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Challenges to the treatment of HIV-infected individuals in the HAART era

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Challenges to the treatment of HIV-infected individuals in the HAART era .

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Abbreviations

AICD	Activation Induced Cell Death
AIDS	Acquired Immune Deficiency Syndrome
APC	Antigen Presenting Cell
ARS	Acute Retroviral Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Azidothymidine
CDC	Centers for Disease Control and Prevention
CTL	Cytotoxic T Lymphocyte
DC	Dendritic Cell
DNA	Desoxy-Ribonucleic Acid
FDA	Food and Drug Administration
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HAART	Highly Active Antiretroviral Treatment/Therapy
HIV	Human Immune Deficiency Virus
IL-2	Interleukin-2
IRD	Immune Recovery Disease
IRIS	Immune Reconstitution Inflammatory Syndrome
KS	Kaposi Sarcoma
MTCT	Mother To Child Transmission
NK	Natural Killer Cell
NKT	Natural Killer T Cell
NRTI	Nucleoside/Nucleotide Reverse Transcriptase Inhibitor
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
OI	Opportunistic Infection
PCD	Programmed Cell Death
PCP	Pneumocystis Carinii Pneumonia
PI	Protease Inhibitor
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
RTE	Recent Thymus Emmigrant
STI	Structured Treatment Interruption
STD	Sexually Transmitted Disease
TB	Tuberculosis
TCR	T Cell Receptor
TREC	T Cell Receptor Excision Circle
VL	Viral Load
WHO	World Health Organization

CHAPTER I: DISEASE HISTORY

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1.1 FIRST HIV/AIDS REPORTS

The HIV/AIDS pandemic started 25 years ago. In 1981 clinicians in New York reported the increased incidence of the previously rare diseases Kaposi Sarcoma (KS) and *Pneumocystis carinii* pneumonia (PCP) in a population of homosexual men[1]. Soon other risk groups were identified (intravenous drug users, heterosexuals with high promiscuity and individuals receiving blood products), which led to the suspicion of an infectious agent.

Omnipresent organisms not normally causing health problems were inexplicably responsible for serious clinical deterioration and even death. It was discovered that these opportunistic infections (OI's) were related to the then unexplained cellular immune failure; called 'Acquired Immune Deficiency Syndrome' (AIDS). Two years later, the chronic infection, leading to AIDS, was proven to be caused by a retrovirus, named Human Immunodeficiency Virus (HIV) [2].

The main explanation for the immune deficiency caused by HIV-infection was found to be the progressive loss of CD4 T cells. These cells, central in the regulation of humoral and cellular immune effector functions, were found to decline due to direct viral cytotoxicity and more importantly indirect apoptosis inducing mechanisms [3]. Once the CD4 T cells fall below the critical level of 200 cells/µl blood, the HIV-infected individual becomes extremely susceptible to develop opportunistic infections [4].

1.1 THERAPY

In 1987, the first antiretroviral (ARV) drug Azidothymidine (AZT) was approved by the Food and Drug Administration (FDA). Two years later it became obvious that AZT alone was unable to control viral replication in the long term. This was due to the rapid development of drug resistance [5].

With time, new ARV drugs, targeting the viral Reverse Transcriptase (RT), were developed: they could be divided into two classes: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI's) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's). In 1996, another class of drugs, inhibiting the activity of the viral protease enzyme (protease

inhibitors: PI's) was introduced. During that period, new hope for long term viral suppression arose: combinations of at least 3 different drugs of 2 distinct classes (NRTI's, NNRTI's or PI's) were shown to suppress viral replication in the long term. This combination therapy was known as 'Highly Active Antiretroviral Therapy' (HAART).

Indeed the introduction of HAART led to a dramatic decline in HIV-related morbidity and mortality: the recovery of immune functions reversed the risk to develop opportunistic infections. At the same time the HIV-transmission rate diminished. These trends, however, were only observed in the rich, western world. In Europe, the incidence of AIDS (late stage HIV-infection) in HIV-infected patients, declined over 4 years (between 1994 and 1998) from 30,7 to 2,5 per 100 patients per year.

Nevertheless, the global HIV pandemic is seriously growing. The World Health Organization (WHO) has set a goal of treating 3 million people with Antiretroviral Therapy (ART) by the end of 2005. By June 2005 only one million people were estimated to be on ART [6]. This represents only 17% of the 5.8 million people that currently need ART. Provision of ART to all those in need will require a massive and unprecedented investment in health care systems of developing countries. In addition, scaling up HIV prevention also is a priority. A newly developed efficient HIV/AIDS vaccine could potentially offer the ultimate solution. However, there are currently no indications that such a vaccine will be available in the near future; the vaccines currently being studied have failed to exert a protective effect.

CHAPTER II: INTRODUCTION

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2.1 EPIDEMIOLOGY

Global AIDS epidemic

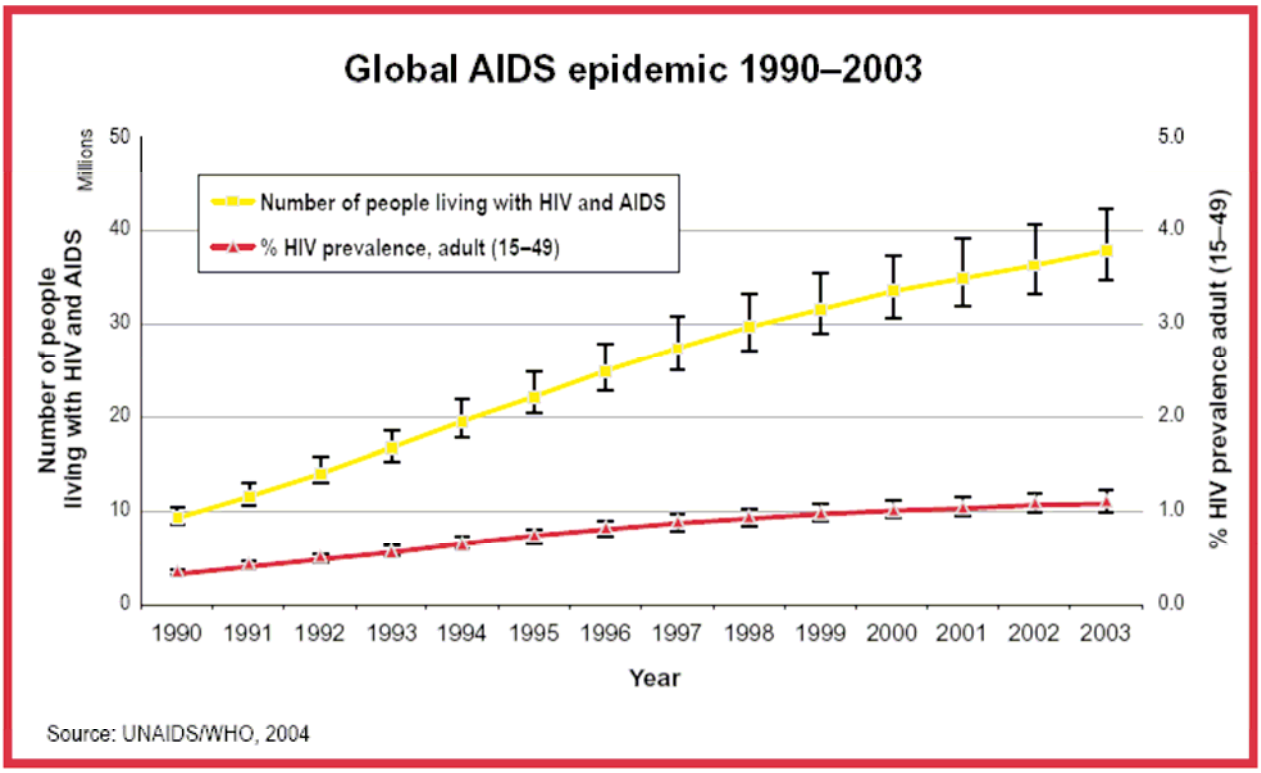

Fig. 1: The global AIDS epidemic. The number of people living with HIV and AIDS and the HIV prevalence in adults (15-49 years) are still increasing despite the introduction of HAART.

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The AIDS epidemic: The spread of a deadly disease in the biotech era

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ABSTRACT: In the last two decades we have witnessed the progression of a newly introduced infection in humans. It is sobering that despite a world-wide effort and the tremendous progression of technical capabilities and scientific knowledge we are still not able to control the global epidemic of HIV. In 2004 more than 40 million people were infected. Educational approaches to modify risk-taking behavior is still the most critical component of prevention and the most important measure to limit the spread of the infection. Vaccine development, which is still far from promising, is probably the only way to control the disease in the future. (J Biol Regul Homeost Agents 2004; 18: 178-82)

KEY WORDS: *HIV, AIDS, Epidemic, Origin, Spread*

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INTRODUCTION

In the late seventies and early eighties, a disease, called “Slim disease”, caused more than 100 deaths in Kasensero, a small village in Uganda (1, 2). Repeated infections and cachexia were followed by a fatal outcome. Because of a bloody guerrilla war investigations was never done. In 1981, dermatologists in New York observed a sudden higher occurrence of a previously extremely rare skin cancer in a population of gay men, which they first called “gay cancer” (3). Shortly after, physicians in New York and San Fransisco were also alarmed by a sudden rise in occurrence of the same skin cancer; formerly known as the Kaposi Sarcoma (KS), and an opportunistic lung infection *Pneumocystis carinii pneumonia* (PCP) (4). In those previously healthy homosexual men an unexplained cellular immune failure was observed: lymphopenia with a decline in number and function of T helper cells and an inversion of the normal ratio T helper cells / T suppressor cells. The term Acquired Immune Deficiency Syndrome (AIDS), was defined later the same year. Other associated opportunistic infections were found in these (AIDS) patients: candidiasis, toxoplasmosis, cryptococcosis, CMV infections, etc. A similar distribution pattern to hepatitis B infection was observed and relationships between different cases were soon made; homosexual contact clearly transmitting the disease. Recipients of blood or blood derived products presenting with AIDS were traced back to their donors dying or already dead from AIDS (5). The modes of transmission of the immune deficiency were found to be sexual intercourse, through blood products or needle sharing and from mother to child, pointing to a transmissible infectious agent. The isolation of the virus responsible for AIDS was accomplished in 1983 by scientists of the Institute Pasteur in France (6). The virus found, was first called HTLV-III /LAV (human T-cell lymphotropic virus-type III/ lymphadenopathy-associated virus). Later an international committee named the virus Human Immunodeficiency Virus (HIV). In 1985 an antibody test was developed and approved by the FDA, so that blood products could be screened. After infection, the virus causes AIDS after a variable time. When a person gets infected by the virus, the immune system will be attacked, leading to a cellular immune deficiency, presented as a decrease in CD4 T cells. This explains the sensibility for opportunistic infections and different cancers, by the presence of which AIDS is defined. The CD4 T cell decline is the consequence of immune activation and the subsequent apoptosis of CD4 T-cells, rather than the cytopathic effect of the virus.

The origin of the virus

A retrospective study searching for the origins of the HIV virus could detect the virus in the blood of a man who died in 1959 (7). The patient had symptoms of sickle cell anemia and lived in Leopoldville, now Kinshasa in the former Belgian Congo. The sequence of the virus detected in this man lies near the ancestral sequence of the subtypes B and D, suggesting that the ancestral HIV-1 must have existed only a few years before that time. Other studies based on mathematical models of the genetic evolution of the virus estimated the ancestral HIV infection between 1915 and 1940 (8). In Europe the first documented patients were a Norwegian family (9); the man, a sailor, probably infected his wife, who in turn transmitted the virus to their child. They all died of

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opportunistic infections. The infection in this man probably dated from before 1966.

The HIV virus is thought to originate from closely related monkey viruses; Simian Immunodeficiency Viruses (SIV). More than 25 species of monkeys harbour SIV (10); as natural host and are not suffering any damage to their immune system. In humans two big classes of HIV can be found: HIV-1, the most prevalent and HIV-2, especially found in West-Africa. HIV-2 is genetically closely related to SIVsm found in the sooty mangabey (11), native to West-Africa, while HIV-1 is related to SIVcpz found in a subspecies of chimpanzees native to West Equatorial Africa (12), which share 98% of their genome with humans. Transmission must in one or another way have taken place. The exact mode of transmission, however, is not known but several hypotheses exist, although most of them do not agree with HIV phylogenetic studies. A first theory is based on the fact that in some regions monkeys are hunted, killed and eaten. Another hypothesis is based on medical research programs; in some studies blood of chimpanzees, sooty mangabeys and macaques was injected in healthy volunteers to study the transmission of malaria (13). A third hypothesis is the introduction of the virus in humans through the use of a polio vaccine, produced in monkeys or on monkey kidney cells (14). The polio-vaccination trials were performed in the former Belgian Congo in the fifties. A fourth hypothesis is the transmission of the virus through an experimental hepatitis B vaccine that was given to homosexual men in New York and to black people in Central Africa (15). Some people believe that the virus was genetically engineered in order to eliminate some populations. All these hypotheses are sources of continuing debate and speculation.

Spread of the virus

Probably the virus originally entered into the human population in Africa. Cross-over, however, could have taken place on several occasions (16). The spread in Africa was favored by the developing health care system after the second world war; syringes, used several times, polygamy, urbanization and modern transport were factors stimulating the spread of the virus. The role of international travel can't be overlooked; the possibly imaginative story of "patient Zero" (5), a flight attendant, illustrating this. "Patient Zero" would have traveled extensively worldwide, while infecting a lot of people. The story says that a lot of the early cases could be traced either directly or indirectly to sexual contacts of this patient. Epidemiological characteristics of the epidemic, however, are different in the developing world compared to industrialized countries: the developing countries showing more heterosexual transmission and a much faster spread of the infection. In the United States and Europe the high risk groups are homosexual males, intravenous drug users and people receiving blood products, while in recent years the contribution of heterosexual infection is relatively rising. In the industrialized world prevalence is still rising also because of the access to medication and the decline in mortality. In the developing world heterosexual and vertical transmission are the most frequent routes of transmission. The probability of transmission being higher from men to women than the reverse, women are infected at a younger age. Vertical transmission, reaching up to 30%, occurs during

TABLE I - REGIONAL HIV/AIDS STATISTICS AND FEATURES, END OF 2003

Region	Adults and children living with HIV/AIDS	Adults and children newly infected with HIV	Adult prevalence (%)*	Adult and child deaths due to AIDS
Sub-Saharan Africa	25.0-28.2 million	3.0-3.4 million	7.5-8.5	2.2-2.4 million
North Africa	470 000-730 000	43 000- 67 000	0.2-0.4	35 000-50 000
South & South-East Asia	4.6-8.2 million	610 000-1.1 million	0.4-0.8	330 000-590 000
East Asia & Pacific	700 000-1.3 million	150 000-270 000	0.1-0.1	32 000-58 000
Latin America	1.3-1.9 million	120 000-180 000	0.5-0.7	49 000-70 000
Caribbean	350 000-590 000	45 000-80 000	1.9-3.1	30 000-50 000
Eastern Europe & Central Asia	1.2-1.8 million	180 000-280 000	0.5-0.9	23 000-37 000
Western Europe	520 000-680 000	30 000-40 000	0.3-0.3	2 600-3 400
North America	790 000-1.2 million	36 000-54 000	0.5-0.7	12 000-18 000
Australia & New Zealand	12 000-18 000	700-1 000	0.1-0.1	<100
Total	40 million (34-46 million)	5 million (4.2-5.8 million)	1.1% (0.9-1.3%)	3 million (2.5-3.5 million)

*The proportion of adults (15 to 49 years of age) living with HIV/AIDS in 2003, using 2003 population figures

The ranges around the estimates in this table define the boundaries within which the actual numbers lie, based on the best available information. These ranges are more precise than those of previous years, and work is under way to increase even further the precision of the estimates that will be published in 2004

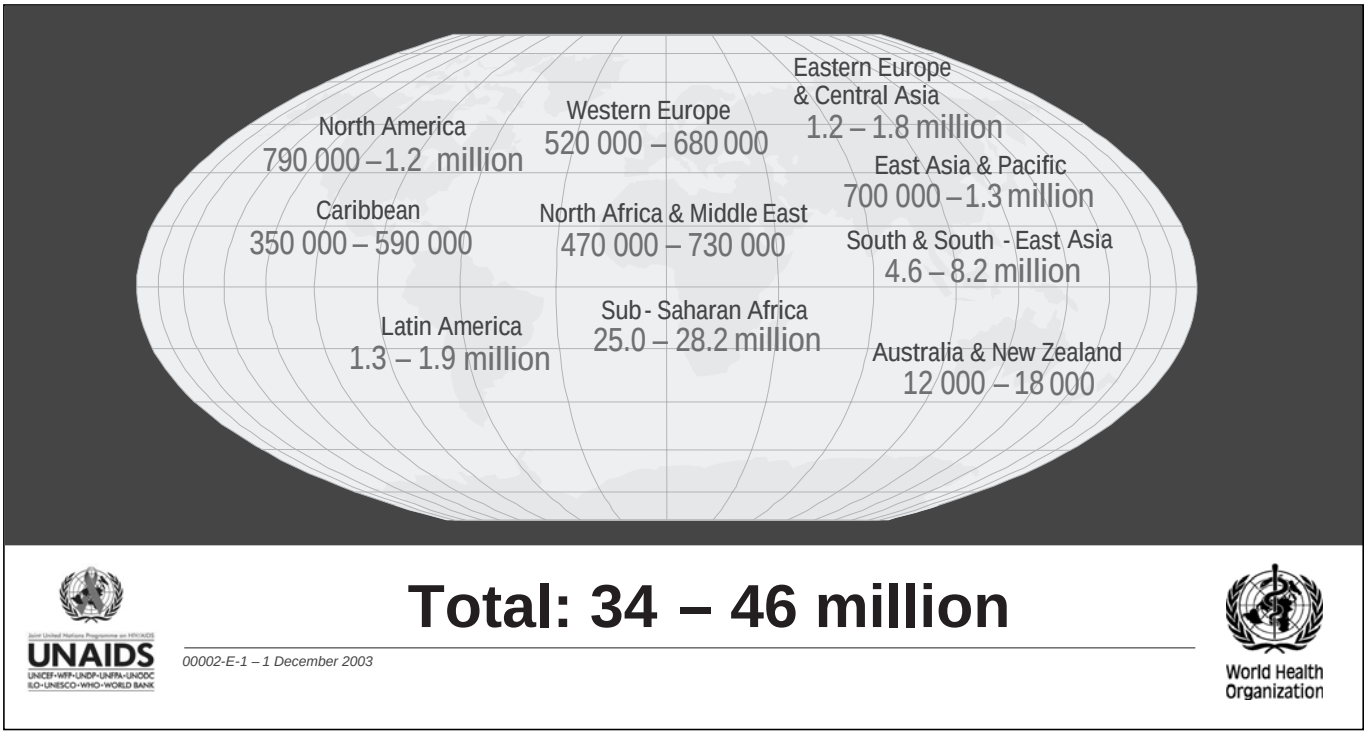

Fig. 1 - Adults and children estimated to be living with HIV/AIDS at the end of 2003.

pregnancy, delivery or breastfeeding when no treatment is available. In the industrialized world vertical transmission dropped to less than 1% through the introduction of therapy.

Sub-Saharan Africa is the most affected region where two thirds of the people with HIV/AIDS are living (16, 17) (Tab. I, Fig. 1). Prevalence varies greatly between the different countries. In South Africa and Zambia the prevalence in adults is 20% while in Gambia and Somalia less than 2% of the adults are infected. In four southern African countries infection rates are even above levels that were thought impossible; Botswana (37.5%), Lesotho (31.5%), Swaziland (38.6%) and Zimbabwe (33.7%).

Trends differ from country to country: in Uganda the prevalence dropped over a 10 year period from 30% to 6% at the beginning of this decade and it continues to decrease.

In Asia the epidemic started later, but exploded during the last decade. The aggressive prevention programs in Thailand and Cambodia showed that reduction of transmission is possible. The infection level is not above 3 to 4% in pregnant women in any Asian country, but there is big concern that because of the present high risk behavior outbreaks can be expected. At this moment the spread of HIV is augmenting especially in South and South East Asia.

In Latin America and the Caribbean the epidemic is also spreading fast. The Caribbean being the second most affected region in the world after Sub-Saharan Africa. In Haiti, the most affected country of this region, the prevalence is 6% in adults. The epidemic there was recognized at the same time as the first cases in the US, probably in relation with the sex tourism of some homosexual men.

In South America, Brazil, a widely affected country, took a leading role in efforts to fight HIV by producing and providing free generic drugs and conducting large education programs.

Eastern European countries show a big rise in prevalence especially through IV drug use. Probably the spread in this region is the fastest in the world.

The social and economic consequences in the developing world are already being felt widely not only in the health care system but also in education, industry, agriculture, transport, human resources and the economy in general. It's difficult to overview all the consequences of this dramatic epidemic.

Treatment of the disease

In the beginning of the pandemic, the first therapeutic options were treatment of opportunistic infections.

In 1987 the FDA approved the first antiretroviral medication zidovudine, AZT, which gave hope (18), but after a year it became clear that resistance developed (19, 20) and it proved to have only a temporary effect. In the early nineties several new drugs, with a different action than AZT were produced. In 1995, a new class of drugs was discovered: the protease inhibitors PI. The great breakthrough came in the same year when studies showed that using a

TABLE II - GLOBAL ESTIMATES OF HIV INFECTION AND AIDS IN ADULTS AND CHILDREN (END OF 2003)		
People living with HIV/AIDS	Gobal	40.0 million
	Africa	29.4 million
	Adults	38.6 million
	Women	19.2 million
	Children	3.2 million
New HIV infections in 2003		5.0 million
Deaths due to HIV/AIDS in 2003		3.2 million
Total deaths from AIDS, 1982-2003		23.0 million
Children orphaned by AIDS		14.8 million

Data from UNAIDS (2003)

combination of three different drugs, viral replication could be suppressed for a long period (21, 22). This strategy, called HAART: Highly Active Antiretroviral Therapy, became the norm in the industrialized world and significantly influenced the mortality and morbidity. In spite of this effective strategy, only 7% of the patients in need of treatment were receiving it thoughout the world in 2003. Cheaper generic drugs are being produced by different countries in order to combat the biggest killer in the world. The WHO has planned to treat 3 million people living with HIV/AIDS with antiretroviral medication in the developing world by the end of 2005; the 3-by-5 project (23).

Knowledge of the immune system and the virus

Scientists worldwide are searching for a better understanding of the immune system. The HIV pandemic led to an explosion in scientific virological and immunological studies. Destruction of the immune system is being understood better and better, while no cure has yet been found. Studying the differences between the immune system of non human primates and humans can help us to understand why the virus is pathogenic in humans without causing devastating effects in the respective natural hosts. In the natural host the virus doesn't cause immune activation. Future therapeutic strategies could try to temper the activation of the immune system. Another point of study is the genetic drift of the HIV virus. The existence of different genetic subtypes, with their respective geographical distribution restricts the possibilities for future vaccination strategies. The HIV virus, showing a fast evolution through continuous mutation, evades the specific immune answer, which always seems a step behind. Moreover the capacity for mutation is the cause of emerging drug resistance, which is of great concern. In the western world, where a lot of different drugs are used, the virus is subjected to enormous genetic pressure. And in the developing world fear exists that the efficacy of the limited drug repertoire will fast be exhausted because of transmission of drug resistant viruses.

Current data

It is estimated that today 34-46 million people are living with HIV/AIDS, 2.5 million of them are under the age of 15 years. Last year 3 million people died of AIDS and 5 million people became infected (Tab. II). In the near future it is expected that even more people will die of AIDS. In 2003 it was estimated that every day 14,000 people become infected with the virus and over 8,200 people died of AIDS every day. The situation is most dramatic in Sub-Saharan-Africa where two thirds of the people infected with HIV are living. One in twelve adults is infected in this region! 95% of the people living with HIV/AIDS are living in the developing world. It is expected that 6 million people will die in the near future if they don't receive treatment. Life expectancy at birth has declined with 15 to 20 years in the most heavily affected countries. From the beginning of the pandemic until now it is estimated that 22 million people have already died of AIDS. 14 million children became orphans due to AIDS, meaning they lost one or both parents from AIDS, before the age of 15. It is estimated that by 2010, at least 44 million children will have lost one or both parents due to AIDS.

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REFERENCES

1. Hooper E. The river. Harmondsworth: Little, Brown, 1999.
2. Serwadda D, Mugerwa RD, Sewankambo NK, et al. Slim disease: A new disease in Uganda and its association with HTLV-III infection. Lancet 1985; 2: 849-52.
3. Gottlieb GJ, Ragaz A, Vogel JV, et al. A preliminary communication on extensively disseminated Kaposi's sarcoma in young homosexual men. Am J Dermatopathol 1981; 3: 111-4.
4. Gottlieb MS, Schroff R, Schanker HM, et al. *Pneumocystis carinii pneumonia* and mucosal candidiasis in previously healthy homosexual men: Evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981; 305: 1425-31.
5. Kanabus A, Fredriksson J. The history of AIDS, 2004. http://www.avert.org
6. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983; 220: 868-71.
7. Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM, Ho DD. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. Nature 1998; 391: 594-7.
8. Yusim K, Peeters M, Pybus OG, et al. Using human immunodeficiency virus type 1 sequences to infer historical features of the acquired immune deficiency syndrome epidemic and human immunodeficiency virus evolution. Philos Trans R Soc Lond B Biol Sci 2001; 356: 855-66.
9. Froland SS, Jenum P, Lindboe CF, Wefring KW, Linnestad PJ, Bohmer T. HIV-1 infection in Norwegian family before 1970. Lancet 1988; 1: 1344-5.
10. Hahn BH, Shaw GM, De Cock KM, Sharp PM. AIDS as a zoonosis: Scientific and public health implications. Science 2000; 287: 607-14.
11. Marx PA, Li Y, Lerche NW, et al. Isolation of a simian immunodeficiency virus related to human immunodeficiency virus type 2 from a west African pet sooty mangabey. J Virol 1991; 65: 4480-5.
12. UAB Center for AIDS. 1999. Origin of HIV-1 discovered, 1999. http://main.uab.edu/show.asp?durki=8535
13. Steinbrook R. A deadly virus hides its past. The latest theories on the origin of HIV, while drawing scepticism, renew interest in the beginnings of the AIDS epidemic. Primate-to-human transmission is the best guess, but exactly how? In Los Angeles Times, Los Angeles, April 7, 1992.
14. Elswood BF, Stricker RB. Polio vaccines and the origin of AIDS. Medical Hypotheses 1994; 42: 347-54.
15. Leonard GH. Emerging viruses: AIDS and Ebola: Nature, accident or intentional? Tetrahedron Inc, 1996.
16. UNAIDS. AIDS epidemic update. Unaids, Geneva, 2003. http://www.unaids.org
17. WHO. 2004 WHO: facts and figures. The world health report 2003. WHO. http://www.who.int/whr/2004/overview/en/
18. Chaisson RE, Allain JP, Leuther M, Volberding PA. Significant changes in HIV antigen level in the serum of patients treated with azidothymidine. N Engl J Med 1986; 315: 1610-1.
19. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science 1989; 246: 1155-8.
20. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science 1989; 243: 1731-4.
21. Nau JY. Les experts préconisent une trithérapie immédiate chez les personnens infectées par le virus du SIDA. Le comité Dorment s' interroge sur la prescription des traitements en urgence. In Le Monde, Paris. 9 October, 1997.
22. Puoti M, Prestini K, Putzolu V, et al. HIV/HCV co-infection: Natural history. J Biol Regul Homeost Agents 2003: 144-7.
23. Fleck F. WHO hopes 3-by-5 plan will reverse Africa's HIV/AIDS epidemic. Bull World Health Organ 2004; 82: 77-8.

2.2 EVOLUTION OF THE HIV INFECTION

2.2.1 Clinical disease progression

During the course of an HIV-infection, distinct clinical stages can be recognised. Immediately after HIV-infection, 40 to 90% of the patients[7,8] experience symptoms such as fever, sore throat, nausea, diarrhoea, rash, myalgy, headache,… Because of the aspecificity of these symptoms, the disease often goes undiagnosed. The severity of this acute retroviral syndrome (ARS) differs from individual to individual. (This is an important factor in predicting the final clinical outcome [9, 10].)

The acute infection stage, which can last for several weeks, is followed by an asymptomatic phase known as the period of 'clinical latency'[11, 12]. This latency can last for several years (with an average of 10 years in the western world). During the asymptomatic stage, the risk for infections in the HIV-infected patient is similar to that of the general population, despite the appearance of enlarged lymph nodes.

The transition to the late stage of the disease is marked by constitutional symptoms such as weight loss, fatigue, fever, sweats and some dermatological infections…

The latest stage or the Acquired Immune Deficiency Syndrome (AIDS) is characterised not only by the occurrence of opportunistic infections specifically observed in severe immune deficient patients but also by central nervous symptoms caused by HIV and by particular cancers (WHO/CDC classification 2005) (Fig. 2).

