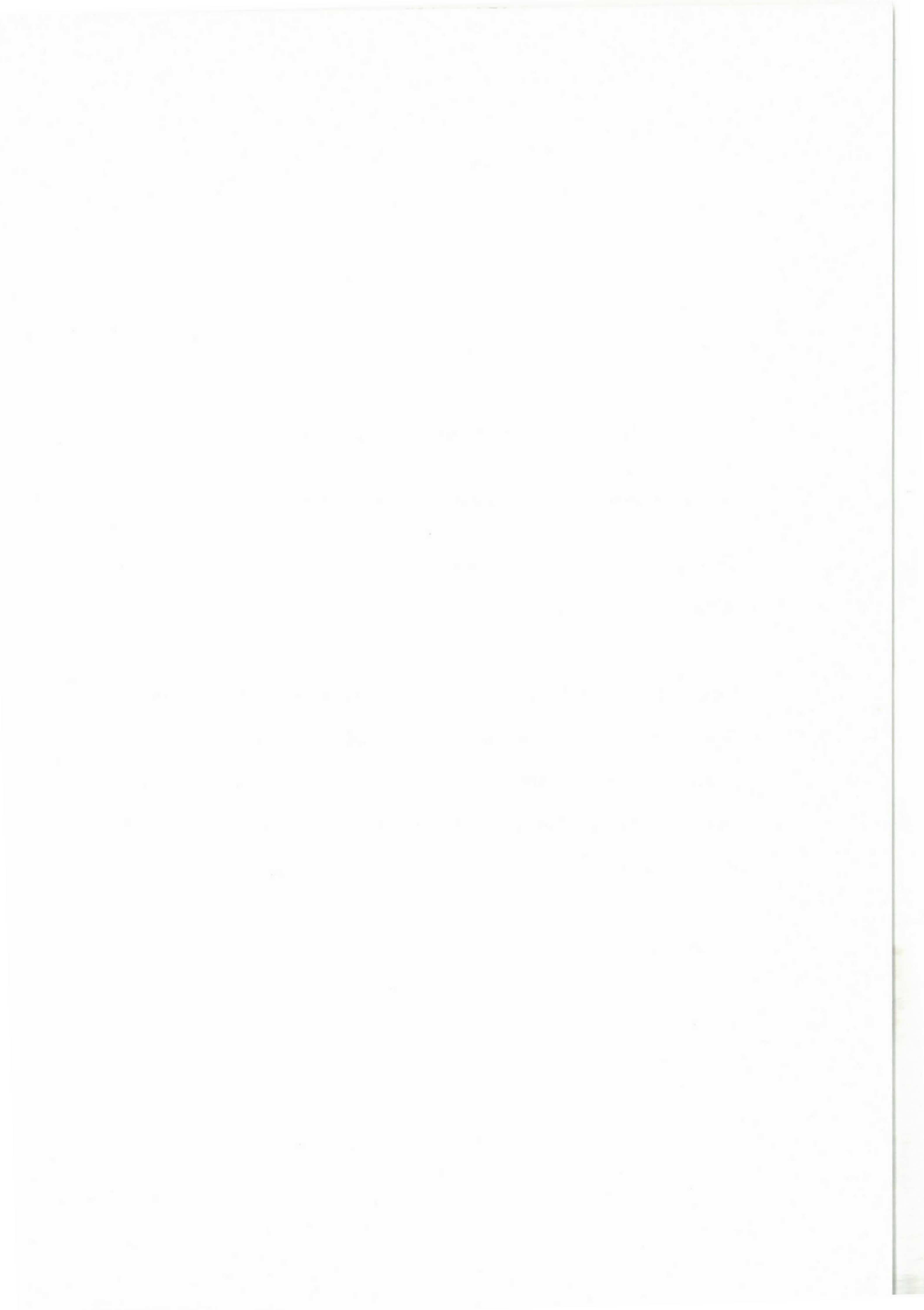
Biopsied Patient No (Series No)	Normal	Demyelin/ Remyelin	Ovoids	"Globules"
1 (1)	77	23	3	0
2 (2)	88	11	4	0
3 (26)	81	17	3	4
4 (36)	25	60	36	10
5 (37)	75	16	0	14
6 (42)	13	0	86	1

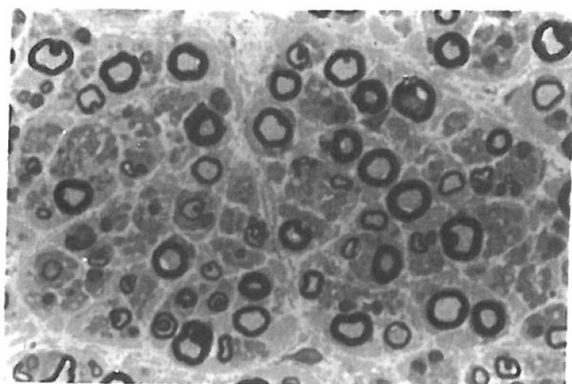
Results expressed as a percentage. Several fibres showed multiple features.

TABLE 6.2: MYELINATED FIBRE DENSITY STUDIES

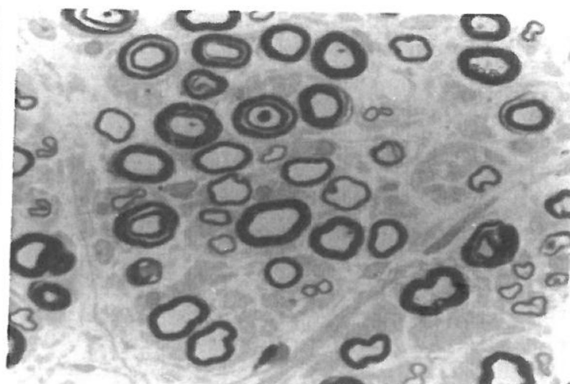
Biopsied Patient No (Series No)	Myelinated Fibres/No. Fascicles	Total Fascicular Area (mm^2)	Myelinated Fibre (density/mm^2)
1 (1)	1400/2	0.3964	3571.8
2 (2)	1457/5	0.2510	5804.7
3 (26)	1547/4	0.4576	3380.8
4 (36)	1497/10	0.6916	2164.5
5 (37)	2432/3	0.3745	6493.9
6 (42)	300/6	0.9825	305.3
Controls*	N/A	N/A	7500 - 10000

N/A = not applicable. Decrease of myelinated fibres noted in all specimens. *Jacobs & Love (1985).

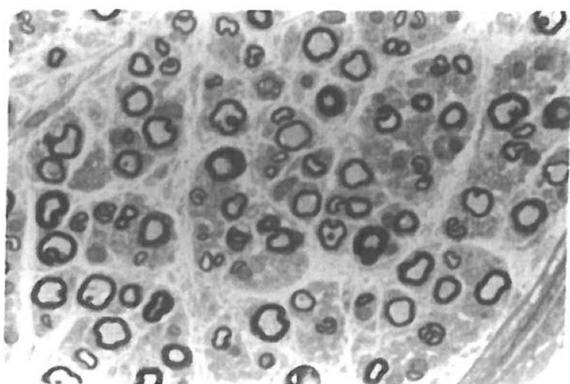




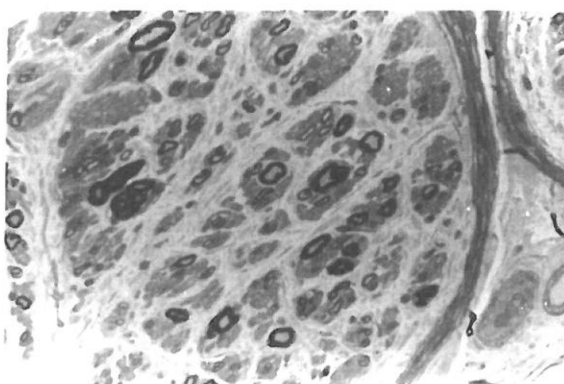
a



b

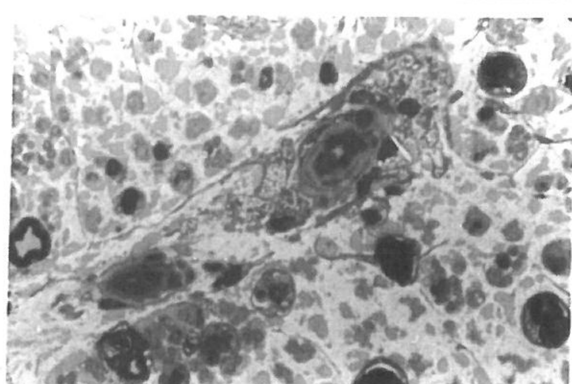


c



d

e



f

PLATE 6.1 Legend on page facing this one

CHAPTER 7

HTLV-I AND INFLAMMATORY MYOPATHY: A CLINICO-PATHOLOGICAL STUDY

7.1 INTRODUCTION

In a HTLV-I seroprevalence study in patients with neurological disease other than HAM/TSP, Mora et al (1988) found that all 7 Jamaican patients with isolated adult onset polymyositis (AOP) tested positive. Their extended studies (Moran et al. 1989) noted that 11 of 13 (85%) patients of AOP had antibodies to HTLV-I. Most subsequent reports of antibody positive polymyositis have been in patients with associated HAM/TSP (Goudreau et al. 1988; Tarras et al. 1989; Francis & Hughes 1989, Evans et al. 1989). Polymyositis with dual HTLV-I and HIV infection has been reported in two further cases (Wiley et al. 1989; McArthur et al. 1990), one of whom had an associated myelopathy.

During the course of the HAM/TSP study 3 patients with disproportionate proximal weakness were identified and studied in greater detail. For comparison 11 other patients with isolated AOP were tested for HTLV-I antibodies.

7.2. MATERIALS AND METHODS

Muscle biopsy specimens were taken under local anaesthesia from either the biceps, deltoid or quadriceps and subjected to the following tests:

1. Routine staining of fresh and paraffin embedded sections with haematoxylin and eosin, ATPase and NADH.

2. Specimens were fixed in 3% phosphate buffered glutaraldehyde and processed for electron microscopy according to standard techniques.
3. Viral protein detection studies: See chapter 5 for methodological details.
4. In-situ hybridization was performed according to the methods of Wiley et al (1986). See chapter 5 for details.
5. Polymerase chain reaction assays were done on DNA extracted from paraffin embedded sections and processed according to the PCR protocols (Wright & Manos 1990). See chapter 5 for details.

7.3. CASE HISTORIES

7.3.1. Case 1 (CM - Series No 43)

This 27 year old Black woman was first examined in April 1989. Apart from evidence of a myelopathy she had disproportionate proximal weakness (MRC grade 4/5) of all four limbs. Electromyography (EMG) of the proximal muscles (deltoid, biceps, quadriceps) showed features of a 'myopathic' disorder. Following a muscle biopsy, prednisone (60 mgs/day) was administered. No definite improvement was noted over a 6 month period when a second muscle biopsy was performed.

7.3.2. Case 2 (TN - series no 47)

This 29 year old Black woman presented with a six week history of lumbar backache, numbness of the legs and progressive weakness of all 4 limbs. She had evidence of a myelopathy with a sensory level at T4 and

disproportionate proximal weakness of arms and legs. There was no weakness of the neck or bulbar muscles. EMG showed features similar to case 1.

7.3.3.**Case 3 (HN - Series no 86)**

This 28 year old Black woman presented with a two year history of pain in the legs, difficulty in walking and urinary urgency. Apart from her myelopathy she had marked proximal weakness of upper and lower limbs. There was no neck or bulbar muscle weakness.

7.4. **RESULTS**

The relevant routine blood tests of the 3 patients are summarised in Table 7.1. In the isolated AOP group (i.e. without HAM/TSP) there were 5 males and 6 females. Of these only one patient exhibited antibodies to HTLV-I. This patient was a 28 year old Black woman who gave a 2 month history of dysphagia, dysarthria, nasal regurgitation and proximal limb weakness. She had mouth ulcers as well. Examination showed facial, jaw, neck and proximal limb weakness. The upper limbs were weaker than the lower limbs. Sensation was intact. Besides the expected raised creatinine kinase (CK) and a positive muscle biopsy, she demonstrated antinuclear antibodies - speckled pattern; titre 1:800. She was started on prednisone (60 mgs/day) and began showing improvement after 3 weeks of treatment.

All the muscle biopsy specimens showed mononuclear inflammatory infiltrates within the fascicles and degenerating muscle fibres (Plate 7.1 a,b). The biopsies from the HAM/TSP patients also showed varying numbers of small fibres, angulated fibres, fibre type grouping and group atrophy - all features suggesting associated neurogenic changes. None of the biopsies showed a vasculitis.

The viral protein studies, the in-situ hybridization tests and the polymerase chain reaction assays all gave negative results.

7.5. **DISCUSSION**

AOP is characterised by subacute to chronic progressive weakness of proximal muscles including the neck and often the bulbar muscles. Cardiac involvement may occur in up to 40% of patients. The CK is nearly always raised several fold, the EMG demonstrates characteristic features and a representative muscle biopsy is diagnostic. Response to steroids, even if only initial or partial, is noted in most patients.

Apart from a sub-group of cases associated with systemic auto-immune disorders (Dalakas 1991), the aetiology of AOP has remained elusive. Reports linking the disorder with viruses such as the picornaviruses (Bowles et al. 1987; Yousef et al. 1990) have not been conclusively proven. Animal (Dalakas et al. 1987) and human (Mora et al. 1988; Dalakas & Pezeshkpour 1988) retroviruses are the first group of viruses that have been consistently associated with polymyositis in adults. In HIV-positive patients, the polymyositis may be the first sign of infection or may be

seen with fully developed AIDS. However, attempts to demonstrate direct viral infection of the muscle fibre by HIV have not been successful (Dalakas 1991).

