# The Amsterdam Cohort Studies

## *on hiv infection and aids*

*A summary of the results* ***1996 - 2000***

SEPTEMBER 2001

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## COLOPHON

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THE COVER REFLECTS THE PROGRESSION IN TIME AND HIGHLIGHTS CELLS,VIRUSES AND THE HUMAN BODY.

PART ONE

# Introduction to the Amsterdam Cohort Studies



**THE AMSTERDAM COHORT STUDIES (ACS) ON HIV INFECTION AND AIDS STARTED SHORTLY AFTER THE FIRST CASES OF AIDS HAD BEEN DIAGNOSED IN THE NETHERLANDS. IN OCTOBER 1984, HOMOSEXUAL MEN WERE ENROLLED IN A PROSPECTIVE STUDY. APPROXIMATELY 9% OF THE MALE POPULATION IN AMSTERDAM IS HOMOSEXUAL AND DUE TO EARLIER FIELD STUDIES AND PREVENTION ACTIVITIES CONCERNING SEXUALLY TRANSMITTED INFECTIONS, GOOD RELATIONSHIPS HAVE BEEN ESTABLISHED WITH THESE MEN, FACILITATING RECRUITMENT. ONE YEAR AFTER THE FIRST ENROLMENT, A SECOND PROSPECTIVE STUDY WAS STARTED AMONG INJECTING AND NON-INJECTING DRUG USERS. ENROLMENT AND FOLLOW-UP OF DRUG USERS IS POSSIBLE DUE TO THE WELL-ORGANIZED HEALTH FACILITY SYSTEM FOR DRUG USERS IN AMSTERDAM. THIS SYSTEM ENABLES ACCESS TO THE MAJORITY OF THE CITY'S DRUG USERS, WHO WOULD OTHERWISE HAVE BEEN DIFFICULT TO REACH.**

## The ACS framework

Within the ACS, different institutes collaborate: the Amsterdam Municipal Health Service (Department of AIDS Research), the Academic Medical Center of the University of Amsterdam (Department of Human Retrovirology, AIDS Unit of the Department of Infectious Diseases and the International Antiviral Therapy Evaluation Center) and CLB Sanquin (Department of Clinical Viro-Immunology and Laboratory of Experimental and Clinical Immunology, University of Amsterdam). From the beginning, the ACS has had a multidisciplinary approach (epidemiology, social science, virology, immunology and clinical medicine) and this unique collaboration has been very fruitful, significantly contributing to the knowledge and understanding of many different aspects of HIV-1 infection. Overall, the aims of the ACS are to study the prevalence, incidence and risk factors of HIV-1 infection and AIDS, the natural history of HIV-1 infection and the effects of intervention. The epidemiology and the natural history of other bloodborne and sexually transmitted infections among the participants of the ACS have also been studied. Over the past 17 years, the aims have remained the same though the emphasis has changed. In the beginning, the epidemiology had to be elucidated whereas later on the research focus was changed towards virological and immunological in-depth studies on the pathogenesis of HIV-1 infection.

The ACS collaborate with many other research groups both in the Netherlands and in other countries. The Ethiopian

Netherlands AIDS Research Program (ENARP) should be mentioned specifically: the collaborative research program on HIV/AIDS between the Ethiopian Health and Nutrition Institute (EHNRI) and the ACS. This program is financed by the Netherlands Ministry of Foreign Affairs. Its aims are to study the epidemiology and natural history of HIV-1 infection in Ethiopia and to contribute to establishing intervention studies especially with HIV vaccines. This project also has an important training component (Ethiopian Ph.D., M.Sc. and M.P.H. students, training of EHNRI staff) and stimulates the development of the infrastructure at EHNRI. ENARP is located in Addis Ababa at EHNRI and has about 60 Ethiopian staff and 3 permanent expatriates.
In 1996, an overview was published of the results of the Amsterdam Cohort Studies 1984 – 1995. The booklet presented here, which was compiled by A.M. de Roda Husman, Ph.D., and K.C. Wolthers, M.D., Ph.D., gives an overview of the most important research outcomes over the subsequent 5 years (1996-2000), along with a listing of all publications and Ph.D. theses over these years.

## The Amsterdam cohort study among homosexual men

The study population consists of homosexual men living mainly in and around the city of Amsterdam, The Netherlands. **table 1** shows how many participate(d) in the ACS among homosexual men and its substudies. The first wave of enrolment took place between October 1984 and April 1985 (Protocol 1). Included were asymptomatic homosexual men aged 18-65 with at least two sexual partners in the six months prior to intake. They were recruited through announcements in the gay press, advertisements and by word of mouth. Between April 1985 and February 1988 only seronegative men could enter the study (Protocol 2). Enrolment was re-opened to HIV-1 infected individuals from February 1988 until December 1998 (6000 numbers). Some of these participants entered the ACS because they were found to be HIV-positive while participating in another Municipal Health Service study or to start with antiretroviral treatment (open and double-blind AZT study). In June 1995, a special recruitment campaign was started among young (≤30 years) homosexual men, a study which is still ongoing. In February 1996, the follow-up of the 'old' HIV seronegative participants was terminated. Finally, a few participants entered the ACS but could not be classified in either of the above mentioned studies (9000 numbers) or were allowed to start their treatment within the ACS from February 1997 onwards (7000 numbers). In February 1999, follow-up of all HIV-infected participants was transferred to the Jan van Goyen clinic in the scope of the National Athena monitoring project **(table 2)**.

**TABLE 1** History of total number of homosexual men ever included in a study until 01-10-2001
Each participant can participate in as many as 4 studies over calendar time

| | total | death | AIDS | HIV antibody status | | |
|---|---|---|---|---|---|---|
| | | | | negative | positive | sero-converter |
| At least 1 visit at the Municipal Health Service | 1987 | 287 | 318 | 1281 | 557 | 149 |
| protocol 1 | 748 | 193 | 200 | 417 | 238 | 93 |
| Protocol 2 | 265 | 21 | 24 | 226 | 1 | 38 |
| Young homosexual men | 681 | | 1 | 633 | 30 | 18 |
| 6000 numbers | 196 | 46 | 54 | | 196 | |
| 7000 numbers | 26 | 1 | 6 | | 26 | |
| 9000 numbers | 28 | 3 | 4 | 1 | 27 | |
| Clinical follow-up *1 | 277 | 221 | 251 | | 215 | 62 |
| Jan van Goyen *2 | 184 | 1 | 15 | | 139 | 45 |
| Open AZT | 26 | 22 | 23 | | 19 | 7 |
| Doubleblind AZT | 56 | 36 | 42 | | 51 | 5 |
| Early antiretroviral treatment | 185 | 43 | 64 | | 140 | 45 |
| Combination Study | 12 | 10 | 9 | | 9 | 3 |
| Delta Study | 10 | 4 | 7 | | 6 | 4 |
| Triple Study | 6 | 1 | 1 | | 4 | 2 |
| AZT/3TC/D4T | 46 | | 4 | | 36 | 10 |
| Native | 12 | | 3 | | 10 | 2 |
| Atlantic | 9 | | 1 | | 8 | 1 |
| RGP 120 Vaccine | 16 | | 2 | | 3 | 13 |
| P24 Vaccine | 21 | 4 | 3 | | 20 | 1 |
| Vaxgen *3 | 14 | | | 14 | | |

*1 Of approximately 40 participants no additional clinical information could be collected due to missing medical records.
*2 Initially, 227 were eligible for follow-up at the Jan van Goyen, however decided to go to another hospital or had other reasons for refusing further follow-up.
*3 These men are also participant of the young homosexual men study.

**TABLE 2** Total number of homosexual men in follow-up in ongoing studies: participants who had a visit at the Municipal Health Service at or after 01-04-2001 or Jan van Goyen Clinic at or after 01-04-2000

| | total in follow-up | HIV antibody status | | | | |
|---|---|---|---|---|---|---|
| | | negative | positive | | seroconverter | |
| | | | ever on ART | ART naive | ever on ART | ART naive |
| Jan van Goyen | 178 | | 136 | | 42 | |
| Young homosexual men | 333 | 330 | | 1 | | 2 |

ART: any antiretroviral treatment.

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## Daily routine

Seropositives and seroconverters are seen every three months. Clinical, epidemiological and social scientific data are collected with standardized questionnaires (six monthly, co-ordinated by the Department of Social Science, University of Utrecht) and by physical examination. Blood is taken for virological and immunological tests and for storage **(tables 3 and 4).**

Seronegatives are seen by a nurse every six months and similar data are collected but no immunological tests are done nor are cells stored. Participants who developed an AIDS event during follow-up were referred to the Academic Medical Center (AMC) and since 1996 much effort has been put into aligning the AMC and the ACS registry regarding events (clinical follow-up). AIDS cases are also ascertained through

**TABLE 3** Overview of the used virological assays in the ACS.

| VIROLOGICAL ASSAYS | COMPANY | PERIOD HM / DU |
|---|---|---|
| **HIV screening assays:** | | |
| HTLV-III screening test | Home made | 1984 -1985 |
| HTLV-III EIA | Abbott | 1985 - 1989 |
| Vironostika anti-HTLV-III ELISA | Organon International | 1985 - 1987 |
| Wellcozyme anti-HTLV-III | Wellcome | ± 1986 |
| recombinant HIV-1/HIV-2 EIA | Abbott | 1989 - 1993 |
| recombinant HIV-1/HIV-2 3rd generation | Abbott | 1993 - 1995 |
| HIV-1/HIV-2 3rd generation plus EIA | Abbott | 1995 - 1998 |
| HIV IMX (MEIA system) | Abbott | 1998 - 2001 |
| VIDAS HIV DUO | Biomerieux | Since 2001 |
| **Other used HIV-antibody assays:** | | |
| HIV-1 anti-CORE EIA | Abbott | 1986 - 1992 |
| ENVACOR HIV-1 EIA | Abbott | |
| Detection of CORE | | 1989 - 1992 |
| Detection of ENV | | 1987 - 1990 |
| HIVAb p24 (rDNA) | Abbott | 1992 - 1997 |
| **HIV-antigen assays:** | | |
| HTLV-III Antigen EIA | Abbott | 1986 - 1992 |
| HIV Ag-1 EIA polyclonal | Abbott | 1990 - 1998 |
| **Confirmation assays:** | | |
| HTLV-III Western Blot | Home made | 1984 - 1985 |
| LiaTek HIV-1/HIV-2 | Organon Teknika | ± 1986 |
| HIV Blot 2.2 (HIV-1 and HIV-2) | Diagnostic | Since 1986 |
| HIV-2 Blot version 1.2 | Diagnostic | Since 1995 |
| **HIV RNA assays:** | | |
| NASBA HIV-1 QT | Organon Teknika | 1996 - 1997 |
| NucliSens HIV-1 QT | Organon Teknika | Since 1997 |

**Hm:** Homosexual men **Du:** Drug users **EIA:** Enzyme Immuno Assay **ELISA:** Enzyme Linked ImmunoSorbent Assay
**Liatek:** Line Immuno Assay Technique **MEIA:** Microparticle Enzyme Immuno Assay **NASBA:** Nucleic Acid Sequence-Based Amplification
Remark: To compare test results, some assays have been used simultaneously.