2.2.2 Virological aspects of HIV infection

Viral load

In the early phase of the HIV-infection, viral RNA concentrations in plasma reach peak levels of 10^5-10^8 copies/ml [13]. This peak viral load (VL) is observed within a few weeks following the onset of infection.

After 6 to12 months, steady-state plasma viral RNA levels are established, which are lower than those found during acute infection (~10^3-10^4 copies/ml plasma) [14]. These steady state plasma levels are a good measure of the rate by which the HIV disease advances [15, 16].

Plasma VL steadily augments as the disease progresses, ultimately reaching levels above 10^6 copies/ml in the late AIDS stage (Fig. 2).

Diversity

The HIV transmission generally occurs by only a restricted number of viruses [17, 18], explaining the low viral diversity early in infection [19]. However, the viral diversity increases fast thereafter, due to the high mutation and replication rate of the virus. The high mutation rate of $3x10^{-5}$ base pairs per replication cycle [20] results from the lack of proofreading activity of the Reverse Transcriptase (RT) [21]. The combination of a high mutation rate and a high replication rate (10^8-10^{10} viral particles per day) [22] generates a huge diversity. All sorts of pressure result in a fast selection of escape viruses. One type of selective pressure is exerted by ART. Low adherence to the therapy and inefficient ART lead to a fast selection of drug resistant viruses. Another type of pressure is exerted by the immune system. Early in the HIV-infection, immune escape viruses are selected [23, 24]. A third type of selective pressure is due to the specific micro-environment at the site of viral replication. Combined with a distinct distribution of target cells in tissues [26] this leads to localised divergence and compartmentalisation [25].

Recombination is another mechanism explaining the viral diversity in the HIV-infected individual. Superinfection (the sequential infection from two sources), or concomitant infection explains co-infection with distinct HIV-subtypes. Inter- and intra-subtype genetic recombination occurs. The recombination rate will be higher intrasubtype compared to intersubtype [27-29], while influence on diversity will be of more importance when recombination occurs intersubtype.

Latency

The major impediment to the total eradication of HIV is the establishment of a viral reservoir. This already occurs in the first days after infection [30]. In the transcriptionally silent condition, HIV evades recognition by the immune system and targeting by ART.

When a cell becomes infected with HIV, the viral genome undergoes reverse transcription. The subsequently transcribed DNA either is inserted into the human genome (integrated DNA = proviral DNA) or remains non-integrated [31]. The non-integrated form is unstable and decreases very fast under ART [32]. On the other hand, the decay rate of the more stable integrated provirus under HAART is very low.

The integration-site is located preferentially in active genes [33] and is supposed not only to determine the efficacy of subsequent viral replication but also to play a role in the latency status of the HIV-virus [34].

The resting memory CD4 T cells harbour the greatest part of the actually known HIV-reservoir. Latency in these cells is supposed to arise from HIV-infection of activated CD4 T cells at the moment that these cells are turning to the resting state. Other cells that contribute to the latent HIV-reservoir are macrophages [25], monocytes [35], dentritic cells [25, 36], γδ T cells [37] and natural killer (NK) cells [38]. The contribution of all these different cell types to the latent pool, depends on the half-live of these cells, their cellular activity, sensitivity to apoptosis and their distribution in the distinct tissues (as the brain [39], testes, kidneys, …). The peripheral blood cells are the best accessible and most frequently studied HIV-infected cells, although, they are supposed to constitute only a minor fraction of the total HIV-reservoir.

Clinical, virological and immunological evolution of an HIV-infection

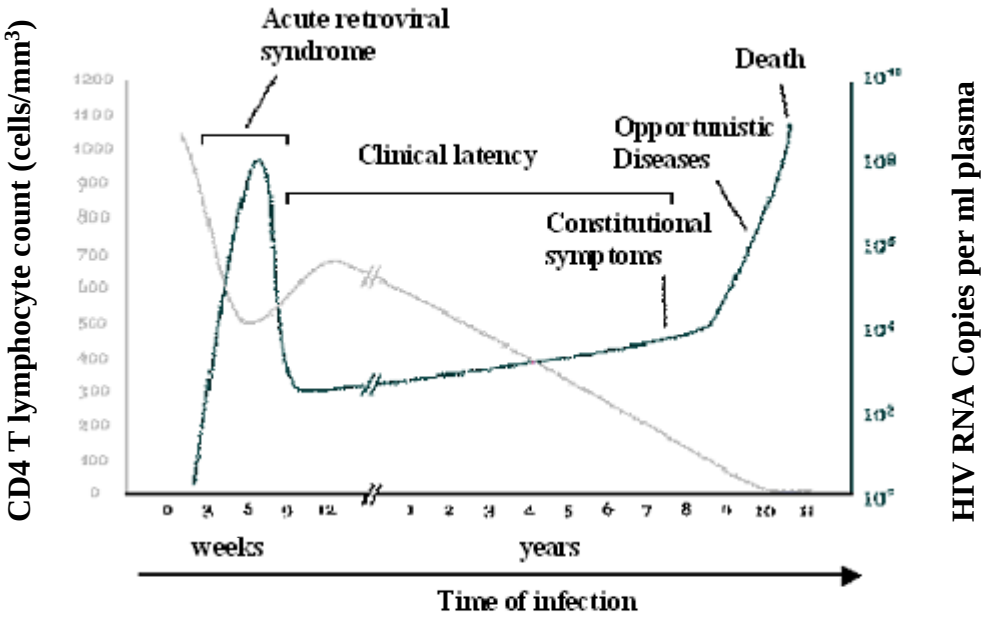

Fig. 2: The distinct phases of the HIV-infection; clinical, viral load and CD4 T cell number evolution is schematically depicted.
(adapted from http://www.nationmaster.com/encyclopedia/HIV).

2.2.3 Immunological evolution

A major number of cells that play a crucial role in the immune system are paralysed from the early start of HIV-infection. The adaptive as well as the innate immune system and the links between these systems are seriously affected.

Adaptive immune system

CD4/CD8 T cell ratio and CD4 T cell counts

Already early in the HIV-infection, the number of CD4 T cells, constituting the main marker of disease progression, is profoundly decreased. At the same time CD8 T cells expand, leading to an inverted CD4/CD8 T cell ratio.

After the early stage there is a spontaneous but incomplete recovery of CD4 T cells (Fig. 2), while the CD4/CD8 T cell ratio generally remains inverted.

Afterwards, CD4 T cells gradually decline over the course of the HIV-infection [11]. When CD4 T cells fall below a critical level (<200 cells/µl blood or <14% of blood lymphocytes), the risk to develop OI's increases considerably. Clinical decisions as when to start preventive treatment for OI's and/or ART will strongly be influenced by the CD4 T cell count.

CD4 T cell decline

HIV and CD4 T Cell loss

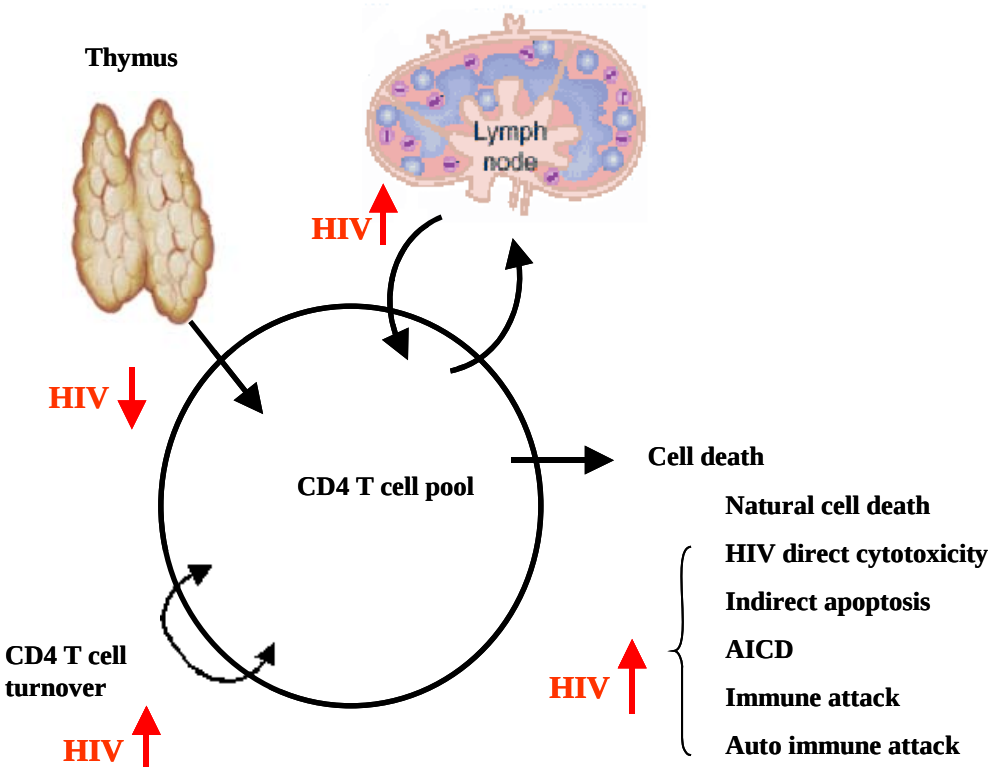

Fig. 3: Mechanisms contributing to the total number of blood CD4 T cells

The progressive loss of the CD4 T cells is the result of the imbalance between the clearance and the renewal of those cells. This implies that the production of the CD4 T cells, which is in the order of $2x10^9$ cells per day (in HIV-infection), is exceeded by its destruction. CD4 T cell destruction is caused by direct cytotoxicity of HIV and by apoptosis of HIV-infected and uninfected CD4 T cells.

In vitro HIV-infection is associated with apoptosis of T cells and T cell lines [40-42]. The activation state of the host cell probably influences its sensitivity to direct viral toxicity, as activated cells faster undergo apoptosis. Direct viral toxicity, however, is probably not the main reason for the CD4 T cell decline, as the number of HIV-infected cells is too low (in the order of 1/1000 to 1/8000) to explain the observed cell death and as only a minor fraction of the apoptotic cells is physically infected by HIV. Apoptosis has indeed been shown to occur especially in bystander cells [43] and less in productively HIV-infected cells [44, 45].

Besides direct viral cytotoxicity, cell death can be explained by apoptosis induced by viral plasma proteins. *In vitro,* the interaction of the viral gp120 envelope protein with CD4 has been shown to impair lymphocyte function [46] and to prime T cells for programmed cell death (PCD) [47]. Subsequent T cell receptor (TCR) engagement is sufficient to induce apoptosis. Alternatively, if the HIV-infected cells are already activated, binding of gp120 to the CD4/CXCR4 molecules induces cell death. In addition, not only gp120, but also other HIV plasma proteins have been reported to play a role in inducing cell death [48].

Apoptosis of lymphocytes also results from continuous immune activation as described in chronic infections with cytomegalovirus, Epstein Barr virus and varicella zoster. This physiological apoptosis is called activation induced cell death (AICD) [49, 50]. AICD is the regulatory mechanism necessary in order to maintain the equilibrium in the repertoire of immune cells after antigen encounter. In HIV-infection a big proportion of the extensive cell death can be attributed to AICD [51, 52].

The immune response against HIV is as well responsible for the induction of cell death. This is mediated by cytotoxic T lymphocytes (CTL's) and Natural Killer cells (NK cells) which lyse HIV-infected cells.

Several scientists suggest that auto-immune mechanisms, provoked by HIV contribute to a progressive decline in CD4 T cell count and cause progression from HIV-infection to AIDS[53].

In addition to the enhanced destruction of CD4 T cells, also the replenishment of those cells is severely affected by HIV-infection because of diminished thymic output. HIV-infection causes a profound reduction in precursor proliferation of thymocytes [54], leading to a serious limitation in CD4 T cell regeneration [55].

Trapping of CD4 T cells in the lymph nodes also contributes to the decline of their number in the blood [56].

CD8 T cells, cytotoxic T cells (CTL)

In the acute HIV-infection, a crucial role is attributed to HIV-specific CTL's. The clonal expansion of these CTL's is supposed to be responsible for the decline in plasma peak VL [57] as it occurs simultaneous. Studies in non-human primates also provide arguments for the pivotal role of these CTL's as depletion of CTL's results in an uncontrolled viral replication and a fast progression to AIDS [58, 59]. The antiviral effect of CTL's relies on their possibility to lyse HIV-infected cells and to release soluble antiviral factors.

Despite the continuous presence of high amounts of HIV-specific CTL's throughout the HIV disease, progression to AIDS is seen (Fig. 4).

It is suggested that the efficacy of the CTL's decreases after the acute phase of HIV-infection as a result of the depletion of sustaining HIV-specific CD4 T cells (Fig. 4; 1).

In addition, the fast occurrence of immune escape HIV variants is responsible for the loss of efficient CTL immune responses [23, 24, 60](Fig. 4; 2). Antigen presentation as well as immune recognition can be affected by these mutations which diminish the sensitivity of HIV-infected cells to CTL-mediated lysis. The observation that over the disease course different HIV peptides are recognised is in line with this hypothesis. Tat and Rev are especially targeted early in HIV-infection and are shown to be related with suppressed VL [61-63].

Another mechanism that explains the persistence of HIV replication despite the presence of HIV-specific CTL's, is the induction of tolerance and clonal exhaustion because of the extremely high concentrations of antigen [64, 65] (Fig. 4; 3).

Deficient co-stimulation, because of an HIV-induced decreased expression of co-stimulatory proteins, is an additional way by which the virus escapes immune responses (Fig. 4; 4).

Causes of CTL dysfunction

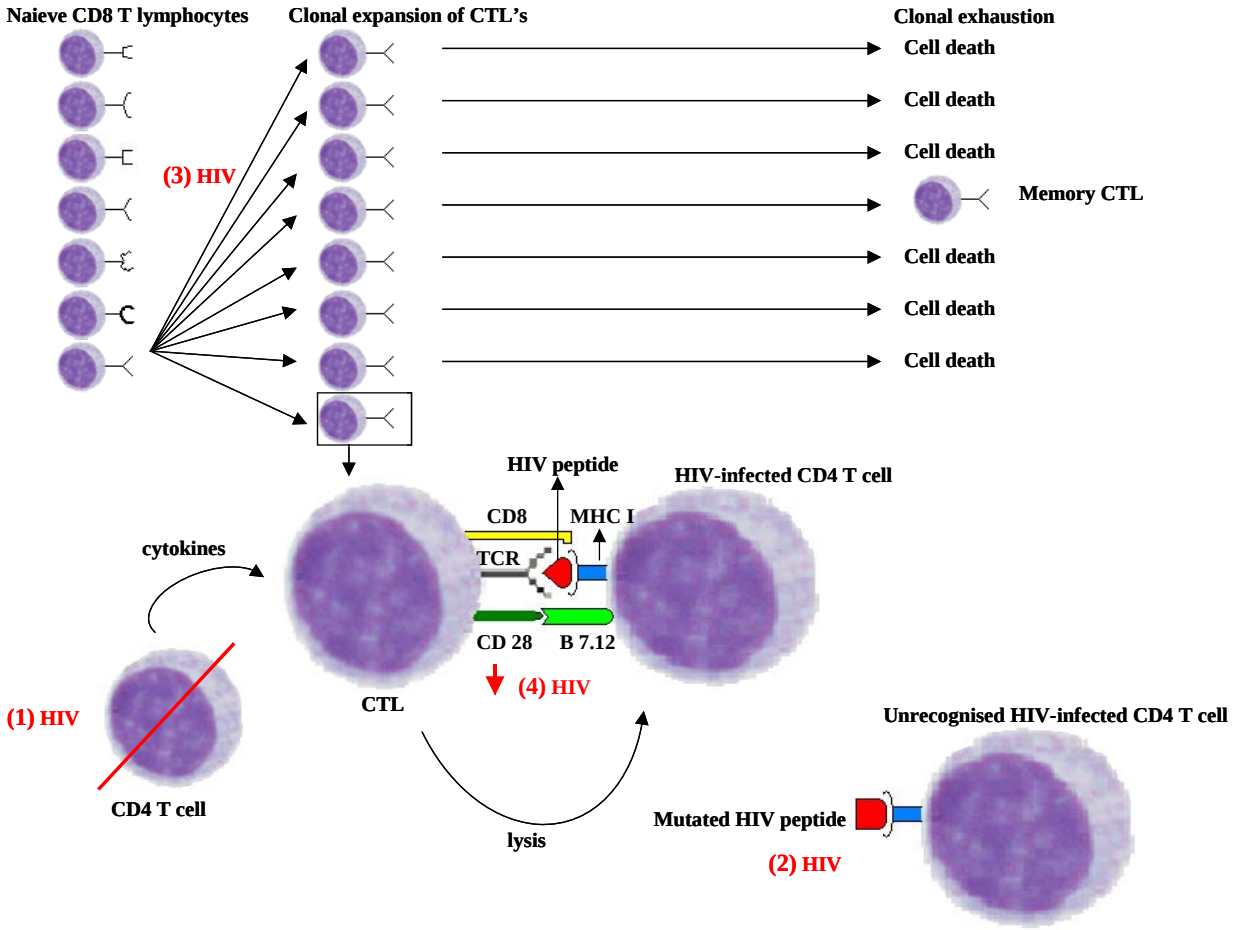

Fig. 4: Different mechanisms that contribute to the dysfunction of CTL's

Innate Immune system

Also the cells of the innate immune system are seriously affected by the HIV virus. The immune dysfunction caused by HIV, in Natural Killer (NK) cells, NKT cells, monocytes, macrophages and Dendritic Cells (DC) (all responsible for the innate immune response), is characterised by serious functional impairment in addition to reduced cell numbers. NK cells are impaired in their cytotoxic activity, which can be explained by a shift in cell subsets; HIV causes an expansion of dysfunctional NK cells ($CD56^-/CD16^+$), which have an upregulated expression of inhibitory NK receptors and a downregulated expression of cytotoxic receptors[66]. Those cells also have an impaired secretion of antiviral cytotoxic cytokines. On the other hand HIV causes a downregulation in HLA class I A and B expression in the infected cells [67], which further increases their escape from NK cell killing. The NKT subset

expressing CD4, is seriously depleted in HIV infection [68]. Monocytes and macrophages can become infected by HIV as they express CD4 and CCR5 coreceptor. Those cells, together with DC's, are spreading the virus as a Trojan horse to the different organs [69]. DC's are impaired in their stimulation of T cells, probably because of a diminished expression of costimulatory molecules and a block in their maturation [70].

2.2.4 Inter-individual differences

Disease progression varies strongly among HIV-infected individuals. A small subset of HIV-infected individuals (1%) is able to maintain high and stable CD4 T cell counts together with low to undetectable plasma VL for more than 10-15 years in the absence of ART [71-73]. These patients are called Long-Term Non-Progressors (LTNP). On the other hand, very fast progression with evolution to AIDS in several months also has been described [74, 75]. In general, in the western world, HIV-infected individuals are without therapy, progressing to AIDS in a mean period of 10 years.

Factors influencing the rate of disease progression in HIV infection vary greatly among individuals and are still poorly defined. Yet, HIV-specific CD4 helper and effector T cell responses are consistently found in LTNP [73]. Certainly viral as well as host dependent mechanisms contribute to the rate of disease progression. Among viral factors deletions in Nef [76] and other rare molecular changes such as deletions in Gag, Env and Nef and insertion in Vpu genes [77], have been related with slow or non-progression . In addition, host dependent mechanisms are the expression of certain HLA class I haplotypes [78-80], expression of the Δ32 mutated CCR5 co-receptor [81] or other co-receptor polymorphisms [82] and certain cytokine and cytokine receptor specificities [83].

Understanding the factors related with better outcome of the HIV-infection will improve our knowledge of the HIV-pathogenesis and will open perspectives for future treatment options.

2.3 INFLUENCE OF HAART ON CLINICAL DISEASE PROGRESSION, VIRAL RESERVOIRS AND IMMUNE FUNCTION

Action sites of antiretroviral drugs

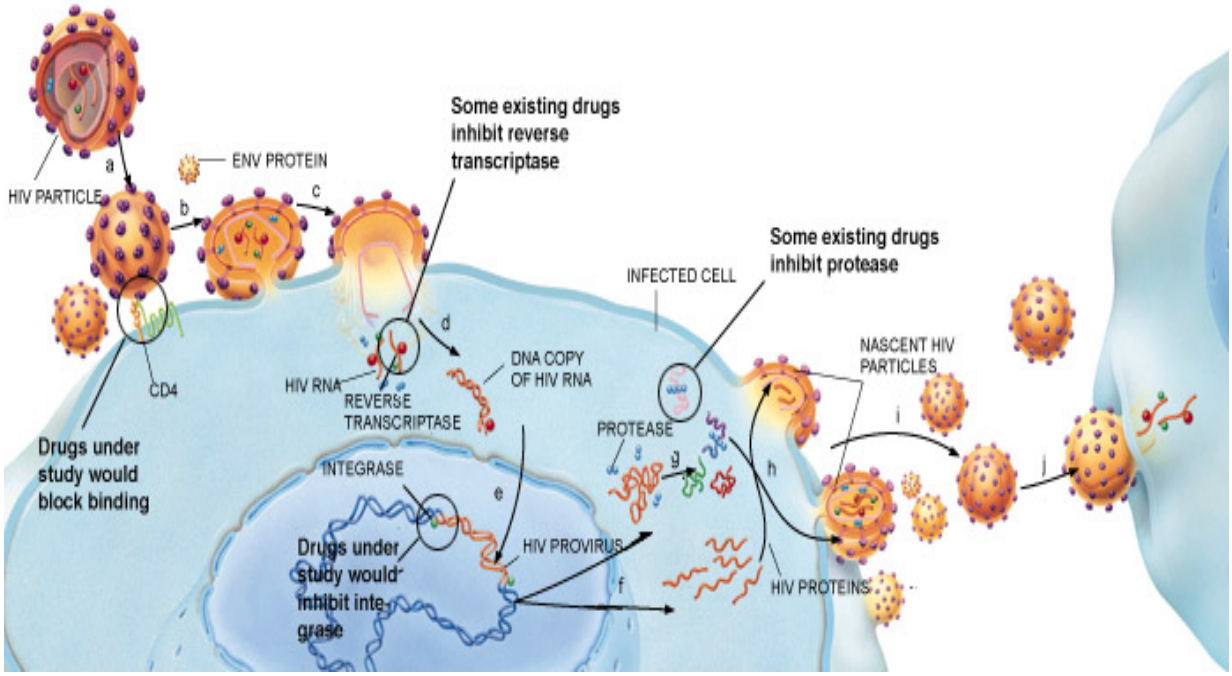

Fig. 5: Viral replication and action sites of different antiretroviral drug classes (from: http://webs.wichita.edu/mschneegurt/biol103/lecture15/hiv_cycle_drugs_best.jpg).

2.3.1 Influence of HAART on clinical disease progression

The clinical impact of HAART strongly depends on the stage of the disease at which HAART is started. It is obvious that in the 'clinical latency' phase no immediate clinical benefit will be seen. Moreover, adverse effects of ART can worsen the patient's quality of life in the short and long term. Depending on the specific antiretroviral drugs complications are lipodystrophy, glucose intolerance, allergies, gastro-intestinal intolerance and neurological inconveniences. Those side effects are responsible for a large proportion of the hospitalisations among the HIV infected patients.

The start of HAART late in the course of the HIV disease, on the other hand, can be followed by a spectacular clinical amelioration. When HIV-infected individuals are consulting with symptoms of AIDS, they will first receive treatment for the OI and ART will be started later. The effect of this combined treatment can be very spectacular and is known as the 'Lazarus

Syndrome'. Patients nearly dying on admission in the hospital, are able to return to work and to lead a normal life after a period of treatment.

After the instauration of HAART, however, some patients are clinically deteriorating despite decreasing VL and rising CD4 T cells. Side effects of medication but also unrecognised OI's or auto-immune diseases can cause the new or aggravated symptoms. Progression to AIDS can easily occur in this late stage disease even after instauration of HAART. The CD4 T cell count at initiation of therapy is the dominant prognostic factor in this progression to AIDS [84].

Another possible cause of clinical deterioration is paradoxically due to the immune recovery on ART. Pathogens, previously present in the tissue but unable to cause an immune reaction because of the profound immunologic failure, are now able to provoke a severe inflammatory response. This phenomenon is known as the immune reconstitution inflammatory syndrome (IRIS). The inflammatory reaction can be extremely strong, leading to death (for example through rise of intracranial pressure) or irreversible sequelae as for example blindness. HIV-infected individuals at risk for IRIS are those with previous apparent or sub-clinical OI's. The pathogens most frequently associated with IRIS are cytomegalovirus, *Mycobacterium tuberculosis, Mycobacterium avium, Cryptococcus neoformans*, hepatitis B and C and herpes zoster. Factors, related with a high occurrence of IRIS are low CD4 T cell count and high plasma VL at the time of starting HAART [85]. IRIS is associated with a strong decrease in plasma VL in the first 3 months after instauration of HAART, starting HAART within the first 30 days after initiating treatment for an OI and the fact of being ARV naïve before starting HAART [86]. Other studies found that a higher rise in CD4 T cell percentage [87] and in CD4/CD8 T cell ratio [88] was especially correlated with a higher incidence of IRIS.

It is difficult to distinguish between the intimately linked inflammation and active infection as the cause of clinical deterioration. Usually non steroidal anti-inflammatory drugs as well as corticosteroids eventually combined with antimicrobial therapy provide relief [89, 90].

It is not known if a certain treatment strategy in late stage HIV-infection is able to prevent IRIS. No consensus exists about the management of HIV-infected individuals presenting with severe immune deficiency and OI's. Therapeutic decisions are taken case by case, relying on clinical presentation, drug related adverse effects, pill burden and the patient's choice.

2.3.2 Influence of HAART on viral load, latency and resistance

HAART is generally able to suppress plasma VL to undetectable levels (<50 HIV copies/ml plasma). The time period after which this undetectable VL is reached is longer in patients with a high plasma VL (>10^5 log copies/ml) at the start of HAART.

Despite prolonged periods of efficient ART resulting in long term undetectable plasma VL, the latent HIV pool decreases very slowly. The half-life time of memory CD4 T cells, which harbour the greatest part of the latent HIV reservoir in the blood, is shown to be at least 44 months [91, 92]. Immune intervention (e.g. with IL-2) [93, 94] and HAART intensification [95] can augment the proviral decay rates. Proviral decay rates are also higher in treated acute HIV-infection [96, 97].

Sustained low level HIV replication is supposed to contribute to the containment or even the expansion of the HIV proviral reservoir [25]. The frequency of detectable viral blips (>50 HIV copies/ml) was found to be two times higher in HIV-infected individuals treated during chronic infection compared to those treated during acute infection [98].

Problems of ARV drug resistance, emerging fast during periods of non-compliance or sub-optimal HAART, compromise the clinical benefits related to ART. Genotyping, detecting specific resistance-linked mutations, is the most frequently used method to detect drug resistance. A large number of mutations associated with resistance to the currently used ARV drugs are identified [99]. These mutations generally emerge in a step-wise manner with a cumulative influence on the sensitivity to the ARV drugs. Different algorithms are used to predict phenotypical resistance based on the observed genotype (Stanford University, REGA Institute). The results of these predictions are guiding the clinicians in their choice for effective HAART combinations.

2.3.3 Immune recovery under HAART

Many cells that mediate important immune functions are affected by HIV: CD4 T cells, HIV-specific CD4 and CD8 T cells, γδ T cells, dendritic cells (DCs), IFNα producing Antigen Presenting cells (APC's), NK cells, Natural Killer T (NKT) cells,… These impaired immune functions cannot always recover with HAART.

The timing of HAART intervention may be a key factor in determining the extent of possible immune restoration. There is a consensus that patients with AIDS or with a CD4 T cell count <200 cells/µl blood should receive HAART. Recent guidelines recommend now intervention with HAART at 350 CD4 T cells/µl blood. This is in line with studies demonstrating that the immune recovery will be more pronounced if therapy is started earlier in the HIV disease course [100-102]. However, studies have shown that even with extremely severe immune deficiency significant increases in CD4 T cell count could be obtained with HAART in combination with IL-2 [103].

When the mean recovery of CD4 T cells is studied, a biphasic process is observed [104]. In the first year, the number of memory CD4 T cells increases rapidly, which is explained by a redistribution of CD4 T cells from the periphery, especially from the lymph nodes to the blood [105, 106]. This is followed by a slow increase in naïve CD4 T cells, which continues for 2 to 3 years after instauration of HAART [107]. Therapy in early HIV-infection, however, can result in an immediate rise in naïve CD4 T cells [108]. When mechanisms responsible for this increase are further investigated a reduction in direct viral cytotoxicity and indirect apoptosis with an increased thymic output is expected. Al-Harthi et al. reported that a significant reduction of apoptosis because of HAART could only be found in HIV-infected patients who had >500 CD4 T cells/µl blood at the start of treatment [109, 110]. Naïve CD4 T cells, rising after the instauration of HAART are supposed to proceed from the thymus. T cell receptor excision circles (TREC's), a marker for recent thymic immigrants (RTE), are rising because of HAART even irrespective of HIV-infection [111]. A longer thymocyte survival is suggested as explanation. Thymus volume is shown to increase in some patients after the instauration of ART. Also diversity of the immune answer, analysed by the T cell receptor (TCR) repertoire, is enhanced for CD8 T cells in HAART treated acute and chronic HIV-infection, while for CD4 T cells amelioration was only seen in HAART treated acute infection [112].