HTLV-I related muscle disease has been seen in association with HAM/TSP or in patients with dual HIV/HTLV-I infection. In the 3 cases described here, all had associated HAM/TSP and the one patient given steroids showed no response. In the comparison group of 11 isolated AOP, only one had HTLV-I antibodies. Of interest, this patient had a significantly raised titre of antinuclear antibodies and showed the expected steroid responsiveness. Attempts to demonstrate the virus, proviral DNA and viral proteins in the patients have been unsuccessful. This may be due to sampling as the direct infection may be patchy. On the other hand the finding may be truly representative and suggests that HTLV-I triggers off an immune mediated cytotoxic damage similar to that postulated for AOP (Dalakas 1991).

7.6 **CONCLUSION**

This study demonstrated that HTLV-I may be associated with an inflammatory myopathy. However, unlike the Jamaican experience an unequivocal association between HTLV-I and classical isolated AOP has not been demonstrated.

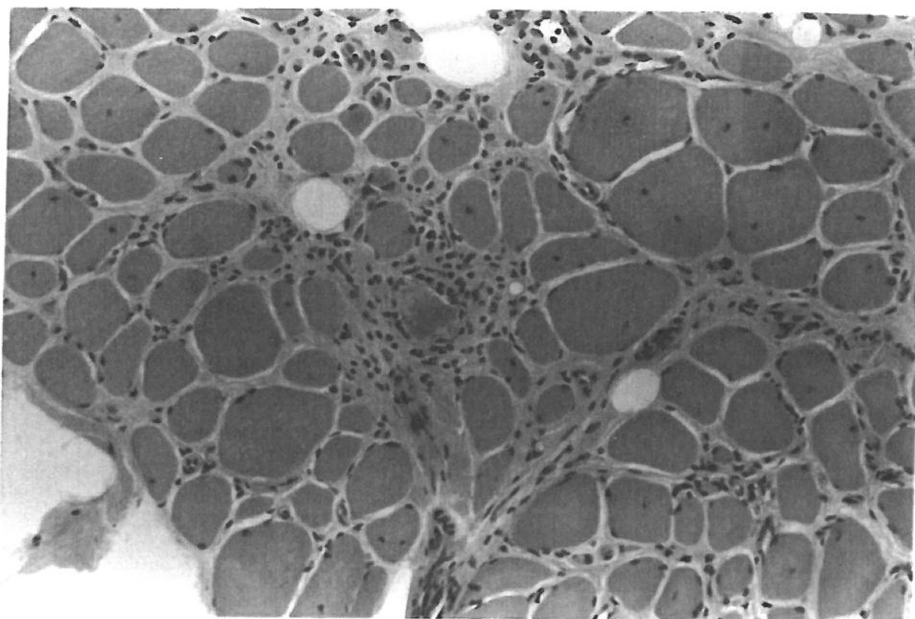


Plate 7.1a

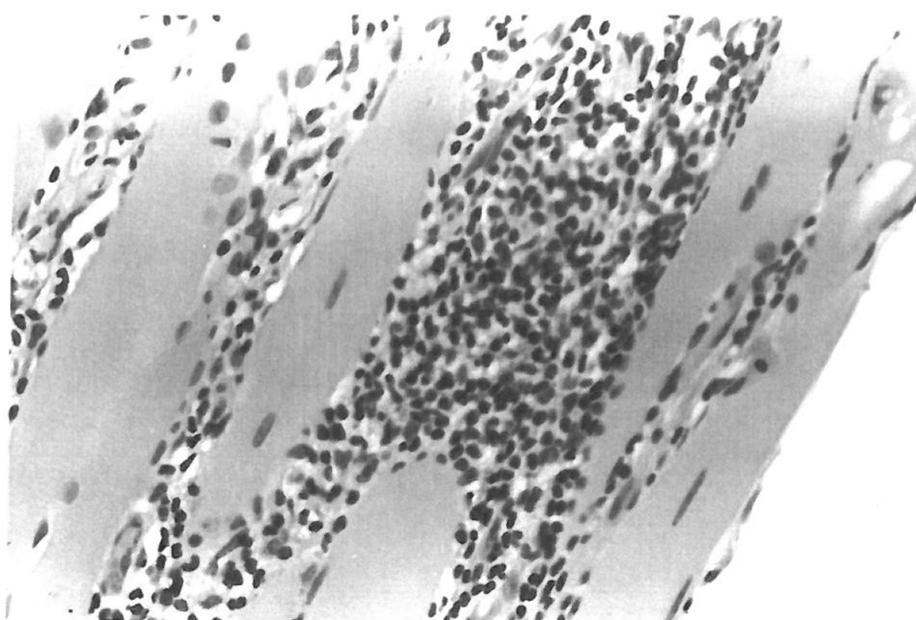


Plate 7.1b

PLATE 7.1 : SECTIONS OF MUSCLE TISSUE SHOWING FEATURES OF AN IMFLAMMATORY MYOPATHY

TABLE 7.1: LABORATORY DATA OF MYOPATHY PATIENTS

TEST	PATIENTS			Isolated Polymyositis Patient
	1	2	3	
Haemoglobin	12.7	4.4	10.0	13.0
White cell count X10^9 ml	10.4	6.1	12.9	8.3
ESR (mm/hr)	94	16	33	21
SPEP	Poly-clonal	Poly-clonal	Poly-clonal	Normal
Antinuclear Antibodies	N	1:50	N	1:800 Speckled
IgG (Norm:7.23-18.88 g/l)	26.0	252 iu	32.5	ND
IgA (Norm : 0.73-12 g/l)	3.16	156 iu	4.41	ND
IgM (Norm: 0.83-177 g/l)	26.0	476 iu	3.78	ND
Creatinine Kinase (Normal 15 - 110 iu)	488 - 729	620 - 895	53	2710
RPR	P(1:16)	N	N	P(1:8)
CSF	Abn	Abn	Abn	Normal

CHAPTER 8

DUAL HTLV-I AND HIV-1 INFECTION: A CLINICO-LABORATORY STUDY OF SIX PATIENTS

8.1 INTRODUCTION

The modes of transmission of HTLV-I and HIV are similar. With the spread of HIV to previously endemic HTLV-I regions, it was not surprising that reports of dual HTLV-I and HIV infection soon followed. Initial studies also noted a high seroprevalence of HTLV-I amongst intravenous drug users (Gradilone et al 1986), but such cases were later shown to be due to HTLV-II infection (Lee et al. 1989). True dual infection has been reported in patients from the USA (Palmar et al. 1985; Harper et al. 1986; Kanner et al. 1987; Chang et al. 1988; Wiley et al. 1989; McArthur et al. 1990; Berger et al. 1991), Zaire (Getchell et al. 1987), the Caribbean (Bartholomew et al. 1987a), Brazil (Cortes et al. 1989) and West Africa (Ramiandrisoa et al. 1991). In the Wentworth Hospital HAM/TSP study, six patients were seropositive for both HTLV-I and HIV. Their clinical and laboratory profiles are presented and the probable pathogenesis of their neurological disorder discussed.

8.2 METHODS

These patients underwent the same range of investigations as the other HAM/TSP cases.

8.3 CASE HISTORIES

8.3.1. **Case 1 (NN - Series no 35)**

A 17 year old Black woman presented to the Neurology Unit in March 1988 with progressive weakness of the legs. After investigation a diagnosis of an inflammatory myelopathy of uncertain cause was made, the patient commenced on antituberculous therapy and discharged.

The patient was lost to follow up until January 1989 when she was readmitted for assessment. She had not continued her antituberculous drugs and felt that her neurological deficit was stable. Examination revealed a spastic quadriparesis. The tendon reflexes were brisk, both plantar responses were extensor and abdominal reflexes absent. The mental state, cranial nerves, coordination and sphincter function were normal. The rest of the clinical examination was normal. Specifically there was no skin rash, lymphadenopathy, hepatosplenomegaly, arthritis or evidence of heart or lung disease.

Investigations done during her most recent admission are summarised in Table 8.1. CSF bacterial (including mycobacterial) and fungal cultures were negative and the cryptococcal antigen was not detected. In vitro T cell function showed slightly reduced response to phytohaemagglutinin stimulation but marked spontaneous lymphocyte transformation. The stimulation index was 5.5 (normal > 100). Delayed hypersensitivity was evaluated using the multitest CMI kit (Institut Merieux). The patient demonstrated

total anergy to all seven antigens tested - tetanus, diphtheria, streptococcus group C, tuberculin, candida, trichophyton and proteus. Following discharge she was lost to follow up.

8.3.2 **Case 2 (FS - Series no 41)**

This 19 year old Black woman presented in 1987 with acute onset of pain in the legs with subsequent weakness progressing over a period of three days to total paralysis. She denied any preceding influenza-like illness, trauma, backache or other neurological symptoms.

Her mental state, cranial nerves and upper limbs were normal. She had a flaccid paraplegia and was incontinent of urine. The tendon jerks at the knees were brisk but absent at the ankles. The plantar responses were equivocal. Apart from mild impairment of joint position sense, sensation was intact. The rest of the physical examination was normal.

The CSF cryptococcal antigen latex agglutination and the cysticercus ELISA tests were negative. Rectal snip demonstrated ova of S. mansoni. The patient was treated with Praziquantel and steroids for presumed spinal schistosomiasis but showed no response. Following discharge she did not return for follow up.

8.3.3. **Case 3 (ZG - Series no 65)**

This 25 year old Black woman presented with a 10 month history of urinary urgency, urge incontinence and progressive weakness of her legs. She denied any other neurological symptoms. She was treated twice in the past for pelvic inflammatory disease.

On examination she was pale and pyrexial. The latter was due to a urinary tract infection and settled on antibiotic treatment. There was no visceromegaly. The mental state and cranial nerves were normal. In the upper limbs the distal power was grade 4/5. There was grade 1 - 2 power in the lower limbs. The tendon jerks in the upper limbs and knees were brisk but were absent at the ankles. The plantar responses were equivocal. She had patchy impairment to pin prick and touch to the T6 dermatome. Joint position and vibration sense were intact.

She was discharged following an improvement in her general condition. She failed to return for review.

8.3.4. **Case 4 (MM - Series no 87)**

This 34 year old Black man developed pain and paraesthesia in his feet, and lumbar backache in July 1990. A month later he developed weakness of the legs which gradually progressed so that he had become wheelchair bound by February 1991. Sphincter dysfunction followed 3 months later.

Examination revealed a normal mental state and cranial nerves. There was mild wasting of the small muscles of the hands but power was normal. The power in legs was graded as 3-4/5 proximally and 0/5 at the ankles and toes. The knee tendon reflexes were brisk but the other jerks were normal. The plantar responses were extensor and the abdominal reflexes absent. Pin prick and light touch was impaired in a graded fashion below the knees. Joint position sense was defective at the toes and vibration sense impaired up to the hips.

8.3.5.**Case 5 (BN - Series no 82)**

This 40 year old Black man presented with a one month history of progressive weakness of the legs, numbness and sphincter dysfunction. He had received blood transfusion 3 years previously following a stab chest.

Examination revealed generalised lymphadenopathy, 2-3 cm in diameter, firm mobile and non tender. The only other abnormality was a spastic paraparesis (grade 4/5) with a T12 sensory level. The tendon reflexes in the legs were brisk and the plantar responses were extensor.

Biopsy of an axillary lymph node showed cortical and paracortical hyperplasia, folliculolysis and sinus histiocytosis. Immunoblasts were numerous and plasma cells were identified in the medulla.

8.3.6. **Case 6 (SM - Series no 67)**

This 26 year old Black man presented with a 2 month history of headache, backache and lower limb weakness. He complained of numbness in the legs but had no sphincter disturbance. He had conjunctivitis and seborrhoeic dermatitis. There was no lymphadenopathy. The mental state and cranial nerves were normal. He had mild distal weakness of the arms. In the legs the power was graded as 2/3 proximally and 0/5 distally. Only the biceps and knee tendon reflexes were elicited. The abdominal reflexes were absent and both plantar responses were extensor. Sensation to all modalities was impaired up to the calves.

The relevant blood tests, the CSF profiles and lymphocyte marker studies of the above cases are summarised in Tables 8.1, 8.2 and 8.3 respectively.

8.4. **DISCUSSION**

In HAM/TSP, besides the myelopathy, the mental state is normal, the cranial nerves are rarely affected, CSF pleocytosis is common and some patients may respond to steroids. Myelopathy in HIV infection may occasionally occur at seroconversion (Denning et al. 1987; unpublished personal data). In these cases the clinical picture is that of an acute transverse myelitis and spontaneous improvement may be noted. Better recognised and more frequent is the vacuolar myelopathy (VM) seen in the advanced stages of HIV infection (Petito et al. 1985; Goldstick et al. 1985). VM is commonly seen in association with dementia and is seldom the sole neurological manifestation of HIV disease. However, subclinical spinal cord

dysfunction has been described in latent HIV infection (Jakobsen et al. 1989). CSF pleocytosis is not a feature.

The pathogenesis of HAM/TSP is unclear and has been discussed in chapter 5. The pathogenesis of VM is equally uncertain. On the one hand there is evidence suggesting direct viral infection (Maier et al. 1989) but on the other Rosenblum et al (1989) found a poor correlation between the presence of productive infection within the cord and VM.

Patients 1 - 4 had a myelopathy as the sole clinical presentation. None had a history or evidence of an AIDS defining illness. The mental state was normal but it must be noted that formal neuropsychological testing was not done. The lymphocytes of patient 1 showed evidence of spontaneous proliferation and a decreased stimulation index. All the features described above would suggest that HTLV-I was responsible for these 4 patients' disease. However, of note is that the CD4/CD8 ratios were low and the absolute CD4 counts were in the low normal range in the patients tested.

Patient 5 had no other neurological manifestations nor a history of opportunistic infection. However, he had generalised lymphadenopathy and biopsy showed features of Primary Generalised Lymphadenopathy (PGL) as seen in HIV infection. His CD4 count was raised and the CD4/CD8 ratio was normal. His neurological problem was probably due to HAM/TSP.