**TABLE 4** Overview of applied immunological assays performed in the ACS.

| PERIOD | PROLIFERATION ASSAYS | | | | | | | | | | T CELL SUBSETS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PHA | | ALS | | ACD3 | | CD228 | | CD328 | | CD2 | | CD3 | | CD4 | | CD8 | | MT-2 (*) | |
| | Hm | Du | Hm | Du | Hm | Du | Hm | Du | Hm | Du | Hm | Du | Hm | Du | Hm | Du | Hm | Du | Hm | Du |
| 1984 | | X | | X | | X | | X | | X | | X | | X | | X | | X | | X |
| 1985 | | | | | | | | | | | | | | | | | | | | |
| 1986 | | | | | | | | | | | | | | | | | | | | |
| 1987 | | | | | | | | | | | | | | | | | | | | |
| 1988 | | | | | | | | | | | | | | | | | | | | |
| 1989 | | | | | | | | | | | | | | | | | | | | |
| 1990 | | | | | | | | | | | | | | | | | | | | |
| 1991 | | | | | | | | | | | | | | | | | | | | |
| 1992 | | | | | | | | | | | | | | | | | | | | |
| 1993 | | | | | | | | | | | | | | | | | | | | |
| 1994 | | | | | | | | | | | | | | | | | | | | |
| 1995 | | | | | | | | | | | | | | | | | | | | |
| 1996 | | | | | | | | | | | | | | | | | | | | |
| 1997 | | | | | | | | | | | | | | | | | | | | |
| 1998 | | | | | | | | | | | | | | | | | | | | |
| 1999 | | | | | | | | | | | | | | | | | | | | |
| 2000 | | | | | | | | | | | | | | | | | | | | |

(*) Although the MT-2 assay is not really an immunological assay, it is presented here because it is developed and routinely determined in the Clinical Viro-Immunological laboratory.

**Hm:** Homosexual men **Du:** Drug users **PHA:** phytohemaglutinin (before 1994, human pooled serum (HPS) was added to the culture medium, after 1994, no HPS was added: PHA responses with and without HPS are not comparable) **ALS:** horse antilymphocyte stimulation test **ACD3:** T cell function measured after stimulation with monoclonal antibodies (mAb) against the CD3 receptor **CD228:** T cell function stimulation with CD2 and CD28 mAb **CD328:** T cell function stimulation with CD3 and CD28 mAb **T cell Immuno-phenotyping CD2, CD3, CD4 and CD8:** before 1988, the single indirect staining, a single indirect immuno-fluorescence staining on Ficoll isolated peripheral blood mononuclear cells (PBMC) was used and replaced by a double direct staining thereafter. Since 1994, lymphocyte immunophenotyping was accomplished in whole blood.

Dark-gray box = performed at every visit; Light-gray box = not performed on a routine basis.

cross-linking with the Amsterdam AIDS registry. Once a year information on survival status is obtained through active follow-up and matching with local population registries. The cause of death is obtained from the Amsterdam AIDS surveillance registry, hospital records and from next of kin.

**Intervention/vaccination studies**

At the beginning of 1987 a preliminary study of zidovudine (AZT) treatment in asymptomatic HIV-infected subjects was started within the ACS, followed one year later by a multi-centered, double-blind, placebo-controlled study. Since then the ACS has participated in several different (multi-centered) trials like Delta, Triple, Atlantic, Prometheus, Native and D4T/3TC study. The ACS has also participated as a study-site in three vaccination studies. Recruitment of the p24-HIV and rgp120 study among HIV-positives started between March 1993 and March 1994. The Vaxgen (multi-centered) double-blind, placebo-controlled study among high-risk HIV-uninfected homosexual men started in 1999 and is still ongoing.

**TABLE 5** History of total number of drug users ever included in a study up to 01 - 10 - 2001

Each participant can participate in more than one study over calendar time

| | | HIV ANTIBODY STATUS AT ENTRY | | |
|---|---|---|---|---|
| | TOTAL | NEGATIVE | POSITIVE | SEROCONVERTER |
| At least 1 visit at the Municipal Health Service | 1598 | 1187 | 319 | 91 |
| Drug user cohort | 1488 | 1078 | 318 | 91 |
| Clinical follow-up | 96[1] | - | 30 | 66 |
| '98 Young cross sectional study | 293[2] | 261 | 21 | - |
| JODAM[3] | 154 | 151 | 3 | 0 |

[1] Originally 110 paticipants participated, however of 14 participants no additional clinical information could be collected due to missing medical records, [2] Originally 452 participated however, part of them appeared not to have met the inclusion criteria. Of 293, 11 provided too little saliva to determine HIV status and 88 are followed within the drug cohort, [3] Follow-up study among young drug users from Amsterdam.

**TABLE 6** Total number of drug users in follow-up in ongoing studies:

had a visit at the Municipal Health Service at or after 01 - 10 -2000

| | | HIV ANTIBODY STATUS AT ENTRY | | |
|---|---|---|---|---|
| | TOTAL IN FOLLOW-UP | NEGATIVE | POSITIVE AT ENTRY | SEROCONVERTER |
| Drug user cohort | 559 | 456 | 64 | 39 |
| JODAM | 148 | 146 | 2 | |

JODAM: Follow-up study among young drug users from Amsterdam.

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## The Amsterdam cohort study among drug users

Participants are recruited at methadone outposts, the weekly sexually transmitted diseases (STD)-clinic for drug-using prostitutes and by word of mouth. HIV-negative and asymptomatic HIV-positive injecting and non-injecting drug users (IDU and non-IDU) are invited to participate. **Table 5** shows how many participate(d) in the ACS among drug users and its substudies.

The first wave of enrolment took place between December 1985 and September 1990 wherefore inclusion stopped until August 1991. Enrolment was then re-opened and in 1998 a special recruitment campaign was started among young drug users (≤30 years) **(table 6)**. Although it was a cross-sectional study design, a quarter is being followed in the drug user cohort. Again, in June 2000, much effort was put into recruiting young drug users. This follow-up study is still ongoing. From February 2001 'old' drug users can no longer enter the study.

**Daily routine**

Participants are seen every four months regardless of HIV status, but many return more irregularly. Clinical, epidemiological and drug use related information is collected at each occasion by interviewing participants using a standardized questionnaire. Since April 1989 this questionnaire was thoroughly revised and all participants were physically examined by a physician at each visit. In January 1999 this examination was terminated for the HIV-negatives. Blood is taken for virological tests and storing, and from April 1989 immunological tests are part of the daily routine in HIV-negative as well as HIV-positive participants. Since 1995 these are limitedly performed in a small subset of the HIV-negatives. Data on hospitalization are collected at each visit from the participants, independently through the Drug Department of the Municipal Health Service and since 1997 for all seroconverters and highly active antiretroviral therapy (HAART) using drug users effort has been put into aligning hospital and ACS event registration (clinical follow-up). Cases of AIDS are also ascertained through cross-linking with the Amsterdam AIDS registry. After AIDS has been diagnosed drug users can still participate.

Yearly, deaths and causes of death are identified by determining participants' vital status at the register of population in their city of residence and through

locating and examining hospital records and coroners' reports.

**Substudies**
Several substudies have been done among the drug users. Participants were tested for Hepatitis A, B and C, HTLV-1 and 2 and haematology. Also in-depth interviews were held to investigate why participants stopped injecting drugs. Furthermore there is a close collaboration with the STD clinic of the Municipal Health Service and the Central Methadone Registry.

## Collaborating institutes

**Department of AIDS Research of the Municipal Health Service of Amsterdam**
*Projectleader Prof. Dr. R.A. Coutinho*
The Municipal Health Service research mainly focuses on the prevalence, incidence and risk factors for HIV infection and AIDS in homosexual men and drug users. Also, studies are done on the clinical course of HIV-1 infection and on the epidemiology of other sexually transmitted and blood-borne infections. Furthermore, this department is involved in intervention and vaccination studies.

**Department of Retrovirology of the Academic Medical Center, University of Amsterdam**
*Projectleader Prof. Dr. J. Goudsmit*
The research of the Department of Retrovirology is well-known for their studies on virological aspects of HIV-1 infection. The viral load assays to monitor disease progression and efficacy of therapy are performed here. Main topics of this department are drug resistance of HIV-1 variants, evolution and classification. Other viruses occurring as coinfections of HIV-1 such as HHV-8 and HCV are also studied in-depth.

**Department of Clinical Viro-Immunology, CLB Sanquin and Laboratory of Experimental and Clinical Immunology, University of Amsterdam**
*Projectleaders Dr. H. Schuitemaker and Prof. Dr. F. Miedema*
Virus and host determinants of HIV-1 infection and AIDS pathogenesis are thoroughly studied at the Department of Clinical Viro-Immunology. The immunological markers for disease progression are determined at this department.
The immunological research focuses on cellular immune responses to HIV-1 and EBV in relation to disease progression. Virological research covers biological properties of HIV-1 variants in relation to the clinical course of HIV-1 infection.

**The International Antiviral Therapy Evaluation Center, Academic Medical Center, University of Amsterdam**
*Projectleader Prof. Dr. J.M.A. Lange*

PART TWO

# Results

## 1996 - 2000



RISK FACTORS FOR HIV-1 INFECTION AND PROGRESSION MARKERS FOR AIDS PATHOGENESIS HAVE BEEN THOROUGHLY STUDIED DURING THE FIRST YEARS OF RESEARCH WITHIN THE FRAMEWORK OF THE ACS. HOWEVER, IN 1995 EFFECTIVE THERAPY WAS STILL NOT AVAILABLE AND EXCEPT FOR A SMALL NUMBER OF SO-CALLED LONG-TERM SURVIVORS, HIV-1 INFECTION WOULD ULTIMATELY LEAD TO INCREASING VIRUS LOAD AND DECREASING $CD4^+$ T CELL COUNTS, INEVITABLY FOLLOWED BY DISEASE AND DEATH. THIS INCREASING VIRUS LOAD OFTEN RESULTED FROM FAST REPLICATING SI VARIANTS OR THERAPY ESCAPE MUTANTS. AT THE END OF 1995, TWO CRUCIAL DISCOVERIES WERE MADE. FIRSTLY, HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY SHOWED THAT NOT ONLY THE VIRUS REPLICATION BUT ALSO TURNOVER OF $CD4^+$ T CELL COUNTS WAS MUCH MORE RAPID THAN PREVIOUSLY THOUGHT. SECONDLY, THE SECOND RECEPTOR NECESSARY BESIDES CD4 FOR HIV-1 ENTRY INTO HUMAN CELLS WAS DISCOVERED WHICH ALSO LED TO NEW INSIGHTS AND POSSIBILITIES FOR THERAPY. THIS PART OF THE SECOND BOOKLET ON THE ACS DESCRIBES THE YEARS OF THESE EXCITING DISCOVERIES AND ENSUING RESEARCH QUESTIONS.

### The HIV-1 epidemic: incidence, prevalence and progression to disease

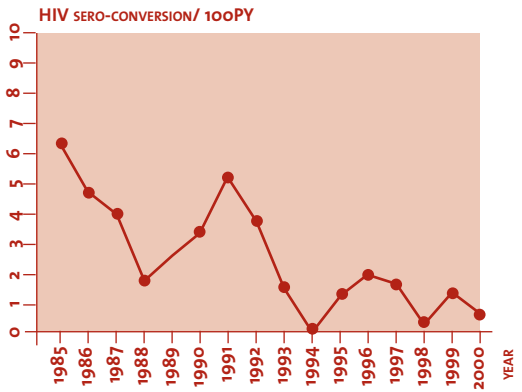
During the first 10 years of the ACS, much insight has been gained in the spread of HIV-1 among risk groups, the risk factors for acquisition and transmission of HIV-1, natural history of HIV-1 infection, and risk factors and prognostic markers for disease progression*. In contrast to developing countries, especially in sub-Saharan Africa, in the industrialized countries HIV-1 did not spread among the general population but remained mainly limited to the two major risk groups of homosexual men and injecting drug users (IDU). The HIV-1 incidence among homosexual men in the ACS stabilized around 1% per year since 1993 while among drug users the incidence became even lower **(Table 7; Figure 1)**.