Some patients fail to exhibit a marked increase in CD4 T cells despite a suppression of their VL to undetectable levels in response to HAART. The opposite, an increase in CD4 T cells without a total viral suppression, is also observed. This discordance between virological and immunological responses is seen in 5-27 % of HIV-infected patients starting HAART and is associated with a poor clinical outcome [113]. Thymus failure [114] or sustained apoptosis [115] are proposed to be the reason of failing immune recovery. PI-containing HAART regimens are associated with better immune recovery [116]. Virological failure with immunological response can be explained by resistance [117] and adherence problems [118] but is also found associated with PI based regimens without drug resistance [119].

References

1. Gottlieb MS, Schroff R, Schanker HM, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med.* Dec 10 1981;305(24):1425-1431.

2. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science.* May 20 1983;220(4599):868-871.

3. Herbeuval JP, Boasso A, Grivel JC, et al. TNF-related apoptosis-inducing ligand (TRAIL) in HIV-1-infected patients and its in vitro production by antigen-presenting cells. *Blood.* Mar 15 2005;105(6):2458-2464.

4. CDC issues guidelines on treating OIs among adults. ART is cornerstone of treatment plan. *AIDS Alert.* Jun 2005;20(6):66-68.

5. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science.* Dec 1 1989;246(4934):1155-1158.

6. Traynor K. WHO, UNAIDS set HIV treatment goal for developing countries. Plan is to treat 3 million with HIV infection by 2005. *Am J Health Syst Pharm.* Jan 15 2004;61(2):128, 130.

7. Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. *N Engl J Med.* Jul 2 1998;339(1):33-39.

8. Huang ST, Lee HC, Liu KH, Lee NY, Ko WC. Acute human immunodeficiency virus infection. *J Microbiol Immunol Infect.* Feb 2005;38(1):65-68.

9. Vanhems P, Beaulieu R. Primary infection by type 1 human immunodeficiency virus: diagnosis and prognosis. *Postgrad Med J.* Jul 1997;73(861):403-408.

10. Vanhems P, Voirin N, Hirschel B, et al. Brief report: incubation and duration of specific symptoms at acute retroviral syndrome as independent predictors of progression to AIDS. *J Acquir Immune Defic Syndr.* Apr 15 2003;32(5):542-544.

11. Differences in CD4 cell counts at seroconversion and decline among 5739 HIV-1-infected individuals with well-estimated dates of seroconversion. *J Acquir Immune Defic Syndr.* Sep 1 2003;34(1):76-83.

12. Michael NL, Burke DS. Natural history of human immunodeficiency virus infection. *Dermatol Clin.* Jul 1991;9(3):429-441.

13. Pilcher CD, Fiscus SA, Nguyen TQ, et al. Detection of acute infections during HIV testing in North Carolina. *N Engl J Med.* May 5 2005;352(18):1873-1883.

14. DeSimone JA, Pomerantz RJ. New methods for the detection of HIV. *Clin Lab Med.* Sep 2002;22(3):573-592, v.

15. Blattner WA, Ann Oursler K, Cleghorn F, et al. Rapid clearance of virus after acute HIV-1 infection: correlates of risk of AIDS. *J Infect Dis.* May 15 2004;189(10):1793-1801.

16. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science.* May 24 1996;272(5265):1167-1170.

17. Verhofstede C, Demecheleer E, De Cabooter N, et al. Diversity of the human immunodeficiency virus type 1 (HIV-1) env sequence after vertical transmission in mother-child pairs infected with HIV-1 subtype A. *J Virol.* Mar 2003;77(5):3050-3057.

18. Ritola K, Pilcher CD, Fiscus SA, et al. Multiple V1/V2 env variants are frequently present during primary infection with human immunodeficiency virus type 1. *J Virol.* Oct 2004;78(20):11208-11218.

19. Altfeld M, Rosenberg ES, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J Exp Med.* Jan 15 2001;193(2):169-180.
20. Havlir DV, Richman DD. Viral dynamics of HIV: implications for drug development and therapeutic strategies. *Ann Intern Med.* Jun 1 1996;124(11):984-994.
21. Mansky LM, Temin HM. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J Virol.* Aug 1995;69(8):5087-5094.
22. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science.* Mar 15 1996;271(5255):1582-1586.
23. Bernardin F, Kong D, Peddada L, Baxter-Lowe LA, Delwart E. Human immunodeficiency virus mutations during the first month of infection are preferentially found in known cytotoxic T-lymphocyte epitopes. *J Virol.* Sep 2005;79(17):11523-11528.
24. Borrow P, Lewicki H, Wei X, et al. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med.* Feb 1997;3(2):205-211.
25. Schrager LK, D'Souza MP. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. *Jama.* Jul 1 1998;280(1):67-71.
26. Fulcher JA, Hwangbo Y, Zioni R, et al. Compartmentalization of human immunodeficiency virus type 1 between blood monocytes and CD4+ T cells during infection. *J Virol.* Aug 2004;78(15):7883-7893.
27. Fang G, Weiser B, Kuiken C, et al. Recombination following superinfection by HIV-1. *Aids.* Jan 23 2004;18(2):153-159.
28. Steain MC, Wang B, Dwyer DE, Saksena NK. HIV-1 co-infection, superinfection and recombination. *Sex Health.* 2004;1(4):239-250.
29. Taylor JE, Korber BT. HIV-1 intra-subtype superinfection rates: estimates using a structured coalescent with recombination. *Infect Genet Evol.* Jan 2005;5(1):85-95.
30. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci U S A.* Jul 21 1998;95(15):8869-8873.
31. Monie D, Simmons RP, Nettles RE, et al. A novel assay allows genotyping of the latent reservoir for human immunodeficiency virus type 1 in the resting CD4+ T cells of viremic patients. *J Virol.* Apr 2005;79(8):5185-5202.
32. Zhou Y, Zhang H, Siliciano JD, Siliciano RF. Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. *J Virol.* Feb 2005;79(4):2199-2210.
33. Schroder AR, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell.* Aug 23 2002;110(4):521-529.
34. Jordan A, Defechereux P, Verdin E. The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transactivation. *Embo J.* Apr 2 2001;20(7):1726-1738.
35. Belmonte L, Bare P, de Bracco MM, Ruibal-Ares BH. Reservoirs of HIV replication after successful combined antiretroviral treatment. *Curr Med Chem.* Feb 2003;10(4):303-312.
36. Patterson S, Helbert M, English NR, Pinching AJ, Knight SC. The effect of AZT on dendritic cell number and provirus load in the peripheral blood of AIDS patients: a preliminary study. *Res Virol.* Mar-Jun 1996;147(2-3):109-114.

37. Imlach S, Leen C, Bell JE, Simmonds P. Phenotypic analysis of peripheral blood gammadelta T lymphocytes and their targeting by human immunodeficiency virus type 1 in vivo. *Virology.* Jan 20 2003;305(2):415-427.
38. Valentin A, Rosati M, Patenaude DJ, et al. Persistent HIV-1 infection of natural killer cells in patients receiving highly active antiretroviral therapy. *Proc Natl Acad Sci U S A.* May 14 2002;99(10):7015-7020.
39. Glass JD, Johnson RT. Human immunodeficiency virus and the brain. *Annu Rev Neurosci.* 1996;19:1-26.
40. Lelievre JD, Petit F, Perrin L, et al. The density of coreceptors at the surface of CD4+ T cells contributes to the extent of human immunodeficiency virus type 1 viral replication-mediated T cell death. *AIDS Res Hum Retroviruses.* Nov 2004;20(11):1230-1243.
41. Martin SJ, Matear PM, Vyakarnam A. HIV-1 infection of human CD4+ T cells in vitro. Differential induction of apoptosis in these cells. *J Immunol.* Jan 1 1994;152(1):330-342.
42. Pandolfi F, Pierdominici M, Oliva A, et al. Apoptosis-related mortality in vitro of mononuclear cells from patients with HIV infection correlates with disease severity and progression. *J Acquir Immune Defic Syndr Hum Retrovirol.* Aug 15 1995;9(5):450-458.
43. Alimonti JB, Ball TB, Fowke KR. Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *J Gen Virol.* Jul 2003;84(Pt 7):1649-1661.
44. Cosenza MA, Zhao ML, Lee SC. HIV-1 expression protects macrophages and microglia from apoptotic death. *Neuropathol Appl Neurobiol.* Oct 2004;30(5):478-490.
45. Mahlknecht U, Deng C, Lu MC, et al. Resistance to apoptosis in HIV-infected CD4+ T lymphocytes is mediated by macrophages: role for Nef and immune activation in viral persistence. *J Immunol.* Dec 1 2000;165(11):6437-6446.
46. Williams MA, Trout R, Spector SA. HIV-1 gp120 modulates the immunological function and expression of accessory and co-stimulatory molecules of monocyte-derived dendritic cells. *J Hematother Stem Cell Res.* Oct 2002;11(5):829-847.
47. Badley AD, Pilon AA, Landay A, Lynch DH. Mechanisms of HIV-associated lymphocyte apoptosis. *Blood.* Nov 1 2000;96(9):2951-2964.
48. James CO, Huang MB, Khan M, Garcia-Barrio M, Powell MD, Bond VC. Extracellular Nef protein targets CD4+ T cells for apoptosis by interacting with CXCR4 surface receptors. *J Virol.* Mar 2004;78(6):3099-3109.
49. Akbar AN, Borthwick N, Salmon M, et al. The significance of low bcl-2 expression by CD45RO T cells in normal individuals and patients with acute viral infections. The role of apoptosis in T cell memory. *J Exp Med.* Aug 1 1993;178(2):427-438.
50. Uehara T, Miyawaki T, Ohta K, et al. Apoptotic cell death of primed CD45RO+ T lymphocytes in Epstein-Barr virus-induced infectious mononucleosis. *Blood.* Jul 15 1992;80(2):452-458.
51. Badley AD, Parato K, Cameron DW, et al. Dynamic correlation of apoptosis and immune activation during treatment of HIV infection. *Cell Death Differ.* May 1999;6(5):420-432.
52. Muro-Cacho CA, Pantaleo G, Fauci AS. Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden. *J Immunol.* May 15 1995;154(10):5555-5566.

53. Bourinbaiar AS, Abulafia-Lapid R. Autoimmunity, alloimmunization and immunotherapy of AIDS. *Autoimmun Rev.* Jul 2005;4(6):403-409.
54. Dion ML, Poulin JF, Bordi R, et al. HIV infection rapidly induces and maintains a substantial suppression of thymocyte proliferation. *Immunity.* Dec 2004;21(6):757-768.
55. Ye P, Kirschner DE, Kourtis AP. The thymus during HIV disease: role in pathogenesis and in immune recovery. *Curr HIV Res.* Apr 2004;2(2):177-183.
56. Hengel RL, Jones BM, Kennedy MS, Hubbard MR, McDougal JS. Cd4+ T cells programmed to traffic to lymph nodes account for increases in numbers of cd4+ T cells up to 1 year after the initiation of highly active antiretroviral therapy for human immunodeficiency virus type 1 infection. *J Infect Dis.* Jul 1 2001;184(1):93-97.
57. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol.* Jul 1994;68(7):4650-4655.
58. Jin X, Bauer DE, Tuttleton SE, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med.* Mar 15 1999;189(6):991-998.
59. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science.* Feb 5 1999;283(5403):857-860.
60. O'Connor DH, Allen TM, Vogel TU, et al. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. *Nat Med.* May 2002;8(5):493-499.
61. Addo MM, Altfeld M, Rosenberg ES, et al. The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals. *Proc Natl Acad Sci U S A.* Feb 13 2001;98(4):1781-1786.
62. van Baalen CA, Pontesilli O, Huisman RC, et al. Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. *J Gen Virol.* Aug 1997;78 (Pt 8):1913-1918.
63. van Baalen CA, Guillon C, van Baalen M, et al. Impact of antigen expression kinetics on the effectiveness of HIV-specific cytotoxic T lymphocytes. *Eur J Immunol.* Sep 2002;32(9):2644-2652.
64. Pantaleo G, Soudeyns H, Demarest JF, et al. Evidence for rapid disappearance of initially expanded HIV-specific CD8+ T cell clones during primary HIV infection. *Proc Natl Acad Sci U S A.* Sep 2 1997;94(18):9848-9853.
65. Schols D, De Clercq E. Human immunodeficiency virus type 1 gp120 induces anergy in human peripheral blood lymphocytes by inducing interleukin-10 production. *J Virol.* Aug 1996;70(8):4953-4960.
66. Mavilio D, Lombardo G, Benjamin J, et al. Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc Natl Acad Sci U S A.* Feb 22 2005;102(8):2886-2891.
67. Cohen GB, Gandhi RT, Davis DM, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity.* Jun 1999;10(6):661-671.
68. Unutmaz D. NKT cells and HIV infection. *Microbes Infect.* Sep 2003;5(11):1041-1047.
69. Verani A, Gras G, Pancino G. Macrophages and HIV-1: dangerous liaisons. *Mol Immunol.* Feb 2005;42(2):195-212.
70. Donaghy H, Stebbing J, Patterson S. Antigen presentation and the role of dendritic cells in HIV. *Curr Opin Infect Dis.* Feb 2004;17(1):1-6.

71. Kootstra NA, Miedema F, Schuitemaker H. Analysis of CD8+ T lymphocyte-mediated nonlytic suppression of autologous and heterologous primary human immunodeficiency virus type 1 isolates. *AIDS Res Hum Retroviruses.* May 20 1997;13(8):685-693.

72. Mendila M, Heiken H, Becker S, et al. Immunologic and virologic studies in long-term nonprogressors with HIV-1 infection. *Eur J Med Res.* Oct 15 1999;4(10):417-424.

73. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science.* Nov 21 1997;278(5342):1447-1450.

74. Demarest JF, Jack N, Cleghorn FR, et al. Immunologic and virologic analyses of an acutely HIV type 1-infected patient with extremely rapid disease progression. *AIDS Res Hum Retroviruses.* Sep 20 2001;17(14):1333-1344.

75. Markowitz M, Mohri H, Mehandru S, et al. Infection with multidrug resistant, dual-tropic HIV-1 and rapid progression to AIDS: a case report. *Lancet.* Mar 19-25 2005;365(9464):1031-1038.

76. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC. Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. *N Engl J Med.* Jan 26 1995;332(4):228-232.

77. Alexander L, Weiskopf E, Greenough TC, et al. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. *J Virol.* May 2000;74(9):4361-4376.

78. den Uyl D, van der Horst-Bruinsma IE, van Agtmael M. Progression of HIV to AIDS: a protective role for HLA-B27? *AIDS Rev.* Apr-Jun 2004;6(2):89-96.

79. Frahm N, Adams S, Kiepiela P, et al. HLA-B63 presents HLA-B57/B58-restricted cytotoxic T-lymphocyte epitopes and is associated with low human immunodeficiency virus load. *J Virol.* Aug 2005;79(16):10218-10225.

80. Migueles SA, Sabbaghian MS, Shupert WL, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci U S A.* Mar 14 2000;97(6):2709-2714.

81. Stewart GJ, Ashton LJ, Biti RA, et al. Increased frequency of CCR-5 delta 32 heterozygotes among long-term non-progressors with HIV-1 infection. The Australian Long-Term Non-Progressor Study Group. *Aids.* Dec 1997;11(15):1833-1838.

82. Burton CT, Gotch FM, Imami N. CCR2/64I mutation detection in a HIV-1-positive patient with slow CD4 T-cell decline and delay in disease progression. *Int J STD AIDS.* May 2005;16(5):392-394.

83. Soriano A, Lozano F, Oliva H, et al. Polymorphisms in the interleukin-4 receptor alpha chain gene influence susceptibility to HIV-1 infection and its progression to AIDS. *Immunogenetics.* Sep 28 2005:1-11.

84. Egger M, May M, Chene G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet.* Jul 13 2002;360(9327):119-129.

85. French MA, Lenzo N, John M, et al. Immune restoration disease after the treatment of immunodeficient HIV-infected patients with highly active antiretroviral therapy. *HIV Med.* Mar 2000;1(2):107-115.

86. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy. *Aids.* Mar 4 2005;19(4):399-406.

87. de Boer MG, Kroon FP, Kauffmann RH, Vriesendorp R, Zwinderman K, van Dissel JT. Immune restoration disease in HIV-infected individuals receiving highly active

antiretroviral therapy: clinical and immunological characteristics. *Neth J Med.* Dec 2003;61(12):408-412.

88. Breton G, Duval X, Estellat C, et al. Determinants of immune reconstitution inflammatory syndrome in HIV type 1-infected patients with tuberculosis after initiation of antiretroviral therapy. *Clin Infect Dis.* Dec 1 2004;39(11):1709-1712.

89. French MA, Price P, Stone SF. Immune restoration disease after antiretroviral therapy. *Aids.* Aug 20 2004;18(12):1615-1627.

90. Hirsch HH, Kaufmann G, Sendi P, Battegay M. Immune reconstitution in HIV-infected patients. *Clin Infect Dis.* Apr 15 2004;38(8):1159-1166.

91. Finzi D, Blankson J, Siliciano JD, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med.* May 1999;5(5):512-517.

92. Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med.* Jun 2003;9(6):727-728.

93. Kulkosky J, Pomerantz RJ. Approaching eradication of highly active antiretroviral therapy-persistent human immunodeficiency virus type 1 reservoirs with immune activation therapy. *Clin Infect Dis.* Dec 15 2002;35(12):1520-1526.

94. Lafeuillade A, Poggi C, Chadapaud S, Hittinger G, Khiri H, Halfon P. Impact of immune interventions on proviral HIV-1 DNA decay in patients receiving highly active antiretroviral therapy. *HIV Med.* Jul 2001;2(3):189-194.

95. Ramratnam B, Ribeiro R, He T, et al. Intensification of antiretroviral therapy accelerates the decay of the HIV-1 latent reservoir and decreases, but does not eliminate, ongoing virus replication. *J Acquir Immune Defic Syndr.* Jan 1 2004;35(1):33-37.

96. Ngo-Giang-Huong N, Deveau C, Da Silva I, et al. Proviral HIV-1 DNA in subjects followed since primary HIV-1 infection who suppress plasma viral load after one year of highly active antiretroviral therapy. *Aids.* Apr 13 2001;15(6):665-673.

97. Strain MC, Little SJ, Daar ES, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis.* May 1 2005;191(9):1410-1418.

98. Di Mascio M, Markowitz M, Louie M, et al. Dynamics of intermittent viremia during highly active antiretroviral therapy in patients who initiate therapy during chronic versus acute and early human immunodeficiency virus type 1 infection. *J Virol.* Oct 2004;78(19):10566-10573.

99. Johnson VA, Brun-Vezinet F, Clotet B, et al. Drug resistance mutations in HIV-1. *Top HIV Med.* Nov-Dec 2003;11(6):215-221.

100. Babiker A, Darbyshire J, Pezzotti P, et al. Short-term CD4 cell response after highly active antiretroviral therapy initiated at different times from seroconversion in 1,500 seroconverters. *J Acquir Immune Defic Syndr.* Mar 1 2003;32(3):303-310.

101. Sterling TR, Chaisson RE, Keruly J, Moore RD. Improved outcomes with earlier initiation of highly active antiretroviral therapy among human immunodeficiency virus-infected patients who achieve durable virologic suppression: longer follow-up of an observational cohort study. *J Infect Dis.* Dec 1 2003;188(11):1659-1665.

102. Garcia F, de Lazzari E, Plana M, et al. Long-term CD4+ T-cell response to highly active antiretroviral therapy according to baseline CD4+ T-cell count. *J Acquir Immune Defic Syndr.* Jun 1 2004;36(2):702-713.

103. Katlama C, Carcelain G, Duvivier C, et al. Interleukin-2 accelerates CD4 cell reconstitution in HIV-infected patients with severe immunosuppression despite highly

active antiretroviral therapy: the ILSTIM study--ANRS 082. *Aids.* Oct 18 2002;16(15):2027-2034.

104. Wu H, Connick E, Kuritzkes DR, et al. Multiple CD4+ cell kinetic patterns and their relationships with baseline factors and virological responses in HIV type 1 patients receiving highly active antiretroviral therapy. *AIDS Res Hum Retroviruses.* Sep 1 2001;17(13):1231-1240.

105. Bucy RP, Hockett RD, Derdeyn CA, et al. Initial increase in blood CD4(+) lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. *J Clin Invest.* May 15 1999;103(10):1391-1398.

106. Lederman MM. Immune restoration and CD4+ T-cell function with antiretroviral therapies. *Aids.* Feb 2001;15 Suppl 2:S11-15.

107. Autran B. Effects of antiretroviral therapy on immune reconstitution. *Antivir Ther.* 1999;4 Suppl 3:3-6.

108. Al-Harthi L, Landay A. Immune recovery in HIV disease: role of the thymus and T cell expansion in immune reconstitution strategies. *J Hematother Stem Cell Res.* Oct 2002;11(5):777-786.

109. Al-Harthi L, Siegel J, Spritzler J, Pottage J, Agnoli M, Landay A. Maximum suppression of HIV replication leads to the restoration of HIV-specific responses in early HIV disease. *Aids.* May 5 2000;14(7):761-770.

110. Al-Harthi L, Voris J, Patterson BK, et al. Evaluation of the impact of highly active antiretroviral therapy on immune recovery in antiretroviral naive patients. *HIV Med.* Jan 2004;5(1):55-65.

111. Graham DB, Bell MP, Huntoon CJ, et al. Increased thymic output in HIV-negative patients after antiretroviral therapy. *Aids.* Sep 23 2005;19(14):1467-1472.

112. Cossarizza A, Poccia F, Agrati C, et al. Highly active antiretroviral therapy restores CD4+ Vbeta T-cell repertoire in patients with primary acute HIV infection but not in treatment-naive HIV+ patients with severe chronic infection. *J Acquir Immune Defic Syndr.* Mar 1 2004;35(3):213-222.

113. Moore DM, Hogg RS, Yip B, et al. Discordant Immunologic and Virologic Responses to Highly Active Antiretroviral Therapy Are Associated With Increased Mortality and Poor Adherence to Therapy. *J Acquir Immune Defic Syndr.* Nov 1 2005;40(3):288-293.

114. Teixeira L, Valdez H, McCune JM, et al. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *Aids.* Sep 28 2001;15(14):1749-1756.

115. Hansjee N, Kaufmann GR, Strub C, Weber R, Battegay M, Erb P. Persistent apoptosis in HIV-1-infected individuals receiving potent antiretroviral therapy is associated with poor recovery of CD4 T lymphocytes. *J Acquir Immune Defic Syndr.* Jun 1 2004;36(2):671-677.

116. Dronda F, Moreno S, Moreno A, Casado JL, Perez-Elias MJ, Antela A. Long-term outcomes among antiretroviral-naive human immunodeficiency virus-infected patients with small increases in CD4+ cell counts after successful virologic suppression. *Clin Infect Dis.* Oct 15 2002;35(8):1005-1009.

117. Nicastri E, Sarmati L, d'Ettorre G, et al. High prevalence of M184 mutation among patients with viroimmunologic discordant responses to highly active antiretroviral therapy and outcomes after change of therapy guided by genotypic analysis. *J Clin Microbiol.* Jul 2003;41(7):3007-3012.

118. Moroni M. Genotypic resistance tests for the management of patients with viro-immunological discordant response to highly active antiretroviral therapy. *Scand J Infect Dis Suppl.* Dec 2003;35 Suppl 106:85-87.

119. Kaplan SS, Ferrari G, Wrin T, et al. Longitudinal assessment of immune response and viral characteristics in HIV-infected patients with prolonged CD4(+)/viral load discordance. *AIDS Res Hum Retroviruses.* Jan 2005;21(1):13-16.

CHAPTER III: AIMS

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Although the benefit of HAART on HIV-related morbidity and mortality is proven, the current ART is unable to eradicate HIV. Moreover, short and long term drug toxicity, adherence problems because of drug intake fatigue and the resulting resistance problems all compromise the efficacy of HAART.

For the optimal use of HAART, a lot of questions remain to be elucidated. When is the optimal time to start HAART? What are the risks and benefits of a temporary HAART regimen in early HIV-infection? Can HAART, started early in HIV-infection, induce a status of long-term non-progression? How long should temporary HAART in early HIV-infection be given? Is recycling of ARV drugs possible after resistance formation?

To answer those questions we studied the effect of HAART in different cohorts of HIV-infected individuals.

1) As an introduction to the different sub-studies that are presented in this work, we assessed in article 1 the global impact of the first decade of HAART on the AIDS epidemic, with special emphasis on the differences between the western and the developing world.

2) From viral as well as immunological point of view, treatment in acute HIV-infection can be very useful. Early treatment of HIV-infection restricts viral diversity and spread and the amount of latently HIV-infected cells. This strategy also preserves HIV-specific CD4 T cells and maintains a larger and broader CD4 T cell repertoire. Most of the studies showing a better immune recovery or a lower burden in viral HIV reservoir with early ART are assessing those factors while patients are still on therapy. As HAART is associated with diverse side effects and risk for drug resistance, it is important to evaluate whether temporary treatment can change the HIV disease evolution.

In article 2, we investigated the benefit of HAART started early after HIV-infection. VL, CD4 T cell count and HIV-specific immunity were analysed longitudinal after treatment interruption in a group of 40 patients. A cohort of 28 acute HIV-infected patients that remained untreated was followed as a control group.

3) A large proportion of HIV-infected patients, however, first consults in the late stage of the HIV disease. In article 3, the start of HAART in late stage HIV-infection was investigated.

The clinical consequences of this strategy are illustrated by a case report of a tuberculosis (TB)/HIV co-infected patient suffering from IRIS.

4+5) Although the theoretical ultimate goal of therapy is the total clearance of HIV from its host, it is more and more clear that this goal will not be reached in HIV-infection with the current HAART. This is mainly due to two factors: (1) the development of ARV drug resistance and (2) the presence of a long living HIV reservoir. ARV medication, exerting its action by blocking viral replication, can not target latent HIV as no replication takes place. The combination of resistance and latency compromising the efficacy of HAART, is addressed in articles 4 and 5. A cohort of patients harbouring resistant HIV was switched to an efficient HAART regimen which suppressed their plasma VL to undetectable levels for several years (mean: 59 months). Genotyping of pro-viral reservoir in those patients, assessed the persistence of the former resistance linked mutations and wild type HIV.

CHAPTER IV: ARTICLES

Influence of temporary treatment in early HIV-1 infection on disease evolution.

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Influence of temporary treatment in early HIV-1 infection on disease evolution.

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Running head: Influence of treatment in early HIV

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Abstract: We investigated the long-term benefit of early temporary antiretroviral therapy in HIV-1 infection. Sixty-eight recently infected individuals were selected of whom forty initiated antiretroviral therapy within the first 6 months of infection. Twenty eight patients remained untreated. Eight patients were excluded for further analysis because they did not achieve an undetectable viral load. The remaining 32 patients stayed on treatment for a mean period of 17,3 months. Blood samples were collected regularly before, during and after treatment, for viral load (VL) determination and CD4 T cell count. HIV specific CD8 and CD4 T cells, producing IFN-γ in response to overlapping HIV peptides, were measured in 23 individuals (13 treated and 10 untreated). The evolution of the different parameters in treated and untreated individuals was compared, after therapy interruption and infection respectively.

Over the 3 years of treatment free follow-up a trend towards lower VL and higher CD4 T cell count was seen in the treated patients compared to the untreated individuals. No loss of HIV-specific immune cells because of treatment was observed. In conclusion, our results indicate that temporary treatment early after infection can result in a delay in disease evolution that extends the strict time on therapy.

Keywords: early treatment, HIV-infection, CD4 T cell count, viral load

Introduction

Acute HIV-1 infection is characterized by an extensive viral replication reaching levels of over 10^6copies HIV-1 RNA/ml. The spontaneous decrease of viral load after the initial burst [1] is mainly attributed to the development of a specific immune response [2, 3], in combination with a profound loss of target CD4 T cells. Cytotoxic CD8 T cells (CTL's), supported in their function by CD4 T cells, are thought to play a pivotal role in the early immune response as their expansion coincides with the decrease in plasma VL. In addition, studies in CD8 T cell depleted macaques demonstrated an uncontrolled replication of the virus and an accelerated clinical progress [4, 5]. The efficacy of the CD8 T cell responses, however, seems strongly diminished in the later stages of the infection, as observations show that disease progresses despite the presence of high numbers of CTL's. The decrease of sustaining CD4 T cells and the selection of immune escape mutants are possible explanations for the failure of the CTL's.