Patient 6 is more problematical and his entire clinical presentation could reflect HIV seroconversion (Denning et al. 1987). Co-infection of HIV and HTLV-I provides an example of virus - virus interaction. There is evidence to suggest that HTLV-I enhances HIV infection. De Rossi et al (1986) demonstrated increased HIV antigen when lymphocytes were co-infected than when infected with HIV alone. Zack et al (1988) noted that HTLV-I virions may exert their effect before or after the cells were infected with HIV-1. In vitro studies have also shown that the HTLV-I transactivator protein tat-1 can activate the long terminal repeats (LTR) of HIV (Siekevitz et al. 1987). Bartholomew et al (1987b) provide clinical evidence for more rapid progression to AIDS in co-infected individuals than in those infected with HIV alone.

Of further interest in the present study is that the reversal óf the CD4/CD8 ratios in two of the three patients tested is in part due to absolute increase in CD8 counts. Early increase in CD8 counts have been noted in HIV infection and may represent an attempt on the part of the immune system to resist HIV or other pathogens (Cooper et al. 1988; Giorgi & Detels 1989). There is evidence that HTLV-I too can cause CD8 proliferation. Harper et al (1988) found that HTLV-I integration of lymphocytes from their dually infected patient was predominantly clonal. The cell lines established by Ehrlich et al (1989) were initially polyclonal but later grew out the same two clones. Their patient also developed nasopharyngeal and pulmonary lymphoid nodules which regressed on steroids and cytotoxic therapy. It could be speculated that destruction of HIV infected CD4 cells may allow unchecked proliferation of CD8 and HTLV-I infected cells.

8.5. CONCLUSION

With the advent of the HIV epidemic in Southern Africa more cases of dual infection are expected and more aggressive HIV-related disease anticipated.

TABLE 8.1: RESULTS OF RELEVANT BLOOD TESTS IN COMBINED HTLV-I/HIV-1 INFECTION

TEST	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4	PATIENT 5	PATIENT 6
Hb g/dl	8.9	12.2	6.6	13.4	13.5	13.1
WCC x 10^9/l	11.0	11.8	3.2	11.6	8.9	7.2
ESR mm/hr	145	46	116	87	36	35
SPEP	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal	-
IgG g/l (7.23-16.8)	82.1	-	32.2	21.5	84.1	-
IgA g/l (0.7-3.12)	2.79	-	4.87	8.92	11.1	-
IgM g/l (0.63-2.77)	6.46	-	1.56	2.19	3.5	-
RPR	Negative	Negative	Pos (1:8)	Pos (1:1)	Negative	Negative

ND = Not done. Pos = positive.
SPEP = serum protein electrophoresis

TABLE 8.2: RESULTS OF CSF TESTS IN COMBINED HTLV-I/HIV-1 INFECTION

CSF TEST	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Polymorphs no/mm^3	2	0	0	0	6	2
Lymphocytes no/mm^3	16	40	2	4	30	30
Total Protein g/l	0.46	1.0	0.45	0.54	1.07	1.00
Globulin	T	+	T	T	T	+ +
Glucose mmol/l	3.0	3.6	2.2	2.8	3.0	2.2
Albumin (0.13 -0.24 g/l	0.11	ND	0.09	ND	ND	0.27
IgG (.055 - 0.06 g/l)	0.49	ND	0.27	ND	ND	0.59
IgG Index (<0.7)	1.3	ND	1.8	ND	ND	1.5
Oligoclonal Bands	Present	ND	Prèsent	Present	Present	ND
FTA	Neg	Neg	Neg	Neg	Neg	Neg

ND = Not done; NEG - Negative; T - Trace

TABLE 8.3: LYMPHOCYTE MARKER STUDIES IN COMBINED HTLV-I/HIV-1 INFECTION

Lymphocyte Test	Patient 1	Patient 3	Patient 4	Patient 5	Control (Mean $\pm$ SD)
Absolute Count	4223	3700	2350	4220	2414 $\pm$ 800
'T' Cells	3013	2828	2046	3782	2030 $\pm$ 568
'B' Cells	1622	936	398	438	605 $\pm$ 235
CD4 Cells	888	573	927	2644	1168 $\pm$ 450
CD8 Cells	2163	2475	1052	1111	903 $\pm$ 406

CHAPTER 9

HTLV-I: SERO-EPIDEMIOLOGICAL OBSERVATIONS IN NATAL/KWA ZULU

9.1 INTRODUCTION

The worldwide prevalence of HTLV-I infection demonstrates marked geographical variation (Table 9.1), ranging from 0% in China to 12% in Japan and 26% in the Melanesia Islands. HTLV-I is also common in Africa (Table 9.2). However, the reliability of some of these estimates is questionable. Gross national estimates may obscure foci of high prevalence. For example the 0.025% seroprevalence in the USA conceals the endemic focus in the South East region of that country. Furthermore, the serological tests have in some hands lacked specificity. The previously reported high seropositivity (37.7%) among the Ethiopian Jews who emigrated to Israel have been shown to be incorrect (Karpas et al. 1986). The initial controversy of the Melanesian studies has been resolved by the occurrence of HAM/TSP (Ajdukiewicz et al. 1989) and the isolation of HTLV-I from that region (Yanagihara et al. 1990). Similarly in Africa Ramiandrisoa et al. (1991) demonstrated lower seroprevalence rates in the Ivory Coast (.18%), Senegal (0.3%), and Burkina Faso (0.8%) than previously reported (De The' et al. 1985).

Seroprevalence of HTLV-I infection in South Africa varies from 0% amongst blood donors of all races to 5.2% amongst Black female staff at the Kruger National Park (Table 9.3). In order to obtain more information about the magnitude of HTLV-I infection in the Natal region, and to evaluate the risk factors for acquiring the virus,

a seroprevalence study was undertaken in the Ngwelezane district of Natal/Kwa Zulu. For comparison, coded samples were tested for HIV 1/2 as well.

9.2 METHODS

The Ngwelezane district was chosen for the survey because (a) cases of HAM/TSP had been seen in this area, (b) the local hospital was conveniently located to act as a base, (c) the area was within easy reach of Durban. Apparently healthy individuals over the age of 15 years were recruited from the Ngwelezane township and nearby shopping complexes. The purpose of the study was explained to groups of individuals. Those who volunteered answered a brief questionnaire (appendix L) and donated a sample of blood.

Blood was collected in a pre-chilled tube and transported to the laboratory in an ice box within 5 hours. Following separation the serum samples were stored at -20°C. Testing was done within 2 months of sample collection. The test kits were the same as those described in Chapter 3. In view of the difficulty in distinguishing HTLV-I from HTLV-II on serological tests all Western Blot HTLV-I positive results were tested for HTLV-II using the Coulter Select-HTLV test kit.

The association between HTLV-I infection and the risk factors of age, gender, age at first sexual experience, blood transfusion and the number of units transfused, number of sexual partners, marital status, occupation and scarifications were assessed using the Chi square (or Fisher's exact) or t-test.

9.3 RESULTS

A total of 1018 individuals, 490 males (48.12%) and 528 females (51.9%), participated in this study. The age range was 15 - 82 years with a mean of 31.5 (SD $\pm$ 12.7) years. Most subjects were unemployed (56.6%), 62.8% were single, 68.7% had evidence of scarification and 13.1% had received blood transfusion in the past. Sixty eight persons declined to provide information about their sexual behaviour and a further 10 indicated that they had never been sexually active. Of the remainder, the mean age at first sexual experience was 17.9 (SD $\pm$ 2.7) years and the mean number of sexual partners was 1.5 (SD $\pm$ 1.07).

A total of 26 (2.6%) (95% confidence interval 1.62 - 3.58) sera, of which 16 were from males, tested positive for anti HTLV-I antibodies. A further two had indeterminate results on Western Blot and for the purposes of analysis were regarded as negative. Another serum demonstrated antibodies to HTLV-II.

Of all the risk factors assessed, only age showed a significant correlation ($p < 0.05$; and figure 9.1). The age-related prevalence rates are summarised in Table 9.4.

Thirty six (3.5%) sera tested positive for anti HIV-1 antibodies. No sample tested positive for antibodies to HIV-2. The relative risk of co-infection with HIV-1 given that the individual was HTLV-I positive was 3.46 (95% confidence interval 1.135 - 10.526).

9.4 DISCUSSION

This study showed the prevalence of HTLV-I infection in the Ngwelezane district to be 2.6%. More males than females were seropositive. The most likely explanation is that the sample contained more older men than older women and there is an age related increase in HTLV-I seropositivity.

The seropositivity increased with age to 6.1% in those over 55 years of age. This finding is consistent with the world-wide experience. The rise with age may relate to the age at exposure to the virus and the time to seroconversion.

The modes of transmission of HTLV-I are from mother to child (predominantly through breast milk), through sexual intercourse, blood transfusion and intravenous drug abuse (Blattner 1989). Obvious parenteral spread can be excluded in the study sample. Intravenous drug abuse is not practised and neither blood transfusion nor scarification showed a significant association with HTLV-I infection. However, the multiply transfused individual will always be at risk.

The problem of mother to child spread is more difficult to evaluate because of the question of latent, seronegative HTLV-I infection. About 20% of children of seropositive mothers convert, usually between 12 and 24 months of age (Hino et al. 1985). The seroprevalence rate in children then remains the same until the teenage years (Kajiyama et al. 1986). An explanation proposed for this observation is that some children of seropositive mothers may seroconvert only decades later as suggested by experimental (Yamada et al. 1991) and some human studies (Ehrlich

& Poeisz 1988; Saito et al. 1989). On the other hand, studies by another Japanese group (Nakashima et al. 1990) and Pate et al (1991) failed to confirm a sero-negative HTLV-I carrier state in children. In a separate paediatric study of 561 healthy Zulu children under the age of 16 years I found no cases of HTLV-I seropositivity (unpublished data). In another small unpublished study 4 offspring of 3 women with HAM/TSP were tested for HTLV-I seropositivity. Only one, a 2 year old, was positive whilst her 10 year old sibling was negative. The interpretation of this finding is made difficult by the practice of multiple sexual partners as the time of maternal acquisition of infection and hence spread to her offspring could be variable. Another factor to consider is that, whilst breast feeding is almost universally practised, economic needs often necessitate premature weaning.

The main mode of transmission in our subjects remains to be defined. However, the relative risk of 3.46 for co-infection with HIV-1 would suggest that sexual transmission is important.

9.5 CONCLUSION

This seroprevalence study, together with the cases of HAM/TSP confirm the Natal region to be an endemic HTLV-I area.

This study also established for the first time serological evidence of HTLV-II in this region.

TABLE 9.1: SELECTED WORLDWIDE HTLV-I SEROPREVALENCE RATES

Country/ area	% +ve	No Tested	Reference	Comment
Japan	6-37%	1028	Hinuma et al 1982	Mixed sample: healthy & non malignant disease
Japan-Kagoshima	11.9	4741	Hanada et al 1989	Healthy residents & blood donors
Melanesian Islands	26	317	Kazura et al 1987 Babona & Nurse 1988	Healthy residents Blood donors
Australian Aborigines	16	653	May et al 1990	Included hospi-talised patients
Australia Blood donors	0.21	4641	Bolton et al 1989	ELISA test only
Brazil	1-13	70	Cortes et al 1989	At risk individuals
Arctic Regions	0-12	905	Robert Guroff et al 1985	Mixed populations. Banked sera
Iran N-E Region	12	208	Meytes et al 1990	Iranian Jews emigrated to Israel
Colombia	10	NS	Rodgers-Johnson et al 1988	not mentioned
Seychelles	6.8	1055	Lavanchy et al 1991	Healthy residents
Caribbean Islands	4	352	Blattner et al 1982	Urban residents
Taiwan	0.9	2545	Pan et al 1985	Blood donors
U.S.A.	0.025	40000	Williams et al 1988	Blood donors
Korea	0.0	373	Hinuma et al 1983	Urban residents
China	0.0	6884	Zeng et al 1984	Urban residents

NS = Not stated

TABLE 9.2: SELECTED HTLV-I SEROPREVALENCE RATES IN AFRICA

Country/ area	% +ve	No Tested	Reference	Comment
North Western Tanzania	16.9	468	De The' et al 1985	Banked sera
Ivory Coast	16.0	100	De The' et al 1985	Banked sera
South Zaire	14.2	187	De The' et al 1985	Banked sera
South Sudan	9.2	76	De The' et al 1985	Banked sera
N-E Uganda	8.0	101	De The' et al 1985	Banked sera
Ghana	8.0	236	Saxinger et al 1984	Normal population
Gabon	6.3	1340	Hunsmann et al 1984	Mixed population
Burkina Faso	4.6	43	De The' et al 1985	Banked sera
Zaire	3.2	62	Hunsmann et al 1984	Village population
Nigeria	2.6	390	Hunsmann et al 1984	Blood donors
Kenya	1.7	231	Hunsmann et al 1984	Normal population
Liberia	1.6	620	Hunsmann et al 1984	Village population
Senegal	1.2	993	Hunsmann et al 1984	Village population
Morocco	0.6	677	De The' et al 1985	Banked sera
Namibia	0.0	704	Lecatsas et al 1988	Healthy residents

TABLE 9.3: SELECTED HTLV-I SEROPREVALENCE IN SOUTH AFRICA

Area	% ve	No Tested	Reference	Comment
Durban	5	20	Hunsmann et al 1984	Black adults
Johannesburg	0	104	Saxinger et al 1984	Black blood donors
Cape Town	5.3	283	Saxinger et al 1984	Black & white blood donors
Natal & Cape Town	3.5	543	Becker et al 1985	Black blood donors
Kruger Park: Male Female	3.2 1.2 5.2	688	Botha et al 1985	Black staff
Natal	0	5603	Neill & Fernandes-Costa 1990	Blood donors - all races.