Decreased unsafe sexual behavior was found among young homosexual men entering the cohort in 1995-1997 compared to young homosexual men entering the cohort in 1984-1985, associated with a strong decline in antibodies against herpes simplex virus [202]. However, in 1999, a strong increase was found in gonorrhea and syphilis among homosexual men attending the Sexually Transmitted Diseases (STD) clinic in Amsterdam compared to 1998, indicating an increase in unsafe sexual behavior, probably related to the availability of effective antiretro-

* *ACS, a summary of the results 1984-1995*

**TABLE 7; FIGURE 1** YEARLY HIV INCIDENCE, NUMBER OF HIV POSITIVES AND NUMBER OF PERSON YEARS PER CALENDAR YEAR FOR HOMOSEXUAL MEN <30 YEARS OF AGE AT ENTRY INTO THE COHORT AND IN FOLLOW-UP (AT RISK FOR HIV INFECTION) UNTIL 35 YEARS OLD

| YEAR | NO. HIV POSITIVES | PERSON YEARS | INCIDENCE |
|---|---|---|---|
| 1985 | 10,00 | 157,24 | 6,36 |
| 1986 | 9,00 | 188,95 | 4,76 |
| 1987 | 7,00 | 175,45 | 3,99 |
| 1988 | 3,00 | 160,91 | 1,86 |
| 1989 | 3,00 | 113,60 | 2,64 |
| 1990 | 5,00 | 148,64 | 3,36 |
| 1991 | 5,00 | 97,22 | 5,14 |
| 1992 | 3,00 | 79,97 | 3,75 |
| 1993 | 1,00 | 63,17 | 1,58 |
| 1994 | ,00 | 47,84 | ,00 |
| 1995 | 1,00 | 73,73 | 1,36 |
| 1996 | 5,00 | 258,87 | 1,93 |
| 1997 | 5,00 | 284,92 | 1,75 |
| 1998 | 1,00 | 293,90 | ,34 |
| 1999 | 5,00 | 358,95 | 1,39 |
| 2000 | 2,00 | 343,25 | ,58 |

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viral therapy [184]. In the ACS it was also found that risk behavior and the incidence of reported STDs increased; evidence was found that this may be related to being successfully treated with highly active antiretroviral therapy (HAART) resulting in a low viral load and higher $CD4^+$ T cells.

The natural history of HIV-1 is characterized by primary infection followed by seroconversion, a variable asymptomatic interval between seroconversion and symptomatic disease leading to AIDS, and time from AIDS to death. In the Tricontinental seroconverter study, which covers data from 403 seroconverters from 5 different cohort studies from 1984 to 1992*, median time from seroconversion to AIDS was estimated to be 8.3 years and from AIDS to death 17 months. In a follow-up of 362 homosexuals over 18 years (1978-1995), time from seroconversion to death was 12.1 years [168]. Time from seroconversion to AIDS ('incubation period') is frequently used as a measure for HIV-1 disease progression.
The length of the incubation period varies from two months to more than 15 years. Older age is the only cofactor that has been consistently shown to be associated with faster disease progression.
Even in seroconverter studies (i.e. studies among individuals for whom the date of the last seronegative and the first seropositive HIV-1 test are known) variability in estimates might additionally be due to methodological issues such as imputation of the seroconversion moment, changes in the definition of AIDS, and competing events such as pre-AIDS mortality among IDU [36]. Furthermore, the fact that definitions of progression/non-progression are not consistent across studies should be taken into account when "non-progression" rates are compared [23, 48, *]. Follow-up of the HIV-positive homosexual men at the Municipal Health Service in Amsterdam was discontinued in 1999, and as many other cohorts among homosexual men also stopped follow-up, the Tricontinental study was terminated in 1998. In order to study progression of HIV-1 infection in HIV-infected individuals without a negative test result (so-called seroprevalent persons), models have been developed to estimate seroconversion dates based on markers of progression as $CD4^+$ T cell counts [20,194]. This type of study will also be of use in future studies on disease progression and therapy decision making.

During the last years, considerable more attention has been paid to transmission of HIV in heterosexuals, in whom the number of HIV-1 infections is gradually rising. Overall HIV-1 prevalence among attenders of a clinic for sexually transmitted diseases was 2.8% in 1996, but less than 1% in the group without IDU. Most heterosexually acquired HIV-1 infections are found in non-Dutch persons [96]. In migrants from Surinam, the Antilles and sub-Saharan Africa living in Amsterdam, the HIV-1 prevalence was 1.1%. More frequent multiple partners and history of STD were reported compared to the Dutch population. Of note, sex in the country of origin during a visit occurred frequently, which may be a potential source for transmission of HIV-1 in the migrant population [163].
Since 1999, the ACS coordinates the European Study of HIV-infected women with a known duration of infection, a collaboration of 31 centers in 12 European countries, in which 487 women participate. Despite known HIV status, STD diagnoses were frequent in these women [183].
Incidence of pregnancies decreased after HIV diagnosis and during HIV progression, and the incidence of induced abortions increased. However, since 1995, the proportions of births after HIV diagnosis is increasing compared to earlier years due to a decrease in induced abortions, whereas pregnancy rates remained constant [183].

In summary, these results clearly show the importance of a good monitoring system to study the HIV epidemic.



* *ACS, a summary of the results 1984-1995, dissertation P.J. Veugelers, 1996*

* *dissertation M.R. Klein, 1997*

## Natural history

The clinical course of HIV-1 infected individuals can be highly variable. Progression to AIDS can occur within one year after infection but symptom-free infection for over 15 years has also been reported. This variation in part will be determined by viral factors such as genotypic and phenotypic variability of the virus and in part by host factors. In this chapter the viral and host factors which may influence HIV-1 infection and pathogenesis are discussed emphasizing the studies performed within the framework of the Amsterdam cohort.

### *Viral factors*

#### *HIV-1 genotype*

The lentivirus HIV is a retrovirus of 100 nm in size. The enveloped icosahedral nucleocapsid contains a single-stranded diploid RNA genome of approximately 9.8kb. The three viral genes *gag, pol* and *env* encode a number of core proteins and viral enzymes that are responsible for virus replication in the human host. The *gag* gene encodes a precursor protein, which is processed into subsequent (nucleo) capsid and matrix proteins. The enzymes necessary for the replication in the host cell originate from the *pol* gene and the *env* gene encodes the envelope proteins.

*Replication cycle*

HIV-1 infection initiates through binding of gp120 to its cellular receptor CD4 and one of the coreceptors (see next section on tropism). This leads to exposure of the fusion peptide of gp41 followed by fusion of the viral and cellular membranes. After release and uncoating of the inner core of HIV-1, the process of reverse transcription may be initiated in the cytoplasm. This step in the HIV-1 replication cycle is impaired in quiescent cells [88]. In activated cells, the produced proviral genome is transported to the nucleus and integrated into the host genome [179]. The matrix protein *vpr* and the viral integrase IN have been suggested as mediators of this process in non-dividing cells and macrophages [133]. Similar to observations in quiescent T lymphocytes, incomplete proviral DNA species were found to be arrested in the cytoplasm of the macrophages [179]. In primary macrophages the intracellular nucleotide pools and other cellular factors that coincide with late G(1) phase of the cell cycle may contribute to efficient reverse transcription and nuclear localization. Nuclear factor of activated T cells (NFATc) is a host cofactor for T cell-tropic and macrophage-tropic HIV-1 replication in quiescent T lymphocytes. Recently, it has been demonstrated that blocking of NFAT in primary macrophages inhibited replication of macrophage-tropic HIV-1, but did not affect the process of reverse transcription.

*Evolution*

HIV-1 strains can be separated into genetic subtypes based on phylogenetic analysis of the envelope gene. The evolution of the V3 domain of HIV-1 subtypes A, B, C, and D is confined to an area in sequence space within a fixed distance to the consensus of a respective subtype [63]. This in turn indicates that each HIV-1 subtype is a distinct viral quasispecies that is well adapted to the present environment, able to maintain its identity in the V3 region over time, and unlikely to merge during progression of the AIDS epidemic.

Phylogenetic analysis of the *vpu, vpr* and *env* V3 showed that HIV-1 isolated from IDU could be distinguished from HIV-1 isolates of homosexual men on the basis of specific, mostly non-silent, mutations [11]. The three genes evolved in a highly independent way with the only stable clustering being based on risk-group distinction. Phylogenetic analysis of the *env* V3 sequences of HIV-1 isolated from homosexual men and IDU showed restricted intra-patient evolution over five and ten years after seroconversion [1]. It could be shown that both homosexual and parenteral transmission give rise to synonymous substitutions in *env* V3 but nonsynonymous substitutions are more frequent in homosexual men than in IDU [46].
Also little variation was seen in HIV-1 *env* V3 sequences from seroconversion and late samples from one donor-recipient pair [1]. The population-wide variation in seroconversion samples from consecutive calendar years is increasing with a stable consensus sequence representing the center of the swarm of variants.
This indicates that the HIV-1 quasispecies in a naive population evolves through unbiased expansion around a stationary consensus sequence. In individual HIV-1 infection, the adaptive evolution of HIV-1 is firstly influenced by transmission bottlenecks followed by the effect of host immune competence, which altogether have an impact on HIV-1 intrahost evolution.

*Selective pressure during treatment*

In 1985, the first therapeutic agent against HIV-1 was identified, named zidovudine (AZT). This agent, as holds true for lamivudine (3TC), didanosine (ddI) and many other compounds, interferes at an early stage of the virus life cycle in the process of reverse transcription. After triphosphorylation of these antiretrovirals, elongation of the DNA chain is terminated when the reverse transcriptase encounters the built-in nucleoside analogue. However, the process of HIV-1 reverse transcription is error-prone resulting in approximately one misincorporation per life cycle. In combination with the enormous production rate of $10^{10}$ virions each day, this results in large genotypic variation. This gives the virus the ability to adapt rapidly to changes in its environment as

occurs in the case of treatment and enables HIV-1 to escape eradication. It was shown that under zidovudine treatment even one mutation in the HIV-1 reverse transcriptase could cause the RNA load to return to baseline [18]. Despite the relative loss of RNA load suppression, selection toward other point mutations in the reverse transcriptase continued. The outgrowth of viruses under selective pressure of treatment does not result in the fittest HIV-1 variants as was shown in a subject newly infected with a zidovudine-resistant HIV-1 strain [24,56]. In absence of the drug, the viral population with the resistance-conferring mutation in the reverse transcriptase was gradually replaced by virus variants with a higher calculated fitness.

Zidovudine has been shown to preferentially benefit individuals infected with only non-syncytium inducing (NSI) HIV-1 variants [33]. Changes in virus load in subjects also carrying syncytium inducing (SI) HIV-1 variants were mainly due to the loss of NSI HIV-1 variants. Resistance mutations emerged at similar rates in both coexisting variants failing to explain the phenotype dependent benefit of zidovudine. In contrast with zidovudine, treatment with didanosine preferentially inhibits SI HIV-1 variants [69]. Treatment with both zidovudine and didanosine or with the protease inhibitor ritonavir equally decreased the numbers of SI and NSI HIV-1 variants. Since these HIV-1 variants display a different cellular tropism and the fact that cellular kinases may be necessary for activation of the reverse transcriptase inhibitors zidovudine and didanosine, this could explain the observed phenotype dependent efficacy which is not shown for the protease inhibitor ritonavir. This phenotype independent efficacy for the protease inhibitor ritonavir could also be shown for lamivudine [104]. Similar kinetics of virus load changes and similar emergence of resistance mutations could be observed in plasma and cells, which were also comparable for the SI and NSI populations of lamivudine treated individuals.

## *HIV-1 phenotype*

Alterations in the HIV-1 genotype, if non-synonymous or non-silent, may alter its phenotype. These changes may be reflected in the tropism, the syncytium-inducing ability or the replicative capacity of the virus, which in turn may affect the virus load in the infected individual. Alternatively, the virus may be more or less neutralization resistant to specific antibodies. These phenotypic properties of HIV-1 and their association with pathogenesis will be discussed below.