The possible benefit of treating patients during acute infection is a matter of controverse. Although studies showed that early ART could preserve HIV-specific immunity [6-8], side effects of the current treatment protocols such as lipodystrophy, glucose intolerance, allergies, gastro-intestinal intolerance and neurological inconveniences limit their long term use. Current guidelines therefore recommend the initiation of ART only in those patients in whom CD4 counts are indicative for a fast progression towards AIDS [9].

Only few studies have evaluated the long-term effect of temporary early treatment on biological parameters as CD4 T cell count and VL. Although this approach might allow the preservation of HIV-specific CD4 T cell responses, and possibly induce a long term immune control, fear exists that after stopping the treatment, viral rebound soon abolishes the possible benefits.

We studied 68 patients with an acute HIV-1 infection. Forty patients were temporary treated within the first six months of their infection, with individual adapted ART. Thirty two were able to reach undetectable viral load levels and were included for further analysis. Longitudinal analysis for VL and CD4 T cell count were performed and the results were compared for the 32 treated and 28 untreated individuals. In 23 patients, 13 treated and 10 untreated, also the presence of HIV specific IFN-γ producing CD8 and CD4 T cells was examined.

Materials and Methods

Patient recruitment

Sixty-eight adult individuals with a documented acute HIV-infection were included in the study. Sixty-six were Caucasian and sixty-four were men. Seventeen had been tested positive for syphilis, one patient had an active hepatitis B infection and 3 had active hepatitis C. Baseline clinical characteristics were comparable in both groups. Early infection was attributed to the individuals with either a documented HIV seroconversion, an isolated high risk exposure, an indeterminate western blot or an acute seroconversion syndrome. Forty patients were consulting the Aids reference Centre (ARC) in the first six months after the presumed infection date and were willing to participate in the study. They all received ART treatment. The ART combination was chosen on an individual basis. Twenty eight patients consulted the ARC later than six months after the presumed infection date and these patients were followed as untreated controls. The study was approved by the Ethical Committee of our institution and the participating patients signed the informed consent form.

PBMC's, CD4 T cell counts, HIV-1 levels:

At each visit CD4 T cells numbers were determined by flow cytometry using the FACScan cytofluorometer and the Cellquest software (Beckton Dickinson Mountain View, California, USA). CD4 T cells numbers were expressed as cells per microliter whole blood. Plasma HIV-1 was measured using the ultrasensitive Amplicor HIV-1 Monitor test (Roche Diagnostic Systems) with a lower detection limit of 1,7 $\log_{10}$ copies/ml and a higher detection limit of 5,0 $\log_{10}$ copies/ml. PBMC's were recovered by centrifugation on Ficoll-Hypaque gradients and cryopreserved in 90% FCS and 10% DMSO. PBMC's were thawed on the day of testing.

Stimulation assay for HIV specific CD4 and CD8 lymphocytes

PBMC's were thawed and used in a stimulation assay as described [10]. PBMC's were suspended at 10^6 /150 µl RPMI medium, together with co-stimulatory antibodies; anti-CD49d and anti-CD28, at a final concentration of 1µg/ml each. Cells were divided in a 96 well round bottom plate and peptides were added at a concentration of 2µg/ml. The peptides were obtained from the NIBSC centralized Facility for AIDS reagents (UK). The peptides used were 20-mers spanning the rev, tat and p24 region and 15-mers spanning the p17 region of HIV-1 type B. As negative control, only co-stimulatory antibodies without peptides were added. As positive control we used SEB 2µg/ml. 10µg/ml BFA was added to each well. The final volume was 200µl/well. Cells were left

at 37°C in a humidified 7% CO2 incubator for 5 hours. After this incubation, cells were placed overnight in a refrigerator, protected from light. Subsequently, cell surfaces were stained with CD4-PerCP, CD3-APC and CD8-PE (Becton Dickinson). Cells were permeabilised with cytofix/cytoperm (BD) and additionally stained intracellular for IFN-γ-FITC (BD). Acquisition was done with the FACScalibur; 500.000 cells per test were acquired. Frequencies of IFN-γ producing cells were reported after subtraction of the frequencies in medium controls. Tests were done at least in two-fold and mean results were taken for presentation.

Statistical analysis

Statitistical analysis was performed using the program SPSS 12.0 for windows. The Mann Whitney test was used for analyzing differences between groups. For longitudinal tests in one group the Wilcoxon rank test was chosen. Kaplan Meier survival curve was used together with Log Rank testing for analyzing a defined endpoint over time. Only p values ≤0.05 were considered significant.

Results

Patient characteristics

Of the 68 individuals with acute HIV-1 infection that were selected, 28 remained untreated. Treatment was initiated within 6 months after the onset of infection in 40. Eight of them did not reach undetectable viral load levels due to non-compliance (in 7) or side effects (in 1) and the therapy in these patients was stopped. Final analysis was performed on the remaining 32 treated (T) and the 28 untreated patients (UT). The treatment-free follow-up time was counted from the treatment interruption in the patients receiving therapy while for the patients who remained untreated the follow-up time was counted from the presumed infection date. The mean treatment free follow-up period was 34 months (5-87 months). The mean age at infection and the baseline CD4 T cell counts were comparable in both groups (UT: 34 years versus T: 36 years; p=0.744; UT: 506 versus T: 492 cells/µl; p=0.578), but a significantly lower mean VL was observed in the untreated individuals (p<0,001) (see Table I).

The effect of ART on viral load

Antiretroviral therapy suppressed plasma viral load to undetectable levels in the 32 patients after a mean period of less than four months. After one year of treatment free follow-up, the mean viral load in the patients who received treatment was lower than the mean viral load in the treatment naïve patients (3,95 log versus 4,42 log; p=0.060). Lower viral loads in the treated group were also observed after 2 and 3 years of follow-up, though differences were not statistically significant (respectively 3,86 log and 4,30 log after 2 years; p=0,108; 3,63 log and 4,05 log after 3 years; p=0.172) (see Table I).

The Kaplan Meier curve (not shown) illustrates the evolution of the VL in the two groups. An endpoint VL of 55 000 copies/ml was chosen. The treated patients were able to suppress their viral load to below this endpoint for a significant longer time compared to the treatment naïve patients (Log Rank: 0,027).

Detailed observation of the evolution of the viral load after treatment cessation in the different patients revealed different patterns. In part of the patients, a peak of virus replication followed by a relative suppression was observed, others showed a viral rebound without any subsequent control and in others no peak levels but a slow continuous increase of the viral load over time was seen. In 2 treated patients VL remained extremely low after treatment interruption (less than 2,00 log). In one of them, the viral load even remained undetectable during the whole follow-up period of 18 months.

The effect of ART on CD4 T cell count

The use of ART during acute infection resulted in a mean increase of 332 CD4 T cells/µl as compared to the pretreatment baseline value ($p<0.001$). One year after treatment interruption the mean CD4 T cell count in the treated group was 666 cells/µl compared to 490 cells/µl in the treatment naïve group one year after infection (p=0.087). After two years the mean CD4 T cell counts were respectively 569 and 488 (p=0.500). After 3 years CD4 T cell counts were 708 and 505 respectively (p=0.010) (table I).

We observed an overall association between a low baseline CD4 T cell count and a fast decline of CD4 cells after treatment interruption. However, in 5 of the 9 treated patients with baseline CD4 T cell counts under 350 cells/µl, no drop below this level was observed during the treatment free follow-up of respectively 9, 30, 30, 33 and 50 months.

The effect of ART on the IFN-γ-producing HIV-specific T cells.

We determined the presence of HIV-1 specific CD8 and CD4 T cell IFN-γ reaction respectively 200 and 500 days after treatment interruption or infection (see fig 2). The mean HIV-specific CD8 reaction was not significantly different for both groups (3.35 $x10^6$ cells/l at 200 days and 5.27 $x10^6$ cells/l at 500 days in the treated group; 1.20 $x10^6$ cells/l at 200 days and 1.95 $x10^6$ cells/l at 500 days in the untreated group) (p=0.108 and p=0.099 respectively). An increase in reactivity over time was observed in both groups.

Equally, no differences in HIV-specific CD4 T cell reactivity were seen, with a mean reactivity in the treated group of 0.77 $x10^6$ cells/l after 200 days and 0.69 $x10^6$ cells/l after 500 days and of 0.41 $x10^6$ cells/l after 200 days and 0.63 $x10^6$ cells/l after 500 days in the treatment naïve group (p=0.23 and p=1). Immune reaction against p24 was observed most frequently in both groups. The second most frequently recognized peptide for CD8 T cell reactivity was p17. No relation could be found between the intensity of the CD8 T cell reaction and the viral load. The CD4 T cell analysis revealed generally lower percentages of cells producing IFN-γ, but in the 5 treated individuals with at least 2000 x 10^3 HIV-specific CD4 T cells/l, long term viral suppression was seen in 3 and temporary viral suppression in 2. In the untreated individuals CD4 T cell reactivity never reached levels of 2000 x 10^3 cells/l.

In 2 of the treated individuals, CD8 T cell reaction to p17 epitopes arose after more than one year of treatment interruption. Moreover, in 2 other patients CD8 T cell reaction against respectively tat and rev arose after more than 3 years of interruption.

The effect of ART on the need to restart treatment

We also compared the number of patients in both the treated and the untreated group who reached the criteria to start or restart HAART. These criteria are a CD4 T cell count of less then 350 cells/µl and/or a VL above 55 000 copies/ml. The period before reaching these criteria was significantly longer in the treated compared to the untreated individuals (Log Rank: 0,033) (Fig 1). Of the 28 untreated patients with a follow-up of more than 2 years, 19 reached the criteria. Only 8 of them effectively started HAART. Of the 26 treated patients that were followed for more than 2 years after treatment interruption, only 10 reached the defined criteria. Five effectively restarted treatment.

Discussion

A relative and durable viral control in early HIV-1 infection has been seen following structured treatment interruptions (STI) [11, 12]. The aim of these controlled on- and off-cycles of drug intake was to stimulate the immune system through natural vaccination. Results, however, were disappointing and the observation in some cases of drug resistant viral variants during the periods of therapy interruption [13] further tempered the enthusiasm. Also the risk of an extreme loss of CD4 T cells was described [14]. Therefore most STI studies are actually stopped. The effect of one single short period of early antiretroviral therapy on the long term outcome of the infection on the other hand, is still rarely studied. Jansen et al. found that only one out of 5 temporary treated acute HIV-infected patients was able to maintain a viral control, one year after treatment interruption [7]. Desquilbet et al. were not able to show a difference in VL, one year after treatment interruption, between 58 temporary treated patients and 116 patients that were never treated [15]. Markowitz et al. also were not able to see a positive effect on the viral load set point, one year after an early antiretroviral treatment either or not combined with an adjunctive vaccine in 16 individuals tested [16]. The aim of the study presented here was to assess the influence of early but temporary treatment in a real-life setting. Despite the lack of restrictions according to the choice of the antiretroviral regimen or the duration of the treatment we were able to show that this approach is definitely not harmful for the patient or for the disease process and that it might even lead to a delayed disease process after therapy interruption.

Sixty eight patients with a documented acute HIV-1 infection and for whom the presumed infection date could be defined, were studied. Patients willing to participate in the study were divided in two groups according to the time of their first consultation. Forty patients first visited the Aids Reference Centre of our hospital within 6 months after the presumed infection date and in these patients therapy was initiated. The drug regimen was individually adapted. Two patients, included during 1997, received bitherapy, the remaining 38 received a HAART regimen composed of 3 or 4 drugs. In the 32 analysed patients, ART was given for a mean period of 17,3 months after which all medication was stopped. Twenty eight patients first visited the ARC 6 months or later after the presumed infection time. These patients remained untreated. Viral load and CD4 T cell counts were determined in both groups as markers of disease progression. The baseline characteristics of both the treated and untreated group were comparable with exception

of the baseline viral load that was significantly higher in the treated compared to the untreated group. This difference is most likely due to the fact that the baseline viral load sample for the treated individuals was obtained within 6 months of the presumed infection time, while in the untreated patients the interval between sampling and infection time was more than 6 months. Therefore most of the samples from the treated individuals have been collected during the peak of viral replication. The differences in baseline viral load between both groups however did not influence the findings and final conclusions of this work.

The ART regimens were well tolerated in all but one patient. Seven patients were unable to reach an undetectable viral load within 6 months of therapy initiation. All 7 admitted bad adherence. For the remaining 32 patients therapy resulted in a fast drop of viral loads to below the limit of detection and a significant increase of the CD4 T cell count. After treatment interruption viral load rebounded in 31. One patient was able to maintain an undetectable viral load during the whole treatment free follow-up period of 18 months. Encouraging was that, despite the renewed virus replication and a decrease of the CD4 T cell count after the treatment stop, lower viral load levels and higher CD4 counts were consistently found after 1, 2 and 3 years follow up in the treated compared to the untreated patients, although, differences were not significant. Viral load levels and CD4 T cell numbers are generally accepted as good prognostic markers for disease progression [17-19]. Our results are therefore indicative for a delay in disease progression in the treated individuals that extends beyond the strict on-treatment period.

The number of patients included in the analysis for the 3 year follow-up time point was significantly reduced in both groups due to a drop out of those patients who needed to start or restart therapy and due the fact that for several patients the actual follow-up time did not exceed 2 years. After 2 years of follow-up, the untreated patients achieved significantly faster the criteria to initiate medication compared to the treated patients. Therapy had to be initiated in 67,9% of the untreated patients, while reinitiation of therapy was needed in only 38,5% of the treated patient. The better outcome of the patients who received temporary treatment is also clearly illustrated in the Kaplan Meier curve in Fig 1, showing a significantly lower drop-out of patients because of viral loads exceeding 55 000 copies/ml in the treated group compared to the untreated

group. We chose 55 000 copies/ml as cut-off value for the VL, as above this level HAART was often started [20].

The difference in conclusion between our study and the former temporary treated patient cohorts reported, could be explained by our study design. We omitted patients not achieving undetectable viral load from further analysis. Only 1 patient in our study had adverse events that required interruption of treatment. The prevalence of adverse events was much higher in the study of Desquilbet et al. (12/58) [15], which could imply a higher risk for low level replication under treatment. However, we found the same mean VL set point after one year treatment interruption (3,95 log). In their study 22% were women, which were related with lower VL set points, while in our study only 9% were women. Their control patients were taken from another study cohort, at a much earlier time point (1989 ↔ 1996-2003), which could provoke a selection bias. Markowitz et al. [16] used vaccines, activating immune cells, and so inducing a higher amount of target cells. This could have a negative effect on the viral rebound. Jansen et al. studied only 5 patients, so it is difficult to make general conclusions [7].

We also analysed the cellular immune responses against several HIV peptides. In agreement with the results presented by others [6-8, 21-23], we were able to show that HIV-specific T cells are preserved through therapy in early HIV-infection. Besides, from the small cohort of patients that we studied we observed that the magnitude and the breadth of the CD4 and CD8 response in the treated patients after treatment interruption were superior to the untreated group. Absolute numbers of HIV-specific CD4 T cells exceeded 2 000 x 10^3 cells/l in all 5 patients with a post-treatment suppression of the viral load; however, in 2 this suppression was only temporary. No relation between HIV-specific CD8 T cells and HIV-specific CD4 T cells or VL was seen. We observed that in most of the patients, HIV-specific CD8 T cell reaction is increasing over time after treatment interruption and that the response is broadening. Rising viral diversity can explain the broader T cell receptor recognition over time. It is also remarkable that even after a long time (> 3 years) of treatment interruption, new HIV peptides can be targeted.

The improved outcome in the treated patients could be explained by efficient activation of a sufficient pool of HIV specific memory cells through viral rebound. Treating early in infection

could also preserve other precious functions of the immune system [24]. Restriction of viral diversity by early treatment might be beneficial for the immune response after treatment stop. A reduction of viral spread and proviral levels can have similar effects.

The study presented here was performed on a relatively low number of patients. Although the results are promising, further analysis on larger patient populations is definitely needed to allow a better insight in the impact of temporary treatment on the disease evolution. Studies, aimed at elucidating the differences between those patients with a favourable outcome after treatment interruption and those with a bad outcome, will improve our understanding of the HIV pathogenesis and might guide the development of alternative intervention strategies. The ideal treatment period is actually unknown and needs further studies. In literature treatment in acute HIV infection varies between some weeks [8] and several years (3 years [16]). Although the number of patients that we studied is small, our results pointed towards an improved outcome after longer treatment (more that 15 months) compared to shorter treatment (results not shown), but this observation definitely needs further evaluation.

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References

1. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med.* Apr 4 1991;324(14):961-964.

2. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol.* Jul 1994;68(7):4650-4655.

3. Musey L, Hughes J, Schacker T, Shea T, Corey L, McElrath MJ. Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. *N Engl J Med.* Oct 30 1997;337(18):1267-1274.

4. Jin X, Bauer DE, Tuttleton SE, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med.* Mar 15 1999;189(6):991-998.

5. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science.* Feb 5 1999;283(5403):857-860.

6. Alter G, Hatzakis G, Tsoukas CM, et al. Longitudinal assessment of changes in HIV-specific effector activity in HIV-infected patients starting highly active antiretroviral therapy in primary infection. *J Immunol.* Jul 1 2003;171(1):477-488.

7. Jansen CA, De Cuyper IM, Steingrover R, et al. Analysis of the effect of highly active antiretroviral therapy during acute HIV-1 infection on HIV-specific CD4 T cell functions. *Aids.* Jul 22 2005;19(11):1145-1154.

8. Oxenius A, Price DA, Easterbrook PJ, et al. Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes. *Proc Natl Acad Sci U S A.* Mar 28 2000;97(7):3382-3387.

9. Wang C, Vlahov D, Galai N, et al. Mortality in HIV-seropositive versus -seronegative persons in the era of highly active antiretroviral therapy: implications for when to initiate therapy. *J Infect Dis.* Sep 15 2004;190(6):1046-1054.

10. Cosma A, Nagaraj R, Buhler S, et al. Therapeutic vaccination with MVA-HIV-1 nef elicits Nef-specific T-helper cell responses in chronically HIV-1 infected individuals. *Vaccine.* Dec 8 2003;22(1):21-29.

11. Kaufmann DE, Lichterfeld M, Altfeld M, et al. Limited durability of viral control following treated acute HIV infection. *PLoS Med.* Nov 2004;1(2):e36.

12. Rosenberg ES, Altfeld M, Poon SH, et al. Immune control of HIV-1 after early treatment of acute infection. *Nature.* Sep 28 2000;407(6803):523-526.

13. Tremblay CL, Hicks JL, Sutton L, et al. Antiretroviral resistance associated with supervised treatment interruptions in treated acute HIV infection. *Aids.* May 2 2003;17(7):1086-1089.

14. Oxenius A, Hirschel B. Structured treatment interruptions in HIV infection: benefit or disappointment? *Expert Rev Anti Infect Ther.* Jun 2003;1(1):129-139.

15. Desquilbet L, Goujard C, Rouzioux C, et al. Does transient HAART during primary HIV-1 infection lower the virological set-point? *Aids.* Dec 3 2004;18(18):2361-2369.

16. Markowitz M, Jin X, Hurley A, et al. Discontinuation of antiretroviral therapy commenced early during the course of human immunodeficiency virus type 1 infection, with or without adjunctive vaccination. *J Infect Dis.* Sep 1 2002;186(5):634-643.

17. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med.* Jun 15 1997;126(12):946-954.

18. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science.* May 24 1996;272(5265):1167-1170.

19. Craib KJ, Strathdee SA, Hogg RS, et al. Serum levels of human immunodeficiency virus type 1 (HIV-1) RNA after seroconversion: a predictor of long-term mortality in HIV infection. *J Infect Dis.* Sep 1997;176(3):798-800.

20. Jacobs B, Neil N, Aboulafia DM. Retrospective analysis of suspending HAART in selected patients with controlled HIV replication. *AIDS Patient Care STDS.* Jul 2005;19(7):429-438.

21. Altfeld M, Rosenberg ES, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J Exp Med.* Jan 15 2001;193(2):169-180.
22. Oxenius A, Fidler S, Brady M, et al. Variable fate of virus-specific CD4(+) T cells during primary HIV-1 infection. *Eur J Immunol.* Dec 2001;31(12):3782-3788.
23. Fidler S, Oxenius A, Brady M, et al. Virological and immunological effects of short-course antiretroviral therapy in primary HIV infection. *Aids.* Oct 18 2002;16(15):2049-2054.
24. George MD, Reay E, Sankaran S, Dandekar S. Early antiretroviral therapy for simian immunodeficiency virus infection leads to mucosal CD4+ T-cell restoration and enhanced gene expression regulating mucosal repair and regeneration. *J Virol.* Mar 2005;79(5):2709-2719.

Fig 1

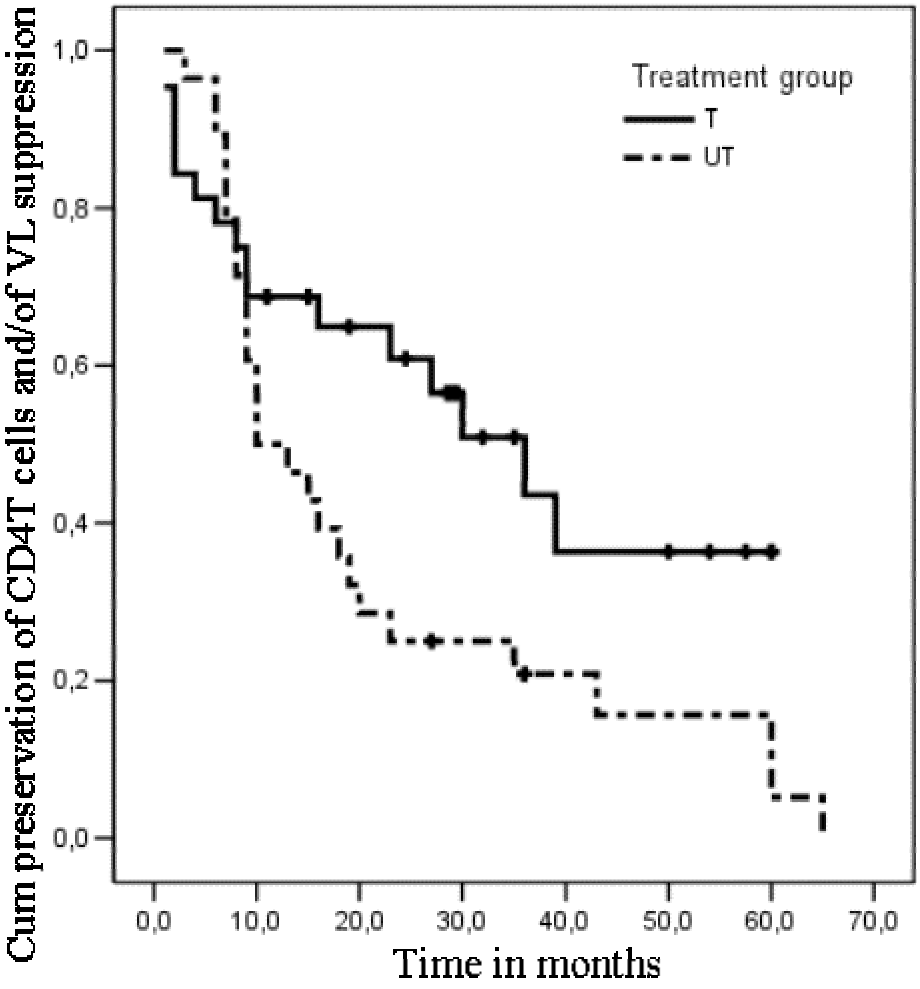

Fig 1:

Kaplan-Meier curve showing the percentage of patients who reach the criteria to start HAART; CD4 T cells <350 cells/ml and/or VL >55000 copies/µl, over time. Time is expressed in months. Log Rank between the T and UT group is 0,033.

Fig 2 a

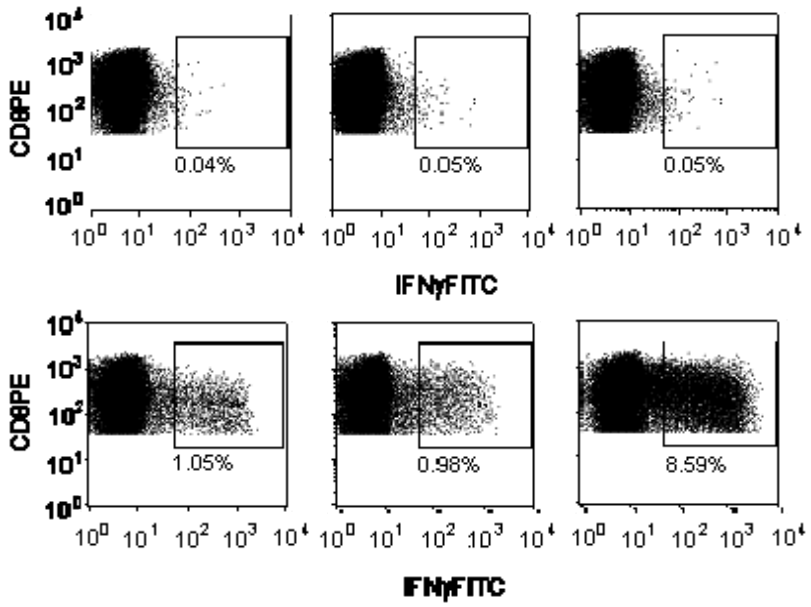

Fig 2 b

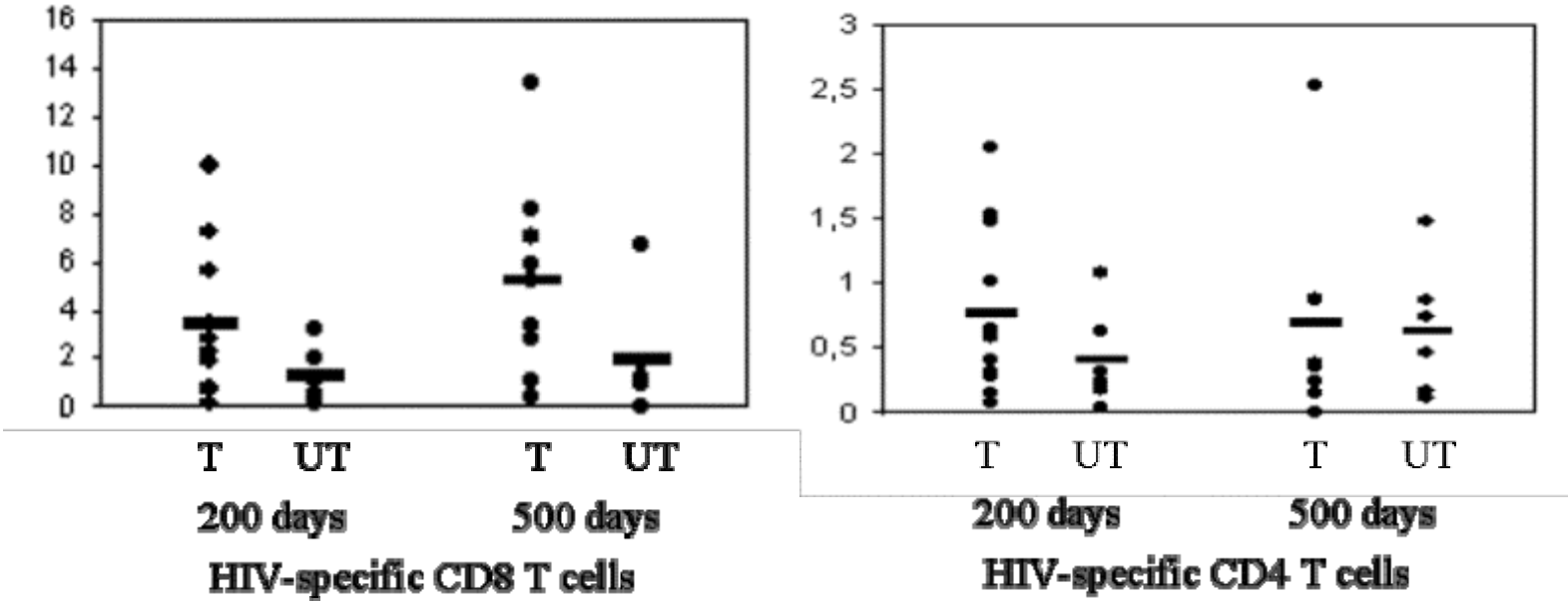

Fig 2a Representative example of dot plots representing the reaction of CD3 CD8 T cells to the negative controle, to HIV-1 tat, rev, p17 and p24 peptides and to SEB.

Fig 2b Cross-sectional absolute numbers (expressed in number x 10^3 cells/l) of HIV-specific CD8 and CD4 T cells at 200 and 500 days of therapy-free follow-up in treated (T) and untreated (UT) individuals. Means are shown with lines.