TABLE 9.4: AGE ADJUSTED HTLV-I SEROPREVALENCE IN DIFFERENT AGE GROUPS

Age Group (years)	Males	Females
15 - 24	0.59	1.42
25 - 34	2.27	2.11
35 - 44	7.58	1.32
45 - 54	4.76	3.70
55+	6.67	4.55
TOTAL (Crude) (%)	3.27	1.90
AGE ADJUSTED	2.65	1.70

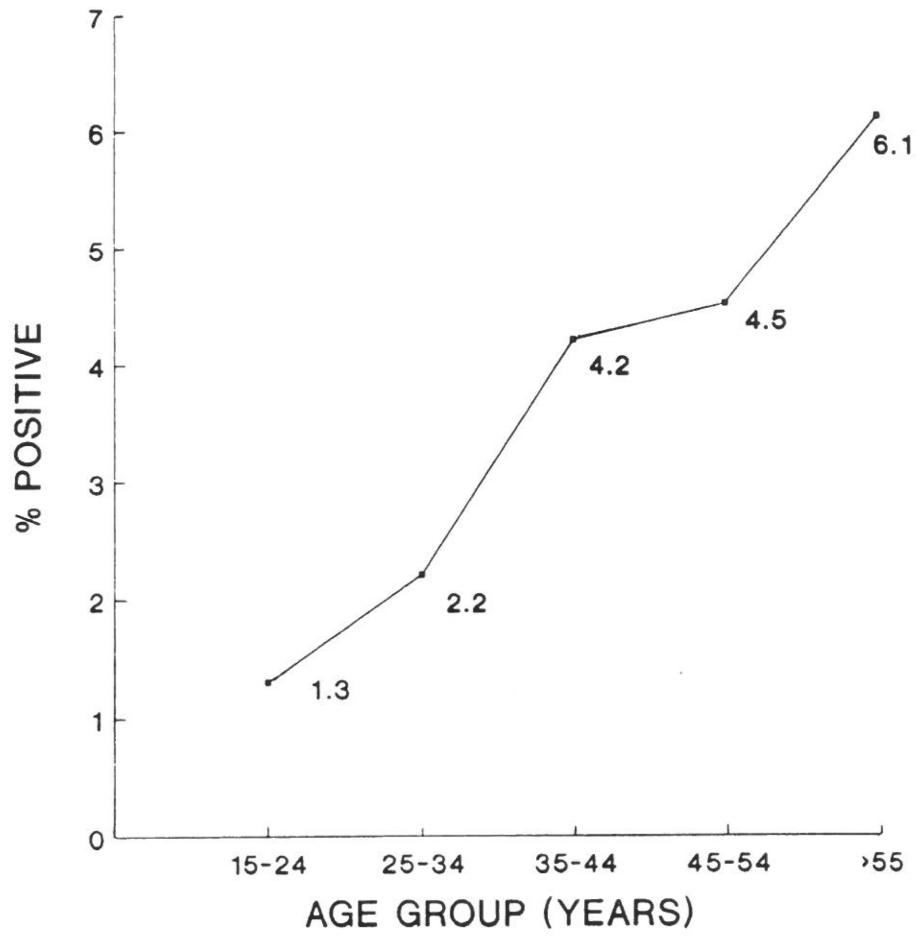


FIGURE 9.1: AGE RELATED RISE IN HTLV-I SEROPOSITIVITY

CHAPTER 10

CONCLUSIONS AND FUTURE HTLV-I RESEARCH

10.1 PATHOGENESIS OF HAM/TSP

Whilst the association between HAM/TSP and HTLV-I is well established, the pathogenesis remains elusive. The current evidence would suggest that a co-factor is necessary for the development of the myelopathy. This co-factor may be a genetic predisposition. MHC restriction has been demonstrated in this regard (Usuku et al 1990; Jacobson et al 1990b). The paucity of post mortem spinal cord tissue has also hampered progress in determining the pathogenesis of HAM/TSP. The presence of gag proviral DNA in the spinal cord has been demonstrated (Chapter 5). This study, however, does not define the cell type harbouring the proviral DNA. The use of in-situ PCR (Bagasra et al 1992) may prove informative in this regard.

There is lack of a suitable animal model. Although experimental animals can be easily infected with HTLV-I, the virus does not produce disease in them. Whether infection of the SCID-HU mice results in disease remains to be seen.

10.2 CLINICAL ASPECTS OF HAM/TSP

10.2.1. Diagnosis

The diagnosis of HAM/TSP is straightforward. Of greater importance are cases resembling HAM/TSP but are antibody negative. These cases may

have a completely different aetiology or may in fact be true seronegative cases of HAM/TSP . Using PCR d'Auriol et al (1990) were able to detect proviral HTLV-I DNA in the PBLs of 18 out of 20 antibody negative cases. This observation requires further study.

10.2.2. **Management**

The successful treatment of HAM/TSP with immunosuppression and plasmapheresis (Osame et al 1987; Matsuo et al 1988) in Japanese patients has not been duplicated elsewhere. The French have tried Zidovudine without success (Gout et al. 1991). High dose methylprednisolone, too, showed little benefit (Duncan & Ridge 1990). A properly randomised double blind placebo controlled trial is required to objectively evaluate the different forms of therapy.

10.3 **EPIDEMIOLOGY**

Seroepidemiological studies have indicated that HTLV-I infection is widespread. Foci of infection have been noted in virtually every continent and the evidence suggests that HTLV-I is an ancient virus. Less certain is the major mode of spread in some of the areas. In Japan, however, screening blood donors has virtually eliminated spread by blood transfusion (Osame et al. 1990b). Mother to child spread can be effectively prevented by prohibiting breast feeding but this would have serious implications for the infant in Third World countries.

10.4. **HTLV-I AND OTHER NEUROLOGICAL DISEASE**

There is no doubt that the demonstration of an association between HTLV-I and neurological disease represents a major advance. It has sparked off the search for viruses in other unexplained neurological disorders. A retrovirus may yet be aetiologically linked to Multiple Sclerosis (Perron et al. 1991) despite the earlier controversy.

10.5 **FUTURE HTLV-I RESEARCH IN SOUTH AFRICA**

As a follow up to this thesis the following HTLV-I related projects are being planned:

(1) The search for MHC restriction in our HAM/TSP using PCR

(2) Determining extent of intrafamilial spread of the virus using serological tests and PCR

(3) The search for HTLV-I proviral DNA in antibody negative cases of non-compressive myelopathy

(4) The setting up of a national register of HAM/TSP cases.

BIBLIOGRAPHY

Ajdukiewicz AL, Yanagihara R, Garruto RM, Gajdusek DC, Alexander SS. HTLV-I myeloneuropathy in the Solomon Islands. *N Engl J Med* 1989; 321(9): 615 - 616.

Akizuki S, Setoguchi M, Makazato O et al. An autopsy case of human T-lymphotropic virus type I - associated myelopathy. *Hum Pathol* 1988; 19: 988 - 990.

Arimura K, Rosales R, Osame M, Igata A. Clinical electrophysiologic studies of HTLV-I associated myelopathy. *Arch Neurol* 1987; 44: 609 - 612.

Babona DV, Nurse GT. HTLV-I antibodies in Papua, New Guinea. *Lancet* 1988; ii : 1148.

Bagasra O, Hauptman SP, Lischner HW, Sachs M, Pomerantz RJ. Detection of human immunodeficiency virus type I provirus in mononuclear cells by in-situ polymerase chain reaction. *N Engl J Med* 1992; 326(21): 1385 - 1391.

Barkhaus PE, Morgan O. Jamaica Neuropathy: an electrophysiological study. *Muscle Nerve* 1988; 11: 380 -385.

Bartholomew C, Saxinger C, Clark JW et al. Transmission of HTLV-I and HIV among homosexual men in Trinidad. *JAMA* 1987a; 257 (19): 2604 - 2608.

Bartholomew C, Blattner W, Cleghorn F. Progression of AIDS in homosexual men co-infected with HIV and HTLV-I in Trinidad. *Lancet* 1987b; ii: 1469.

Becker WB, Becker MLB, Homma T, Brede HD, Kurth R. Serum antibodies to human T-cell leukaemia virus type-I in different ethnic groups and in non-human primates in South Africa. *S Afr Med J* 1985; 67: 445 - 449

Becker WB, Botha MC, Engelbrecht S, Becker MLB. Isolation of human T-lymphotropic virus type I (HTLV-I) from a black South African with Kaposi's sarcoma. *S Afr Med J* 1988; 73: 481 - 483.

Behse F. Morphometric studies on the human sural nerve. *Acta Neurol Scand* 1990; 82 (suppl): 1 - 38.

Berger JR, Raffanti S, Svenningson A, McCarthy M, Snodgrass S, Resnick L. The role of HTLV in HIV-1 neurological disease. *Neurology* 1991; 41: 197 - 202.

Bhagavati S, Ehrlich G, Kula RW et al. Detection of human T-cell lymphoma/leukemia virus Type I DNA and antigen in spinal fluid and blood of patients with chronic progressive myelopathy. *N Engl J Med* 1988; 318(18): 1141 - 1437.

Blattner WA, Kalyanaraman VS, Robert-Guroff M et al. The human type-C retrovirus, HTLV, in blacks from the Caribbean region and relationship to adult T-cell leukemia/lymphoma. *Int J Cancer* 1982; 30: 257 - 264.

Blattner WA. Retroviruses. In: Evans AS, ed. *Viral infections and human epidemiology and control.* 3rd ed. New York: Plenum Press, 1989: 545 - 592.

Bolton WV, Wylie BR, Kenrick KGX et al. HTLV-I and blood donors. *Lancet* 1989; i : 1324 - 1325.

Botha MC, Jones M, De Klerk WA, Yamamoto N. Distribution and possible spread of human T-cell leukaemia virus type I in human communities in northern and eastern Transvaal. *S Afr Med J* 1985; 67: 668 - 671.

Bowles NE, Dubowitz V, Sewry CA, Archard LC. Dermatomyositis, polymyositis and Coxsachie-B-virus infection. *Lancet* 1987; 1: 1004 - 1007.

Casadei E, Jansen P, Rodrigues A, Molin A, Rosling H. (1) Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. (2) Nutritional factors and hydrocyanic acid content of cassava products. *Bull World Health Organ* 1984; 62: 485 - 492.

Ceroni M, Piccardo P, Rodgers-Johnson P et al. Intrathecal synthesis of IgG antibodies to HTLV-I supports an aetiological role for HTLV-I in tropical spastic paraparesis. *Ann Neurol* 1988; 23(suppl): S188 - S191.

Chang KSS, Wang LC, Gao C et al. Concomitant infection of HTLV-I and HIV-1: prevalence of IgG and IgM antibodies in the Washington DC area. *Eur J Epidemiol* 1988; 4: 426 - 434.

Cooper DA, Tindall B, Wilson EJ et al. Characterisation of T lymphocyte responses during primary infections with human immunodeficiency virus. *J Infect Dis* 1988; 157: 889 - 896.

Cortes E, Detels R, Aboulafia D et al. HIV-1, HIV-2, HTLV-I infection in high risk groups in Brazil. *N Engl J Med* 1989; 320: 953 - 958.

Cosnett JE. Unexplained spastic myelopathy : 41 cases in a non-European hospital. *S Afr Med J* 1965; 39: 592 - 595.

Cruickshank EK. A neuropathic syndrome of uncertain origin, review of 100 cases. *W Indian Med J* 1956; 5: 147 - 158.

Cruickshank JK, Rudge P, Dalgleish AG et al. Tropical spastic paraparesis and human T-cell lymphotropic virus type I in the United Kingdom. *Brain* 1989; 112: 1057 - 1090.

d'Auriol L, Vernant J-C, Ouka M et al. Diagnosis of HTLV-I infected seronegative neurological patients by polymerase chain reaction amplification in Martinique. *Nouv Rev Fr Hematol* 1990; 32: 113 - 116.

Dalakas MC, Gravell M, London WT, Cunningham G, Sever JL. Morphological changes of an inflammatory myopathy in rhesus monkeys with simian acquired immunodeficiency syndrome. *Proc Soc Exp Biol Med* 1987; 185: 368 - 376.

Dalakas MC, Pezeshkpour GH. Neuromuscular disease associated with human immunodeficiency virus infection. *Ann Neurol* 1988; 23(Suppl) :S38 - S48.

Dalakas MC. Polymyositis, dermatomyositis and inclusion body myositis. *N Engl J Med* 1991; 325(21): 1487 - 1498.

Dalgleish A, Richardson J, Matutes E et al. HTLV-I infection in tropical spastic paraparesis: lymphocyte culture and serological response. *Aids Res Hum Retro* 1988; 4(b): 475 - 485.

Danilovs PI, Ayoub G, Terasaki PI. B lymphocyte isolation by thrombin-nylon wool. In: Terasaki PI ed. *Histocompatibility Testing 1980* . Los Angeles: University of California Press, 1980: 287 - 288.

De The G, Gessain A, Gazzalo L et al. Comparative sero-epidemiology of HTLV-I and HTLV-III in the French West Indies and some African countries. *Cancer Res (Suppl)* 1985; 45: 4633s - 4646s.

De Rossi A, Franchini G, Aldovini A et al. Differential response to cytopathic effects of human T-lymphotropic virus type III (HTLV III) superinfection in T4 (helper) and T8 (Suppressor) T-cells transformed by HTLV-I. *Proc Natl Acad Sci USA* 1986; 83: 297 - 301.

Denning DW, Anderson J, Rudge P, Smith H. Acute myelopathy associated with primary infection with human immunodeficiency virus. *BMJ* 1987; 294: 143 - 144.

Duncan J, Rudge P. Methyl prednisolone therapy in tropical spastic paraparesis. *J Neurol Neurosurg Psychiatr* 1990; 53: 173 - 174.

Eeg-Olofsson O, Link H, Wigertz A. Concentrations of CSF proteins as a measure of blood brain barrier function and synthesis of IgG within the CNS in "normal" subjects from age of 6 month to 30 years. *Acta Paediatr Scand* 1981; 70: 167 - 170.

Ehrlich G, Poeisz B. Clinical and molecular parameters of HTLV-I infection. *Clinics Lab Med* 1988; 8: 65 - 84.

Ehrlich GD, Davey FR, Kirshner JJ et al. A polyclonal CD4+ and CD8+ lymphocytosis in a patient doubly infected with HTLV-I and HIV-1: A clinical and molecular analysis. *Am J Hematol* 1989; 30: 128 - 139.