*Tropism and syncytium-inducing ability*

Many studies have been performed within the framework of the ACS to explain the observed differences in cell tropism between different HIV-1 variants. Two major viral phenotypes could be distinguished based on their capacity to induce syncytia, the SI and NSI variants. NSI variants were found to be more capable of infecting macrophages than SI variants. In contrast, the SI variants appeared to be more T cell line-tropic as compared with the NSI variants [16,37]. In HIV-1 infected individuals who developed SI variants, it was found that the proportion of infected $CD4^+$ T cells was higher than in carriers of NSI variants only [6,*]. Moreover, the emergence of SI HIV-1 variants can occur at relatively low numbers of infected cells frequently coinciding with increasing virus load [6]. This was found to be due to the expansion of both SI and NSI variants but with a significantly increasing proportion of SI variants after SI conversion. The emergence of SI HIV-1 variants is an independent predictor for progression to AIDS [109].

During progression to disease more T cell tropic HIV-1 variants appear which in about 50% of infected persons are associated with the emergence of SI variants. These HIV-1 variants replicate more rapidly than those isolated from the early asymptomatic phase of infection. In two SI converters from the ACS, it was shown that early SI variants either had slow or rapid replication kinetics [99].

Though retained in one subject, the more slowly replicating NSI variants were lost in the other subject with the emergence of rapidly replicating SI variants. Phylogenetic analysis of the *env* V3 domain showed early and unique branching of the SI variants from the NSI tree.

In 1995, it was shown that β-chemokines could inhibit *in vitro* replication of NSI variants** followed by the discovery of the coreceptor for T cell line-tropic SI variants CXCR4*** and macrophage-tropic NSI variants****. Studies on the coreceptor usage of NSI variants from either progressors or long-term survivors in the ACS showed that these HIV variants selectively use CCR5 as a coreceptor [164,203]. The CCR5 using HIV-1 variants were reclassified as R5 HIV-1 variants whereas X4 variants use CXCR4 as their coreceptor [84]. The results concerning R5 NSI variants were confirmed by the inability of these variants to replicate in peripheral blood mononuclear cells (PBMCs) that were derived from a blood donor with a deletion in the CCR5 gene (also see next section on host factors – coreceptor). Apparently, an expanded coreceptor repertoire of HIV-1 is not a prerequisite for a progressive clinical course of HIV-1 infection. Moreover, CCR5 coreceptor usage of NSI primary HIV-1 was found to be independent for phylogenetically distinct global HIV-1 isolates [77]. Delineation of a consensus motif in the V3 domain was shown to

*dissertation M.Th.L. Roos, 1998 **Cocchi, Science 1995; 270:1811 ***Feng, Science 1996; 272:872 ****e.g. Deng, Nature 1996; 381:661

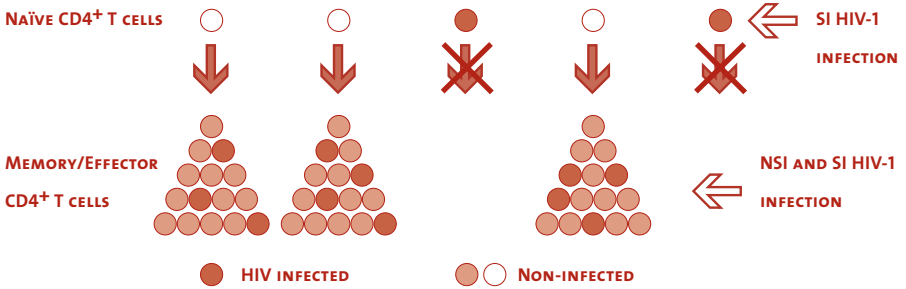
predict CCR5 usage. Since specific NSI and SI variants use different coreceptors for entry of target cells, altered tropism might offer an explanation for increased pathogenesis associated with SI HIV-1 infection. It was found that SI variants were equally distributed over memory ($CD45RO^+$) and naive ($CD45RA^+$) cells in contrast to NSI variants that were mainly present in $CD45RO^+$ cells [178] **(Figure 2)**. Infection of memory cells by both NSI and SI HIV-1 and infection of naive cells primarily by SI HIV-1 corresponded closely with the differential cell surface expression of CXCR4 and CCR5. The frequency of SI-infected $CD45RA^+$ $CD4^+$ T cells, but not the frequency of NSI- or SI-infected $CD45RO^+$ $CD4^+$ T cells, correlated with the rate of $CD4^+$ T cell depletion. Infection of naive cells by SI HIV-1 may interfere with $CD4^+$ T cell production and thus account for rapid $CD4^+$ T cell depletion.

Further analysis on coreceptor expression revealed that even within the memory T cell subset, CXCR4 and CCR5 are differentially expressed. From CXCR4 expressing memory T cells mainly SI HIV-1 variants could be isolated whereas from CCR5 expressing memory T cells only NSI variants were obtained. The differential expression of CXCR4 and CCR5 in naive and memory T cell subsets provide cellular niches that may support the co-existence of NSI and SI virus populations within single individuals [203].

*Load and replicative capacity*

In 1995, with the development of effective multidrug therapy against HIV-1, it became apparent that virus production could be as high as up to $10^{10}$ virus particles per day*. The high viral replication rates in HIV-1 infected individuals lead to high viral loads measured as cell-free RNA in plasma or serum, proviral DNA or cell-associated infectious virus or mRNA.

**FIGURE 2** The impact of HIV-1 infection on T cell ontogeny and peripheral T cell numbers




Many different assays have been described and compared for their efficiency, reproducibility and cost-effectiveness in determining the HIV-1 load levels in infected individuals [3,130,204]. Within the ACS it was possible to distinguish rapid progressors, intermediate progressors, slow progressors and non-progressors on the basis of their viral loads [19]. Rapid progressors show persistently high viral RNA loads from seroconversion on, while all other HIV-1 infected individuals show a steady decline after seroconversion. Subsequent rises in viral RNA levels herald disease progression in later stages of infection. Unintegrated circular DNA shows similar, but somewhat delayed kinetics. These results indicate that the distribution of AIDS and the average length of the symptom-free period in an HIV-1 infected host population is determined by the steady state levels of genomic RNA and of replication intermediates that are produced by a particular HIV-1 virus population in the average seropositive individual. A statistically significant temporal relationship was found between HIV-1 RNA levels in serum and cellular infectious load in peripheral blood [71]. In addition, analysis of longitudinally obtained samples from long-term survivors and slow and rapid progressors either or not undergoing antiviral therapy showed similar kinetics of changes in both measures of virus load. It could be concluded that PBMC and serum represent closely related, if not the same, viral compartments. As reported in many different studies, no significant decline relative to baseline in HIV-1 DNA load or HIV-1 RNA load was found in AZT treated ACS participants [110]. Under combination therapy (AZT/ddC or AZT/ddI), HIV-1 infected individuals showed a maximum mean decline in HIV-1 DNA load of 0.6 log without significant differences from baseline levels. This is in contrast to plasma HIV-1 RNA load that declined earlier and steeper and that remained significantly below baseline for 80 weeks. Although one third of the subjects had prolonged decreased plasma RNA levels, the proviral HIV-1 DNA remained present in the cells throughout the study follow-up of all study subjects indicating renewed active infection after cessation of therapy.

Viral load in long-term survivors and progressors to AIDS was found to correlate to the *in vitro* replicative capacity of NSI HIV-1 variants [89]. However, not all long-term survivors have viruses with low replicative capacity and low virus load indicating that other factors also play a role in containment of HIV-1 infection. In this respect mutations in the HIV-1 *nef* regulatory gene were analyzed, but absence of disease progression could not be correlated with aberrations found in the *nef* sequences of the virus isolates from the long-term survivors. In addition, aberrations in other genes such as *vpr*

* *Ho, Nature 1995; 373:123*

and *vpu* have been studied as possible determinants in HIV-1 pathogenesis [45]. At the gene as well as protein level no gross defects in *vpr* or *vpu* were detected in either long-term asymptomatics with very low RNA copy number or progressors to AIDS.

Longitudinal studies have shown that the viral load in the periphery gradually increases during the asymptomatic phase of infection. It was shown that relatively high infectious cellular load determines whether HIV-1 infected individuals transmit the infection to their partners [78]. Moreover, besides this viral factor, also a host factor was described to play a major role in HIV-1 transmission to the partner in homosexual couples. Relatively high susceptibility of PBMC of the recipient was found to be another important determinant for HIV-1 transmission. In turn, high cellular susceptibility of *in vitro* stimulated PBMC to NSI HIV-1 infection was found to be positively correlated with CCR5 cell surface expression but inversely correlated to β-chemokine production [182] (also see next section on host factors – coreceptor).

The lymphoid tissues are thought to be the primary sites of viral replication since lymph nodes harbor viruses fingerprinted with specific mutations that appear only after some time in peripheral blood mononuclear cells. In tissues other than from lymphoid origin or peripheral blood, e.g. kidney and lung, were also found to be infected with HIV-1 [83]. Infiltration of HIV-1 infected lymphocytes and/ or macrophages to sites of opportunistic infection most likely explains infection of non-lymphoid tissues.

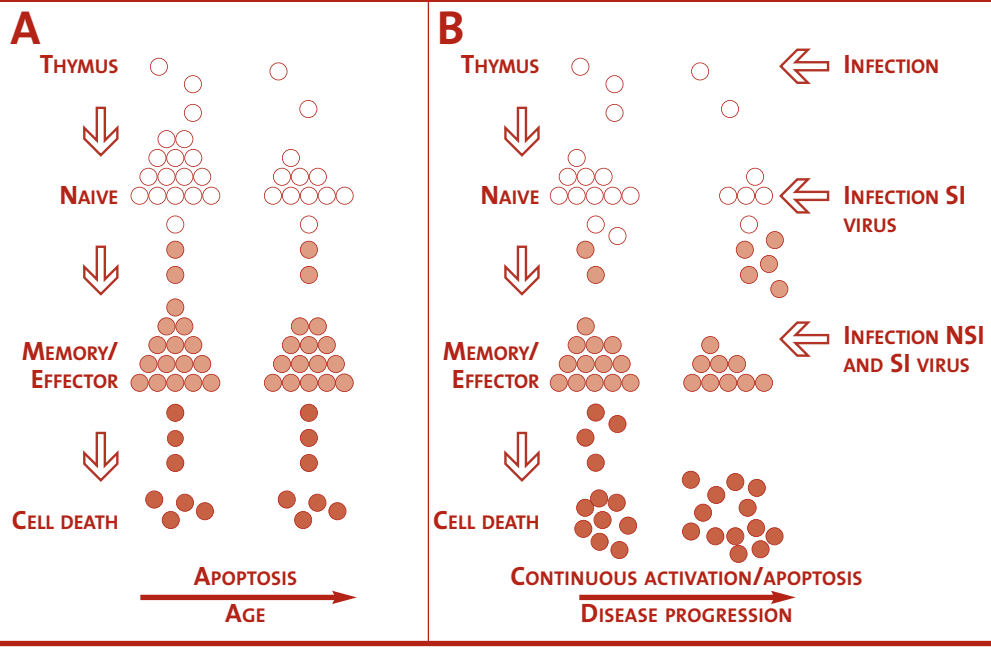
## *Host factors*

### *$CD4^+$ T cell depletion and turnover*

During HIV-1 infection, $CD4^+$ T cell numbers decline, and $CD4^+$ T cell counts are used as a marker for disease progression. In addition, loss of T cell function, as measured by proliferative responses of T cells to mitogens, correlates with disease progression* [29,118]. A long-standing line of research of the ACS is to understand the mechanism by which HIV causes $CD4^+$ T cell depletion and loss of T cell function. From previous results in the ACS** it was postulated that defective interaction between antigen presenting cells and T cells would lead to T cell defects. Altered patterns of costimulatory molecule expression and a disturbed balance between Th1 and Th2 cytokine expression supported this hypothesis [26,31,43,60].
In 1995, two articles released in Nature*** showed that virus production was much higher than previously thought, and a high turnover of $CD4^+$ T cells was postulated as the explanation for exhaustion and finally depletion of $CD4^+$ T cells.