Table I

	UT group	T group	p values
(re)starting therapy in first 12 months of follow-up	6	3	
(re)starting therapy after 12-24 months of follow-up	2	2	
follow-up of less than 24 months	0	5	
loss to follow-up	0	1	
CD4 at baseline*	506 (31-1051) n=28	492 (199-1168) n=32	p=0,578
CD4 at end therapy		821 (385-1408) n=32	
rise in CD4 after therapy			
CD4 1 year after stop therapy or infection**	490 (44-882) n=22	666 (270-1310) n=29	p=0,087
CD4 2 years after stop therapy or infection	488 (245-868) n=19	569 (256-1110) n=20	p=0,500
CD4 3 years after stop therapy or infection	505 (213-1440) n=13	708 (324-1040) n=10	p=0,011
VL at baseline*	4,31 (2,04-5,00) n=28	4,87 (4,00-5,00) n=32	p<0,001
VL 1 year after stop therapy or infection**	4,42 (2,33-5) n=25	3,95 (1,70-5,00) n=29	p=0,060
VL 2 years after stop therapy or infection	4,30 (2,04-5,00) n=18	3,86 (1,70-5,00) n=20	p=0,108
VL 3 years after stop therapy or infection	4,05 (1,80-5,00) n=13	3,63 (1,70-4,80) n=10	p=0,172

mean values (min-max)
* different time point after infection for treated and untreated individuals
** CD4 and VL values were taken from the period between 8 and 12 months of follow-up

Table I. Characteristics of untreated (UT) and treated (T) individuals over time.

Development of multiple abscesses in an HIV/TB co-infected patient after initiation of antituberculous and highly active antiretroviral therapy.

Acta Clinica Belgica 2002; 57-4; 219-222.

Case report

DEVELOPMENT OF MULTIPLE ABSCESSES IN AN HIV/TB CO-INFECTED PATIENT AFTER INITIATION OF ANTITUBERCULOUS AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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Key words : HIV infection, HAART, Immune Restoration Disease, *Mycobacterium tuberculosis.*

ABSTRACT

Since the use of Highly Active Antiretroviral Therapy (HAART) for HIV infection, there have been increasing reports of systemic manifestations of immune restoration. This new clinical syndrome among HIV-infected patients is associated with underlying co-infections with mycobacteria, cytomegalovirus, hepatitis B and C infections, etc... We report on an HIV/tuberculosis (TB) co-infected patient who developed an immune restoration inflammatory syndrome after initiation of HAART and anti-TB treatment. She developed fever, large abscesses and pleural and peritoneal effusions. Systemic symptoms decreased during corticosteroid treatment, but abscesses only disappeared 8 months after the start of the anti-TB treatment.

INTRODUCTION

Immune Restoration Inflammatory Syndrome (IRIS), also called Immune Restoration Disease (IRD), is now a well recognized disorder among HIV-infected patients after initiation of highly active antiretroviral therapy (HAART). It is postulated that HAART, by restoring immune functions, may promote the clinical expression or exacerbation of previously quiescent diseases (1,2). This phenomenon has been especially described in patients with underlying *Mycobacterium tuberculosis* (3-5), and *Mycobacterium avium* Complex (MAC) infections (6-9). According to a recent review by DeSimone et al. (1), systemic inflammatory reactions associated with HAART have been also reported in cytomegalovirus infection, hepatitis B and C infection, *Herpes zoster* infection, in patients with progressive multifocal leukoencephalopathy, cryptococcal meningitis, and even in patients with AIDS-related malignant conditions or auto-immune diseases. Symptoms related to IRIS (fever, general malaise, lymphadenopathies,...) are often aspecific, and could also be caused by other conditions such as opportunistic infections or drug-related adverse events.

Various clinical syndromes have been related to IRIS associated with mycobacterial diseases, including lymphadenitis and abscess formation, mostly caused by MAC infections (6-9). We report here on an HIV/TB co-infected patient who developed multiple lymph node abscesses, as well as pleural and peritoneal effusions during anti-TB treatment and HAART.

CASE REPORT

A 32-year-old Belgian woman, living in Indonesia for 6 years, was repatriated and admitted in February 2000 for recurrent diarrhoea and weight loss since 3 months, associated with abdominal pain and fever of

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one week duration. On physical examination she was cachectic (44 kg and 168 cm), and had a temperature of 38.8°C, several cervical and axillary painless lymph nodes (diameter of 2 cm), oral candidiasis and diffuse abdominal pain.

Laboratory data revealed an haemoglobin of 9.3 g/dl, a leukocyte cell count of 3260/ml, with 14,6 % lymphocytes. The sedimentation rate was increased to 73 mm/hour and C-reactive protein to 13.6 mg/dl. Biochemistry showed a slight elevation of the liver enzymes (aspartate amino-transferase: 139 IU/L; alanine amino-transferase: 71 IU/L; alkaline phosphatasis : 146 IU/L and gamma-glutamyltransferase: 251 IU/L) and a high level of lactacte dehydrogenase (1392 IU/L). She was diagnosed as having an HIV-1 infection, with a CD4+ lymphocyte count of 19/mm3 (3.4 %) and a viral load of > 750000 copies/ml. Serological tests for hepatitis A, B, C and for syphilis were negative. A blood smear was negative for malaria. Blood and urine cultures remained negative while stool culture yielded an infection with *Salmonella enteritidis.*

A chest X-ray was normal. A Computed Tomographic (CT) scan of the abdomen revealed multiple enlarged sub-hepatic and para-aortic homogenous lymphadenopathies (diameter of 2 cm). Intravenous ciprofloxacin was started for the *S. enteritidis* infection. Co-trimoxazole prophylaxis and antiretroviral therapy were initiated, including stavudine, lamivudine and indinavir. As fever persisted, a liver and bone marrow biopsy were performed. On histological examination granulomatous lesions containing acid-fast bacilli were observed and on culture a multi-drug sensitive *Mycobacterium tuberculosis* was grown. Two weeks after the initiation of HAART, anti-TB treatment was started, including rifabutin (150 mg daily), isoniazid (300 mg daily), ethambutol (1200 mg daily) and pyrazinamide (1500 mg daily). The daily dose of indinavir was increased to 1000 mg TID. The fever resolved completely 5 days later and the patient was discharged.

Two weeks later she was readmitted with high fever and abdominal pain. Physical examination revealed enlarged (3-4 cm) painful cervical, axillar, inguinal and epitrochlear lymph nodes and a painful epigastric tumefaction. An abdominal CT scan showed a necrotising sub-hepatic abscess (diameter of 7 cm) and increased generalized abdominal lymph nodes. A large amount of ascitis and a large left pleural effusion were present, requiring evacuation by puncture. Mycobacterial cultures of these fluids remained negative. On the other hand aspiration of an axillary abscess 6 weeks after initiation of the anti-TB treatment showed the presence of acid-fast bacilli and a positive culture for *Mycobacterium tuberculosis*. Because of persistent fever HAART was stopped 7 weeks after its initiation.

The clinical condition of the patient improved and the fever disappeared. After a 2 week interruption of HAART the rifabutin was switched to rifampicin and stavudine, lamivudine and abacavir treatment was started. One week later, new abscesses developed and the fever reappeared. Prednisone (32 mg/day) was then initiated, with a rapid clinical improvement. However, abscesses continued to appear during the following months (Figure 1), some with spontaneous discharge, some requiring aspiration. Acid-fast bacilli were still found on direct examination of the pus 8 months after the start of the anti-TB treatment, but cultures remained negative. Corticosteroids were tapered slowly according to clinical symptoms. In December 2000 the patient's general condition was good, she had gained 12 kg, her CD4+ lymphocyte count was 180/mm3 (13.9%) and the viral load 1600 copies/ml. In total the patient was treated with 2 months of quadritherapy followed by 10 months of bitherapy. No relapse has been observed during the year following the stop of the anti-TB treatment.

DISCUSSION

A "paradoxical response" during anti-TB treatment is a well recognized phenomenon occasionally reported in HIV-uninfected patients (5,10), and in AIDS patients before the introduction of HAART (11,12). Clinical presentations include paradoxical increases of tuberculous lymph nodes (13), or of cerebral tuberculomas (10,14), respiratory distress in patients with miliary or widespread pulmonary tuberculosis and transient worsening of TB meningitis (15). It has been suggested that the paradoxical response may be related to an immunologic process involving altered cell-mediated responsiveness in the context of mycobacterial killing during chemotherapy (16). Since the introduction of HAART, paradoxical responses in HIV/TB co-infected patients have been reported by many authors (4,5,17). These reactions are now considered as manifestations of IRIS, but the differences between the "old" paradoxical responses (not following HAART) and the "new" immune restoration diseases have never been clarified. Until now, only one prospective study has demonstrated a higher incidence of paradoxical reactions

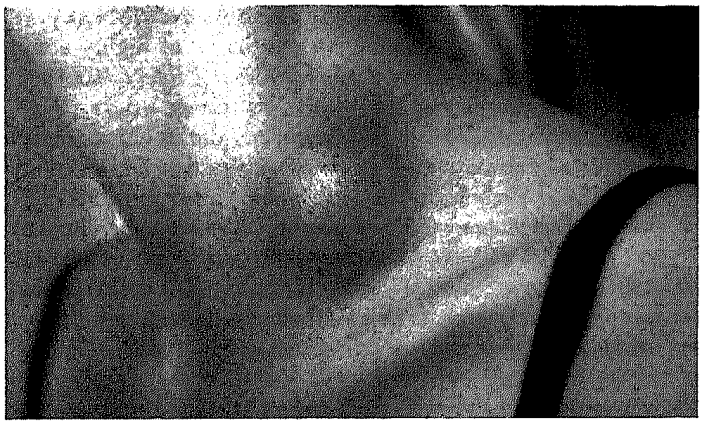

Figure 1 : Two cervical abscesses which appeared during antituberculous and highly active antiretroviral treatment.

among AIDS patients treated simultaneously with anti-TB therapy and HAART (36%), than among those treated only with anti-tuberculous drugs (7%) (5). The same study also showed a temporal relationship between these reactions and the initiation of HAART. However, the link between HAART and paradoxical worsening of TB has been recently questioned in another prospective study, where no significant differences could be found (18). IRIS manifestations occur often before a substantial change in CD4+ lymphocyte counts is observed, suggesting that other factors may be involved, such as the redistribution of antigen-specific T-cells and/or Th2 cytokine-mediated reversal of immunosuppressive T-cells (19).

IRIS is generally considered as a benign and transient condition, even if severe complications have been occasionally reported (CMV vitritis, reactivation of hepatitis B or C infection). Our patient also presented with severe clinical manifestations, including abdominal abscesses, and a life-threatening pleural effusion. A purulent discharge of lymph nodes was noted up to 8 months after the start of anti-TB therapy and HAART, which is an exceptional observation. Several factors may explain such an unusual long course, including the high mycobacterial burden related to the disseminated tuberculosis, the delay in starting anti-TB treatment, the severe immune deficiency before the start of HAART and the vigorous immunological response to HAART in this antiretroviral treatment naive patient.

An important lesson of this observation is that patients, particularly those with severe immune deficiency should be screened carefully for the presence of opportunistic infections before initiating HAART. If tuberculosis is diagnosed TB treatment should be started before initiating HAART. However, in patients with a very low CD4+ count concomitant treatment with HAART should be considered in order to avoid development of new opportunistic infections. Such patients should be carefully monitored for development of IRIS and when IRIS manifestations occur corticosteroid treatment should be considered.

As antiretrovirals are now increasingly used in developing countries where TB/HIV co-infection is highly prevalent, it is likely that clinicians will be increasingly confronted with TB-related IRIS manifestations. Clinical and epidemiological studies are needed to determine the incidence of IRIS, to define diagnostic criteria and prognostic factors, and to investigate appropriate preventive measures and treatment.

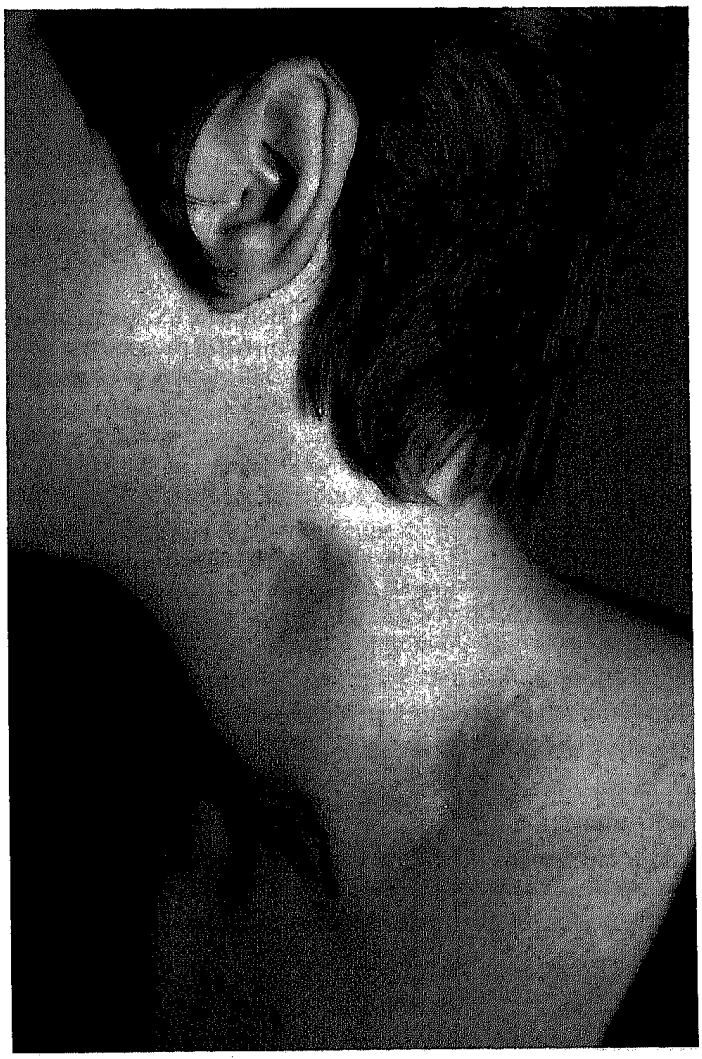

Figure 2 : Scars of cervical abscesses, 1 year after the start of antituberculous treatment.

SAMENVATTING

Sinds het gebruik van «Highly Active Antiretroviral Therapy (HAART)» voor de behandeling van HIV infectie wordt een stijgend aantal klinische verschijnselen beschreven te wijten aan een immuunrestitutie fenomeen. Dit nieuw klinisch syndroom bij personen met HIV infectie komt voor bij onderliggende co-infecties met mycobacteria, cytomegaalvirus, hepatitis B en C infecties, enz... Wij beschrijven een HIV/tuberculose (TB) gecoïnfecteerde patiënt die een inflammatoire syndroom ontwikkelde t.g.v. immuunrestitutie na het starten van HAART en anti-TB behandeling. Zij ontwikkelde koorts, belangrijke abcessen en een pleurale en peritoneale uitstorting. Algemene symptomen regresseerden tijdens behandeling met corticosteroïden maar de abcessen verdwenen slechts 8 maanden na het starten van de anti-TB behandeling.

REFERENCES

1. DeSimone JA, Pomerantz RJ, Babinchak TJ. Inflammatory reactions in HIV-1-infected persons after initiation of highly active antiretroviral therapy. *Ann Intern Med.* 2000; 33:447-54.
2. French MA. Antiretroviral therapy. Immune restoration disease in HIV-infected patients on HAART. *AIDS Reader.* 1999; 9:548-62.
3. Fishman JE, Saraf-Lavi E, Narita M, Hollender ES, Ramsinghani R, Ashkin D. Pulmonary tuberculosis in AIDS patients: transient chest radiographic worsening after initiation of antiretroviral therapy. *Am J Roentgenol.* 2000; 174:43-9.
4. Furrer H, Malinverni R. Systemic inflammatory reaction after starting highly active antiretroviral therapy in AIDS patients treated for extrapulmonary tuberculosis. *Am J Med* 1999; 106:371-2.
5. Narita M, Ashkin D, Hollender ES, Pitchenik AE. Paradoxical worsening of tuberculosis following antiretroviral therapy in patients with AIDS. *Am J Respir Crit Care Med* 1998; 158:157-61.
6. Cabie A, Abel S, Brebion A, Desbois N, Sobesky G. Mycobacterial lymphadenitis after initiation of highly active antiretroviral therapy. *Eur J Clinl Microbiol Infect Dis* 1998; 17:812-3.
7. Dworkin MS, Fratkin MD. Mycobacterium avium complex lymph node abscess after use of highly active antiretroviral therapy in a patient with AIDS. *Arch Intern Med* 1998; 158:1828.
8. Price LM, O'Mahony C. Focal adenitis developing after immune reconstitution with HAART. *Int J STD AIDS.* 2000; 11:685-6.
9. Race EM, Adelson-Mitty J, Kriegel GR, et al. Focal mycobacterial lymphadenitis following initiation of protease-inhibitor therapy in patients with advanced HIV-1 disease. *Lancet.* 1998; 351:252-5.
10. Afghani B, Lieberman JM. Paradoxical enlargement or development of intracranial tuberculomas during therapy: case report and review. *Clin Infect Dis.* 1994; 19:1092-9.
11. Hill AR, Mateo F, Hudak A. Transient exacerbation of tuberculous lymphadenitis during chemotherapy in patients with AIDS. *Clin Infect Dis.* 1994; 19:774-6.
12. Ramdas K, Minamoto GY. Paradoxical presentation of intracranial tuberculomas after chemotherapy in a patient with AIDS. *Clin Infect Dis.* 1994; 19:793-4.
13. Campbell IA, Dyson AJ. Lymph node tuberculosis: a comparison of various methods of treatment. *Tubercle* 1977; 58(4):171-9.
14. Hejazi N, Hassler W. Multiple intracranial tuberculomas with atypical response to tuberculostatic chemotherapy: literature review and a case report. *Infection.* 1997; 25:233-9.
15. Dooley DP, Carpenter JL, Rademacher S. Adjunctive corticosteroid therapy for tuberculosis: a critical reappraisal of the literature. *Clin Infect Dis.* 1997; 25:872-87.
16. Immune reactions in tuberculosis. *Lancet.* 1984; 2:204.
17. Crump JA, Tyrer MJ, Lloyd-Owen SJ, Han LY, Lipman MC, Johnson MA. Miliary tuberculosis with paradoxical expansion of intracranial tuberculomas complicating human immunodeficiency virus infection in a patient receiving highly active antiretroviral therapy. *Clin Infect Dis.* 1998; 26:1008-9.
18. Wendel KA, Alwood KS, Gachuhi R, et al. Paradoxical Worsening of tuberculosis in HIV-Infected persons. *Chest* 2001; 120: 193-7.
19. French MA, Price P. Immune Restoration Disease in HIV-Infected patients after antiretroviral therapy. *Clin Infect Dis.* 2001; 32:325-6.

Drug resistant variants that evolve during non-suppressive therapy persist in HIV-1 infected PBMC after long-term HAART.

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Drug-Resistant Variants That Evolve During Nonsuppressive Therapy Persist in HIV-1–Infected Peripheral Blood Mononuclear Cells After Long-Term Highly Active Antiretroviral Therapy

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Abstract: The aim of this study was to determine whether drug-resistant virus persists in peripheral blood mononuclear cells (PBMCs) after long-term suppression of virus replication. Proviral DNA was extracted from the PBMCs of 11 patients on long-term highly active antiretroviral therapy (HAART). Genotyping of the reverse transcriptase (RT) and protease gene of several proviral variants was performed using limiting dilution polymerase chain reaction and single-copy sequencing. All patients were on successful HAART for a mean period of 59 months but had a history of suboptimal therapy and genotypic drug resistance before. Comparison of the amino acid sequence of the RT and protease gene in the different proviral variants, with that of the plasma virus isolated before HAART treatment, revealed that the different drug-resistant viral variants that evolved during the process of gradually building up resistance were still detectable in the PBMCs in 10 of the 11 patients tested. The proportion of resistant variants was found to correlate with the time that the resistant variants had been able to replicate. These data clearly show that virus variants that are able to replicate for a certain period enter the latent reservoir and remain archived in the PBMCs for a very long period.

Key Words: drug resistance, provirus, latent reservoir, persistence of drug resistance

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INTRODUCTION

Despite a decrease in plasma viral RNA to below the level of detection after starting highly active antiretroviral therapy (HAART), virus persists for a very long period in what is called the latent reservoir.[1–6] Our current knowledge of this reservoir (i.e., how it originates, how it is maintained and eventually renewed) is still very limited. An important stable long-term viral reservoir in patients on HAART is thought to be composed of resting memory $CD4^+$ T cells carrying replication-competent viral genomes.[1,2] The proportion of these cells is supposed to be low, but as demonstrated recently, they persist for many years even in the absence of active virus replication.[7] Finzi et al.[3,4] showed that a latently infected $CD4^+$ T-cell compartment becomes established very early in infection, but the factors that are involved in the maintenance and eventual replenishment of this compartment are still largely unknown. Moreover, much debate continues about the importance of residual viral replication as a mechanism of replenishment of the latent reservoir during HAART.[4,8–10]

If not fully suppressive, any antiretroviral treatment used today will result in the development of resistance. We and others have shown that in the majority of patients with drug-resistant virus, treatment interruption results in the reemergence of drug-susceptible HIV-1.[11–13] These observations have evoked large interest in structured treatment interruptions as a way to reduce the amount of resistant virus to very low levels, thereby possibly increasing the chance of durable viral suppression on subsequently resumed therapy.[13–16] The results of these structured treatment interruption studies are still controversial but a fast reemergence of resistant variants under the selective pressure of the new antiretroviral regimen has been demonstrated already.[17,18] If and to what extent resistant virus enters the latent reservoir, and for how long it persists in this reservoir, are still unknown. A better insight into the kinetics of the latent virus reservoir and its composition with regard to wild-type and resistant virus might help to improve the strategies for successful treatment of heavily exposed individuals.

To study the persistence of proviral sequences carrying drug-resistant mutations, we selected 11 patients who were on fully suppressive HAART for several years but who had been exposed to suboptimal therapy previously. The main objective was to see whether many years of selection for drug-resistant

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virus followed by a long period of suppression of replication would lead to the disappearance of the wild-type drug-sensitive virus and replacement by mutant drug-resistant virus in the reservoir. Also, we wanted to examine the value of sequencing of the cellular provirus as a way to obtain information about previous drug resistance. We used limiting dilution polymerase chain reaction (PCR) followed by sequencing of the single-copy PCR products to genotype the reverse transcriptase (RT) and protease gene of different proviral variants.

MATERIALS AND METHODS

Study Population

Eleven patients were selected from the patient cohort of the AIDS Reference Center of the University Hospital in Ghent, Belgium. Patients from this cohort are followed intensively. Enrollment was based on the following criteria: the patients had received suboptimal treatment before HAART, they carried drug-resistant virus as revealed by sequencing at the time of HAART initiation, and they had currently been on HAART for >4 years. Viral load determinations during HAART were performed at least every 2–3 months. Viral load remained undetectable (<50 copies/mL) during the whole period with exception of 1 occasional positive result (206 copies/mL) after 48 months of HAART in patient 1. Viral load returned to <50 copies/mL in a sample taken from this patient 1 month later. Before HAART, the patients were treated with either zidovudine (AZT), zalcitabine (ddC), didanosine (ddI), or lamivudine (3TC) as monotherapy or in combination. One patient participated in a nonnucleoside reverse transcriptase inhibitor (NNRTI) trial and received the drug loviride. Suboptimal therapy was given for a mean period of 46 months. Most patients received a total of 2 or 3 drugs. During the pre-HAART treatment, plasma was collected every 3–6 months for viral load determination. In all patients, the viral load remained detectable during the whole pre-HAART treatment period.

At the time the peripheral blood mononuclear cell (PBMC) samples were taken, all patients were on HAART for a mean period of 59 months (range 54–68) with a combination of 2 nucleoside reverse transcriptase inhibitors (NRTIs) and 1 or 2 protease inhibitors (PIs). All patients were white and infected with subtype B virus. Their mean age was 43 years (range 32–54).

HIV RNA Quantification

Plasma samples were obtained at each visit and stored at −70°C. HIV RNA quantification was performed using the Ultrasensitive Cobas Amplicor HIV-1 Monitor Test (Roche Molecular Systems, Branchburg, NJ) with a detection limit of 50 copies/mL.

DNA Extraction and Limiting Dilution PCR

DNA was extracted from freshly isolated PBMCs using the QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). DNA samples were diluted 10-fold and 5 μL of this dilution was added to each of at least 40 identical PCR mixes containing the outer primer set (sense 5′-ATGATGCAGAGAGGCAATTT-3′; antisense 5′-TTCTGTATGTCATTGACAGTCCAGC-3′) to amplify an approximately 1200-bp fragment spanning the protease and the first 240 amino acids of the RT gene. Amplification was performed for a total of 35 cycles (20 seconds at 94°C, 20 seconds at 50°C, and 1 minute at 72°C), after which 2 μL of the amplified products were transferred to 48 μL of 2 reaction mixes containing either a primer set to amplify the protease gene (sense 5′-AGAGCCAACAGCCCCACCA-3′; antisense 5′-GGGCCATCCATTCCTGGCTT-3′) or a primer set to amplify the first part of the RT gene (sense 5′- CCAAAAGTTAAACAATGGCCATTGACAGA-3′; antisense 5′-AGTTCATAACCCATCCAAAG-3′). Nested PCR amplification was performed for 30 cycles (20 seconds at 94°C, 20 seconds at 57°C, and 30 seconds at 72°C). Positive PCR products were selected for sequence analysis only if less than one-third of the replicate reactions were found positive. DNA samples for which more than one-third of the reactions were positive were diluted 10-fold further, and the PCR reactions were repeated until a dilution was found for which no more than one-third of the reactions were positive. Both positive and negative controls were included in all PCR assays to assess the sensitivity of the reaction and to detect possible contamination. The lower limit of detection of the PCR assay was equivalent to 1 copy per reaction. All positive PCR products were sequenced.

Sequencing

Direct sequencing of both sense and antisense strands of the inner PCR products was done with the dRhodamine Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). The sequencing reaction was performed with the same primers as the ones used in the inner PCR reactions, but to obtain a full sequence of both strands of the RT gene fragment, 2 additional sequencing reactions were run with internal primers (sense 5′- GGGNGAYGCATATTTTTCARTWCC-3′; antisense 5′- CCTGGTGTYTCATTRTTTRYACTT-3′). Sequencing reaction products were analyzed on an ABI 310 Genetic Analyzer (Applied Biosystems). A minimum of 11 (range 11–18) different PCR products were sequenced for each patient sample. The principle of limiting dilution sequencing is based on the mathematical calculation that if no more than one-third of replicate PCR reactions are positive, the likelihood that the PCR products are the result of the amplification of only 1 molecule is ~70%. Limiting dilution sequencing allows us to obtain an accurate profile of the distribution of different variants in a single sample.[19]

The likelihood of comparing single provirus sequencing data is further enhanced by withdrawing all sequencing products for whom visual inspection of the electropherograms revealed nucleotide mixtures at ≥1 positions. Sequences containing stop codons or frame shifts indicating defective virus were also withdrawn. Sequencing results were only used in the analysis if the sequence of both strands was available and fully concordant.

Genotypic Analysis of Plasma Viral RNA

RT-PCR of the RT and protease genes was performed on stored plasma viral RNA using the Titan One Tube RT-PCR System (Roche Molecular Systems). Direct sequencing of the PCR product was done as described for the proviral DNA samples. Amino acid substitutions were identified by comparison of the plasma RNA sequences with a consensus HIV-1 subtype B sequence.

Phylogenetic Analysis

Nucleotide sequences were assembled using the BioEdit package (www.mbio.ncsu.edu/BioEdit). Phylogenetic analyses and neighbor-joining tree reconstructions were performed using programs from version 3.6 of the PHYLIP package (http://evolution.genetics.Washington.edu/phylip), with a maximum likelihood distance matrix and a transition to transversion ratio of 2.0. Approximate confidence limits for individual branches were assigned by bootstrap resampling with 1000 replicates. Tree diagrams were plotted with Treeview v1.4 (http://taxonomy.zoology.gla.ac.uk/rod/treeview).

Nucleotide Sequence Accession Numbers

The nucleotide sequences reported in this paper have been submitted to GenBank and were given accession numbers AY356748 to AY357066.

RESULTS

Response to HAART

Table 1 summarizes the treatment history and response to HAART for the patients enrolled in the study. Patients are ordered according to the time on suboptimal therapy. All patients showed a rapid decline in plasma viral load to below the levels of quantification (<50 copies/mL) after initiation of HAART. Values all remained undetectable on repeated measurements during the whole treatment period. HAART also resulted in an important increase in CD4 count (mean CD4 rise: 575, range 203–837).