Evans BK, Gore I, Harrel LE, Arnold T, Oh SJ. HTLV-I associated myelopathy and polymyositis in a US native. *Neurology* 1989; 39: 1572 - 1575.

Forcier NJ, Mizisin AP, Rimmer MA, Powell HC. Cellular pathology of the nerve micro-environment in galactose intoxication. *J Neuropathol Exptl Neurol* 1991; 50: 235 - 255.

Francis DA, Hughes RAC. Polymyositis and HTLV-I antibodies. *Ann Neurol* 1989; 25(3): 311.

Gallo RC, Sliski A, Wong-Staal F. Origin of human T-cell leukemia-lymphoma virus. *Lancet* 1983; ii: 962 - 963.

Gessain A, Barin JF, Vernant JC et al. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 1985; ii: 407 - 410.

Getchell JP, Heath JL, Hicks DR, Sporborg C, Mann JM, McCormick JB. Detection of human T-cell lymphotropic virus type I and human immunodeficiency virus in cultured lymphocytes of Zairian man with AIDS. *J Infect Dis* 1987; 155(4): 612 - 616.

Giorgi JC, Detels R. T-cell subset alterations in HIV-infected homosexual men: NIAID multicentre AIDS cohort study. *Clin Immunol Immunopathol* 1989; 52: 10 - 18.

Goldstick L, Mandybur TI, Bode R. Spinal cord degeneration in AIDS. *Neurology* 1985; 35: 103 - 105.

Goudreau G, Karpati G, Carpenter S. Inflammatory myopathy in association with chronic myelopathy in HTLV-I seropositive patients. *Neurology* 1988; 38(Suppl 1): 206.

Gout O, Banlac M, Gessain A et al. Rapid development of myelopathy after HTLV-I infection acquired by transfusion during cardiac transplantation. *N Engl J Med* 1990; 322: 383 - 388.

Gout O, Gessain A, Ibn-Zizen M et al. The effect of Zidovudine on chronic myelopathy associated with HTLV-I. *J Neurol* 1991; 238: 108 - 109.

Gradilone A, Zani M, Barillari G et al. HTLV-I and HIV infection in drug addicts in Italy. *Lancet* 1986; i: 753 - 756.

Grafe GM, Wiley CA. Spinal cord and peripheral nerve pathology in AIDS: the roles of cytomegalovirus and human immunodeficiency virus. *Ann Neurol* 1989; 25: 561 - 566.

Hanada S, Uematsu T, Iwahashi M et al. The prevalence of human T-cell leukaemia virus type-I infection in patients with haematologic and non-haematologic diseases in an adult T-cell leukemia endemic area of Japan. *Cancer* 1989; 64: 1290 - 1295.

Harper ME, Kaplan MH, Marselle LM et al. Concomitant infection with HTLV-I and HTLV-III in a patient with T8 lymphoproliferative disease. *N Engl J Med* 1986; 315(17): 1073 - 1078.

Hino S, Yomaguchi K, Katamine S et al. Mother-to-child transmission of human T-cell leukemia virus type-I. *Jpn J Cancer Res* 1985; 76: 478 - 480.

Hinuma Y, Komoda H, Chosa T et al. Antibodies to adulŕ T cell leukemia virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide seroepidemiologic study. *Int J Cancer* 1982; 29: 631 - 635.

Hinuma Y, Chosa T, Komoda H et al. Sporadic retrovirus (ATLV) -seropositive individuals outside Japan. *Lancet* 1983; i: 824 - 825.

Hunsmann G, Bayer H, Schneider J et al. Antibodies to ATLV/ HTLV-I in Africa. *Med Microbiol Immunol* 1984; 173: 167 - 170.

Ishida T, Hinuma Y. The origin of Japanese HTLV-I. *Nature* 1986; 332: 504.

Iwasaki Y. Pathology of chronic myelopathy associated with HTLV-I infection (HAM/TSP). *J Neurol Sci* 1990; 96: 103 -123.

Jacobs JM, Love S. Qualitative and quantitative morphology of the human sural nerve at different ages. *Brain* 1985; 108: 897 - 924.

Jacobson S, Gupta A, Mattson D, Minigioli E, McFarlin DE. Immunological studies in tropical spastic paraparesis. *Ann Neurol* 1990a; 27: 149 - 156.

Jacobson S, Shida H, McFarlin DE et al. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* 1990b; 348: 245 -248.

Jakobsen J, Smith T, Gaub J, Helwag-Larsen S, Trojaberg W. Progressive neurological dysfunction during latent HIV infection. *BMJ* 1989; 299: 225 - 228.

Jogessar VB, De Bruyn CC, Bhigjee AI, Naicker VL, Bill PLA, Tait D. Adult T-cell leukaemia/lymphoma associated with HTLV-I in Natal. *S Afr Med J* 1992; 81: 528 - 529.

Kajiyama W, Kashiwagi S, Hayashi J et al. Intra-familial clustering of anti-ATLA-positive persons. *Am J Epidemiol* 1986; 124: 800 - 806.

Kajiyama Wl, Kashiwagi S, Ikematsu H, Hayashi J, Nomura H, Okochi K. Intrafamilial transmission of adult T-cell leukemia virus. *J Infect Dis* 1986; 154: 851 - 857.

Kakigi R, Shibasaki H, Kuroda Y et al. Multimodality evoked potentials in HTLV-I associated myelopathy. *J Neurol Neurosurg Psychiatr* 1988; 51: 1094 - 1096.

Kanner SB, Parks ES, Scott GB, Parks WP. Simultaneous infections with human T-cell leukemia virus type I and the human immuno-deficiency virus. *J Infect Dis* 1987; 155(4): 617 - 625.

Karpas A, Maayan S, Raz R. Lack of antibodies to adult T-cell leukaemia virus and to AIDS virus in Israeli Falashas. *Nature* 1986; 319: 794.

Kazura JW, Saxinger WC, Wenger J et al. Epidemiology of human T-cell leukemia virus type I infection in East Sepik Province, Papua, New Guinea. *J Infect Dis* 1987; 155(6): 1100 - 1107.

Kelly R, De Mol B. Paraplegia in the islands of the Indian Ocean. *Afr J Neurol Sci* 1982; 1: 5 - 7.

Kermode AG, Rudge P, Thompson AJ, Du Boulay EPGH, McDonald WI. MRI of thoracic cord in tropical spastic paraparesis. *J Neurol Neurosurg Psychiatr* 1990; 53 : 1110.

Kier G, Luxton RW, Thompson EJ. Isoelectric focusing of cerebrospinal fluid immunoglobulin G: an annotated update. *Ann Clin Biochem* 1990; 27: 436 - 443.

Kira J, Minato S, Itoyama Y, Goto I, Kato M, Hasuo K. Leukoencephalopathy in HTLV-I associated myelopathy: MRI and EEG data. *J Neurol Sci* 1988; 87: 221 - 232.

Lavanchy D, Bovet P, Hollanda J, Shamlaye CJF, Burezak JD, Lee H. High seroprevalence of HTLV-I in the Seychelles. *Lancet* 1991; 337: 248 - 249.

Lecatsas G, Joubert JJ, Schutte CHJ, Taylor MB, Swanevelder C. HTLV-I seropositivity in east Caprivi, SWA/Namibia. *S Afr Med J* 1988; 74: 643 - 644.

Lee H, Swanson P, Shorty VS, Zack JA, Rosenblatt JE, Chen IS. High rates of HTLV-II infection in seropositive IV drug abusers in New Orleans. *Science* 1989; 244: 471 - 475.

Lefvert AK, Link H. IgG production within the central nervous system: a critical review of proposed formulae. *Ann Neurol* 1985; 17 : 13 - 20.

Liberski PP, Rodgers-Johnson P, Char G, Piccardo P, Gibbs CJ, Gajdusek DC. HTLV-I-like viral particles in spinal cord cells in Jamaican tropical spastic paraparesis. *Ann Neurol* 1988; 23(Suppl): S185 - S187.

Ludolph AC, Hugon J, Roman GC et al. A clinical neurophysiologic study of tropical spastic paraparesis. *Muscle Nerve* 1988; 11: 392 - 397.

Mani KS, Mani AJ, Montgomery RD. A spastic paraplegic syndrome in South India. *J Neurol Sci* 1969; 9: 179 - 199.

Manns A, Blattner WA. The epidemiology of the human T-cell lymphotropic virus type I and II: etiologic role in human disease. *Transfusion* 1991; 31: 67 - 75.

Matsuo H, Nakamura T, Tsujihata T et al. Plasmapheresis in treatment of human T lymphotropic virus type-I associated myelopathy. *Lancet* 1988; ii: 1109 - 1113.

Mattiuz PL, Ihde D, Piazza A et al. New approaches to the population genetics and segregation analysis of the HLA system. In: Terasaki ed. *Histocompatibility Testing* Copenhagen: Munksgaard 1970: 193 - 206.

May JT, Stent G, Bishop F, Schnagel D. Prevalence of antibody of human T-lymphotropic virus type-I (HTLV-I) in Australian Aborigines and detection in Indonesian sera. *Acta Virol* 1990; 34: 80 - 84.

McArthur JC, Griffin JW, Cornblath DR et al. Steroid responsive myeloneuropathy in a man dually infected with HIV-1 and HTLV-I. *Neurology* 1990; 40: 938 - 944.

McLean BN, Rudge P, Thompson EJ. Viral specific IgG and IgM antibodies in the CSF of patients with tropical spastic paraparesis. *J Neurol* 1989; 236: 351 - 352.

Meytes D, Schochat B, Lee H et al. Serological and molecular survey for HTLV-I infection in a high risk Middle Eastern group. *Lancet* 1990; 336 (i); 1533 - 1536.

Montgomery RD, Cruickshank EK, Robertson WB, McMenemey WH. Clinical and Pathological observations on Jamaican neuropathy: A report on 206 cases. *Brain* 1964,: 87: 425 - 462.

Mora CA, Garruto RM, Brown P et al. Seroprevalence of antibodies to HTLV-I in patients with chronic neurological disorders other than tropical spastic paraparesis. *Ann Neurol* 1988; 23(suppl): S192 - S195.

Morgan O StC, Rodgers-Johnson P, Mora C, Char G. HTLV-I and polymyositis in Jamaica. *Lancet* 1989; ii: 1184 - 1187.

Mori M, Kinoshita K, Ban N, Yamada Y, Shiku H. Activated T-lymphocytes with polyclonal gammopathy in patients with human T-lymphotropic virus type I associated myelopathy. *Ann Neurol* 1988; 24: 280 - 282.

Nakashima M, Itagaki A, Yamada O et al. Evidence against a seronegative HTLV-I carrier rate among children. *Aids Res Hum Retroviruses* 1990; 6: 1057 - 1058.

Nakazato O, Mori T, Okajima T. Sural nerve pathology in HTLV-I associated myelopathy. *Neurol Neurobiol* 1989: 51: 269 - 274.

Neill AGS, Fernandes-Costa FJTD. Anti HTLV-I in blood donors in Natal *S Afr Med J* 1990; 78: 376.

Newton M, Cruickshank JK, Miller D et al. Antibodies to HTLV-I in West Indian born UK resident patients with spastic paraparesis. *Lancet* 1987; i: 415 - 416.

Nishimoto N, Yoshizaki I, Eiraku N et al. Elevated levels of interleukin-6 in serum and cerebrospinal fluid of HTLV-I associated myelopathy/tropical spastic paraparesis. *J Neurol Sci* 1990; 97: 183 - 194.

Nishimura Y, Okubo R, Minato S et al. A possible association between HLA and HTLV-I associated myelopathy (HAM) in Japanese. *Tissue Antigens* 1991; 37: 230 - 231.

Osame M, Matsumoto M, Usuku K et al. Chronic progressive myelopathy associated with elevated antibodies to human T-lymphotropic virus type I and adult T-cell leukemia like cells. *Ann Neurol* 1987; 21: 117 - 122.

Osame M, Igata A, Matsumoto M, Kohka M, Usuku K, Izumo S. HTLV-I associated myelopathy (HAM): treatment trials, retrospective survey and clinical laboratory findings. *Hematol Rev* 1990a; 3: 271 - 284.

Osame M, Jansen R, Kubota H et al. Nation wide survey of HAM/TSP in Japan: association with blood transfusion. *Ann Neurol* 1990b; 28: 50 - 56.

Osuntokun BO. An ataxic neuropathy in Nigeria: a clinical, biochemical and electrophysiological study. *Brain* 1968; 91: 215 - 248.

Palmer EL, Harrison AK, Ramsey RB et al. Detection of two human T-cell leukemia/lymphotropic viruses in cultured lymphocytes of a haemophiliac with acquired immunodeficiency syndrome. *J Infect Dis* 1985; 151: 559 - 563.

Pan IH, Chung CS, Komada H, Hinuma Y. Sero-epidemiology of adult T-cell leukemia virus in Taiwan. *Gann* 1985; 76: 9 - 11.

Pate EJ, Wiktor SZ, Shaw GM, Champagnie E, Murphy EL, Blattner WA. Lack of viral latency of human T-cell lymphotropic virus type I. *N Engl J Med* 1991; 325(4): 284.

Perron H, Lalande B, Gratacap B et al. Isolation of retrovirus from patient with multiple sclerosis. *Lancet* 1991; 337: 862 -863.

Petito CK, Navia BA, Cho E-S, Jordan BD, George DC, Price RS. Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with acquired immunodeficiency syndrome. *N Engl J Med* 1985; 312(14): 874 - 879.

Piccardo P, Ceroni M, Rodgers-Johnson P et al. Pathological and immunological observations on tropical spastic paraparesis in patients from Jamaica. *Ann Neurol* 1988; 23(suppl): S156 - S160.

Poeisz BJ, Ruscetti FW, Gazdar AF et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980; 77: 7415 - 7419.

Power C, Weinshenker BG, Dekaban GA, Kaufmann JC, Shandling M, Rice GP. Pathological and molecular biological features of a myelopathy associated with HTLV-I infection. *Can J Neurol Sci* 1991; 18(3): 352 - 355.