Since then, a new line of research focused on dynamics of T cells by combining biological assays with mathematical models. T cell turnover was initially studied by change in telomere lengths, leading to the highly debated conclusion that $CD4^+$ T cell turnover was not substantially increased in HIV-1 infection [39,156]. Later studies showed, by using expression of Ki-67 as a direct marker for cell proliferation, that there is indeed increased proliferation of mainly memory $CD4^+$ and $CD8^+$ T cells. However, this cell division is rapidly lost during HAART, suggestive for immune activation and not homeostasis as the driving force for T cell turnover [172]. A new model was proposed that fitted most of the relevant aspects of HIV-1 infection as known. It was hypothesized that HIV-1 infection depletes T cell supplies (that are not replaced because of low and static thymic function) by direct infection and killing of cells and through hyperactivation of the immune system [205] **(Figure 3)**.

FIGURE 3 T cell depletion by persistent immune activation




A. In healthy adults, thymic output of naive T cells is low and constant. With age, the naive T cell pool reduces in size because upon encountering their cognate antigen, naive T cells are primed, acquire a memory phenotype and ultimately die by apoptis.
B. In HIV-1 infection, this process occurs at a faster pace because of the continuous attendance of pathogens. Various factors may contribute to the effect of continuous immune activation. In early stage of infection, patients are infected with NSI variants, only infecting memory T cells. In later stage SI variants could increase loss of both naive and memory T cells by infecting and killing naive T cells. Infection of the thymus may lead to reduced thymic output. (N)SI: (non)-syncytium inducing.

*dissertation M.Th.L.Roos,1998 **ACS, a summary of the results 1984-1995 ***Ho and Wei, Nature 1995; 373:117,123

Repopulation of T cells is extensively studied since the introduction of protease inhibitors in 1995. For the first time it was possible to suppress viral load to undetectable levels for a prolonged time. It was observed that the initial rapid increase in $CD4^+$ T cells was mainly due to memory $CD4^+$ T cells, while naive $CD4^+$ T cells showed a slow but continuous repopulation* [87]. Furthermore, T cell function improved during HAART, as was shown by increased *in vitro* proliferative responses to recall antigens as Candida albicans, tetanus toxoid, and PPD [138]. However, restoration of $CD4^+$ T cell counts does not invariably occur during HAART, but correlates with the numbers of naive T cells at start of therapy [136]. With the results from studies on virus and T cell dynamics, and repopulation of naive and memory T cells after start of HAART, the role of the thymus in the pathogenesis of HIV infection was again put to our attention. Two new techniques were introduced. First, T cell development capacity of progenitor cells could be determined by using fetal thymic organ cultures. It was shown that progressors to AIDS had a dramatic loss in T cell development capacity, in contrast to individuals who remained asymptomatic for a prolonged time [195]. Next, the quantification of T cell receptor excision circles (TRECs) in a cell population is used as a marker for thymic output. Several groups have shown decreased TRECs in cells from HIV-infected individuals, suggestive for impairment in thymic function**. A recent study from the ACS however shows that not thymic impairment, but increased division rates of naive T cells can best explain the loss in TREC content in the naive T cell population in HIV-1 infection [199].

### *Antiviral immunity: Role of $CD8^+$ T cells*

$CD8^+$ cytotoxic T cells (CTLs) play an important role in control of viral infections. In HIV-1 infection, CTLs initially control viral replication, but eventually CTLs fail to prevent disease progression. HIV-specific CTLs kill HIV-infected cells by MHC class I restricted recognition. Studies on the kinetics of CTL responses against different viral epitopes during asymptomatic versus progressive HIV-1 infection within the ACS showed convincingly the beneficial effects of CTLs. Persistent HIV-specific CTL responses are associated with long-term non-progression of HIV-1 infection*** [59,117]. With progression to AIDS, CTL activity and T cell function deteriorated, while viral load increased. The regulatory proteins Rev and Tat are expressed early in the HIV-1 replication cycle, and CTL against these proteins are more frequently found in individuals with a long-term non-progressive course of infection. However, individuals progressing towards AIDS also frequently show vigorous CTL responses during their asymptomatic period [117], and epitopes recognized by CTLs from long-term non-progressors can also be recognized by CTLs from progressors [66]. Virulence of the virus and host response factors such as certain HLA types, which are associated with time to disease progression [155], may determine the efficacy of the CTL response. Indeed, several HLA types are associated with either rapid progression or longer time to AIDS [41]. HLA-B57 is strongly associated with slow disease progression in HIV-1 infected individuals from the ACS. The dominant HLA-B57 restricted CTL epitopes for Gag and RT were characterized [114].

Failure of the CTL response to prevent progression to disease may either be due to functional failure or to physical depletion of HIV-1 specific cells. In 1996, a new technique was described, allowing direct visualization of virus specific T cells by staining with HLA-peptide tetramers, combined with other markers*. $CD8^+$ T cells can be divided into a naive ($CD45RA^+$ $CD27^+$), memory ($CD45RA^-$ $CD27^+$) and effector ($CD45RA^+$ $CD27^-$) subset [146,196]. A positive correlation between high frequencies of Gag/Pol tetrameric-binding cells early after seroconversion and slow disease progression was shown in a longitudinal study, together with an inverse correlation with viral load, and loss of HIV-1 tetramer-binding cells preferentially due to loss in the memory subset of $CD8^+$ T cells [167]. Follow-up studies combining HIV-tetrameric staining with measuring HIV-specific interferon $\gamma$ (IFN$\gamma$) production will differentiate between functional failure or physical loss of HIV-specific immune responses, analogous to the study on EBV-specific CTLs in HIV-infected individuals (see section on co-infections).

### *HIV-1 coreceptors*

After the discovery of the chemokine receptors as coreceptors for HIV-1 (also see previous section on tropism), mutations in the encoding genes were shown to be associated with susceptibility to HIV-1 infection and AIDS pathogenesis. At first, a homozygous 32-bp deletion in the CCR5 gene ($\Delta32/\Delta32$) was identified in some individuals who remained uninfected despite high-risk sexual behaviour**. This homozygous deletion occurs in approximately 1% of the Caucasian population whereas the heterozygous deletion occurs in approximately 18%. Some individuals with the protective homozygous CCR5 genotype did however get infected***, presumably by viruses, which are able to use alternative coreceptors such as CXCR4 [98]. Among participants of the ACS, it was found that CCR5 $\Delta32$ heterozygotes showed significantly delayed disease progression, slower decrease in $CD4^+$ T cell counts and lower viral RNA load as compared with CCR5 wild type



*Autran, Science 1997; 277:112 ** e.g. Douek, Nature 1998; 396:690 *** dissertation M.R. Klein, 1997

* Altman, Science 1996; 274:94 ** e.g. Samson, Nature 1996; 382:722 *** e.g. Biti, Nature Med. 1997; 3:252

homozygous individuals [72]. Moreover, though both groups had the same prevalence of SI HIV-1 at the end of the study period, the SI conversion rate was clearly delayed in CCR5 Δ32 heterozygotes. The protective effect of CCR5 Δ32 heterozygosity was stronger in the presence of NSI variants only. The CCR5 genotype predicted disease progression independent of viral RNA load, $CD4^+$ T cell count, T cell funtion and HIV-1 biological phenotype.

The CCR5 cell surface expression on $CD4^+$ T cells was studied amongst CCR5 wild type (CCR5 +/+) and CCR5 Δ32 heterozygous (CCR5 Δ32/+) individuals as a possible explanation for the protective effect of CCR5 Δ32 heterozygosity from disease progression [166]. Indeed, it was found that HIV-1 infected CCR5 +/+ individuals had higher percentages of CCR5 expressing $CD4^+$ T cells than HIV-1 infected CCR5 Δ32/+ individuals. Moreover, HIV-1 infected individuals had higher percentages of CCR5 expressing $CD4^+$ T cells as compared with uninfected individuals in both genotypic groups. High percentages of CCR5 expressing $CD4^+$ T cells were associated with low $CD4^+$ T cell counts, high viral RNA load and low T cell function in HIV-1 infected persons. Individuals who progressed to AIDS had higher percentages of CCR5 expressing $CD4^+$ T cells than non-progressors with similar $CD4^+$ T cell counts. This difference was also noticed pre-seroconversion with higher percentages of CCR5 expressing $CD4^+$ T cells in progressors as compared with non-progressors within similar time frames. Apparently, with progression to AIDS CCR5 expression increases, which may further accelerate the disease.

Associations between other coreceptor alterations and HIV-1 pathogenesis were subsequently described. A point mutation in the CCR2b gene affecting the amino acid position 64 (CCR2 64I) was shown to be associated with prolonged AIDS-free survival* . CCR5 and CCR2b were analysed as compound genotypes in the ACS since the CCR2 64I mutation was previously found to be invariably linked to the CCR5 wild type genotype. CCR5 Δ32 heterozygosity and CCR2b genotype were found to protect independently against disease progression [126]. Probably due to lack of power in the studies, conflicting results have been reported on the association between a polymorphism in the 3' untranslated region of the SDF-1 gene encoding the CXCR4 binding chemokine and its effect on the rate of HIV-1 disease progression**. A slightly accelerated progression to AIDS and death was found for participants in the ACS carrying the SDF-1 gene alteration [101]. Combining the data derived from the ACS on AIDS and the French SEROCO study however did not show any additional progressive effect to AIDS for carriers of the SDF-1 gene variation [140]. In CCR5 Δ32 heterozygotes a reduced prevalence of AIDS dementia cases were found as compared with CCR5 Δ32 wild type individuals [159]. This may point at the reduced or absent reservoir for NSI HIV-1 variants in the brain of heterozygotes.

Another host genetic factor of which the presence has been studied amongst ACS participants were the variant mannose-binding lectin (MBL) alleles [127]. Indeed indications could be demonstrated for a weak pre-AIDS protective effect of variant MBL alleles. Although HIV-1 infected men were found to progress somewhat slower to AIDS and death these data were not significant. Stronger evidence was derived from data on $CD4^+$ T cell counts at AIDS onset which were significantly lower among persons with the mutation.