Proviral DNA Sequencing

Table 2 summarizes the results of the sequencing analysis of the RT gene of proviral DNA variants isolated from PBMCs collected after an average HAART period of 59 months and the results of the sequencing analysis of the RT gene of plasma virus isolated from consecutive blood samples during the pre-HAART period. Baseline plasma samples were not always available (missing for patients 6, 8, 9, 10, and 11). The proviral DNA was shown to be constituted of a mixture of wild-type proviruses and drug-resistant proviruses in 9 of the 11 patients studied. Only in patient 1 were no drug-resistant proviral variants detected. This patient had been on suboptimal therapy for the shortest period (11 months). In patient 9, only variants with resistant mutations were found. This patient had been on suboptimal therapy for a long period (5 years), indicating a possible association between the time on suboptimal therapy and the amount of resistant proviral variants. This assumption is further strengthened by the observed overall correlation between the time on suboptimal therapy and the rela-

TABLE 1. Treatment History and Viral and Cellular Response to HAART

		Suboptimal Therapy		HAART		Viral Load (log)		CD_4	
Patient	Age (y)	Drugs Taken	Time on Therapy (mo)	Regimen	Time on HAART (mo)	At HAART Initiation	At PTMC Sampling	At HAART Initiation	At PBMC Sampling
1	32	AZT	11	d4T + 3TC + RTV + SQV	60	4.41	<1.70	297	781
2	47	AZT-ddC-ddl	15	d4T + 3TC + RTV + SQV	68	4.39	<1.70	<100	781
3	49	AZT-ddC-ddl-3TC	20	AZT + 3TC + RTV + SQV	59	4.59	<1.70	144	455
4	54	AZT-3TC	22	d4T + RTV + SQV	62	5.12	<1.70	324	979
5	34	AZT-ddC-ddl	27	d4T + 3TC + RTV + SQV	60	4.89	<1.70	121	737
6	41	AZT-ddC-LV	48	d4T + ddl + RTV + SQV	54	4.64	<1.70	347	735
7	43	AZT-ddl	50	d4T + 3TC + RTV + SQV	55	4.82	<1.70	168	1005
8	45	AZT-ddC	60	d4T + 3TC + IDV	60	4.31	<1.70	305	1034
9	47	AZT-ddl	60	d4T + ddl + RTV + SQV	60	4.32	<1.70	388	591
10	41	AZT-ddC	67	d4T + 3TC + NFV	57	4.08	<1.70	249	871
11	41	AZT-ddC	96	d4T + 3TC + RTV + SQV	55	4.03	<1.70	594	1388

AZT, zidovudine; ddC, zalcitabine; ddl, didanosine; LV, loviride; 3TC, lamivudine; d4T, stavudine; RTV, ritonavir; SQV, saquinavir; IDV, indinavir; NFV, nelfinavir.

TABLE 2. Results of the Sequence Analysis of the RT Gene in Viral RNA Isolated From Consecutive Plasma Samples Taken During the Pre-HAART Period (Left) and in Proviral DNA Isolated From a Single PBMC Sample Taken After Several Years of Successful HAART (Right)

			Plasma Virus									
Patient	**Date**	**Therapy**	**41 M**	**67 D**	**69 T**	**70 K**	**103 K**	**184 M**	**210 L**	**215 T**	**219 K**	**HAART Regimen**
1	01-18-96	no	—	—	—	—	—	—	—	—	—	d4T + 3TC + RTV + SQV
	03-11-96	AZT	—	—	—	—	—	—	—	—	—	
	04-05-96	AZT	—	—	—	—	—	—	—	—	—	
	05-31-96	AZT	—	N	—	R	—	—	—	—	—	
	07-26-96	AZT	—	N	—	R	—	—	—	Y	—	
	09-23-96	AZT	—	N	—	R	—	—	—	Y	—	
	12-12-96	AZT	—	N	—	R	—	—	—	Y	—	
	12-13-96	Start HAART										
2	04-19-95	no	—	—	—	—	—	—	—	—	—	d4T + 3TC + RTV + SQV
	06-19-95	AZT	—	—	—	—	—	—	—	—	—	
	08-01-95	AZT + ddC	—	—	—	—	—	—	—	Y	—	
	09-21-95	AZT + ddC	—	N	—	R	—	—	—	Y	—	
	11-09-95	AZT + ddC	—	N	—	R	—	—	—	Y	—	
	12-12-95	ddI	—	—	—	—	—	—	—	—	—	
	02-14-96	ddI	—	N	—	R	—	—	L/W	Y	K/E	
	04-09-96	ddI	—	N	—	R	—	—	L/W	Y	K/E	
	08-01-96	ddI	—	N	—	K/R	—	—	—	Y	E	
	09-03-96	Start HAART										
3	05-23-95	no										AZT + 3TC + RTV + SQV
	06-13-95	AZT	—	—	—	—	—	—	—	—	—	
	08-01-95	AZT + ddC	—	—	—	—	—	—	—	—	—	
	09-25-95	AZT + ddC	—	—	—	—	—	—	—	Y	—	
	12-14-95	AZT + ddC	—	N	—	R	—	—	—	Y	—	
	01-15-96	AZT + ddC	—	N	—	R	—	—	—	Y	—	
	06-06-96	AZT + ddI	—	—	—	—	—	—	—	Y	—	
	07-25-96	AZT + ddI	—	—	—	—	—	—	—	Y	—	
	08-21-96	AZT + ddI	L	—	—	—	—	—	W	Y	—	
	10-18-96	AZT + 3TC	L	—	—	—	—	V	W	Y	—	
	01-27-97	Start HAART										
4	05-09-95	no	—	—	—	—	—	—	—	—	—	d4T + RTV + SQV
	06-15-95	AZT + 3TC	—	—	—	—	—	M/V	—	—	—	
	07-13-95	AZT + 3TC	—	—	—	—	—	V	—	—	—	
	10-11-95	AZT + 3TC	—	—	—	—	—	V	—	—	—	
	12-13-95	AZT + 3TC	—	D/N	—	K/R	—	V	—	—	—	
	03-20-96	AZT + 3TC	—	N	—	R	—	V	—	—	—	
	05-15-96	AZT + 3TC	—	N	—	R	—	V	—	—	—	
	09-18-96	AZT + 3TC	—	N	—	R	—	V	—	I	Q	
	11-19-96	AZY + 3TC	—	N	—	R	—	V	—	I/F	Q	
	03-27-97	Start HAART	—	N	—	R	—	V	—	F	Q	
5	10-28-94	no	—	—	—	—	—	—	—	—	—	d4T + 3TC + RTV + SQV
	03-23-95	AZT + ddC	—	—	—	—	—	—	—	—	—	
	05-15-95	AZT + ddC	—	—	—	—	—	—	—	—	—	
	08-16-95	AZT + ddC	—	N	—	R	—	—	—	Y	—	
	02-26-96	AZT + ddI	—	—	—	K/R	—	—	—	Y/F	—	
	07-17-96	AZT + ddI	—	D/N	—	R	—	—	L/W	Y/F	E	
	12-16-96	AZT + ddI	—	N	—	R	—	—	W	Y/F	E	
	03-24-97	AZT + ddI	L	N	—	R	—	—	W	Y	E	
	04-07-97	Start HAART	L	N	—	R	—	—	W	Y	E	

TABLE 2. *(continued)* Results of the Sequence Analysis of the RT Gene in Viral RNA Isolated From Consecutive Plasma Samples Taken During the Pre-HAART Period (Left) and in Proviral DNA Isolated From a Single PBMC Sample Taken After Several Years of Successful HAART (Right)

			Provirus								
Patient	**Date Sampling PBMC**	*n**	**41 M**	**67 D**	**69 D**	**70 K**	**103 K**	**184 M**	**210 L**	**215 T**	**219 K**
1	13-03-01	18	—	—	—	—	—	—	—	—	—
2	05-23-02	5	—	—	—	—	—	—	—	—	—
		1	—	—	—	—	—	—	—	Y	—
		4	—	N	—	R	—	—	—	Y	E
		1	—	N	—	R	—	—	W	Y	E
3	12-03-01	14	—	—	—	—	—	—	—	—	—
		1	—	—	—	—	—	—	—	Y	—
		1	—	N	—	R	—	—	—	Y	—
		1	L	—	—	—	—	V	W	Y	—
4	05-15-02	10	—	—	—	—	—	—	—	—	—
		1	—	N	—	R	—	V	—	—	—
		1	—	N	—	R	—	V	—	F	Q
5	04-08-02	8	—	—	—	—	—	—	—	—	—
		1	—	—	—	—	—	I	—	—	—
		1	—	N	—	R	—	—	—	—	—
		3	—	N	—	R	—	—	—	F	E
		2	—	N	—	R	—	—	W	Y	E
		1	L	N	—	R	—	—	W	Y	E
		1	L	N	N	R	—	—	W	Y	E

TABLE 2. *(continued)* Results of the Sequence Analysis of the RT Gene in Viral RNA Isolated From Consecutive Plasma Samples Taken During the Pre-HAART Period (Left) and in Proviral DNA Isolated From a Single PBMC Sample Taken After Several Years of Successful HAART (Right)

			Plasma Virus									
Patient	Date	Therapy	41 M	67 D	69 T	70 K	103 K	184 M	210 L	215 T	219 K	HAART Regimen
6	02-28-94	AZT	L	—	—	—	—	—	L/W	Y	—	d4T + ddI + RTV + SQV
	05-24-94	AZT + ddC + LV	L	—	—	—	—	—	L/W	Y	—	
	06-29-94	AZT + ddC + LV	L	—	—	—	—	—	W	Y	—	
	08-05-94	AZT + ddC + LV	L	—	—	—	—	—	W	Y	—	
	01-23-95	AZT + ddC + LV	L	—	—	—	—	—	W	Y	—	
	05-23-95	AZT + ddC + LV	L	—	—	—	N/K	—	W	Y	—	
	05-30-96	AZT + ddC + LV	L	—	—	—	N	—	W	Y	—	
	08-23-96	Start HAART										
7	02-03-93	no	—	—	—	—	—	—	—	—	—	
	01-12-94	AZT	M/L	—	—	—	—	—	—	T/Y	—	
	06-15-94	AZT	—	—	—	—	—	—	—	T/Y	—	
	08-17-94	AZT	M/L	—	—	—	—	—	—	Y	—	
	03-15-95	AZT	L	—	—	—	—	—	L/W	Y	—	
	03-13-96	AZT + ddI	L	—	—	—	—	—	W	Y	—	
	07-04-96	AZT + ddI	L	—	—	—	—	—	W	Y	—	
	11-20-96	AZT + ddI	L	—	—	—	—	—	W	Y	—	
	04-09-97	Start HAART	L	E	—	—	—	—	W	Y	—	
8	03-17-94	AZT	—	—	—	R	—	—	—	—	—	d4T + 3TC + IDV
	03-08-95	AZT	—	—	—	R	—	—	—	Y	—	
	04-26-95	AZT	—	D/N	—	R	—	—	—	Y/T	Q/K	
	05-16-95	AZT + ddC	—	D/N	—	R	—	—	—	Y/T	—	
	10-16-95	AZT + ddC	—	N	—	R	—	—	—	Y	Q	
	03-26-96	AZT + ddC	—	N	—	R	—	—	—	Y	Q	
	06-24-96	AZT	—	N	—	R	—	—	—	Y/T	—	
	09-03-96	AZT	—	N	—	R	—	—	—	Y	Q	
	01-17-97	Start HAART										
9	09-11-95	AZT	—	D/E	—	R	—	—	—	—	Q/K	d4T + 3TC + RTV + SQV
	11-13-95	AZT	—	E	—	R	—	—	—	—	—	
	01-10-96	AZT + ddI	—	D/N	—	R	—	—	—	T/F	Q	
	09-30-96	AZT + ddI	—	N	—	R	—	—	—	F	Q	
	03-24-97	Start HAART	—	N	—	R	—	—	—	F	Q	
10	03-11-93	AZT	—	—	—	—	—	—	—	—	—	d4T + 3TC + NFV
	01-05-95	AZT	—	—	—	—	—	—	—	—	—	
	01-09-96	AZT	—	—	—	R	—	—	—	—	—	
	02-06-96	AZT	—	—	—	R	—	—	—	I	—	
	05-06-96	AZT	—	—	S/T	K/R	—	—	—	T/Y	—	
	10-07-96	AZT	—	—	—	R	—	—	—	—	—	
	11-12-96	AZT + ddC	—	—	—	—	—	—	—	Y	—	
	01-13-97	AZT + ddC	—	—	—	—	—	—	—	Y	—	
	04-01-97	Start HAART	—	—	—	—	—	—	—	Y	—	
11	11-23-92	AZT	—	—	N	R	—	—	—	—	—	d4T + 3TC + RTV + SQV
	04-21-93	AZT	—	—	N	R	—	—	—	—	—	
	09-22-93	AZT	—	—	N	R	—	—	—	—	—	
	03-01-94	AZT	—	—	N	R	—	—	—	—	—	
	07-25-94	AZT + ddC	—	—	N	R	—	—	—	—	—	
	04-10-95	AZT + ddC	—	—	N	R	—	—	—	—	—	
	08-07-95	AZT + ddC	—	G/D	N	R	—	—	—	—	—	
	11-13-95	AZT + ddC	—	G	N	R	—	—	—	—	Q	
	03-12-96	AZT + ddC	—	G	N	R	—	—	—	—	Q	
	04-07-97	Start HAART	—	G	N	R	—	—	—	—	Q	

TABLE 2. *(continued)* Results of the Sequence Analysis of the RT Gene in Viral RNA Isolated From Consecutive Plasma Samples Taken During the Pre-HAART Period (Left) and in Proviral DNA Isolated From a Single PBMC Sample Taken After Several Years of Successful HAART (Right)

			Provirus								
Patient	Date Sampling PBMC	*n**	41 M	67 D	69 D	70 K	103 K	184 M	210 L	215 T	219 K
6	02-05-01	2	—	—	—	—	—	—	—	—	—
		2	L	—	—	—	—	—	—	Y	—
		3	L	—	—	—	—	—	W	Y	—
		2	L	—	—	—	N	—	W	—	—
		3	L	—	—	—	N	—	W	Y	—
7	11-21-01	2	—	—	—	—	—	—	—	—	—
		2	L	—	—	—	—	—	—	Y	—
		2	L	—	—	—	—	—	W	Y	—
		7	L	E	—	—	—	—	W	Y	—
8	03-12-02	4	—	—	—	—	—	—	—	—	—
		4	—	N	—	R	—	—	—	—	—
		4	—	—	—	R	—	—	—	Y	—
		1	—	N	—	R	—	—	—	Y	—
		1	—	N	—	R	—	—	—	Y	Q
9	03-06-02	3	—	—	—	R	—	—	—	—	—
		1	—	E	—	R	—	—	—	—	
		1	—	N	—	R	—	—	—	—	—
		1	—	N	—	R	—	—	—	—	Q
		1	—	G	—	R	—	—	—	F	Q
		8	—	N	—	R	—	—	—	F	Q
		1	L	N	—	R	—	—	—	F	Q
10	01-07-02	4	—	—	—	—	—	—	—	—	—
		7	—	—	—	R	—	—	—	—	—
		4	—	—	S	R	—	—	—	—	—
11	11-21-01	2	—	—	—	—	—	—	—	—	
		3	—	—	—	R	—	—	—	—	—
		7	—	—	N	R	—	—	—	—	—
		2	—	G	N	R	—	—	—	—	—
		4	—	G	N	R	—	—	—	—	Q

*Number of proviral variants with the corresponding mutational pattern.

AZT, zidovudine; ddC, zalcitabine; ddI, didanosine; 3TC, lamivudine; d4T, stavudine; LV, loviride; RTV, ritonavir; SQV, saquinavir; IDV, indinavir; NFV, nelfinavir.

tive amount of mutant sequences between the proviral variants (logistic regression coefficient $r^2 = 0.6308$; $P = 0.004$) (Fig. 1).

The number of different proviral variants that were detected in 1 sample varied from 3 to 8. Proviral variants with resistant mutations were heterogeneous in all patients; variants with either different numbers of mutations or different combinations of mutations were detected in the same patient. Results of sequencing analysis performed retrospectively on stored plasma samples revealed that most of these variants had been circulating in the plasma transiently during the period of suboptimal therapy (Table 2). Proviral variants with additional mutations as compared with the variants found in plasma were seen in only 1 patient (patient 5). This patient carried a variant with an additional M184I mutation and a variant with an additional T69N mutation. Proviral variants with PI-associated primary mutations were not observed (data not shown).

Phylogenetic Analysis

After aligning the whole nucleotide sequence of about 1000 base pairs (protease gene and part of the RT gene) of all proviral and viral variants, a phylogenetic tree was constructed for each patient. The codons associated with resistance were removed from the alignment before the phylogenetic analysis was performed so that the tree topology was not determined by the resistance mutations. All trees showed a pronounced intermingling of viral and proviral sequences. No indications for a separate evolution within the provirus population were found. Despite removal of codons associated with resistance mutations before the analysis, variants with the same resistance pattern always clustered together. Two representative trees are shown in Figure 2. One tree is constructed from the results of patient 3. This patient had been on suboptimal therapy for 20 months. The proportion of wild-type variants in the provirus is high. Drug-resistant viral and proviral variants cluster together but bootstrap support for clustering was low (<50%). The second tree is constructed from the results of patient 7. This patient had been on suboptimal therapy for 50 months. The proportion of wild-type variants in the provirus is low. An intermingling of viral and proviral variants can be observed, and there is no evidence for a separate evolution of the provirus population.

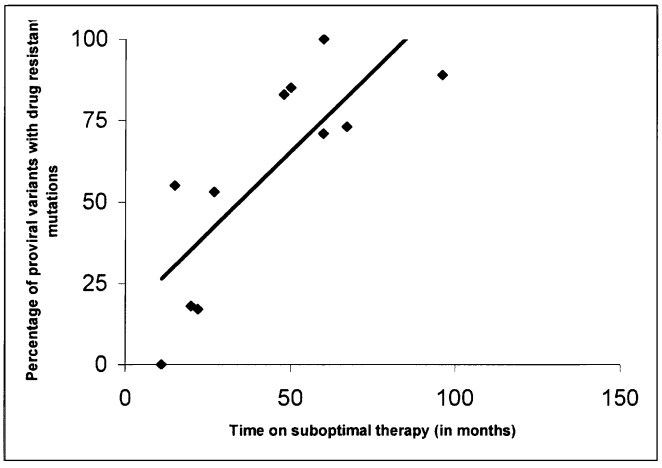


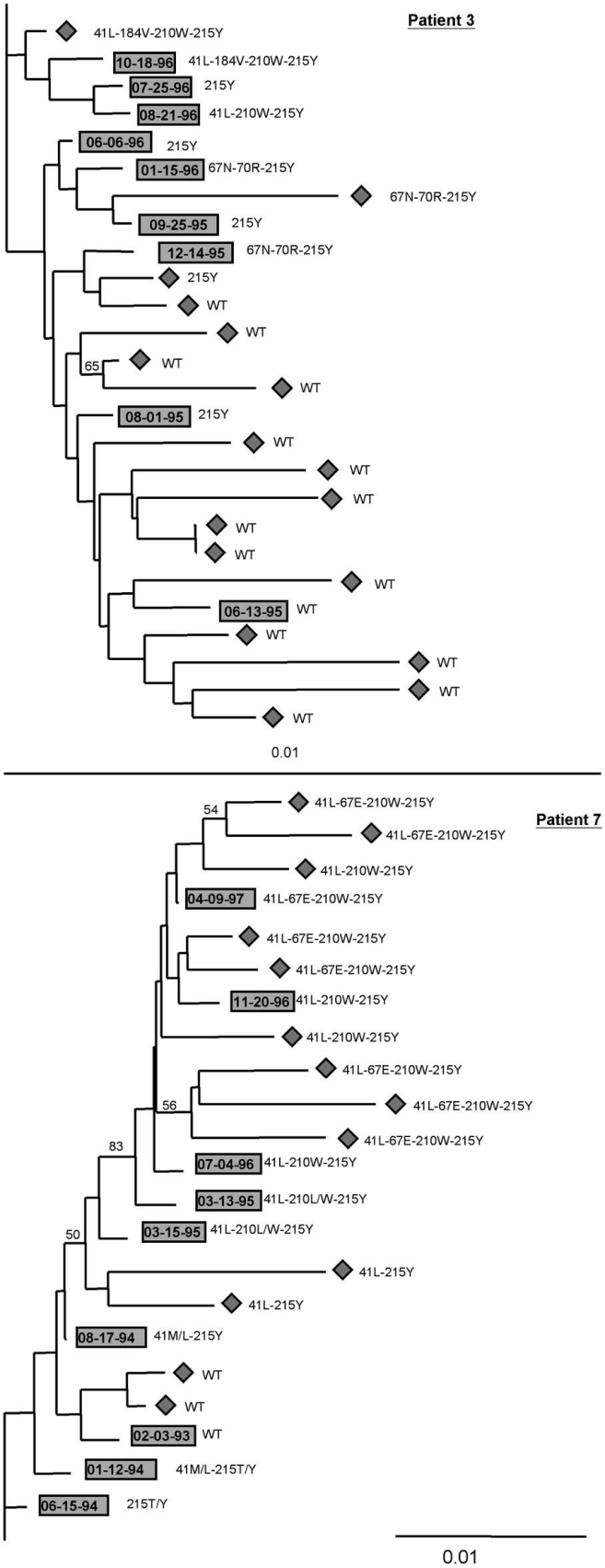
FIGURE 1. Association between the time on suboptimal therapy and the proportion of proviral sequences with drug resistance–associated mutations in the RT gene. The proportion of proviral sequences with drug resistance–associated mutations is expressed as percentage of the total number of analyzed proviral sequences for that patient. Logistic regression coefficient $r^2 = 0.6308$; $P = 0.004$.

DISCUSSION

Although advances in HIV treatment have reduced the morbidity and mortality rates among HIV-infected individuals, all currently prescribed antiretroviral drugs fail to eliminate the latent reservoir and it is clear that, with the current treatment strategies, eradication of the virus will never be possible. Therapy has to be taken for life, and this is complicated due to the adverse effects of the drugs and due to the emergence of drug resistance. HIV-infected individuals in whom drug regimens have repeatedly failed often harbor virus with multiple drug resistance–associated mutations. Although it has been shown that stopping therapy or switching from one class of drugs to another leads to the disappearance of the resistant strains from the plasma in the majority of cases, the question of whether this also will enable recycling of these drugs in the future is not yet clearly answered.[11,12] Although viral latency under HAART is the subject of several studies, the mechanisms of HIV persistence and reservoir establishment remain largely unknown.[2–5,7,8,20,21]

We studied the variability of the RT and protease gene in the provirus of patients who were under long-term HAART but who had a history of suboptimal therapy in the past. Our results confirm the observations of others that cells containing HIV-1 provirus remain detectable for periods extending several years.[7,10] Proviral sequences with a fully wild-type RT gene were found in 10 of the 11 patients despite the fact that in all these patients drug-resistant mutants have been favored by the selective conditions for many years. For the 1 patient in whom no wild-type proviral variants were detected, no pretreatment plasma samples were available so we cannot exclude the presence of the 70R mutation as a polymorphism already before starting medication.

In accordance with the findings in HIV-1–infected children, our results show that viruses in the latent reservoir are diverse and reflect selection by the pre-HAART regimens.[21] From the results of this study we have arguments to support the observation also made recently by Strain et al.[10] that the maintenance of the cellular reservoir is a dynamic process. New variants that are able to replicate for a certain period enter the



reservoir to be conserved for longer periods. With a few exceptions, all mutant virus variants that were found in the plasma during the process of gradually building up resistance were still detectable several years later in the provirus. From the correlation that we observed between the period on suboptimal therapy before HAART and the proportion of mutant proviral sequences in the PBMCs, we can conclude that the quantity that a certain variant occupies within the reservoir will depend in part on the period that this variant has been able to replicate. However, the slow fading out of the oldest variants—in these cases the wild-type variants—might also contribute to the observed correlation.

We were not able to find indications for a further virus evolution under HAART. Only in patient 5, two observations might reflect some evolution: the detection of a 184I-carrying proviral variant and a 69N variant in provirus but not in plasma. The patient was on a combination of stavudine (d4T) + 3TC + ritonavir (RTV) + saquinavir (SQV) and selection of a 69N by this combination is possible, although it is more likely that this mutant arose during the pre-HAART bitherapy with AZT and ddI but was missed in plasma. The 184I mutation is known to be a 3TC-resistant transient intermediate stage between the wild-type 184M and the 3TC-resistant 184V.[22] Because 3TC is a component of the HAART regimen and the patient has never taken 3TC before, the chance is high that this variant arose during the HAART period. However, we cannot exclude the occurrence of 184I as a natural polymorphism. In this regard it is important to note that the 184I mutation was detected in a proviral variant with an otherwise completely wild-type background. 3TC was a component of HAART in 9 of the 11 patients, but no other patients showed proviral sequences with mutations at codon position 184.

PIs were a component of the HAART regimen in all patients but no additional PI-associated mutations compared with the secondary mutations already present in the plasma

FIGURE 2. Rooted neighbor-joining trees of the HIV-1 protease and RT gene from viral RNA (*bars*) and proviral DNA (*squares*) of patients 3 and 7. Viral RNA was isolated from plasma at different time points during nonsuppressive therapy (date of sampling is indicated in the bar). Proviral DNA was isolated from a single PBMC sample taken after 59 months (patient 3) and 55 months (patient 7) of successful HAART. The resistance pattern of each isolate is indicated but the codons associated with drug resistance mutations were removed from the nucleotide alignment before phylogenetic analysis. Trees were rooted with a reference subtype B strain (B.FR.83.HX). The numbers at the nodes indicate proportion of support in 1000 bootstrap resamplings. Only bootstrap proportions of ≥50% are indicated. WT, wild-type, no resistance-associated mutations detected.

virus before HAART initiation were detected (results not shown). An additional argument against further evolution of proviral sequences is the fact that phylogenetic analysis revealed intensive intermingling of proviral and viral variants in all patients.

Currently, plasma is the only compartment used routinely for drug resistance testing and studies that address the role of the cellular reservoir with regard to emerging drug resistance and conservation of drug resistance are limited.[21] Our results show that infected PBMCs of patients under HAART contain a heterogeneous mixture of different viral variants. Because of this heterogeneity, population-based sequencing of provirus will presumably only detect major variants and will not provide valuable information about the resistance potential. Limiting dilution sequencing, conversely, was shown to allow detection of archived viral resistance, but the method is time consuming and expensive and therefore not suitable for large-scale use. It also remains to be examined to what extent the archived viruses remain replication competent. In this study, 10 sequences with stop codons, frame shifts, or hypermutations were found on the total of 173 sequences that were analyzed and they were removed from the analysis. However, our observations are limited to the HIV-1 protease and part of the RT gene and we cannot exclude the occurrence of mutations resulting in defective virus elsewhere in the genome. Despite the fact that we have no evidence for the replication competence of the archived proviral sequences, we consider the lack of any selective pressure that might be induced by in vitro culture as an important advantage, allowing a better estimate of the quantitative distribution of the different proviral variants present.

The results described here have important clinical implications because they confirm the long-term persistence of any drug-resistant virus once it has arisen, thereby permanently jeopardizing certain treatment options. In the patients studied here, HAART was initiated at a time when resistance testing was not performed. It is important to notice that, with the current knowledge, several of the drug combinations used at that time would no longer be prescribed in these patients, considering the observed genotypic resistance patterns. However, even with a suspected "less active" HAART, all patients showed a long-term virologic and immunologic response to the treatment, indicating that drugs to which resistance is predicted can still have therapeutic value in a combination regimen. Besides the fact that resistance is seldom an all-or-nothing phenomenon and low-grade resistance can be overcome by high drug concentrations, the results of this study point to another possible explanation for this observation, the fact that despite the detection of fully resistant virus in plasma, the majority of infected cells might still contain wild-type virus. As part of a combination regimen, drugs to which resistance has been developed can still add to the activity of the combination by preventing the replication of this latent wild-type, drug-sensitive, virus pool.

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REFERENCES

1. Chun TW, Finzi D, Margolick J, et al. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat Med*. 1995;1:1284–1290.
2. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A*. 1997;94:13193–13197.
3. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*. 1997; 278:1295–1300.
4. Finzi D, Blankson J, Siliciano JD, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med*. 1999;5:512–517.
5. Persaud D, Pierson T, Ruff C, et al. A stable latent reservoir for HIV-1 in resting CD4(+) T lymphocytes in infected children. *J Clin Invest*. 2000; 105:995–1003.
6. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997;278:1291–1295.
7. Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4(+) T cells. *Nat Med*. 2003;9:727–728.
8. Ramratnam B, Mittler JE, Zhang L, et al. The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. *Nat Med*. 2000;6:82–85.
9. Blankson JN, Persaud D, Siliciano RF. The challenge of viral reservoirs in HIV-1 infection. *Annu Rev Med*. 2002;53:557–593.
10. Strain MC, Gunthard HF, Havlir DV, et al. Heterogeneous clearance rates of long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong persistence. *Proc Natl Acad Sci U S A*. 2003;100: 4819–4824.
11. Devereux HL, Youle M, Johnson MA, et al. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. *AIDS*. 1999; 13:F123–F127.
12. Verhofstede C, Wanzeele F, Van Der Gucht B, et al. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors resulted in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. *AIDS*. 1999;13:2541–2546.
13. Deeks SG, Wrin T, Liegler T, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *N Engl J Med*. 2001;344:472–480.
14. Hance AJ, Lemiale V, Izopet J, et al. Changes in human immunodeficiency virus type 1 populations after treatment interruption in patients failing antiretroviral therapy. *J Virol*. 2001;75:6410–6417.
15. Izopet J, Massip P, Souyris C, et al. Shift in HIV resistance genotype after treatment interruption and short-term antiviral effect following a new salvage regimen. *AIDS*. 2000;14:2247–2255.
16. Miller V, Sabin C, Hertogs K, et al. Virological and immunological effects of treatment interruptions in HIV-1 infected patients with treatment failure. *AIDS*. 2000;14:2857–2867.
17. Delaugerre C, Valantin MA, Mouroux M, et al. Re-occurrence of HIV-1 drug mutations after treatment re-initiation following interruption in patients with multiple treatment failure. *AIDS*. 2001;15:2189–2191.