Prasad LS, Sharan RK. Lathyrism. In Vinken PJ, Bruyn GW, eds. *Handbook of Clinical Neurology*. Amsterdam: North-Holland, 1979; 36: 505 - 514.

Ramiandrisoa H, Dumas M, Giordano C et al. Human retroviruses HTLV-I, HIV-1, HIV-2 and neurological disease in West Africa. *J Trop Geogr Neurol* 1991; 1: 39 - 44.

Robert-Guroff M, Clarke J, Lanier AP et al. Prevalence of HTLV-I in Arctic regions. *Int J Cancer* 1985; 36: 651 - 655.

Rodgers-Johnson PE, Garruto RM, Gajdusek DC. Tropical myeloneuropathies - a new aetiology. *TINS* 1988; 11 (12): 526 - 532.

Rodgers-Johnson P, Morgan O StC, Mora C et al. The role of HTLV-I in tropical spastic paraparesis in Jamaica. *Ann Neurol* 1988; 23(suppl): S121 - S126.

Roman GC, Roman LN, Spencer PS, Schoenberg BS. Tropical spastic paraparesis: a neuroepidemiological study in Colombia. *Ann Neurol* 1985; 17: 361 - 365.

Roman GC, Schoenberg BS, Madden DL et al. Human T-lymphotropic virus type I antibodies in the serum of patients with tropical spastic paraparesis in the Seychelles. *Arch Neurol* 1987; 44: 605 - 607.

Roman G. The neuroepidemiology of tropical spastic paraparesis. *Ann Neurol* 1988; 23(suppl): S113 - S120.

Said G, Goulon-Goeau C, Lacroix C et al. Inflammatory lesion of peripheral nerve in a patient with human T-lymphotropic virus type I - associated myelopathy. *Ann Neurol* 1988 24: 275 - 277.

Saito S, Ando Y, Furuki J et al. Detection of HTLV-I genome in seronegative infants born to HTLV-I seropositive mothers by polymerase chain reaction. *Jpn J Cancer Res* 1989; 80: 808 - 812.

Saxinger W, Blattner WA, Levine PH et al. Human T-cell Leukemia virus (HTLV-I) antibodies in Africa. *Science* 1984; 225: 1473 - 1476.

Siekevitz M, Josephs SF, Dukovick M, Peffer J, Wong-Staal F, Goene JWC. Activation of HIV-1 LTR by T cell mitogens and the transactivator protein of HTLV-I. *Science* 1987; 238: 1575 - 1578.

Sugimura K, Takahashi A, Watanabe M et al. Demyelinating changes in sural nerve biopsy of patients with HTLV-I associated myelopathy. *Neurology* 1990; 40: 1263 - 1266.

Svejgaard A, Jersild L, Staub-Nielsen L, Bodmer WF. HLA antigens and disease: Statistics and genetical considerations. *Tissue Antigens* 1974; 4: 95 - 105.

Svejgaard A, Platz P, Ryder LP et al. HLA and disease associations - a survey. In: Moller G ed. *HLA and disease. Transplant Review 22* . Copenhagen: Munksgaard, 1975: 3 - 44.

Tarras S, Sheremata WA, Snodgrass S, Aygar DR. Polymyositis and chronic myelopathy associated with presence of serum and cerebrospinal fluid antibody to HTLV-I. *Neurol Neurobiol* 1989; 51: 57 -63.

Terasaki PM, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature* 1964; 204: 988.

Todd JA, Acha-Orbea H, Bell JI et al. A molecular basis for MHC class II associated autoimmunity. *Science* 1988; 240: 1003 - 1009.

Usuku K, Sonoda S, Osame M et al. HLA haplotype-linked high immune responsiveness against HTLV-I in HTLV-I associated myelopathy: comparison with adult T-cell leukemia/lymphoma. *Ann Neurol* 1988; 23(Suppl): S143 - S150.

Usuku K, Nishizawa M, Matsuki K et al. Association of a particular amino acid sequence of HLA-DR beta 1 chain with HTLV-I associated myelopathy. *Eur J Immunol* 1990; 20: 1603 - 1606.

Vernant JC, Maurs L, Gessain A et al. Endemic tropical spastic paraparesis associated with human T lymphotropic virus type I: a clinical and sero-epidemiological study of 25 cases. *Ann Neurol* 1987; 21: 123 - 130.

Vernant JC, Bellance R, Buisson GG, Havard S, Mikol J,´Roman G. Peripheral neuropathies and myositis associated with HTLV-I infection and Martinique. In Blattner, ed. *Human Retrovirology* New York: Raven Press, 1990: 225 - 235.

Wallace ID, Cosnett JE. Unexplained spastic paraplegia. *S Afr Med J* 1983; 63: 689 - 691.

Watabe K, Saida T, Kim SV. Human and simian glial cells infected by human T-lymphotropic virus type I in culture. *J Neuropathol Exp Neurol* 1989; 48: 610 - 619.

Wayne Moore GR, Traugott U, Schienberg LC, Raine CS. Tropical spastic paraparesis: a model of virus induced cytotoxic T-cell mediated demyelination ? *Ann Neurol* 1989; 26: 523 - 530.

Wiley CA, Nerenberg M, Cros JD, Soto-Aguilhar MC. HTLV-I polymyositis in a patient also infected with the human immuno-deficiency virus. *N Engl J Med* 1989; 320(15): 992 - 995.

Wiley CA, Schrier RD, Nelson JA, Lampest PW, Oldstone MBA. Cellular localisation of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 1986; 83: 7089 - 7093.

Williams AE, Fang CT, Slamon DJ et al. Seroprevalence and epidemiological correlates of HTLV-I infection in U.S. blood donors. *Science* 1988; 240: 643 - 646.

Woolf B. On estimating the relation between blood groups and disease. *Ann Hum Gen* 1955; 19: 251 - 253.

Wright DK, Mano MM. Sample preparation from paraffin-embedded tissues. In: Innes MA, Gelfand DH, Sninsky JJ, White TJ, Eds. *PCR Protocols* San Diego: Academic Press, 1990: 153 - 158.

Yanagihara R, Garruto RM, Millar MA et al. Isolation of HTLV-I from members of a remote tribe in New Guinea. *N Engl J Med* 1990; 323(14): 993.

Yamada I, Ishiguro T, Seto A. Infection without antibody response in mother-to-child transmission in rabbits. *J Med Virol* 1991;33: 268 - 272.

Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterisation of retrovirus from cell lines of human adult T-cell leukaemia and its implication in the disease. *Proc Natl Acad Sci USA* 1982; 79: 2031 - 2035.

Yoshida M, Osame M, Usuku K et al. Viruses detected in HTLV-I associated myelopathy and adult T-cell leukaemia are identical on blotting. *Lancet* 1987; i: 1085 - 1086.

Yousef GE, Isenberg DA, Mowbray JF. Detection of entero-specific RNA sequences in muscle biopsy specimens from adult onset myositis. *Ann Rheum Dis* 1990; 49: 310 - 315.

Zack JA, Cann AJ, Lugo JP, Chen ISY. HIV-1 production from infected peripheral blood T-cells after HTLV-I induced mitogen stimulation. *Science* 1988; 240: 1026 - 1029.

Zaninovic V, Arango C, Biojo R et al. Tropical spastic paraparesis in Colombia. *Ann Neurol* 1988; 23(Suppl): S127 - S132.

Zeng Y, Lan X, Fang J et al. HTLV antibody in China. *Lancet* 1984; i: 799 - 800.

APPENDIX A: MYELOPATHY PROTOCOL*

BLOOD TESTS

Full blood count

Erythrocyte sedimentation rate

Urea and electrolytes

Liver function tests

Serum protein electrophoresis

Serum immunoglobulins

Serum angiotensin converting enzyme

Serum folate and Vitamin B12

Serological tests for syphilis

Bilharzial immunohaemagglutination test

Cysticercosis ELISA

Serological tests for mycoplasma

Viral antibody studies: EBV, cytomegalovirus, herpes simplex, herpes zoster, measles, influenza HTLV-I and HIV 1/2

STOOL AND URINE EXAMINATION AND RECTAL MUCOSAL BIOPSY

For detection of bilharzial ova

CEREBROSPINAL FLUID TESTS

Cell count, protein, globulin, glucose concentration

Bacterial (including mycobacterial) culture

Serological tests for syphilis

Antischistosomal ELISA

Cysticercosis ELISA

Cryptococcal antigen test

Viral antibody studies: as per blood tests

CSF IgG index

Isoelectric focusing for IgG oligoclonal bands

RADIOLOGICAL STUDIES

Radiographs of chest and spine

Conventional myelography followed by CT

CT scan of the brain

* not all tests done in every patient

APPENDIX B: MRC POWER GRADING SYSTEM

GRADE		
	0	No contraction
	1	Flicker or trace of contraction
	2	Active movement with gravity eliminated
	3	Active movement against gravity
	4	Active movement against gravity and resistance
	5	Normal power

APPENDIX C: METHODOLOGY FOR THE DETECTION OF IgG OLIGOCLONAL BANDS IN CSF

PREPARATION OF GEL

d-Sorbitol (3.6g) and 0.3g Agarose IEF were dissolved in 27 ml 10% aqueous glycerol using a boiling water bath. The liquid gel was equilibrated in a water bath at 65°C. Two ml Pharmalyte 3-10 and 0.5 ml Pharmalyte 8-10.5 were then added to this, allowed to cool and then stored in a moist chamber at 4°C until use.

ISOELECTRIC FOCUSING

After the frame was removed the gel was placed on the cooling platten. Surface fluid was removed with a sheet of nitrocellulose membrane (NCM), which was then discarded.

Paired serum and CSF samples were then applied and focused using a voltage of 1200 and power of 20w for a total of 1000V-hrs.

BLOTTING

The gel was pre-blotted with another sheet of NCM which was also discarded. A third sheet of NCM was applied and overlayed with filter paper, several layers of

blotter and a glass slab on which was placed a weight of 1kg. Transfer of immunoglobulins from gel to NCM was allowed to proceed for 30 minutes.

The NCM was then placed in 2% dried milk in saline for 30 minutes and then rinsed in tap water. Fifty ml of 0.2% milk in saline containing 50 μl goat anti-human IgG Fc was added for 30 minutes. The NCM was then washed in several changes of tap water. The second antibody (50 μl horse-radish peroxidase conjugated rabbit antigoat serum) was allowed to react with the NCM for a further 30 minutes. The NCM was washed once more and developed with a solution of 20 mg ethylamino carbazole dissolved in 20 ml methanol and added to 100 ml 0.02M acetate buffer (pH 5.1), containing 100 μl of hydrogen peroxide. The colour was allowed to develop for approximately 20 minutes. The NCM was washed and dried.

INTERPRETATION

A result was positive if there were two or more bands present in the CSF and not in the serum.

APPENDIX D: RESULTS OF ROUTINE CSF STUDIES

Patient No	P	L	Protein g/l	Globulin	Glucose mmol/l
1	6	12	0.80	T	2.50
2	8	68	0.74	+	1.90
3	14	2	0.88	+	2.10
4	16	8	0.44	+	2.80
5	0	48	0.56	T	2.70
6	0	0	0.88	+	2.30
7	0	0	0.18	ND	2.00
8	28	8	0.94	T	2.70
9	2	32	0.30	T	2.40
10	4	2	0.18	ND	2.90
11	0	0	0.20	T	2.80
12	0	6	0.20	ND	2.10
13	0	8	0.44	ND	2.40
14	4	8	0.32	ND	2.10
15	2	9	0.40	ND	3.30
16	0	0	0.27	ND	2.80
17	40	2	0.70	+	2.20
18	0	0	0.16	ND	2.50
19	0	8	0.40	T	3.20
20	0	0	0.24	T	2.40
21	2	34	0.42	ND	2.20
22	0	0	0.33	T	2.30
23	6	8	0.56	+	2.50
24	0	4	0.20	ND	2.90
25	6	0	0.76	+	2.40
26	0	0	0.30	ND	2.20

APPENDIX D CONTINUED					
27	2	16	0.26	T	2.80
28	0	0	0.16	ND	2.60
29	0	50	0.70	+	0.00
30	0	4	0.00	ND	0.00
31	32	51	0.75	+	2.50
32	0	8	0.16	ND	2.80
33	33	16	0.56	T	2.60
34	2	4	0.24	ND	3.30
35	2	16	0.46	T	3.00
36	28	0	0.40	T	2.50
37	12	8	0.74	ND	2.90
38	0	0	0.00	ND	0.00
39	0	0	0.04	ND	2.80
40	6	10	0.25	T	2.50
41	2	16	0.44	T	2.80
42	0	30	0.22	NI	3.10
43	0	10	0.52	T	2.40
44	0	8	0.40	T	2.60
45	0	4	0.22	NI	3.00
46	0	0	0.00	ND	0.00
47	0	24	1.40	+	2.70
48	0	0	0.10	ND	3.40
49	0	32	0.35	NI	2.80
50	0	14	0.19	ND	8.30
51	0	22	0.32	ND	2.90
52	0	10	0.26	+	3.50
53	0	14	0.32	ND	3.10
54	0	4	0.15	ND	3.10
55	0	24	0.56	T	3.30

APPENDIX D CONTINUED					
56	0	12	0.23	ND	3.30
57	0	4	0.50	T	3.10
58	4	0	0.62	T	2.90
59	0	8	0.23	NI	2.90
60	8	16	0.26	NI	3.40
61	0	0	0.18	NI	2.70
62	12	68	0.35	NI	2.30
63	0	6	0.30	NI	2.40
64	0	28	0.58	T	2.00
65	0	2	0.40	T	2.10
66	0	22	0.25	NI	3.00
67	2	30	1.00	+ +	2.20
68	0	2	0.20	T	3.10
69	0	0	0.10	ND	2.70
70	0	20	0.22	T	2.30
71	0	40	0.20	ND	3.20
72	0	32	0.28	ND	3.10
73	0	20	0.00	ND	3.00
74	0	0	0.42	T	3.60
75	0	0	0.14	ND	3.30
76	4	56	0.76	T	3.00
77	6	10	0.66	T	2.80
78	0	6	0.58	T	2.60
79	0	4	0.49	T	2.70
80	4	2	0.36	NI	2.80
81	6	30	1.07	+	3.00
82	8	120	1.22	+	3.20
83	4	46	0.55	T	3.60
84	0	0	0.36	NI	5.80

APPENDIX D CONTINUED					
85	0	0	0.36	T	2.70
86	0	0	0.27	NI	2.20
87	0	4	0.54	T	2.80
88	0	52	1.54	+	2.00
89	6	38	0.00	+	0.00
90	0	6	0.98	T	2.90

P = polymorph /μl, L = lymphocytes /μl; normal protein $\leq$ 0.4g/l, Globulin: NI - not increased, T = trace, ND = not done.