## CO-INFECTIONS

### *Epstein-Barr virus*

Epstein-Barr Virus (EBV) is a human gamma herpes virus, that is prevalent in about 90% of the adult population. After primary infection, the virus persists for life in a latent form in B cells. This latency is thought to be controlled by specific CTLs. Acquired immunodeficiency can lead to reactivation of EBV infection and to uncontrolled lymphoproliferation. In HIV-1 infection, the incidence of non-Hodgkin's lymphoma (NHL) is increased and the majority of these tumors is EBV positive. The ACS started studying EBV-specific immunity in cooperation with the Academic Medical Center in 1994. Longitudinal analysis showed loss of EBV-specific CTL-precursors preceding NHL diagnosis [49]. By combining the newly available techniques for measuring the number of virus-specific T cells by tetrameric HLA-peptide complexes and measuring the function of virus-specific T cells by IFNγ production with the ELI-SPOT technique, physical loss or dysfunction of specific T cells could be differentiated. EBV-specific $CD8^+$ T cells were not physically lost but over time a decrease in capability to produce IFNγ was shown, indicative for loss of function*. Loss of function correlated with loss of $CD4^+$ T cell numbers. In HIV-infected individuals remaining asymptomatic for prolonged time, IFNγ producing EBV-specific $CD8^+$ T cells were stable. Therefore, functional loss of EBV-specific CTLs may be important in the pathogenesis of AIDS-NHL and this loss may be secondary to lack of $CD4^+$ T cell help. Whether EBV viral load is related to the occurrence of AIDS-NHL is currently under investigation. Furthermore, individuals developing AIDS-NHL showed low numbers of CD27-negative EBV-specific $CD8^+$ T cells, whereas long-term asymptomatic individuals showed accumulation of these so-called effector T cells. Based on these observa-



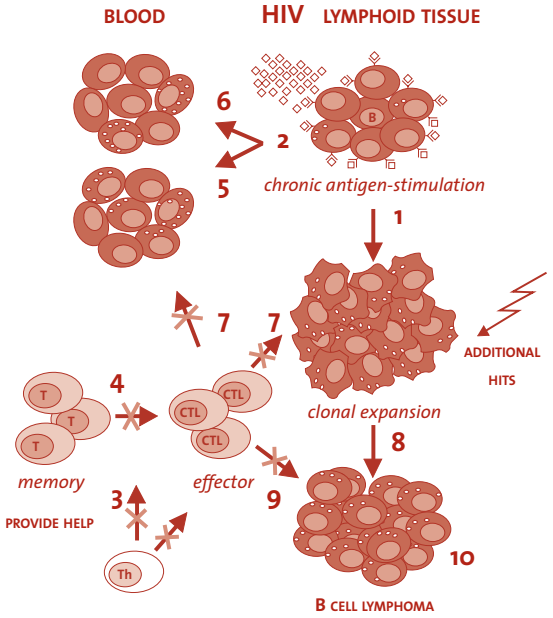
* *Smith, Science 1997; 277:959 ** e.g. Winkler, Science 1998; 279:389*

* *dissertation D. van Baarle, 2000*

tions a model was proposed to explain EBV-induced lymphomagenesis in HIV infection. **(Figure 4)**.

Two types of EBV have been described, EBV type 1 and 2. Both viruses can be transmitted orally and infect epithelial cells and B cells. However, EBV type 2 has less transforming capacity than type 1. Type 1 strains are more prevalent in Caucasian and Oriental populations, whereas both types are common in Africa and New Guinea. It has been suggested that the occurrence of AIDS-NHL is related to infection with EBV type 2. Therefore, a PCR was developed that analyzed the presence of EBV subtypes directly in PBMC [145]. In this study, published in 1999, it was shown that EBV type 2 is not associated with a higher risk for the development of AIDS-NHL. HIV-1 infected individuals show high prevalence of EBV type 2 infection and dual infection with type 1 but this is found already early in HIV-1 infection and does not correlate with loss of immune function. This merely reflects the higher prevalence of EBV type 2 infections among homosexual men [191]. Data from the latter study support the conclusion that EBV type 2 is transmitted sexually.

**FIGURE 4** Model of EBV-induced lymphomagenesis in HIV infection




A model is depicted to explain the development of EBV-related non-Hodgkin's lymphomas in HIV-infected individuals. Chronic antigen-stimulation in the lymphoid tissue can lead to clonal expansion of EBV-infected B cells (1) and increased EBV load in the peripheral blood. (2) Because of lack of CD4+ T cell help (3), as a consequence of HIV infection, memory CD8+ T cells do not differentiate into CD27- effector T cells. (4) EBV-specific CD8+ T cell function decreases, whereby EBV load in the blood can increase, either by increased numbers of EBV-infected cells (5), or increased numbers of EBV copies per B cell (6). Dysfunctional EBV-specific T cells can not control the expansion of EBV-infected B cells. (7) Additional genetic hits may subsequently result in a malignant outgrowth of these EBV-infected B cells (8), which can also not be controlled by specific T cells (9), resulting in an AIDS-related non-Hodgkin's lymphoma (10).

### *Human herpes virus-8 and Kaposi's sarcoma*

Since 1994 there is evidence that human herpes virus-8 (HHV-8) plays a causative role in the pathogenesis of Kaposi's sarcoma (KS)*. HIV-1 infected homosexual men are at risk for developing KS. The ACS provides an excellent opportunity to study HHV-8 infection in relation to HIV-1 infection and development of KS. An Enzyme Immuno Assay (EIA) was developed for the rapid detection of antibodies against HHV-8. The assay detects antibodies against recombinant latency-associated nuclear antigen (ORF73) and recombinant lytic capsid antigen (ORF65) [128]. It was shown that the incidence of HHV-8 infection is higher in homosexual men than in drug users. HHV-8 seroprevalence in the group of homosexual males was 21% [180]. HHV-8 seroprevalence was highest among those infected with HIV-1, no steady partner, and Southern-European or Latin-American origin. In HIV-1 infection, HHV-8 is a risk factor for the development of KS. The risk for KS was highest when seroconversion for HHV-8 antibodies occurred in a person already infected with HIV. Risk factors for HHV-8 seroconversion are orogenital sex with >5 partners in the past 6 months, older age, and HIV-1 infection. The most likely transmission route for HHV-8 in homosexual men therefore is via orogenital contact [128,180]. In HIV-infected individuals, seroconversion to the latent antigen ORF73 precedes seroconversion to ORF65, and antibody levels were higher compared to HIV-seronegative individuals. Antibody levels against ORF65 increase with declining $CD4^+$ T cell counts, and peak with KS development. Only in 10% of HHV-8 seroconversions, transient viremia could be detected. It is assumed that primary infection is reflected by seroconversion for HHV-8 antibodies [188].

### *Hepatitis C*

Hepatitis C virus (HCV) is an important cause of parenterally transmitted hepatitis and is widespread among intravenous drug users, of which a subset is infected with HIV-1. However, the interaction between HIV-1 and HCV is still unclear. From the cohort of drug users, HIV-positive and-negative HCV seroconverters were selected to investigate the effect of HIV-1 on HCV replication. Nineteen HCV seroconverters were identified, of whom four were infected with HIV-1 before HCV seroconversion, and five seroconverted for HIV-1 later [95]. HCV RNA levels were higher in the HIV-positive group.

* *Chang, Science 1994; 266:1865*

In persons seroconverting for HIV-1, HCV RNA levels increased significantly. HCV RNA levels inversely correlated with $CD4^+$ T cell counts, for HIV-positive as well as HIV-negative individuals. No correlation was found between HIV-1 RNA and HCV RNA levels. No evidence was found for retrotranscription of HCV RNA into DNA in PBMCs co-infected with HIV-1 [185].

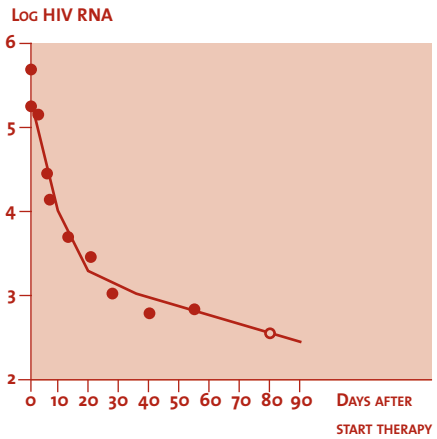
Of the six HCV genotypes that have been distinguished, HCV types 1, 2 and 3a are predominant in the Western world. In our IDU cohort, HCV 1a is predominant (53%), followed by HCV 3a (37%) [90]. When this cohort of IDU was serotyped for HCV it was noticed that of those in which serotyping was not possible, a higher percentage was co-infected with HIV-1, suggesting that co-infection with HIV-1 could influence the antibody response to HCV [119]. However, of the five individuals with a prolonged interval between the detection of HCV RNA and HCV antibodies (> 12 months), none was HIV-1 positive [157]. Thus in a low number of drug users, independent from their HIV status, low levels of HCV RNA may be present without eliciting an antibody response.

## INTERVENTION

### *Therapy*

Since 1987, the ACS is involved in several trials and vaccination studies. The early studies showed already that dual therapy with nucleoside analogue reverse transcriptase (RT) inhibitors was superior compared to monotherapy*.
In 1995, the use of a new class of agents, the protease inhibitors (PI), was approved. Since 1996, new strategies for treatment of HIV-1 infection were implemented combining the use of a protease inhibitor with 2 RT-inhibitors. At the same time, the importance of HIV-1 RNA load in plasma in addition to $CD4^+$ T cell counts as a marker of disease progression became clear**. Thus, efficacy of therapy could be monitored directly by HIV-1 RNA plasma load. The NATEC started its first trial with a triple combination of one protease inhibitor (ritonavir) combined with the two RT inhibitors zidovudine (AZT) and lamivudine (3TC) in the beginning of 1996. Results were spectacular, with decreases in viral load below detection limits and increased $CD4^+$ T cell counts during the first 24 weeks [85]. After 2 years, suppression in viral load sustained, but $CD4^+$ T cell counts did not increase any further after 72 weeks, even when normal values were still not reached [162]. The same phenomenon occurred in viral responders to combination therapy containing nevirapine [136].

Intervention of HIV-1 replication with the powerful regime of the Triple study was used as a tool to study the dynamics of viral replication and T cell repopulation. During triple therapy, a faster rate of decline in plasma RNA load was found compared to monotherapy. Decay of viral particles was observed to take place in two phases **(Figure 5).**




Two phase decline in plasma HIV-1 RNA from one HIV-infected individual after start of triple therapy. The initial rapid decline during the first two weeks of therapy is followed by a second phase of slower decline, and finally the virus load is below the detection limit of the assay (open circle).

During the first 3 weeks of therapy there is a fast clearance of the virus, presumably achieved by fast elimination of productively infected cells which are responsible for >97% of plasma virus. The second phase is slower, probably due to elimination of virus from a compartment of longer-lived cells [87,111,*]

Another study from the ACS compared the effect of AZT + 3TC with stavudine (d4T) + 3TC, and showed that the combination of d4T with 3TC is at least as good as AZT with 3TC, however, 3TC resistance emerged in all patients [112]. Furthermore, decrease in viral load is of short duration with dual therapy, but after adding the protease inhibitor indinavir to the regimen viral loads could be decreased below detection limits. Importantly, both drug combinations were equally well effective in reducing viral load in cerebrospinal fluid [97]. In patients with advanced HIV-1 infection, a regimen containing indinavir led to improvement of chronic diarrhoea [81]. HAART leads to restoration of specific immunity, as shown by increase in *in vitro* mycobacteria-specific proliferative responses of PBMC in patients with manifestations of mycobacteria infections during HAART [135].

Suppression of viral load in the first phase of HAART is more effective in a regimen containing 3 drugs compared to 1 PI (Triple study), and is even more effective when 5 drugs are used [108]. However, daily pill burden of these regimens is large and require strict daily routine and sometimes dietary restrictions, furthermore toxicity frequently occurs, which make it difficult to adhere to therapy in the long term. Therefore, an induction-maintenance concept was designed, where a quadruple regimen was followed



* *ACS, a summary of the results 1984-1995 • Delta Study, Lancet 1996; 248:283* ** *Mellors, Science 1996; 272:1167*

* *Cavert, Science 1997; 276:960 • Zhang, PNAS 1998; 95:1154*

by dual therapy [105]. In this ADAM study, induction therapy included 2 nucleoside analogue RT inhibitors and 2 PI for 26 weeks, followed by maintenance therapy consisting of either 2 PI or 1 PI and 1 RT inhibitor, or a prolonged induction regimen. Although the quadruple therapy provided a rapid decrease of viral load below the detection limit, suppression of viral replication could not be maintained in all patients on maintenance therapy, and therefore it was concluded that simplified maintenance therapy is not advisable.

### *Vaccination*

It has been hypothesized that immunization with HIV-1 antigens could slow down the progression of HIV-1 disease by increasing the immunity against HIV-1. The ACS participated in a multicenter study on the safety of and the immune response to HIV p17/p24: Ty virus like particles in HIV-positives. This therapeutic vaccine induced humoral and cellular immune responses in HIV-negative individuals, however, in the placebo-controlled trial no increase in p24 antibody levels was found and no effect on $CD4^+$ T cell counts or viral load was observed in the 74 asymptomatic HIV-positive individuals [34]. Since 1999 the ACS participates in a large multicenter phase III trial on the efficacy of the gp120 vaccine (Genentech/Vaxgen). This randomized double blind, placebo-controlled study among homosexual men and high risk heterosexual women is held in 50 centers, mostly located in the USA, and over 5000 persons participate, of whom 120 from Amsterdam.