18. Izopet J, Souyris C, Hance A, et al. Evolution of human immunodeficiency virus type 1 populations after resumption of therapy following treatment interruption and shift in resistance genotype. *J Infect Dis*. 2002;185: 1506–1510.
19. Becker-Pergola G, Mellquist JL, Eshleman JR, et al. Improved detection of human immunodeficiency virus type 1 variants by analysis of replicate amplification reactions: relevance to studies of human immunodeficiency virus type 1 vertical transmission. *Mol Diagn*. 1999;4:261–268.
20. Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Annu Rev Immunol*. 2000;18:665–708.
21. Ruff CT, Ray SC, Kwon P, et al. Persistence of wild-type virus and lack of temporal structure in the latent reservoir for human immunodeficiency virus type 1 in pediatric patients with extensive antiretroviral exposure. *J Virol*. 2002;76:9481–9492.
22. Frost SD, Nijhuis M, Schuurman R, et al. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. *J Virol*. 2000;74:6262–6268.

The latent HIV reservoir in patients undergoing HAART: an archive of pre-HAART drug resistance.

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The latent HIV-1 reservoir in patients undergoing HAART: an archive of pre-HAART drug resistance

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Recent studies on patients with a history of pre-HAART drug resistance, but currently on a successful regimen, provided new insights into the dynamics of the latent cellular viral reservoir. Results indicated that the latent reservoir is an archive, composed of a mixture of wild-type and drug-resistant strains. The studies showed that, even after years of successful HAART, the wild-type viral strains that circulated before the initiation of the therapy as well as all the different drug-resistant viral strains that evolved over time during eventual periods of non-suppressive treatment, remain detectable in the proviral reservoir. These findings support the hypothesis that during active viral replication, new variants, including drug-resistant ones, continuously enter the latent viral reservoir. It can be concluded that, as a consequence of the lifelong conservation of this latent reservoir, the potency of drugs for which resistance once developed will remain reduced, even after years of withdrawal of the drug.

Keywords: reservoirs of resistance, mechanisms of resistance, mutations

Introduction

Since the availability of sensitive assays for viral load quantification in plasma, it has become clear that HIV-1 infection is characterized by a continuous massive virus replication, even during the asymptomatic phase of the disease. Although the immune system exerts some control, it generally fails to completely arrest the replication or reduce it to a form of true latency as is seen for other viral infections. The process of continuous virus replication can be interrupted or at least significantly reduced by HAART. But, although the introduction of HAART results in undetectable levels of plasma virus in the majority of patients, HAART fails to completely eradicate the virus *in vivo*, even after years of uninterrupted therapy. Viral persistence is thought to be the result of the long-term survival of a pool of infected, resting CD4 cells. Recent studies in patients on successful HAART but with a history of pre-HAART drug resistance, provided new evidence for the dynamic nature of the latent reservoir and showed that any viral variant, including any drug-resistant variant that has been allowed to replicate for a certain time during the infection, will enter the reservoir and remain conserved.

Long-term latent reservoir

Finzi *et al.*[1] showed the establishment of a latently infected CD4 cell compartment already very early in infection. The cellular reservoir is found predominantly in resting DR^-CD4 cells with a memory phenotype.[2,3] The half-life of these cells is long (44 months) and this long lifespan, combined with the possibility of self-renewal by proliferation, ensures their lifelong presence. How these cells originate is still a matter of controversy, but it has been postulated that the reservoir of latently infected cells is generated when lymphoblasts that are in the process of reverting to a resting state, become infected.[4,5] Whether the long half-life of the infected cells is the only reason for the persistence of these cells during HAART treatment or whether the latent pool is fully or partly maintained by ongoing low-level viral replication despite treatment, has long been unclear. But the lack of detectable evolution in the envelope and polymerase sequences of viral strains in the cellular reservoir during HAART, argues against entry of new genotypes in the latent pool during successful treatment, and supports the belief that persistence depends primarily on the intrinsic stability of the infected cells.[6]

The HIV reservoir during HAART treatment

In patients on successful HAART, the presence of resting CD4 cells harbouring replication-competent virus has been demonstrated by several groups.[1,7–9] In line with these observations is the consistent clinical finding of a quick rebound of plasma virus in all patients who stop treatment, indicating the presence of a latent reservoir that enables quick reinitiation of replication whenever the drug pressure is removed.[10,11]

But perhaps the most convincing argument for long-term conservation of viral strains comes from the observation that a cessation of treatment or a switch of antiretroviral drugs in patients treated for more than 2 years with suboptimal drug regimens,

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resulted in the replacement of the resistant virus in the plasma by wild-type variants.[12–14] In the majority of the patients studied, the replacement of the mutant by the wild-type virus was abrupt and fast, indicating that it was the result of the reappearance of archived wild-type virus and not of the reversal of mutations in the resistant variants. This finding was remarkable since drug-resistant virus predominated in the plasma of these patients for several years and at least an important reduction in the population of wild-type virus through a natural process of cell death could be presumed. If wild-type virus persists in the latent reservoir for such a long time, then it could be postulated that drug-resistant strains too will be conserved.

Ruff *et al.*[15] were the first to demonstrate that drug-resistant viruses, selected by non-suppressive regimens in infected children, entered the reservoir and persisted during HAART. Subsequently, Lambotte *et al.*[16] compared a polymorphic region of the *env* gene and part of the reverse transcriptase gene in pre-HAART plasma and in the reservoir lymphocytes, in nine treated patients with long-term undetectable plasma viral load. They observed archiving of pre-HAART plasma clones in six patients and confirmed the co-existence of wild-type and drug-resistant virus in reservoir T cells in two. We studied the variability of the RT and protease gene in the provirus of 11 patients on successful HAART for years, but with a period of suboptimal regimens before.[17] Not only could we confirm the co-existence of wild-type and drug-resistant virus in the proviral reservoir after 5 years or more of HAART, but we were also able to show that, with a few exceptions, all mutant virus variants that were detected in the plasma before HAART initiation, during the process of gradually building up resistance, were still present in the latent reservoir. Moreover, we observed a correlation between the time period on suboptimal therapy before HAART and the proportion of mutant, drug-resistant, proviral sequences in the cells, indicating that the quantity that a certain variant occupies within the reservoir depends in part on the period that this variant has been able to replicate. These data prove that the reservoir of latently HIV-1-infected cells is dynamic, and that newly infected cells continuously turn into latency to enter the reservoir. These data also indicate the extreme long-term conservation of all variants that have ever entered the reservoir. The latent reservoir can be considered as the life-long archive of whatever viral strain that ever evolved and replicated.

Clinical implications of the persistence of drug resistance

Once it has arisen, long-term persistence of any drug-resistant virus jeopardizes, in a permanent manner, the use of drugs to which resistance has developed. This finding again emphasizes the importance of considering the whole treatment history of a patient whenever a new combination therapy is initiated. Recycling of any drug that was part of a non-suppressive treatment regimen, even if the drug was taken years ago, might result in the reactivation of archived resistant strains and must be avoided unless there are no valid alternatives.

However, the observation that the viral reservoir contains a heterogeneous mix of wild-type variants and mutant variants with different degrees of drug resistance, also indicates that even drugs to which resistance has developed may still 'add' to the activity of a combination therapy by preventing the replication of the drug-sensitive virus pool in the reservoir. Since the wild-type virus is believed to be the fittest variant, suppressing the replication of these wild-type strains can be important, especially in patients with limited treatment options, and might contribute to the reduced viral-load set-point as is observed in many treated patients with drug-resistant viraemia.[18,19] On the other hand, continuation of a failing regimen risks the further accumulation of drug-resistance mutations and an expansion of the reservoir of cells infected with drug-resistant variants, and is not advisable. Whether the proportion of cells infected with drug-resistant strains in the latent reservoir has any impact on the success or failure of subsequent salvage regimens still remains to be examined.

Currently, plasma is the only compartment used routinely for drug resistance testing. However, the observation that the proviral compartment contains an archive of the different strains, wild-type and drug-resistant, that have evolved during the infection, makes this proviral reservoir the ideal substrate for analysis of the 'resistance-potential' in a patient. This can be of special importance in those patients from whom no samples have been conserved and no historical data are available.

Conclusion

An important number of data indicate that the long-lived cellular reservoirs of HIV in patients reflect a heterogeneous population of replication-competent viral strains. The diversity of the reservoir is dynamic and results from successive archiving of circulating plasma viruses during the course of HIV infection, including the drug-resistant variants. Archived variants are assumed to remain life-long, thereby precluding the successful recycling of any drug towards which resistance has arisen.

Our knowledge of the latent HIV-1 latent reservoir is rapidly increasing. Only a thorough understanding of the development and maintenance of the latent reservoir will allow the development of new therapeutic strategies, aimed at a combined effect of arresting viral replication and eliminating the latent reservoir.

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References

1. Finzi, D., Hermankova, M., Pierson, T. *et al.* (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* **278**, 1295–300.

2. Chun, T. W., Carruth, L., Finzi, D. *et al.* (1997). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* **387**, 183–8.

3. Pierson, T., Hoffman, T. L., Blankson, J. *et al.* (2000). Characterization of chemokine receptor utilization of viruses in the latent reservoir for human immunodeficiency virus type 1. *Journal of Virology* **74**, 7824–33.

4. Blankson, J. N., Persaud, D. & Siliciano, R. F. (2002). The challenge of viral reservoirs in HIV-1 infection. *Annual Review of Medicine* **53**, 557–93.

5. Persaud, D., Zhou, Y., Siliciano, J. M. *et al.* (2003). Latency in human immunodeficiency virus type 1 infection: no easy answers. *Journal of Virology* **77**, 1659–65.

6. Frost, S. D. W., Leigh-Brown, A. J., Ignacio, C. C. *et al.* (1999). Evolution of envelope sequences of Human Immunodeficiency Virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. *Journal of Virology* **73**, 9404–12.

7. Chun, T. W., Stuyver, L., Mizell, S. B. *et al.* (1997). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proceedings of the National Academy of Sciences, USA* **94**, 13193–7.

8. Finzi, D., Blankson, J., Siliciano, J. D. *et al.* (1999). Latent infection of $CD4^+$ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nature Medicine* **5**, 512–7.

9. Persaud, D., Pierson, T., Ruff, C. *et al.* (2000). A stable latent reservoir for HIV-1 in resting $CD4^+$ T lymphocytes in infected children. *Journal of Clinical Investigation* **105**, 995–1003.

10. Ruiz, L., Martinez-Picado, J., Romeu, J. *et al.* (2000). Structured treatment interruption in chronically HIV-1 infected patients after long-term viral suppression. *AIDS* **14**, 397–403.

11. Chen, R. Y., Westfall, A. O., Raper, J. L. *et al.* (2002). Immunologic and virologic consequences of temporary antiretroviral treatment interruption in clinical practice. *AIDS Research and Human Retroviruses* **18**, 909–16.

12. Devereux, H. L., Youle, M., Johnson, M. A. *et al.* (1999). Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. *AIDS* **13**, F123–7.

13. Verhofstede, C., Van Wanzeele, F. V., Van Der Gucht, B. *et al.* (1999). Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors results in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. *AIDS* **13**, 2541–6.

14. Deeks, S. G., Wrin, T., Liegler, T. *et al.* (2001). Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *New England Journal of Medicine* **344**, 472–80.

15. Ruff, C. T., Ray, S. C., Kwon, P. *et al.* (2002). Persistence of wild-type virus and lack of temporal structure in the latent reservoir for human immunodeficiency virus type 1 in pediatric patients with extensive antiretroviral exposure. *Journal of Virology* **76**, 9481–92.

16. Lambotte, O., Chaix, M. L., Gubler, B. *et al.* (2004). The lymphocyte HIV reservoir in patients on long-term HAART is a memory of virus evolution. *AIDS* **18**, 1147–58.

17. Verhofstede, C., Noë, A., Demecheleer, E. *et al.* (2004). Drug-resistant variants that evolve during nonsuppressive therapy persist in HIV-1-infected peripheral blood mononuclear cells after long-term highly active antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes* **35**, 473–83.

18. Bates, M., Wrin, T., Huang, W. *et al.* (2003). Practical applications of viral fitness in clinical practice. *Current Opinion in Infectious Diseases* **16**, 11–18.

19. Deeks, S. G., Barbour, J. D., Martin, J. N. *et al.* (2000). Sustained $CD4^+$ T cell response after virologic failure of protease inhibitor-based regimens in patients with human immunodeficiency virus infection. *Journal of Infectious Diseases* **181**, 946–53.

CHAPTER V: GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

CHAPTER V: GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

Although HAART dramatically slows the progression of HIV disease and decreases the transmission rate, the HIV/AIDS prevalence is still rising worldwide. This rise can be explained by the fact that currently (in 2005) only 17% of the patients in need of HAART are effectively treated (http://www.unaids.org/epi/2005/doc/report_pdf.asp).

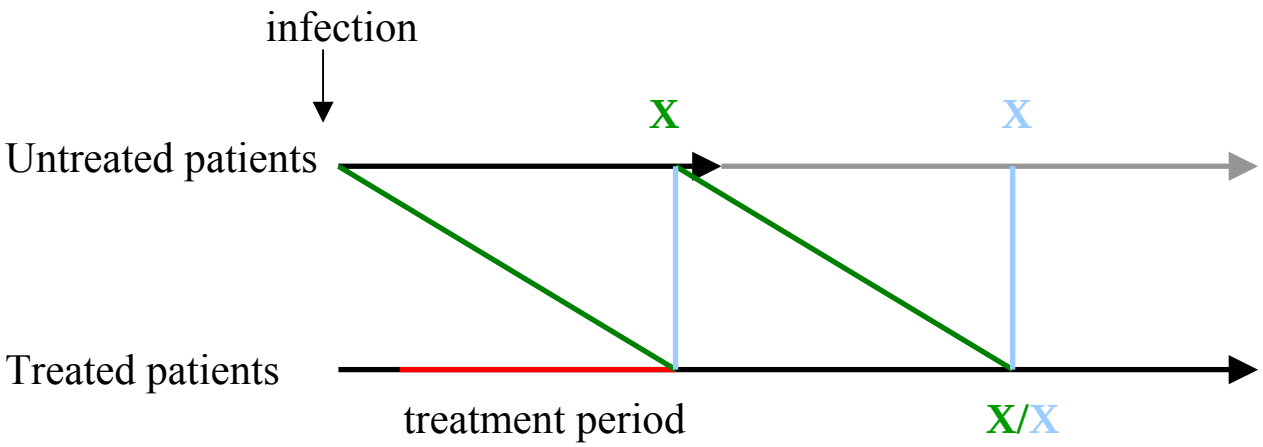
For the worldwide implementation of HAART the economical and logistic needs are enormous. At this moment, hope for a future stabilisation of the HIV/AIDS pandemic is based on these ARV programs which form an essential support for prevention strategies[1]. We must encourage implementation of ARV as it has been proven valuable in resource poor settings, despite the multiple challenges[2]. The WHO developed treatment guidelines, adapted to resource limited settings in order to treat large numbers of patients despite restricted logistic means (http//www.who.int/3by5/publications/documents /arv_guidelines/en/). Currently, an exponentially rising number of patients is treated with affordable drug regimens through several ARV implementation projects. However, fear exists that the improved access to treatment will result in the emergence and spread of resistant viruses. Efforts to prevent resistance are especially focused on counselling as good compliance is the best way to prevent its occurrence. CD4 T cell count and especially viral load determination help to evaluate the efficacy of an antiretroviral regimen and they allow the early recognition of therapy failure. Early detection of resistance enables a fast intervention, thereby reducing the risk of accumulated resistance mutations, of cross resistance and of multi drug resistance. Assays for CD4 determination and certainly for viral load quantification are still unaffordable in most developing countries. Cheap tests and strategies are urgently needed for follow-up[3]. Fear for resistance may not jeopardize the efforts to fight the virus in these resource poor settings, as yet no evidence exists that the risk for resistance is higher in these regions compared to the Western world [4].

As access to and use of ART are improving, it is critical to define regimens which offer the highest benefit with a minimum of adverse effects. One approach might be to search for a specific treatment strategy that enables the induction of a status of long term non progression (LTNP). In LTNP a high number of functional HIV-specific CD4 T cells can consistently be found [5]. A comparable conservation of the CD4 T cell responses has been reported in some

patients treated early in HIV-infection [6-11]. In addition, a lower proviral load is observed in LTNP and in patients treated during acute HIV-infection, compared to patients treated during chronic HIV-infection. [12] Part of this thesis aimed to investigate the effect of early but temporary ART on HIV-infection. The effect of treatment on HIV-specific CD4 T cells and proviral load in acute HIV infection has in most studies been analysed while patients are still on medication. As ARV treatment has considerable short and long term side effects, it is of interest to examine the benefit of a short treatment period. Are HIV-specific CD4 T cells preserved after the treatment interruption? What is the evolution of proviral load after treatment interruption? Can HIV disease progression be delayed with a temporary treatment? What is the impact of side effects?

We investigated the impact of temporary ART in acute HIV-infection on the two most generally used markers of disease progression: the number of CD4 T cells and the VL. We also studied the effect of the treatment on the immune function and we paid attention to the frequency of serious side effects of the medication. On the recent CROI (February 8, 2006, Early, uninterrupted treatment for HIV infection reduces complications, E. Susman) a study was presented in which over 2300 patients were evaluated for the occurrence of drug related side effects. According to these investigators, treatment in early infection was related with 60% less side effects compared to treatment in chronic infection. Also in terms of immune recovery early treatment was beneficial. However, they advise to continue the antiretroviral treatment without interruption. The better tolerance to therapy in early infection is in line with the observation of a low occurrence of side effects, in our patient group; only one patient in 40 had to stop the treatment because of side effects.

When we compared the VL and CD4 T cell count between untreated and treated patients at a same period of time after infection and treatment interruption respectively, we observed lower mean plasma VL and higher mean CD4 lymphocyte counts in the treated patients (**X**). This indicates that early temporary treatment might delay disease progression for a time period that exceeds the course of treatment. Because of a limited follow-up period in the untreated patients, we unable to compare the same time points after infection in both groups (X).



In one of the treated patients, no viral presence could be found after stopping the antiretroviral therapy even for a follow-up period, up to this moment, of 18 months. Obtaining a better insight in the underlying mechanisms that explain this efficient virus control could be very interesting. In this patient, viral characteristics, proviral load, HIV-specific immunity, and other host factors are further being studied.

Whether the preserved HIV-specific immunity contributes to the better outcome is not unequivocally proven. In agreement with the results presented in different other studies [6-11], we found that ART in early infection sometimes preserves HIV-specific immunity. However, we did not find a correlation between the amount and diversity of HIV-specific CD8 T cell reactivity and plasma VL. But, our results are in favour of a possible correlation between high HIV-specific CD4 T cell response and suppressed plasma VL. All the individuals studied for HIV-specific immunity, who suppressed for a long time their viral replication, had high amounts of HIV-specific CD4 T cells. The same numbers of immune cells, however, were also found in patients, who were able to only temporary suppress their viral levels. So the question remains if the HIV-specific CD4 T cells are suppressing viral replication or if VL determines the fate of the HIV-specific CD4 T cells.

High HIV-specific CD8 T cell responses were not seen at baseline in the 5 patients that were tested before treatment initiation. This can be due to the fact that we addressed this parameter in the first 6 months after infection, before the immune response was fully developed. However, it is

also possible that the role of CD8 T cells might have been overestimated in the literature, as their response is too late and too weak [13]. Also of importance is the fact that we only studied circulating blood cells, while most HIV-specific CD8 T cells are attracted to the lymph nodes, where they exert their antiviral function [14]. Another possibility is that due to the baseline activation status of the CD8 T cells, those cells are unable to produce extra IFN-γ in response to an *in vitro* stimulation.

We analysed the number of HIV-specific CD8 and CD4 T cells by peptide stimulation and measuring intracellular IFN-γ expression. Because of the restricted number of cells available, we were not able to address the relation between VL suppression and IL-2 secretion or proliferation capacity.

We also analysed the genetic background of our tested patients. An association between certain HLA antigens and a better outcome of the patient has been described. The genetic background is of importance as it is linked to the strength of the immune responses. Some HLA Class I antigens (HLA B27, B 63, B57, B58) are related with a better clinical outcome[15-17], while HLA B35 is related with a faster progression of the disease[18]. In our cohort of acute infected patients we were not able to find any relation between HLA class I antigens and the immune responses or the disease outcome.

In literature, two studies evaluating the effect of 'Structured Treatment Interruption' (STI) in early infection, have shown a limited long-term benefit after stopping the treatment [19, 20]. These STI may not have the same impact on viral spread and proviral HIV reservoir compared to continuous HAART, because of the repetitive viral rebound and viral spread associated with each therapy interruption. The strategy of STI also includes the risk of drug-resistance [21] and of an enhanced loss of CD4 T cells [22].

The proviral load might be a good prognostic marker to predict viral rebound after treatment interruption [23]. Therefore several strategies have been developed aiming to reduce the amount of latent HIV-infected cells. Starting HAART early after HIV-infection limits HIV proviral spread[24]. The decay rate of proviral load is observed to be higher in treated acute compared to

treated chronic infection. Moreover, HAART in early HIV-infection has been shown to decrease cell associated infectivity (CAI) [24] and gives hope to seriously limit viral rebound.

Lafeuillade showed that a longer treatment period early in infection resulted in lower proviral load [25]. In our study we also found a correlation between better outcome and a longer period of treatment. Studies evaluating the influence of the length of the treatment period in PHI on the final outcome are not done yet[26]. In the different studies that we found in literature, the treatment period varies between some weeks [11] and several years (3 years [27]).
Criteria to analyse the ideal time to stop HAART in acute HIV-infection should be defined. The usefulness of the total CD4 T cell number or the number of HIV-specific CD4 T cells or the proviral load as prognostic markers for predicting viral rebound and/or clinical outcome after treatment interruption must be evaluated[32]. Besides, larger studies with longer follow-up are needed to analyse the possible relation between those factors and long time control of virus replication after treatment stop

Studies have shown that immune activation therapies, with T cell activators and/or IL-2 fail to eradicate HIV infection [28]. On the contrary, those therapies induce an increase in T cell turnover and susceptible target cells thereby augmenting the viral replication. Recently, encouraging results are obtained with more selective strategies, which aim at activating the quiescent proviral genome without influencing the activation status of the immune cells by using prostratin and valproic acid [29-31]. A possible interesting strategy to deplete HIV provirus from resting cells could consist of a combination of early antiretroviral treatment and prostratin and valproic acid.

As only a small percentage of HIV-infected patients are diagnosed during acute HIV-infection, it can be argued that the studied strategy might have a limited impact on the overall HIV-infected population. However, in our hospital 9,7% of all the HIV-infected patients that were followed over the period of inclusion, were seen within the first six months of infection. Also, if a combination of different strategies would have a proven beneficial effect, more efforts could be put in programs for the active tracing of early infection. Detection of acute HIV-infection, through pooling of sera, has been shown feasible and cost effective [33, 34]. In high risk populations, a significant number of patients with acute HIV-infection can be detected [35]. Pilcher et al. studied

the prevalence of acute HIV-infection in a population consulting a clinic for sexually transmitted diseases (STD) in Malawi. They found a prevalence of acute HIV-infection of 5% among antibody-negative individuals [35]. Detection of acute HIV-infection is not only important for the possible benefit related with ARV treatment, but also for prevention of HIV-transmission which is extremely high during acute HIV-infection [36-42]. In addition, study of acute HIV-infection can help us to better understand factors related with protection and with a lower VL set point, which is of importance in future vaccine development and drug design.

A rising problem related to the improved availability of HAART is the management of side effects. In the western world, a high percentage of the hospitalisations in HIV-infected individuals are due to side effects. Next to those side effects, also IRIS is directly linked with therapy intake. Fear exists that the incidence of IRIS, will seriously rise in the developing countries as access to treatment is improving. This relies especially on the high prevalence of pathogens associated with IRIS in the developing countries (*Mycobacterium tuberculosis, Mycobacterium avium, Cryptococcus neoformans,* CMV, hepatitis B and C). TB, the second infectious killer worldwide after HIV, is in developing countries often seen in co-infection with HIV. Both infections, separately and combined, are associated with IRIS after treatment instauration. Mycobacteria are the pathogens most frequently implicated in IRIS after instauration of HAART, causing approximately 40% of the IRIS cases, reported up to 2002 [43]. Especially extrapulmonary TB is a strong risk factor for paradoxical reactions [44-46]. The distinction between inflammation and active infection as the cause of deteriorating clinical symptoms after treatment instauration is often not clear. Breen et al. suggested that HAART could induce and/or aggravate clinical symptoms of active TB infection [47].

Little is known about the incidence of IRIS in the developing world [48]. The benefits of HAART on HIV and the incidence of TB infection are much higher than the possible risks. It is known that because of HAART, the incidence of tuberculosis decreased by approximately 70-90% in treated cohorts living in high and low income countries [49-52].

Interaction of HIV with other highly prevalent tropical diseases can have a high impact on worldwide morbidity and mortality. HIV induced immune dysfunction increases the incidence of malaria [53] and severe malaria [54, 55] while *Plasmodium falciparum* parasitaemia on the other hand, causes higher plasma VL [56]. The effect of malaria on HIV disease progression and HIV

transmission rate remains to be elucidated [57]. No IRIS has been described related to *Plasmodium falciparum.*

The incidence of IRIS can possibly be reduced by early treatment of OI's in immune compromised patients and/or by early ARV treatment of HIV-infection, thereby avoiding serious immune dysfunction. This also requires broader use of screening methods and a better access to medical care to detect infections in an earlier or clinical latent stage. This is extremely difficult as in these immune compromised persons, expression of symptoms is often delayed, while at the time of detection the TB infection is more likely to be multibacillary.
For the many HIV-infected patients consulting in an advanced stage of the HIV-infection, a better understanding of the mechanisms responsible for IRIS can be useful to enable an adaptation of treatment strategies and guidelines for TB/HIV co-infected patients. Anti-inflammatory drugs and glucocorticosteroids have been shown to give relieve [58, 59]. Also surgery and therapeutic aspiration are often needed. Pires et al. observed a clinical and immunological benefit of concomitant administration of HAART and immune therapy with IL-2 plus GM-CSF [60]. They suggested that IRIS is associated with an inadequate recovery of immune functions and found the absence of lymphoproliferative responses and IL-2 production in response to recall/viral antigens which could be induced by immune therapy with IL-2 and GM-CSF. Those immune modulating treatments, however, are actually still inaccessible for the developing world.

Another major problem related to the use of HAART is caused by resistance. In our study on the latent HIV-reservoir we show the archiving of all replication competent HIV variants. Retrieving an individuals' therapy history is possible by analysing HIV-proviral sequences. Archiving implicates that once resistance arose, it can permanently jeopardize future treatment options. This emphasizes the importance of preventing resistance through adherence and optimal treatment regimens. World wide programs trying to prevent mother to child transmission (MTCT) with mono-or bi-therapy, fast elicit resistance linked mutations [61-69]. Depending on the time this sub-optimal treatment is given and on the antiretroviral drugs used, these mutations will develop with a different rate. Moreover, this strategy is not only associated with much higher HIV transmission rate, but also transmission of resistant virus to the child [70]. Future treatment options of those HIV-infected women and children can hereby seriously be restricted. Studies have to investigate

if previous used preventive MTCT programs are decreasing the efficacy of later started HAART in these HIV infected patients. Far most the best option is to use triple-drug MTCT prophylaxis, which has been proven highly efficient despite the standardized approach in the resource poor setting [61].

Big concern exists about infection with resistant viruses [71]. Reported percentages of HIV-infection with resistant viruses are between 10 and 30% [72-74]. This percentage gives an idea of the efficiency of the ARV used in a certain community.

References

1. Harries AD. Management of HIV in resource-poor countries, with a focus on sub-Saharan Africa. *Lepr Rev.* Sep 2002;73(3):268-275.

2. Krain A, Fitzgerald DW. HIV antiretroviral therapy in resource-limited settings: experiences from Haiti. *Curr HIV/AIDS Rep.* Jun 2005;2(2):98-104.

3. Colebunders R, Moses KR, Laurence J, et al. A new model to monitor the virological efficacy of antiretroviral treatment in resource-poor countries. *Lancet Infect Dis.* Jan 2006;6(1):53-59.