APPENDIX E: RESULTS OF IgG INDEX AND OLIGOCLONAL BANDS

Pt. No	CSF Albumin	Se Albumin	CSF IgG	Se IgG	IgG Index	Oligo-clonal Bands
2	0.48	31.5	0.25	19.6	0.84	ND
3	0.22	38.5	0.31	34.5	1.59	P
4	0.15	43.0	0.07	13.8	1.46	ND
5	0.70	39.7	0.02	22.1	0.04	ND
6	0.56	40.2	0.03	29.1	0.07	N
7	0.09	25.3	0.12	37.9	0.80	ND
10	0.09	40.0	0.10	19.8	2.24	ND
11	0.12	36.9	0.05	32.5	0.43	ND
13	0.35	40.0	0.06	12.9	0.53	N
14	0.20	33.0	0.17	19.5	1.48	P
15	0.26	45.8	0.18	24.7	1.28	ND
16	0.13	40.0	0.08	26.4	0.88	ND
17	0.26	41.5	0.55	24.9	3.60	ND
18	0.08	42.0	0.06	28.5	0.97	ND
19	0.20	38.8	0.12	18.3	1.27	ND
21	0.01	38.1	0.08	11.1	15.90	ND
22	0.04	39.4	0.03	6.6	4.40	ND
23	0.31	42.7	0.34	34.0	1.39	P
24	0.08	36.2	0.11	16.7	2.92	P
29	0.40	35.7	0.16	20.6	0.69	ND
32	0.11	25.7	0.15	41.2	0.85	ND
34	0.17	35.6	0.07	36.0	0.40	ND
35	0.11	25.00	0.49	82.1	1.36	P
36	0.12	34.0	0.18	31.6	1.61	P
42	0.17	37.3	0.13	24.6	1.16	p
43	0.10	43.8	0.25	24.6	4.50	ND
48	0.09	43.0	0.03	27.8	0.51	P

APPENDIX E CONTINUED						
50	0.12	42.0	0.05	16.9	1.00	ND
51	0.22	50.0	0.08	36.3	0.50	ND
52	0.12	41.0	0.15	24.3	2.20	ND
54	0.10	40.0	0.05	24.1	0.80	N
55	0.51	46.0	0.31	20.4	3.30	P
57	0.21	46.0	0.34	19.00	3.90	ND
58	0.01	38.0	0.05	27.3	7.00	ND
61	0.14	35.7	0.14	25.3	1.41	ND
62	0.60	16.0	0.27	26.6	0.30	P
63	0.09	45.0	0.14	17.8	3.90	P
64	0.33	45.0	0.15	18.5	1.10	P
65	0.09	22.0	0.27	39.2	1.68	ND
66	0.20	47.0	0.13	18.6	0.80	ND
67	0.27	18.7	0.59	27.6	1.50	ND
68	0.23	37.0	0.13	20.1	0.96	ND
69	0.11	39.0	0.11	18.6	0.47	ND
70	0.12	25.6	0.09	15.5	0.81	P
71	0.30	56.0	0.16	24.6	0.82	ND
72	0.27	29.5	0.08	16.1	1.84	ND
73	0.30	30.4	0.21	19.8	0.93	N
74	0.27	36.2	0.21	24.3	0.66	P
75	0.08	38.0	0.08	30.1	1.26	P
76	0.61	37.0	0.32	28.4	1.46	P
77	0.17	27.2	0.33	23.4	0.44	ND
78	0.21	22.0	0.19	23.1	1.10	ND
79	0.22	30.0	0.21	18.3	0.64	P
80	0.12	31.2	0.12	37.6	1.21	P
82	0.82	42.0	0.30	15.3	0.99	P
85	0.30	28.9	0.27	19.3	0.74	P

APPENDIX E CONTINUED						
86	0.05	28.0	0.12	35.8	0.53	P
89	0.39	22.0	0.33	38.0	0.49	P
90	0.49	40.0	0.31	22.0	1.20	P

IgG Index normal < 0.7. Oligoclonal bands:
P = Positive N = Negative ND = Not Done

APPENDIX F: SERUM AND CSF $\beta 2$-MICROGLOBULIN LEVELS

Patient No	Se $\beta 2$ Microglobulin	CSF $\beta 2$ Microglobulin	Albumin Ratio
6	3.8	4.9	13.7
13	2.2	4.5	8.7
31	12.2	2.3	-
50	2.3	3.6	2.8
51	1.6	2.7	4.4
52	2.1	2.9	2.9
54	4.5	3.1	2.5
55	2.5	5.1	11.0
59	3.3	4.5	-
60	3.6	2.0	-
61	2.3	2.7	4.0
62	12.7	3.6	37.5
63	3.5	4.9	2.0
64	2.1	7.5	7.3
65	6.0	4.7	4.0
66	3.0	3.0	4.2
68	5.5	6.5	6.2
70	2.6	6.0	4.6
71	3.1	7.0	5.3
75	6.8	1.7	2.1
82	2.2	5.1	10.7
85	2.5	10.7	1.7
86	14.8	16.9	-
88	3.5	9.4	17.7
89	3.6	3.0	-

Serum & CSF $\beta 2$ Microglobulin: normal 1.1 - 2.0 mg/l

Albumin ratio= CSF/Se ALB (Value > 6.5 abnormal: Eeg-Olofsson et al 1981)

APPENDIX G: SURAL NERVE CONDUCTION STUDIES

Patient No	Latency (m/sec)	Velocity (m/sec)	Amplitude (μV)
1	4.1	34.1	3.4
3	2.6	53.8	7.2
5	3.6	38.8	1.2
6	4.4	31.8	6.4
9	3.4	41.1	8.2
10	NR	-	-
11	3.2	43.7	5.6
12	2.5	56.0	16.0
13	1.8	61.1	16.8
14	NR	-	-
16	2.9	38.8	17.0
21	3.2	43.7	3.0
22	4.0	35.0	2.6
25	3.2	43.7	18.4
26	NR	-	-
27	3.6	38.8	5.8
28	NR	-	-
29	3.2	43.7	6.0
31	3.4	41.4	9.4
34	3.2	43.7	4.4
35	2.8	50.0	5.6
36	NR	-	-
37	NR	-	-
39	NR	-	-
40	2.8	50.0	2.4
42	NR	-	-
43	2.6	53.8	2.2
44	3.4	41.1	6.4

APPENDIX G CONTINUED			
45	3.2	43.7	4.8
46	3.0	46.6	4.2
47	NR	-	-
48	NR	-	-
49	NR	-	-
50	2.9	48.2	4.0
51	3.2	0.0	36.8
52	3.6	0.0	7.8
53	2.8	50.0	5.0
54	2.4	58.3	2.6
55	2.4	58.3	7.0
56	3.2	43.7	11.2
57	NR	-	-
59	NR	-	-
60	2.8	50.0	17.4
61	2.8	50.0	3.8
63	2.4	50.0	9.6
64	3.8	36.8	4.8
65	2.9	48.2	6.8
66	2.5	56.0	4.6
67	2.6	53.8	7.4
68	3.2	45.1	6.6
69	2.4	58.3	3.4
70	3.3	42.2	5.0
71	2.9	48.2	18.0
73	3.1	45.1	13.2
74	NR	-	-
75	2.8	50.0	6.0
76	4.4	31.8	3.6

APPENDIX G CONTINUED			
77	NR	-	-
78	2.8	50.0	8.8
79	3.2	43.7	3.6
80	3.0	46.6	2.3
81	3.1	45.1	8.8
Normal	< 3.4	> 41.2	> 5.0

NR = no response: N = normal

APPENDIX H: COMMON PERONEAL NERVE CONDUCTION STUDIES

Patient no	Latency (msec)	Velocity (m/sec)	Amplitude (μV)	'F'Wave latency (msec)
1	5.0	36.4	0.96	ND
3	5.6	37.5	0.48	ND
5	4.2	50.0	4.40	49.2
6	4.4	41.6	0.71	50.8
9	4.5	36.4	4.40	55.6
10	4.2	43.1	3.16	54.8
11	3.6	25.5	19.00	42.8
12	4.6	43.1	4.50	54.0
13	3.9	56.4	3.68	ND
14	4.0	45.9	0.64	NR
16	3.8	47.4	18.00	46.8
21	4.0	53.5	1.01	0.0
22	3.5	15.8	5.8	ND
25	3.2	40.1	2.24	49.0
26	4.6	43.4	0.84	ND
27	4.6	41.8	8.7	ND
28	3.8	40.2	1.0	54.0
29	4.4	45.6	3.12	ND
31	8.8	38.6	3.40	62.0
34	3.8	44.5	10.40	49.2
35	5.4	40.7	2.68	57.0
36	5.6	45.0	1.15	48.0
37	NR	-	-	-
39	4.3	47.7	17.40	32.4
40	4.0	45.0	1.90	54.8
42	NR	-	-	-

APPENDIX H CONTINUED				
44	3.8	41.2	6.40	48.0
45	4.8	50.0	9.20	50.0
46	5.6	43.6	6.36	52.6
47	NR	-	-	-
48	6.2	77.8	3.32	NR
49	5.8	31.8	4.10	60.8
53	4.8	38.0	0.89	61.0
54	3.8	46.8	2.02	ND
55	4.6	45.1	3.10	54.4
56	3.6	36.0	0.79	64.0
57	3.0	45.1	6.10	53.0
59	NR	-	-	-
60	3.7	49.3	3.76	48.0
61	4.2	40.5	8.00	53.0
63	3.4	44.5	1.17	47.6
64	6.2	38.3	10.20	53.0
65	5.4	37.0	0.28	56.0
66	3.0	46.3	9.20	52.0
67	4.4	39.6	12.60	ND
68	5.2	39.2	1.50	55.6
69	3.1	58.0	2,50	ND
70	4.2	36.9	1.28	NR
71	5.4	46.3	10.50	53.2
73	5.0	46.8	7.60	48.0
74	3.2	50.0	6.10	46.4
75	4.0	47.8	11.00	44.8
76	NR	-	-	-
77	5.0	30.0	0.27	58.0
78	6.6	38.6	23.20	44.4

APPENDIX H CONTINUED				
79	4.4	39.2	0.91	NR
80	5.2	43.1	1.70	56.0
81	5.0	42.3	8.30	56.0
Normal	< 5.1	> 41.0	> 4.17	< 60.0

NR = no response ; ND = not done

APPENDIX I : VISUAL EVOKED RESPONSE STUDIES

Patient No	Right eye Latency (msec)	Right eye Amplitude (μV)	Left eye Latency (msec)	Left eye Amplitude (μV)
1	99.0	9.20	97.0	12.80
4	102.0	3.60	102.0	3.20
5	108.0	6.60	108.0	6.80
6	97.0	14.60	100.0	13.00
9	99.0	20.40	98.0	16.40
10	92.0	8.00	114.0	6.40
16	86.0	0.00	87.0	0.00
19	99.0	0.00	94.0	0.00
21	94.0	0.00	96.0	0.00
25	102.0	7.80	104.0	7.80
26	108.0	9.80	108.0	9.80
27	96.0	11.40	97.0	11.40
28	94.0	6.20	99.0	5.20
29	105.0	12.80	108.0	5.80
31	100.0	0.40	103.0	0.30
35	100.0	5.60	104.0	7.40
36	99.0	17.40	98.0	12.40
37	90.0	6.20	91.0	7.20
39	102.0	10.40	104.0	8.60
40	97.0	11.40	94.0	11.40
44	92.0	14.80	92.0	14.80
45	72.0	11.00	74.0	10.80
46	90.0	15.00	93.0	16.00
47	92.0	20.00	97.0	18.40
49	72.0	18.60	72.0	19.60
50	81.0	0.00	85.0	0.00

APPENDIX I CONTINUED				
51	68.0	10.20	67.0	9.60
52	116.0	0.00	121.0	0.00
53	72.0	12.40	72.0	12.50
54	73.0	11.20	72.0	10.40
55	109.0	9.40	111.0	7.60
56	106.0	7.00	106.0	7.00
57	105.0	11.00	99.0	11.20
58	108.0	9.20	111.0	8.60
59	98.0	4.00	98.0	4.00
60	94.0	10.00	94.0	10.80
61	76.0	15.20	76.0	15.20
66	90.0	8.40	99.0	7.00
68	102.0	9.60	103.0	9.00
69	93.0	14.20	93.0	14.20
71	108.0	6.30	106.0	7.20
72	88.0	3.10	NE	NE
73	94.0	10.20	94.0	7.80
74	100.0	11.80	96.0	11.00
77	100.0	6.80	100.0	5.40
79	114.0	10.00	109.0	6.80
81	93.0	8.40	96.0	6.60
83	97.8	0.00	98.0	0.00
85	110.0	0.00	115.0	0.00

NE = no eye.
Abnormal P100: Right eye latency > 109.8 msec (Mean + 3 SD) and left eye latency > 110.7 msec (mean + 3 SD)