### The Amsterdam Cohort Study among drug users

Next to homosexual men, injecting drug users (IDU) are a major risk group for HIV-1 infection. In many parts of the world, including a number of Asian and Latin-American countries and parts of Eastern- and Southern-Europe, injecting drug use accounts for most HIV-1 infections. While in Europe and the USA the absolute numbers of AIDS cases have decreased in both risk groups, the relative contribution of IDU to the newly diagnosed AIDS cases has increased. In Europe, IDU have become the largest group of individuals with AIDS, although there are considerable differences between countries. In North-Western Europe, the percentage IDU among AIDS cases is much lower compared to the Southern-European countries (11% in the Netherlands versus 65% in Spain). Although IDU contribute significantly to the HIV-1 epidemic, most studies on the natural history of HIV-1 infection have been performed in cohorts of homosexual men. In 1985, one year after the start of the Amsterdam Cohort Studies on homosexual men, the Amsterdam Cohort Study among drug users was set up. Since then, prevalence and incidence of HIV-1 infection and AIDS could be monitored in relation to drug use, sexual behavior, morbidity and mortality, and intervention programs. By November 2000, 1442 drug users had been enrolled. HIV-1 prevalence among IDU enrolled in 1985-1987 was 34%, and has decreased now to 12% at entry*,[68]. HIV-1 incidence in this cohort decreased from almost 9% per year in 1986 to 1-2% per year after 1990 **(Table 8; Figure 6).**

**FIGURE 6** Yearly HIV incidence for all drug users and among injectors only

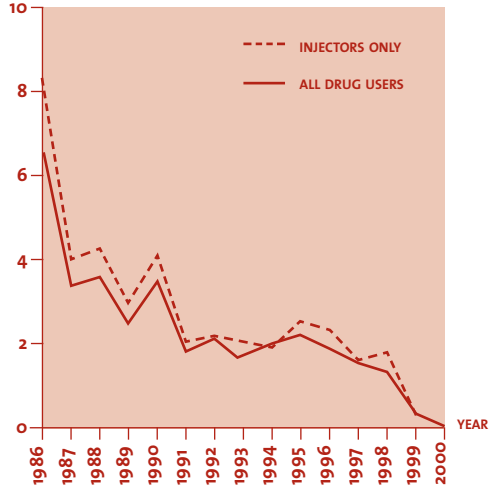



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**TABLE 8** Yearly HIV incidence, number of HIV-positives and number of person years per calendar year according to injecting status at entry

| Year | Injectors only | | | All drug users | | |
|---|---|---|---|---|---|---|
| | No. HIV positives | Person years | Incidence | No. HIV positives | Person years | Incidence |
| 1986 | 5,00 | 58,05 | 8,61 | 5,00 | 74,05 | 6,75 |
| 1987 | 5,00 | 139,41 | 3,59 | 6,00 | 182,22 | 3,29 |
| 1988 | 9,00 | 205,90 | 4,37 | 10,00 | 265,14 | 3,77 |
| 1989 | 7,00 | 239,78 | 2,92 | 8,00 | 312,00 | 2,56 |
| 1990 | 12,00 | 266,37 | 4,50 | 13,00 | 350,52 | 3,71 |
| 1991 | 4,00 | 252,91 | 1,58 | 5,00 | 339,02 | 1,47 |
| 1992 | 5,00 | 271,84 | 1,84 | 7,00 | 371,15 | 1,89 |
| 1993 | 5,00 | 291,90 | 1,71 | 5,00 | 395,07 | 1,27 |
| 1994 | 5,00 | 302,95 | 1,65 | 7,00 | 412,02 | 1,70 |
| 1995 | 8,00 | 312,38 | 2,56 | 9,00 | 433,85 | 2,07 |
| 1996 | 7,00 | 308,25 | 2,27 | 7,00 | 442,16 | 1,58 |
| 1997 | 3,00 | 302,84 | ,99 | 4,00 | 439,18 | ,91 |
| 1998 | 4,00 | 305,84 | 1,31 | 4,00 | 448,80 | ,89 |
| 1999 | ,00 | 312,67 | ,00 | 1,00 | 484,56 | ,21 |
| 2000 | ,00 | 292,24 | ,00 | ,00 | 460,27 | ,00 |

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* *dissertation M. Prins, 2000*

Declines in HIV-1 related risk behaviors have been reported. A significant reduction in injecting risk behaviors was found in the period of 1986-1991, which stabilized from 1991-1997 [93]. In drug using prostitutes, both reduction of commercial as well as non-commercial sexual risk behavior was reported, in agreement with a decline in overall rates of sexually transmitted diseases [35,57]. In contrast, HIV-1 prevalence was relatively high among young and recent-onset IDU, up to 24% in the period of 1989-1995, probably due to more frequent needle sharing [68]. Harm-reduction based methadone treatment is related to lower rates of needle sharing, and, at an individual level, increase in methadone usage leads to cessation of injecting drugs [160,186]. Therefore, methadone treatment programs can be successful in preventing HIV-1 infection.

Injecting drug use is associated with higher rates of infection compared to the general population. Infections transmitted by needle sharing such as hepatitis B, hepatitis C (see section on co-infections) and HIV-1, infections due to injecting such as skin abcesses and bacterial endocarditis, community acquired pneumonia and tuberculosis (TB) are commonly found. Changes in morbidity and mortality due to the introduction of HIV-1 in the cohort of drug users are monitored by the ACS. HIV-positive IDU are at higher risk for skin abcesses and endocarditis than HIV-negative IDU [32]. Bacterial pneumonia is found nearly 4-times more in HIV-positive IDU than in HIV-negative IDU, and this risk increases with decreasing $CD4^+$ T cell counts [17]. HIV-1 infection increases the risk for active tuberculosis in IDU 13-fold. TB occurs relatively early in the course of HIV-1 infection and is mostly due to reactivation [173]. Thus, the HIV-1 epidemic among IDU has a considerable impact on the morbidity of IDU. In addition, the spectrum of AIDS-defining illness in IDU varies from that in homosexual men, in that IDU develop bacterial pneumonia, TB and HIV-1 encephalopathy much more frequently while Kaposi's sarcoma is found almost exclusively in homosexual men. Furthermore, death before AIDS is diagnosed (pre-AIDS mortality) occurs more frequently in IDU than in homosexual men **(Table 9)**.

It was estimated that eventually 24% of the IDU die without an AIDS diagnosis [113]. Consequently, in the period before HAART became generally available the median time from seroconversion to death in IDU is shorter than from seroconversion to AIDS (8.3 vs. 10.5 years), and is faster than progression from seroconversion to death in the cohort of homosexual men (9.6 years). In IDU pre-AIDS mortality from natural causes is related to disease progression, while pre-AIDS mortality due to overdose and suicide is not [8,73].

**TABLE 9** Number of AIDS and death cases among HIV positive and seroconverted drug users per calendar year

| year | | | death | |
|---|---|---|---|---|
| | AIDS | total deaths | with AIDS | without AIDS |
| 1986 | | 2 | | 2 |
| 1987 | 1 | 3 | | 3 |
| 1988 | 7 | 9 | 3 | 6 |
| 1989 | 7 | 6 | | 6 |
| 1990 | 6 | 14 | 3 | 11 |
| 1991 | 5 | 11 | 7 | 4 |
| 1992 | 10 | 11 | 7 | 4 |
| 1993 | 14 | 24 | 14 | 10 |
| 1994 | 20 | 17 | 9 | 8 |
| 1995 | 21 | 20 | 17 | 3 |
| 1996 | 14 | 14 | 9 | 5 |
| 1997 | 6 | 12 | 7 | 5 |
| 1998 | 1 | 16 | 6 | 10 |
| 1999 | 1 | 12 | 5 | 7 |
| 2000 | 4 | 13 | 5 | 8 |
| total | 117 | 184 | 92 | 92 |

Created dd 04 - 10 - 2001, GG&GD Amsterdam

Differences in the spectrum of AIDS-defining conditions and in pre-AIDS mortality have to be taken into account when disease progression rates between risk groups are compared and largely explain, next to age, the reported slower progression in IDU compared to homosexual men [36,73].

Although risk group differences in time from seroconversion to AIDS have been studied over the last decade, differences in markers between risk groups have hardly been studied. $CD4^+$ T cell count is a good prognostic marker in all risk groups, but the absolute level differs by gender, in that women consistently show higher $CD4^+$ T cell counts at seroconversion and with ongoing HIV-1 infection [170]. Earlier studies within the ACS showed a lower occurrence of NSI-SI phenotype switch in IDU compared to homosexual men, which is still not explained*. The prevalence of CCR5 $\Delta 32$ heterozygous genotype in IDU was comparable to that in homosexuals, but there was no protective effect of this mutation on disease progression. Therefore, the marker paths, their prognostic value and mechanism responsible for the observed differences need to be further explored in IDU.

* *dissertation I.J.B. Spijkerman, 1998*

To study natural history of HIV-1 infection in IDU in the Amsterdam cohort, many more years of follow-up would have been required, since there are relatively small numbers of (known) seroconverters and clinical endpoints. Therefore, a multi-cohort study was initiated to pool data of IDU with documented HIV-1 seroconversion from several prospective cohort studies in Europe, which was named the European Seroconverter Study. The ESS started in 1994. Participating cohort studies are The Edinburgh City Hospital Cohort , The Edinburgh Drug Addict Study, The Scottish Collaborative HIV Testing Study, The French SEROCO Cohort, The Innsbruck AIDS Study, The Geneva HIV Cohort Study, The Valencian HIV Seroconversion Study, and the ACS. In total, 750 IDU have been registered in the ESS. Data from the ESS already formed a major contribution to the comparison of natural history of HIV-1 infection and pre-AIDS mortality between the different risk groups*, and will be in the future, especially in relation to HAART, which is used at a low rate in IDU [103].

As the IDU cohort is aging and as there is limited information on the risk behavior and the HIV-prevalence and -incidence in young drug users, the ACS started a new longitudinal study among young (<30 years) drug users in June 2000. Already 140 young drug users – both injectors and non-injectors - have entered this study at this moment (July 2001). Quantitative epidemiological studies as well as qualitative studies will be done to elucidate determinants for transition from non-injecting to -injecting drug use among the young.



* *dissertation M. Prins, 2000*

PART THREE

# Publications and Dissertations 1996 - 2000

# Future research 2000



THE YEARS 1996 TO 2000 HAVE BEEN VERY FRUITFUL IN TERMS OF PUBLICATIONS AND DISSERTATIONS WRITTEN ON RESULTS OF HIV-1 RESEARCH CONDUCTED WITHIN THE FRAMEWORK OF THE ACS. NOT ONLY 208 PAPERS WERE SUBMITTED TO AND ACCEPTED FOR PUBLICATION IN PEER-REVIEWED JOURNALS, IN TOTAL 22 STUDENTS FINISHED THEIR PH.D. EDUCATION SUCCESSFULLY. FROM THE FIVE YEARS DESCRIBED HERE, FOUR TO FIVE PH.D. STUDENTS WERE ABLE TO GRADUATE EACH YEAR AFTER COMPILING SEVERAL FIRST-AUTHOR AND SOME SECOND-AUTHOR PAPERS INTO A THESIS. THESE PUBLICATIONS AND DISSERTATIONS ARE AVAILABLE FOR INTERESTED READERS FROM THE SECRETARIAT OF THE INSTITUTES INVOLVED.