4. Kuritzkes DR. Extending antiretroviral therapy to resource-poor settings: implications for drug resistance. *Aids.* Jun 2004;18 Suppl 3:S45-48.

5. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science.* Nov 21 1997;278(5342):1447-1450.

6. Alter G, Hatzakis G, Tsoukas CM, et al. Longitudinal assessment of changes in HIV-specific effector activity in HIV-infected patients starting highly active antiretroviral therapy in primary infection. *J Immunol.* Jul 1 2003;171(1):477-488.

7. Altfeld M, Rosenberg ES, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J Exp Med.* Jan 15 2001;193(2):169-180.

8. Fidler S, Oxenius A, Brady M, et al. Virological and immunological effects of short-course antiretroviral therapy in primary HIV infection. *Aids.* Oct 18 2002;16(15):2049-2054.

9. Jansen CA, De Cuyper IM, Steingrover R, et al. Analysis of the effect of highly active antiretroviral therapy during acute HIV-1 infection on HIV-specific CD4 T cell functions. *Aids.* Jul 22 2005;19(11):1145-1154.

10. Oxenius A, Fidler S, Brady M, et al. Variable fate of virus-specific CD4(+) T cells during primary HIV-1 infection. *Eur J Immunol.* Dec 2001;31(12):3782-3788.

11. Oxenius A, Price DA, Easterbrook PJ, et al. Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes. *Proc Natl Acad Sci U S A.* Mar 28 2000;97(7):3382-3387.

12. Pires A, Hardy G, Gazzard B, Gotch F, Imami N. Initiation of antiretroviral therapy during recent HIV-1 infection results in lower residual viral reservoirs. *J Acquir Immune Defic Syndr.* Jul 1 2004;36(3):783-790.

13. Altfeld M, van Lunzen J, Frahm N, et al. Expansion of pre-existing, lymph node-localized CD8+ T cells during supervised treatment interruptions in chronic HIV-1 infection. *J Clin Invest.* Mar 2002;109(6):837-843.

14. Folkvord JM, Anderson DM, Arya J, MaWhinney S, Connick E. Microanatomic relationships between CD8+ cells and HIV-1-producing cells in human lymphoid tissue in vivo. *J Acquir Immune Defic Syndr.* Apr 15 2003;32(5):469-476.

15. den Uyl D, van der Horst-Bruinsma IE, van Agtmael M. Progression of HIV to AIDS: a protective role for HLA-B27? *AIDS Rev.* Apr-Jun 2004;6(2):89-96.

16. Frahm N, Adams S, Kiepiela P, et al. HLA-B63 presents HLA-B57/B58-restricted cytotoxic T-lymphocyte epitopes and is associated with low human immunodeficiency virus load. *J Virol.* Aug 2005;79(16):10218-10225.

17. Stewart-Jones GB, Gillespie G, Overton IM, et al. Structures of three HIV-1 HLA-B*5703-peptide complexes and identification of related HLAs potentially associated with long-term nonprogression. *J Immunol.* Aug 15 2005;175(4):2459-2468.
18. Tomiyama H, Miwa K, Shiga H, et al. Evidence of presentation of multiple HIV-1 cytotoxic T lymphocyte epitopes by HLA-B*3501 molecules that are associated with the accelerated progression of AIDS. *J Immunol.* May 15 1997;158(10):5026-5034.
19. Hoen B, Fournier I, Lacabaratz C, et al. Structured Treatment Interruptions in Primary HIV-1 Infection: The ANRS 100 PRIMSTOP Trial. *J Acquir Immune Defic Syndr.* Nov 1 2005;40(3):307-316.
20. Kaufmann DE, Lichterfeld M, Altfeld M, et al. Limited durability of viral control following treated acute HIV infection. *PLoS Med.* Nov 2004;1(2):e36.
21. Schweighardt B, Ortiz GM, Grant RM, et al. Emergence of drug-resistant HIV-1 variants in patients undergoing structured treatment interruptions. *Aids.* Nov 22 2002;16(17):2342-2344.
22. Roger PM, Durant J, Ticchioni M, et al. Apoptosis and proliferation kinetics of T cells in patients having experienced antiretroviral treatment interruptions. *J Antimicrob Chemother.* Aug 2003;52(2):269-275.
23. Hatzakis AE, Touloumi G, Pantazis N, et al. Cellular HIV-1 DNA load predicts HIV-RNA rebound and the outcome of highly active antiretroviral therapy. *Aids.* Nov 19 2004;18(17):2261-2267.
24. Strain MC, Little SJ, Daar ES, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis.* May 1 2005;191(9):1410-1418.
25. Lafeuillade A, Poggi C, Hittinger G, Counillon E, Emilie D. Predictors of plasma human immunodeficiency virus type 1 RNA control after discontinuation of highly active antiretroviral therapy initiated at acute infection combined with structured treatment interruptions and immune-based therapies. *J Infect Dis.* Nov 15 2003;188(10):1426-1432.
26. Dybul M, Fauci AS, Bartlett JG, Kaplan JE, Pau AK. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. *Ann Intern Med.* Sep 3 2002;137(5 Pt 2):381-433.
27. Markowitz M, Jin X, Hurley A, et al. Discontinuation of antiretroviral therapy commenced early during the course of human immunodeficiency virus type 1 infection, with or without adjunctive vaccination. *J Infect Dis.* Sep 1 2002;186(5):634-643.
28. Fraser C, Ferguson NM, Ghani AC, et al. Reduction of the HIV-1-infected T-cell reservoir by immune activation treatment is dose-dependent and restricted by the potency of antiretroviral drugs. *Aids.* Apr 14 2000;14(6):659-669.
29. Biancotto A, Grivel JC, Gondois-Rey F, et al. Dual role of prostratin in inhibition of infection and reactivation of human immunodeficiency virus from latency in primary blood lymphocytes and lymphoid tissue. *J Virol.* Oct 2004;78(19):10507-10515.
30. Lehrman G, Hogue IB, Palmer S, et al. Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. *Lancet.* Aug 13-19 2005;366(9485):549-555.
31. Rullas J, Bermejo M, Garcia-Perez J, et al. Prostratin induces HIV activation and downregulates HIV receptors in peripheral blood lymphocytes. *Antivir Ther.* Aug 2004;9(4):545-554.
32. Goujard C, Bonarek M, Meyer L, et al. CD4 cell count and HIV DNA level are independent predictors of disease progression after primary HIV type 1 infection in untreated patients. *Clin Infect Dis.* Mar 1 2006;42(5):709-715.

33. Quinn TC, Brookmeyer R, Kline R, et al. Feasibility of pooling sera for HIV-1 viral RNA to diagnose acute primary HIV-1 infection and estimate HIV incidence. *Aids.* Dec 1 2000;14(17):2751-2757.
34. Pilcher CD, McPherson JT, Leone PA, et al. Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population. *Jama.* Jul 10 2002;288(2):216-221.
35. Pilcher CD, Price MA, Hoffman IF, et al. Frequent detection of acute primary HIV infection in men in Malawi. *Aids.* Feb 20 2004;18(3):517-524.
36. Janssen RS, Holtgrave DR, Valdiserri RO, Shepherd M, Gayle HD, De Cock KM. The Serostatus Approach to Fighting the HIV Epidemic: prevention strategies for infected individuals. *Am J Public Health.* Jul 2001;91(7):1019-1024.
37. Cates W, Jr., Chesney MA, Cohen MS. Primary HIV infection--a public health opportunity. *Am J Public Health.* Dec 1997;87(12):1928-1930.
38. Flanigan T, Tashima KT. Diagnosis of acute HIV infection: it's time to get moving! *Ann Intern Med.* Jan 2 2001;134(1):75-77.
39. Jacquez JA, Koopman JS, Simon CP, Longini IM, Jr. Role of the primary infection in epidemics of HIV infection in gay cohorts. *J Acquir Immune Defic Syndr.* Nov 1994;7(11):1169-1184.
40. Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *Jama.* Jul 1 1998;280(1):42-48.
41. Leynaert B, Downs AM, de Vincenzi I. Heterosexual transmission of human immunodeficiency virus: variability of infectivity throughout the course of infection. European Study Group on Heterosexual Transmission of HIV. *Am J Epidemiol.* Jul 1 1998;148(1):88-96.
42. Pilcher CD, Eron JJ, Jr., Vemazza PL, et al. Sexual transmission during the incubation period of primary HIV infection. *Jama.* Oct 10 2001;286(14):1713-1714.
43. Shelburne SA, 3rd, Hamill RJ, Rodriguez-Barradas MC, et al. Immune reconstitution inflammatory syndrome: emergence of a unique syndrome during highly active antiretroviral therapy. *Medicine (Baltimore).* May 2002;81(3):213-227.
44. Breen RA, Smith CJ, Bettinson H, et al. Paradoxical reactions during tuberculosis treatment in patients with and without HIV co-infection. *Thorax.* Aug 2004;59(8):704-707.
45. Cheng VC, Ho PL, Lee RA, et al. Clinical spectrum of paradoxical deterioration during antituberculosis therapy in non-HIV-infected patients. *Eur J Clin Microbiol Infect Dis.* Nov 2002;21(11):803-809.
46. Cheng VC, Yam WC, Woo PC, et al. Risk factors for development of paradoxical response during antituberculosis therapy in HIV-negative patients. *Eur J Clin Microbiol Infect Dis.* Oct 2003;22(10):597-602.
47. Breen RA, Smith CJ, Cropley I, Johnson MA, Lipman MC. Does immune reconstitution syndrome promote active tuberculosis in patients receiving highly active antiretroviral therapy? *Aids.* Jul 22 2005;19(11):1201-1206.
48. Lawn SD, Bekker LG, Miller RF. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. *Lancet Infect Dis.* Jun 2005;5(6):361-373.
49. Badri M, Wilson D, Wood R. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet.* Jun 15 2002;359(9323):2059-2064.

50. Girardi E, Antonucci G, Vanacore P, et al. Impact of combination antiretroviral therapy on the risk of tuberculosis among persons with HIV infection. *Aids.* Sep 8 2000;14(13):1985-1991.
51. Ledergerber B, Egger M, Erard V, et al. AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy: the Swiss HIV Cohort Study. *Jama.* Dec 15 1999;282(23):2220-2226.
52. Santoro-Lopes G, de Pinho AM, Harrison LH, Schechter M. Reduced risk of tuberculosis among Brazilian patients with advanced human immunodeficiency virus infection treated with highly active antiretroviral therapy. *Clin Infect Dis.* Feb 15 2002;34(4):543-546.
53. Patnaik P, Jere CS, Miller WC, et al. Effects of HIV-1 serostatus, HIV-1 RNA concentration, and CD4 cell count on the incidence of malaria infection in a cohort of adults in rural Malawi. *J Infect Dis.* Sep 15 2005;192(6):984-991.
54. Chirenda J, Siziya S, Tshimanga M. Association of HIV infection with the development of severe and complicated malaria cases at a rural hospital in Zimbabwe. *Cent Afr J Med.* Jan 2000;46(1):5-9.
55. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. HIV infection as a cofactor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. *Aids.* Feb 20 2004;18(3):547-554.
56. Kublin JG, Patnaik P, Jere CS, et al. Effect of Plasmodium falciparum malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet.* Jan 15-21 2005;365(9455):233-240.
57. Whitworth JA, Hewitt KA. Effect of malaria on HIV-1 progression and transmission. *Lancet.* Jan 15-21 2005;365(9455):196-197.
58. French MA, Price P, Stone SF. Immune restoration disease after antiretroviral therapy. *Aids.* Aug 20 2004;18(12):1615-1627.
59. Hirsch HH, Kaufmann G, Sendi P, Battegay M. Immune reconstitution in HIV-infected patients. *Clin Infect Dis.* Apr 15 2004;38(8):1159-1166.
60. Pires A, Nelson M, Pozniak AL, et al. Mycobacterial immune reconstitution inflammatory syndrome in HIV-1 infection after antiretroviral therapy is associated with deregulated specific T-cell responses: beneficial effect of IL-2 and GM-CSF immunotherapy. *J Immune Based Ther Vaccines.* Sep 25 2005;3:7.
61. Chersich MF, Gray GE. Progress and Emerging Challenges in Preventing Mother-to-Child Transmission. *Curr Infect Dis Rep.* Sep 2005;7(5):393-400.
62. Clarke JR, Braganza R, Mirza A, et al. Rapid development of genotypic resistance to lamivudine when combined with zidovudine in pregnancy. *J Med Virol.* Nov 1999;59(3):364-368.
63. Eastman PS, Shapiro DE, Coombs RW, et al. Maternal viral genotypic zidovudine resistance and infrequent failure of zidovudine therapy to prevent perinatal transmission of human immunodeficiency virus type 1 in pediatric AIDS Clinical Trials Group Protocol 076. *J Infect Dis.* Mar 1998;177(3):557-564.
64. Ekpini RA, Nkengasong JN, Sibailly T, et al. Changes in plasma HIV-1-RNA viral load and CD4 cell counts, and lack of zidovudine resistance among pregnant women receiving short-course zidovudine. *Aids.* Mar 8 2002;16(4):625-630.
65. Frenkel LM, Wagner LE, 2nd, Demeter LM, et al. Effects of zidovudine use during pregnancy on resistance and vertical transmission of human immunodeficiency virus type 1. *Clin Infect Dis.* May 1995;20(5):1321-1326.

66. Kully C, Yerly S, Erb P, et al. Codon 215 mutations in human immunodeficiency virus-infected pregnant women. Swiss Collaborative 'HIV and Pregnancy' Study. *J Infect Dis.* Mar 1999;179(3):705-708.

67. Palumbo P, Holland B, Dobbs T, et al. Antiretroviral resistance mutations among pregnant human immunodeficiency virus type 1-infected women and their newborns in the United States: vertical transmission and clades. *J Infect Dis.* Nov 1 2001;184(9):1120-1126.

68. Welles SL, Pitt J, Colgrove R, et al. HIV-1 genotypic zidovudine drug resistance and the risk of maternal--infant transmission in the women and infants transmission study. The Women and Infants Transmission Study Group. *Aids.* Feb 18 2000;14(3):263-271.

69. Mandelbrot L, Landreau-Mascaro A, Rekacewicz C, et al. Lamivudine-zidovudine combination for prevention of maternal-infant transmission of HIV-1. *Jama.* Apr 25 2001;285(16):2083-2093.

70. Nolan M, Fowler MG, Mofenson LM. Antiretroviral prophylaxis of perinatal HIV-1 transmission and the potential impact of antiretroviral resistance. *J Acquir Immune Defic Syndr.* Jun 1 2002;30(2):216-229.

71. Tozzi V, Corpolongo A, Bellagamba R, Narciso P. Managing patients with sexual transmission of drug-resistant HIV. *Sex Health.* 2005;2(3):135-142.

72. Grossman Z, Lorber M, Maayan S, et al. Drug-resistant HIV infection among drug-naive patients in Israel. *Clin Infect Dis.* Jan 15 2005;40(2):294-302.

73. Masquelier B, Bhaskaran K, Pillay D, et al. Prevalence of transmitted HIV-1 drug resistance and the role of resistance algorithms: data from seroconverters in the CASCADE collaboration from 1987 to 2003. *J Acquir Immune Defic Syndr.* Dec 15 2005;40(5):505-511.

74. Metzner KJ, Rauch P, Walter H, et al. Detection of minor populations of drug-resistant HIV-1 in acute seroconverters. *Aids.* Nov 4 2005;19(16):1819-1825.

CHAPTER VI: SUMMARY

CHAPTER VI: SUMMARY

5.1 EPIDEMIOLOGY

HAART has been of great benefit in reducing morbidity and mortality related to HIV/AIDS in the western world. However, as worldwide only 17% of the HIV-infected patients in need of therapy are receiving HAART, the pandemic is still expanding. Especially in the developing world, where 95% of the people with HIV/AIDS are living, the situation is dramatic. Our epidemiologic overview shows the urgency to enhance efforts against this number one killer worldwide. There is an urgent need for broader preventive strategies, higher access to treatment and in the future, hopefully, an efficient vaccine or new therapies leading to a harmless symbiosis of the virus with his host or to a total HIV eradication.

5.2 HAART TREATMENT IN EARLY HIV-INFECTION

Studies suggested that early treatment of an HIV-infection can be useful and sometimes lead to a control of viral replication after stopping therapy. We studied a cohort of 68 recently HIV-infected patients. In 40 of them HAART was started in the first 6 months of their HIV-infection. Thirty-eight patients were retained for analysis. They were treated for different periods of time (mean: 17,3 months). The 28 remaining patients remained untreated and were followed as controls. VL, CD4 T cell percentages and HIV-specific immunity were followed after treatment interruption. Values were compared between treated (n=32) and untreated acute HIV-infected individuals (n=28).

The treated patient group was found to have a longer VL suppression and higher CD4 T cell numbers after treatment interruption. This effect was seen throughout the follow-up period of 3 years after treatment stop. HIV-specific immunity was not lost by early HAART. Moreover, our results show a correlation between high HIV-specific CD4 T cell IFN-γ production and VL suppression.

Criteria for safely stopping HAART remain to be elucidated: candidate markers are CD4 T cell numbers, HIV-specific CD4 T cells and proviral load. This, however, needs to be addressed in a study with treatment over a longer time and with longer follow-up.

5.3 HAART TREATMENT IN LATE HIV-INFECTION

The clinical impact of HAART is most pronounced in late stage HIV-infection when HIV-related symptoms are present. However, a controversial clinical deterioration after treatment instauration can be observed in some late stage HIV-infected patients. Symptoms, due to increased inflammatory responses are related to treatment induced immune restoration.
We illustrated this IRIS in an HIV/TB co-infected patient. Serious symptoms developed after the instauration of TB and HIV-treatment. Prednisolone gave relieve of general symptoms, while lymph node abscesses continued to appear and persisted up to 8 months after treatment start. After 1 year TB treatment was stopped. The patient remained in good clinical condition for 3 years. She then developed again lymph node abscesses, which were Ziehl Nielsen positive, while specific culture was negative. She was again treated for 9 months with tuberculostatic drugs. Symptoms disappeared again and since 2 years she is in good clinical condition.
The incidence of IRIS is increasing as access to care is rising. By treating HIV-infection earlier in the disease course, IRIS could be avoided. This would need an earlier diagnosis, a higher awareness of possible HIV-infection and a better health education. For people consulting with extremely low CD4 T cell counts, immune modulating therapies could be useful in the prevention or management of IRIS.

5.4 HAART AND LATENT RESISTANT HIV

Viral latency is the major impediment to total eradication of HIV with the current ART. Resistance, on the other hand, compromises the efficacy of HAART. We studied the behaviour of the HIV-reservoir in a cohort of 11 patients in whom resistant virus evolved after sub-optimal ART. The patients were switched to an efficient HAART regimen resulting in viral suppression to undetectable levels. After a mean treatment period of 59 months, blood samples of these 11 patients were analysed for HIV proviral sequences. The presence of resistant HIV variants could be revealed in 10 of the 11 patients. The patient in whom no resistant HIV variants were found had been on sub-optimal therapy for the shortest period of time (11 months). A positive correlation was found between the period on sub-optimal HAART and the percentage resistant HIV variants in the HIV proviral pool.

We conclude that archiving of all replication competent HIV variants over the patient's disease history, takes place. Recycling of drugs to which resistance arose seems therefore impossible.

HOOFDSTUK VI: SAMENVATTING

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5.1 EPIDEMIOLOGIE

Het introduceren van Highly Active Antiretrovirale Therapie (HAART) heeft een groot aandeel gehad in het verminderen van de morbiditeit en de mortaliteit veroorzaakt door HIV/AIDS in de westerse wereld. Nochtans neemt de pandemie nog steeds toe, vooral omdat wereldwijd slechts 17% van de HIV-geïnfecteerde patiënten die behandeling nodig hebben, HAART krijgen. Voornamelijk in de ontwikkelingslanden, waar 95% van de patiënten met HIV/AIDS leven, is de situatie dramatisch. Ons epidemiologisch overzicht benadrukt de noodzaak om de inspanningen tegen deze wereldwijde nummer één doodsoorzaak te verhogen. Er is een dringende nood aan uitbreiding van preventieve strategieën en toegankelijkheid tot medische zorgen. Voor de toekomst wordt gehoopt op een efficiënt vaccin of nieuwe therapieën die tot een onschadelijke symbiose leiden van het virus met zijn gastheer of tot de volledige eliminatie van het virus.

5.2 HAART BEHANDELING IN DE VROEGE HIV-INFECTIE

Studies suggereren dat vroege behandeling van de HIV infectie nuttig kan zijn en soms leidt tot langdurige onderdrukking van HIV na onderbreking van de behandeling. We bestudeerden een cohorte van 68 recent HIV-geïnfecteerde patiënten. In 40 van hen werd HAART gestart in de eerste 6 maanden van hun HIV-infectie. Tweeëndertig patiënten werden weerhouden voor analyse. Deze patiënten werden behandeld gedurende verschillende tijdsperiodes (gemiddeld: 17,3 maanden). Achtentwintig patiënten bleven onbehandeld en werden gevolgd als controles. Virale load (VL), CD4 T cel percentages en HIV-specifieke immuniteit werden opgevolgd na onderbreking van de behandeling. Waarden werden vergeleken tussen behandelde patiënten (n=32) en niet behandelde acuut HIV-geïnfecteerde individuen (n=28).
De behandelde patiënten groep bleek een langduriger suppressie van de VL en hogere CD4 T cel aantallen te hebben na therapie onderbreking. Dit verschil werd gezien gedurende de volledige follow-up periode van 3 jaar na het stoppen van de behandeling. HIV-specifieke immuniteit ging niet verloren door vroege HAART. Onze resultaten toonden een overeenkomst tussen een hoge IFN-γ productie door HIV-specifieke CD4 T cellen en de suppressie van de VL.

Criteria voor het veilig stoppen van HAART moeten nog gedefinieerd worden: mogelijke parameters zijn CD4 T cel aantallen, HIV-specifieke CD4 T cellen en proviral lading. Dit moet echter onderzocht worden in een studie met langere behandelingsperiode en met langere follow-up tijd.

5.3 HAART BEHANDELING IN DE LATE HIV-INFECTIE

De klinische invloed van HAART is meest uitgesproken in de late fase van de HIV-infectie wanneer HIV-gerelateerde symptomen aanwezig zijn. Nochtans kan men in sommige HIV-geïnfecteerde patiënten in deze late fase een tegenstrijdige klinische achteruitgang zien na het instellen van de behandeling. Symptomen, toegeschreven aan een toegenomen inflammatoir antwoord worden veroorzaakt door het immuun herstel geïnduceerd door de behandeling.
We illustreren deze IRIS in een HIV/TB gecoïnfecteerde patiënte. Ze ontwikkelde ernstige symptomen na het instellen van de TB en HIV-behandeling. Prednisolone gaf verlichting van de algemene symptomen, terwijl lymfe knoop abcessen bleven verschijnen en aanwezig bleven tot 8 maand na het opstarten van de behandeling. Na 1 jaar werd de TB behandeling gestopt. De patiënt bleef in goede conditie gedurende 3 jaar. Dan ontwikkelde zij terug lymfe knoop abcessen, die Ziehl Nielsen positief waren, terwijl de specifieke cultuur negatief was. Ze werd opnieuw behandeld met tuberculostatica gedurende 9 maand. Symptomen verdwenen terug en ze is nu in goede algemene conditie sinds 2 jaar.
De incidentie van IRIS neemt toe aangezien de toegang tot medische zorgen stijgt. Door HIV-infectie in een vroeger stadium te behandelen, kan IRIS vermeden worden. Dit vergt een vroegere diagnose, een hoger vermoeden van een mogelijke HIV-infectie en een betere gezondheidsopvoeding. Voor mensen die consulteren met een extreem laag aantal CD4 T cellen, kan immuun modulerende behandeling nuttig zijn in de preventie of de behandeling van IRIS.

5.4 HAART EN LATENTE, RESISTENTE HIV-INFECTIE

Virale latentie is de voornaamste struikelsteen voor volledige uitroeiing van HIV met de huidige ART. Resistentie, aan de andere kant, vermindert de doeltreffendheid van HAART. We bestudeerden het gedrag van het HIV-reservoir in 11 patiënten die resistentie ontwikkelden na sub-optimale behandeling. De patiënten werden vervolgens behandeld met een efficiënt HAART regime wat resulteerde in onderdrukking van het virus tot niet te

detecteren plasma spiegels. Na een gemiddelde behandelingsperiode van 59 maanden, werden bloedstalen van deze 11 patiënten geanalyseerd voor HIV provirale sequenties. De aanwezigheid van resistente HIV varianten kon aangetoond worden in 10 van de 11 patiënten. De patiënt bij wie geen resistente HIV varianten werden gevonden was voor de kortste periode behandeld met sub-optimale therapie (11 maanden). Een positieve relatie werd gevonden tussen de periode van sub-optimale HAART en het percentage resistente HIV varianten in de provirale pool.

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Who turns to the sun leaves his shadows behind

Hemingway

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Publications:

HIV en opportunistische infecties. Medisfeer januari 2001 R. Colebunders, A. Noë

A multidrug, including voriconazole, resistant oral candida infection in an AIDS patient effectively treated with echinocandin. B. Ostyn, A. Noë, D. Swinne, M. Ieven, R. Colebunders Journal of Infection 2002; 44(1) 57-58.

Tuberculeuze meningitis bij personen met HIV infectie. Dohmen, B. Ostyn, I. Coulier, A. Noë, I. Kint, R. Colebunders Tijdschrift voor Geneeskunde 58 nr21, 2002 S.

Development of multiple abscesses in an HIV/TB co-infected patient after initiation of antituberculous and highly active antiretroviral therapy. Bottieau E, Noe A, Florence E, Ostyn B, Colebunders R. Acta Clinica Belgica 2002; 57-4; 219-222.

Multiple tuberculous brain abscesses in an HIV-infected patient successfully treated with HAART and antituberculous treatment. E Bottieau, A. Noë, E. Florence, R. Colebunders Infection 2003; 31(2): 118-20.

verslag van 2nd IAS Conference on HIV Pathogenesis and treatment. Interface Virology and transplantation 16, 2003. A. Noë

Drug Resistant Variants That Evolve During Nonsuppressive Therapy Persist in HIV-1 Infected Peripheral Mononuclear Cells After Long Term Highly Active Antiretroviral Therapy. Verhofstede*

C, Noë* A, Demecheleer E, De Cabooter N, Van Wanzeele F, Van Der Gucht B, Vogelaers D, Plum J. J. Acquir Immune Defic Syndr 2004 Mar 15; 35(5): 473-483. * equally contributed

The AIDS epidemic: The spread of a deadly disease in a biotech era. A Noë, C Verhofstede, J Plum. The Journal of Biological Regulators and Homeostatic Agents. 2004 Apr-Jun; 18(2):178-82.

The latent HIV-1 reservoir in patients undergoing HAART: an archive of pre-HAART drug resistance. Ann Noë, Jean Plum and Chris Verhofstede. J Antimicrob Chemother. 2005 55(4): 410-2.

Submitted to J. Acquir Immune Defic Syndr: Influence of temporary treatment in early HIV-1 infection on disease evolution. Ann Noë, Chris Verhofstede, Bea Van Der Gucht, Filip Van Wanzeele, Dirk Vogelaers and Jean Plum.

Congressen:

Fifth International Congress on Drug Therapy in HIV infection. Glasgow, Schotland 22-26 October 2000. Poster presentation; A multidrug resistant oral Candida Glabrata infection in an AIDS patient effectively treated with voriconazole. A Noë, E Florence, I Vreelust, D Swinne, B Ostyn, R Colebunders

Changing Virus- Adapting Therapy a SMART living programme; Budapest May 26 2001

Belgian Rheumatologic congres: 27 September 2001. Poster presentation; A case of tuberculeous spondylodiscitis at two different cervical levels Noe A, Baeten D, Zmierczak H, Mielants H, Veys E

The 8th European conference on clinical aspects and treatment of HIV infection: 28-31 October 2001, Athens

Symposium on auto-immunity: the Belgian Immunological Society (BIS), Friday 30 November 2001

Treatment Challenges: Moving Beyond Clinical Trial Results and into Clinical Practice; Lugano, Switzerland April 5-6 2002

The XIV International AIDS Conference, Barcelona, Spain.7-12 July 2002.

Joint meeting of the Belgian and Dutch Societies for Immunology: 18-20 December 2002.

Keystone Symposia 29-03-03: abstract; Influence of early but temporary HAART on viral and immunological markers in patients with primary HIV infection. A Noë, C Verhofstede, B Van Der Gucht, F Van Wanzeele, B Verhasselt, J Plum.

2nd IAS Conference on HIV Pathogenesis and treatment; Paris, France: 13-16 July 2003.

Warsaw, Poland: 25-29 October 2003; HIV/AIDS congres

Keystone Symposia 12-18 April 2004: poster presentation; Longitudinal determination of HIV-1 proviral load in different subsets of cells in a cohort of acutely infected patients: A Noë, C Verhofstede, B Van Der Gucht, F Van Wanzeele, D Vogelaers, R Peleman, J Plum.