APPENDIX J(1): RIGHT EAR BRAINSTEM AUDITORY EVOKED RESPONSES: ABSOLUTE LATENCIES

Patient No	Wave (msec)				
	I	II	III	IV	V
1	1.32	2.40	3.64	4.92	6.20
3	1.56	2.72	3.80	4.76	5.28
4	1.52	2.68	3.88	5.48	6.04
5	1.36	2.92	4.12	5.52	6.48
6	1.48	2.60	3.72	5.04	5.88
9	1.28	2.64	3.56	4.88	5.76
10	1.60	2.72	3.56	4.84	5.84
15	1.40	2.68	3.92	5.20	6.00
16	1.76	2.68	3.52	4.80	5.68
19	1.64	2.76	3.76	4.96	4.72
21	1.44	2.80	4.04	5.36	6.04
24	1.60	2.44	3.50	NWI	5.32
25	1.72	2.40	3.52	4.76	5.68
26	1.52	2.52	3.10	4.12	5.64
27	1.72	2.68	3.88	5.04	5.72
29	1.76	2.76	3.84	5.12	6.04
31	1.68	2.40	3.76	4.64	5.72
35	1.64	2.96	4.04	5.44	6.20
36	1.80	2.84	3.64	5.08	5.84
37	1.48	2.44	3.48	4.80	5.88
39	1.44	3.20	5.28	6.32	7.44
40	1.60	2.88	4.00	5.44	6.08
44	1.60	2.68	3.80	5.12	5.84
45	1.68	2.84	4.08	5.76	6.08
46	1.56	2.56	3.76	4.76	5.44
47	1.48	2.52	3.56	5.08	5.76
48	1.68	2.76	4.48	5.20	6.24

APPENDIX J(1) CONTINUED					
49	1.44	NWI	3.96	5.40	6.20
50	1.64	2.76	4.12	5.00	5.68
51	1.56	2.92	3.88	5.04	5.68
52	1.52	2.52	3.64	5.60	6.04
53	1.68	2.56	4.20	5.36	6.24
54	1.44	2.96	4.00	5.16	6.04
55	1.68	2.56	3.60	0.00	0.00
56	1.56	2.72	3.84	5.12	5.88
57	1.36	2.44	3.60	4.80	5.92
58	NWI	2.76	3.92	5.28	5.76
59	1.56	2.80	3.92	4.88	5.72
61	1.48	2.72	3.92	4.96	5.84
64	1.36	2.68	3.88	5.16	5.76
65	1.36	2.72	3.84	4.92	5.80
66	1.52	2.48	3.68	4.72	5.52
68	1.52	2.80	3.84	5.08	6.00
69	1.76	2.88	3.80	4.96	6.64
70	1.72	2.80	4.16	5.16	5.76
71	1.60	2.68	3.88	4.80	5.96
72	1.88	2.92	3.92	5.32	6.16
73	1.96	3.12	4.04	5.16	5.84
75	1.60	2.80	3.80	4.92	5.56
76	1.44	3.12	4.24	5.56	6.68
77	1.56	2.72	3.96	5.28	6.16
79	1.40	2.88	3.76	4.36	5.32
80	2.36	3.36	4.40	5.80	6.48
81	1.64	2.88	4.00	5.32	6.00
Normal	< 1.79	< 2.98	< 4.07	< 5.26	< 5.91

Mean + 3SD NWI = no wave form identified

APPENDIX J (2): RIGHT EAR BRAINSTEM AUDITORY EVOKED RESPONSES: INTERVAL LATENCIES

Patient no	Intervals (msec)		
	I - III	III - V	I - V
1	2.88	2.56	4.88
3	2.20	1.56	3.72
4	2.16	2.12	4.56
5	2.76	2.36	5.16
6	2.20	2.16	4.40
9	2.32	2.16	4.48
10	1.69	2.24	4.24
15	2.48	2.05	4.56
16	1.80	2.16	3.92
19	2.12	1.96	4.08
21	2.60	2.08	4.60
24	1.96	1.68	3.72
25	1.80	2.16	3.96
26	1.84	2.20	4.12
27	2.16	1.84	4.00
29	2.08	2.24	4.32
31	2.08	4.04	1.96
35	2.40	2.16	4.56
36	1.84	2.20	4.04
37	2.00	2.40	4.40
39	3.84	2.16	6.00
40	2.36	2.16	4.48
44	2.20	2.04	4.24
45	2.40	2.00	3.80
46	2.20	1.68	3.88
47	2.08	2.20	4.28

APPENDIX J (2) CONTINUED			
48	2.80	1.76	4.56
49	2.52	2.24	0.00
50	2.48	1.56	4.04
51	2.32	2.80	4.12
52	2.12	2.40	4.52
53	2.52	2.04	4.56
54	2.56	2.04	4.60
57	2.24	2.32	4.56
58	-	1.84	-
59	2.36	1.80	4.16
61	2.40	1.92	4.36
64	2.52	1.88	4.40
65	2.48	1.96	4.44
66	2.16	1.84	4.00
68	2.32	2.16	4.48
69	2.04	2.84	3.88
70	2.44	1.60	4.04
71	2.28	2.08	4.36
72	2.04	2.84	4.18
73	2.08	1.80	3.88
75	2.20	1.76	3.96
76	2.80	2.44	5.24
77	2.40	2.20	4.60
79	2.36	1.56	3.92
80	2.04	2.08	4.12
81	2.36	2.00	4.36
Normal	< 2.44	< 2.30	< 4.52

APPENDIX J(3): LEFT EAR BRAINSTEM AUDITORY EVOKED RESPONSES: ABSOLUTE LATENCIES

Patient no	Wave (msec)				
	I	II	III	IV	V
1	1.52	2.48	3.68	4.60	5.20
3	1.52	2.76	3.76	4.88	5.44
4	1.56	2.80	3.88	5.28	6.16
5	1.28	2.68	4.00	5.28	6.16
6	1.44	2.56	3.52	4.84	5.84
9	1.36	2.44	3.60	4.76	5.84
10	1.36	2.56	3.56	4.64	5.80
15	1.52	2.80	3.84	5.16	6.00
16	1.72	2.80	3.80	4.92	5.84
21	1.52	2.68	4.04	5.32	6.04
24	1.60	NWI	3.72	4.76	5.88
25	1.76	2.52	3.72	4.76	4.52
26	1.52	2.72	4.16	5.68	7.12
27	1.56	2.84	4.04	5.20	5.80
29	1.72	2.56	4.00	5.12	6.08
31	1.76	2.60	3.40	5.00	5.48
35	1.60	2.96	4.08	5.28	6.16
36	1.68	2.64	3.64	4.72	5.76
37	1.56	2.52	3.56	5.00	5.76
39	1.32	2.88	4.12	5.00	5.88
40	1.40	2.80	4.08	6.64	6.12
44	1.60	2.48	3.76	5.12	5.84
45	1.28	3.00	4.04	5.48	6.04
46	1.48	2.56	3.72	5.25	6.24
47	1.52	2.72	5.84	5.08	6.08
48	1.72	2.56	3.88	5.16	5.72

APPENDIX J (3) CONTINUED					
49	1.48	NWI	3.96	5.60	6.28
50	1.52	NWI	4.28	5.16	5.60
51	1.20	2.92	4.04	5.04	5.36
52	1.36	2.44	3.44	4.76	3.20
53	1.44	2.44	3.64	4.80	5.92
54	1.68	2.76	3.80	4.80	5.72
55	1.52	2.92	0.00	0.00	0.00
56	1.08	2.52	3.64	5.00	5.80
57	1.60	2.56	3.72	4.96	5.92
61	1.48	2.72	3.84	5.00	5.80
64	1.56	2.64	3.76	4.80	5.80
65	1.35	2.68	3.88	4.96	5.84
66	1.40	2.48	3.44	4.52	5.36
68	1.52	2.80	3.84	5.20	6.00
69	1.68	2.96	3.72	5.04	5.76
70	1.56	2.92	4.28	5.64	6.52
71	1.36	2.68	4.04	5.12	5.84
72	1.72	2.80	4.00	5.12	5.88
73	1.84	2.92	4.04	5.08	5.96
74	1.20	2.56	3.84	5.24	5.88
75	1.52	2.72	3.84	4.96	5.60
76	1.48	3.04	4.12	5.52	6.36
77	1.36	2.80	3.88	5.08	5.88
79	1.40	2.76	3.68	4.64	5.28
80	2.48	3.80	5.00	6.28	6.84
81	1.56	2.72	3.93	5.40	6.16
Normal	< 1.78	< 2.98	< 4.07	< 5.26	< 5.91

NWI: No wave identified.

APPENDIX J(4).: LEFT EAR BRAINSTEM AUDITORY EVOKED RESPONSES: INTERVAL LATENCIES

Patient No	Interval (msec)		
	I - III	III - V	I - V
1	2.16	1.48	3.68
3	2.24	1.68	3.92
4	2.32	2.24	4.68
5	2.76	2.12	4.88
6	2.12	2.32	4.40
9	2.28	2.20	4.44
10	2.24	2.24	4.44
15	2.32	2.12	4.48
16	2.08	2.08	4.12
21	2.52	2.04	4.52
24	2.12	2.16	4.36
25	1.96	2.00	3.96
26	2.64	3.00	5.60
27	2.48	1.76	4.24
29	2.28	2.08	4.36
31	1.60	3.76	2.16
35	2.48	2.08	4.56
36	1.96	2.12	4.08
37	2.00	2.22	3.20
39	2.80	1.76	4.56
40	2.64	1.92	4.68
44	2.16	2.08	4.24
45	2.74	2.00	4.76
46	2.24	2.52	4.76
47	2.32	2.24	4.56

APPENDIX J (4) CONTINUED			
48	2.16	1.84	4.00
49	2.48	2.32	4.80
50	2.76	1.32	4.08
51	2.84	1.46	4.30
52	2.08	1.76	3.84
53	2.20	2.28	4.48
54	2.12	1.92	4.04
57	2.12	2.20	4.32
61	2.36	1.90	4.32
64	2.20	2.04	4.24
65	2.52	1.96	4.48
66	2.04	1.92	3.96
68	2.32	2.16	4.48
69	2.04	2.04	4.08
70	2.72	2.24	4.96
71	2.68	1.80	4.48
72	2.28	1.88	4.16
73	2.20	1.92	4.12
74	2.64	2.04	4.68
75	2.32	1.76	4.08
76	2.64	2.24	4.88
77	2.52	2.00	4.52
79	2.28	1.60	3.88
80	2.52	1.84	4.36
81	2.36	2.24	4.60
Normal	< 2.44	< 2.30	< 4.52

APPENDIX K: METHODOLOGY FOR PCR FROM PARAFFIN EMBEDDED TISSUE

DNA EXTRACTION

Total cellular DNA was extracted from formalin fixed paraffin embedded blocks of the patient's spinal cord. Two 5 - 10 micron thick sections were cut using a new dry microtome knife and placed in a sterile 1.5ml microfuge tube. One ml of Xylene was added to each tube and thoroughly mixed for 30 minutes. The mixture was pelleted with a microfuge for 5 minutes and the Xylene extraction repeated. To the pellet 0.5ml of 100% ethanol was added and mixed. Ethanol was removed and the sample re-extracted with ethanol and dried under vacuum. One hundred to 200ml of digestion buffer containing 200 ug/ml of proteinase K (in 50mM Tris (pH8.5), 1 mM EDTA, plus 1% Laureth 12 (Mazer Chemicals, Gurne Il) or 0.5% Tween 20) was added to the mixture and incubated for 3 hours at 55°C. Digestion was stopped with 10 minute incubation at 95°C before pelleting and storage of supernatant at -20°C.

POLYMERASE CHAIN REACTION ASSAY

The primers used to detect proviral HTLV-I were targeted towards the viral "gag" glue. These primers, 5'-CCGCGACCGCCCCGGGGGCTGG-3' (WW10) and 5'-TCACCCGGCCGGGGTATCCT-3' (WW11) define a 205 bp fragment extending from +860 to + 1064 in the HTLV-I proviral genome. Primer WW10 was ^{32}P-end labelled using T4 polynucleotide kinase (specific activity 2 x 10^7

cpm/pM) and 1.5 x 10^6 cpm (0,1pM) was added to the PCR assay mixture. The PCR mixture (50 ul final volume) contained 10mM Tris pH8.3; 30mM KCl; 2.0mM MgCl. 0.01% gelatin; 0.1% Triton x-100, 12pM of each primer including the labelled ww10 primer, 0.2mM each of alpha ATP, alpha CTP, alpha GTP and alpha TTP, and the extracted template DNA. PCR was performed using the Ericomp (San Diego CA) temperature cycler for 30 cycles (denaturing at 94°C, annealing and extension at 65°C). Following the first denaturing step 2.5 U of Taq DNA polymerase (Promega Corp; Madison WI) was added to each reaction and the sample was overlayed with a drop of mineral oil.

To normalise for the amount of DNA contained in each sample PCR was performed to assay for the glyceraldehyde 3-phosphate dehydrogenase gene. The PCR assay reaction mixture and conditions were identical to that described for HTLV-I gag, except for the absence of Triton X-100 and adjustment of the final concentration of MgCl to 14mM.

The PCR products were resolved on a 6% acrylamide gel, dried and exposed to Kodak XAR film at -80°C for 0.25 to 16 hours. Hae III cut GX-174 DNA ^{32}P-end labelled fragments served as molecular weight markers.

APPENDIX L: HTLV-I SEROEPIDEMIOLOGY QUESTIONNAIRE

STUDY NUMBER

NAME: SURNAME

FIRST NAMES

ADDRESS

SEX: 1 = MALE 2 = FEMALE

AGE

MARITAL STATUS: 1 = SINGLE 2 = MARRIED
3 = SEPARATED/DIVORCED

BLOOD TRANSFUSION: YES = 1 NO = 2

NO. OF UNITS

TRIBAL SCARIFICATIONS; YES = 1 NO = 2

OCCUPATION

<u>OPTIONAL QUESTIONS:</u>

AGE AT FIRST SEXUAL EXPERIENCE

NUMBER OF SEXUAL PARTNERS

ANY NEUROLOGICAL PROBLEM: YES = 1 NO = 2