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**DISSERTATIONS** (See next page)



## *1996*

**J-W. Mulder**
Evaluation of the efficacy of zidovudine treatment in HIV-1 infected subjects
**J. Veenstra**
Studies on clinical aspects and interventions in HIV-1 infection
**P.J.E. Bindels**
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**P.J. Veugelers**
Epidemiological evaluations of HIV disease progression

## *1997*

**J.S.A. Fennema**
HIV infection among drug users and the potential for heterosexual spread
**M.R. Klein**
The role of HIV-1 specific cytotoxic T lymphocytes in the pathogenesis of AIDS
**A.B. van 't Wout**
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**M. Koot**
The role of HIV-1 syncytium-inducing variants in AIDS pathogenesis

## *1998*

**I.J.B. Spijkerman**
Progression of HIV infection: risk group differences and markers
**M.Th.L. Roos**
Development and evaluation of immunological prognostic markers in HIV infection
**K.C. Wolthers**
T cell function and T cell dynamics in HIV-1 infection
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## *1999*

**N.A. Foudraine**
Tangible effects of antiretroviral therapy in HIV-1 infected patients
**J.C.M. Hendriks**
The incubation period of AIDS. Staged disease progression and prevalent cohort studies
**N. Kootstra**
HIV-1 replication in macrophages
**H. Blaak**
Virus and host determinants of HIV-1 infection and AIDS pathogenesis
**M.G.H.M. Beld**
Natural history of hepatitis C virus among injecting drug users

## *2000*

**J.J. Maas**
Markers of HIV-1 infection and its pathogenesis
**D. van Baarle**
Viro-immunological studies on the role of Epstein-Barr virus in the development of AIDS-related non-Hodgkin's Lymphoma
**M. Prins**
HIV disease progression in injecting drug users: epidemiological studies
**M.P. de Baar**
The impact of HIV-1 subtypes on molecular diagnostics
**M.W. Langendam**
The impact of harm reduction based methadone treatment on HIV infection and mortality

## FUTURE DIRECTIONS FOR RESEARCH

### *Department of AIDS Research of the Municipal Health Service of Amsterdam*

There is clear evidence that sexual risk behavior and STDs are on the rise among homosexual men (and probably also among heterosexuals). The ongoing prospective studies among young homosexual men make it possible to closely monitor these changes, to study the reasons for these changes and to see what impact this has on the HIV incidence. As it appears that young drug users behave very differently from older users, a new cohort of young (<30 years) injecting and non-injecting drug users was recently started. In this group, we aim to monitor the HIV prevalence, incidence and risk behavior and the determinants of transition from non-injecting to injecting use, using both quantitative and qualitative methods. We will also study how drug careers are developing. Monitoring of risk behavior and STD and HIV incidence will also be done in other groups e.g. STD clinic attendees, pregnant women and migrants.

In collaboration with the virologists and immunologists, we will continue our studies on prognostic markers.
One example is the development of an algorithm using available markers (e.g. age, $CD4^+$ T cell count, viral load, SI/NSI, genetic markers) to predict the clinical course of HIV/AIDS. Such a model could be used by clinicians to take decisions when to start treatment in the individual patients. Another example is the study on the progression of HIV infection in women as compared to men. Studying the epidemiology and the natural history of other bloodborne and sexually transmitted infections will also be done in collaboration. As became clear from our previous studies on HCV and HHV-8, these studies do not only depend on the availability of longitudinal samples, but also on new technologies. Within one of our collaborative studies, we will compare through sequencing the HCV strains from several cities in Europe to get a better insight in the spread of this virus. We also plan to compare the natural history of HIV infection in Ethiopian HIV-positives and (historical) Dutch HIV-positives.

At present, we are participating in a phase III efficacy study with a first generation HIV-1 vaccine (gp120). Moreover, plans have been made to get involved in other phase I,II or III HIV-1 vaccine studies, possibly at the same time in the Netherlands and in Ethiopia. Up till now, we were not involved in studying the progression of HIV after HAART. However, such studies are now ongoing in the HIV-positive homosexual men who participate in the ACS and are followed at the Jan van Goyen clinic, possibly using data from the large database of HIV-positives at the AMC.

### *Department of Retrovirology of the Academic Medical Center, University of Amsterdam*

Research within the context of the ACS at this department focuses on five directions, namely: 1) Transmission and spread of antiretroviral drug-resistant HIV, 2) Pathogenesis and natural history of Kaposi's Sarcoma and HHV-8/HIV coinfection, 3) Pathogenesis and natural history of hepatitis B virus (HBV) and HCV infections, 4) Pathogenesis of AIDS-related dementia, and 5) Virus rebound in long-term non-progressors and escape from T-cell immune control.

Transmission of drug-resistant HIV is extremely rare in the cohort. We observed only the transmission of AZT-resistant virus in the years just prior to the wide introduction of triple therapy. A model we developed suggested that the proportion of the HIV-infected population receiving insufficient or failing therapy determines the level of spread of drug-resistant HIV. The more people receive adequate therapy sustaining a significant virus load reduction, the lower the number of people newly infected with drug-resistant HIV.
We subsequently noted, as we had anecdotally reported previously, that the fitness of drug-resistant HIV is significantly less than that of drug-sensitive HIV in the absence of the drug. Within a matter of months drug-resistant HIV is replaced by drug-sensitive mutants that are however only one mutational step removed from a drug-resistant conferring genotype. We have documented the danger associated with these new wild type viruses in a patient receiving d4T. Currently, we are monitoring all newly infected individuals and studying the evolutionary mechanisms involved.

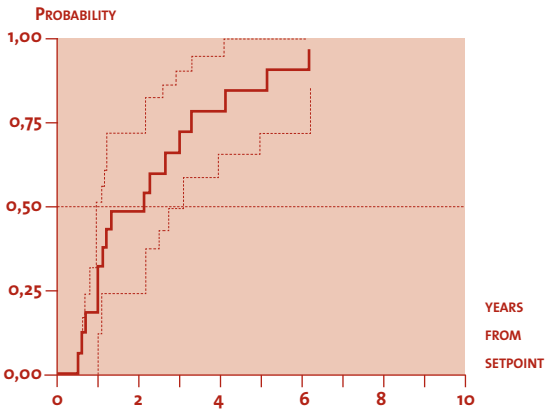
Kaposi's Sarcoma is caused by a gamma-herpes virus HHV-8. Most recently we demonstrated that HHV-8 is transmitted by the oral route and only rarely, if at all, by the parenteral route. Currently, we are focussing on host genes involved in the pathogenesis of KS and upregulated by HHV-8 infection. In addition we are looking into other herpes infections promoting HHV-8 replication.

Our hepatitis studies focus on HBV/HIV coïnfections. We identified a new marker for HBV replication other than HBV-DNA or HBeAg. We are investigating the predictive value of this marker. We subsequently are planning to study the impact of flavivirus infections like GB-C on the progression rate of AIDS.
One of the big surprises of AIDS research was and is the sudden disappearance of

AIDS-dementia following the introduction of AZT. We are initiating studies to elucidate this phenomenon.

Lastly we observed after 17 years of cohort follow-up that virtually all long-term non-progressors that started out with low virus loads and normal stable CD4+ T-cell counts, showed a rebound of virus load **(Figure 7)**.
Our hypothesis is that the cause of this deterioration lies in progressive dysfunction of the HIV-specific immune system or escape of the virus from a relatively intact HIV-specific immune system.

**FIGURE 7A** Time to viremia increase above 1,000 HIV-1 RNA cp/ml



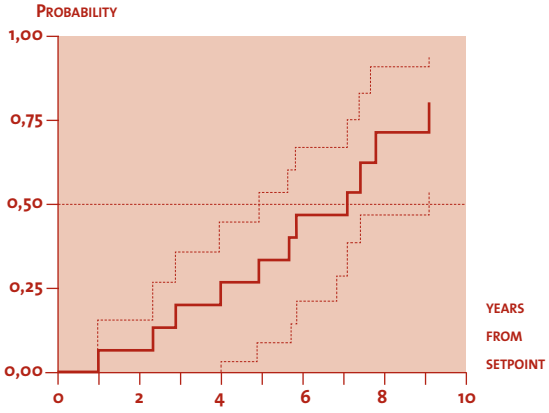

## *Department of Clinical Viro-Immunology, CLB Sanquin and Laboratory of Experimental and Clinical Immunology, University of Amsterdam*

This institute foresees developments specifically in the field of cellular immunity, T cell renewal and biological properties of HIV-1.

*Characterization and dynamics of HIV, EBV and CMV specific $CD4^+$ T lymphocyte responses in relation to $CD8^+$ T cell responses and disease progression*

The magnitude and specificity of specific $CD4^+$ T helper cells in HIV-1 infected individuals will be compared to healthy individuals but also between long-term survivors and rapid progressors. We will study against which proteins these responses are directed, which peptides are recognized by these T helper cells and through which MHC class II molecules these responses are restricted. We will study whether a possible loss of specific $CD4^+$ T cells are accompanied by increasing viral loads, whether the $CD4^+$ T cell loss is a physical or functional loss and whether specific T helper cell and $CD8^+$ T cell immunity can be restored by antiretroviral combination therapy.

**FIGURE 7B** Time to viremia increase above 10,000 HIV-1 RNA cp/ml




Cumulative incidence (and 95% confidence intervals, thin lines) of plasma HIV-1 RNA increase to above 1,000 (panel A) or 10,000 (panel B) copies per ml among HIV-1 seroconverters; the observation time started one year after seroconversion (virological setpoint).

*T cell renewal in HIV infection*
We will perform comparative studies on the renewal capacity of the immune system in adult and young patients with HIV infection in comparison with patients without HIV related T cell depletion. In great detail, we will study the role of thymic output and peripheral T cell division in the restoration of the peripheral T cell pool. These studies will be compared to studies performed in experimental settings in mice that were depleted of T cells and were followed for thymic output and peripheral restoration. In-depth analysis of the rebuilding of a broad repertoire of T cell receptor specificities will be pursued. This will be related to restoration of novel T cell specificities to HIV and other common viruses or pathogens.

*Biological properties of HIV-1*
It is still unclear why SI HIV-1 emerge in only 50% of HIV-1 infected individuals. Therefore the relation between the production of β-chemokines (RANTES, MIP-1α, MIP-1β, the natural ligands for CCR5), differences in CCR5 expression and the rate of disease progression in individuals with or without SI HIV-1 variants will be studied.
The capacity of SI HIV-1 to infect naive T cells will be studied in more detail. As naive T cells are being considered non- or only low-proliferating, we will study the nature of, and the cellular requirements for HIV-1 infection in naive T cells. The cellular factors that are absolutely required for naive T cell infection will be characterized. It will be determined whether the increasing proportion of naive cells that harbour SI HIV-1 after SI conversion is associated with increased affinity for CXCR4.
Viral evolution of SI HIV-1 variants isolated from individuals treated with a combination of 5 antiretroviral drugs will also be studied.

## EPILOGUE

From this overview over the years 1996-2000 and the previous one over the years 1984-1995, it is evident that the Amsterdam Cohort Studies have been a rich source for research: about 450 publications in peer-reviewed journals over 17 years. The 51 (young) students who did their Ph.D. within the context of the ACS did most of the research. We hope that this experience contributed not only to their research training but also to the understanding of how research can contribute in helping to solve one of the biggest public health challenges of our time, the HIV/AIDS pandemic. When we started our studies, the pandemic just started and no one could foresee the devastating scope with tens of millions of people infected throughout the world. The research work done within the context of the ACS contributed to solving small pieces of the scientific puzzle on which thousands of researchers worked. Nevertheless it took more than ten years before the first effective drugs against HIV became available and up till now, we have no effective vaccines.

Over 2500 men and women were followed in the Amsterdam Cohort Studies: they have been willing to regularly visit us, patiently filling in questionnaires and giving blood for the studies. For many HIV positives the effective drugs came too late and we could do little to help them. We will never forget their invaluable contribution to science.

(RAC, september 2001)