The Amsterdam cohort studies on HIV infection and AIDS

A summary of the results 1984–1995

CLB

The Amsterdam Cohort Studies on HIV infection and AIDS represent a collaborative effort between:

- Department of Public Health and Environment of the Municipal Health Service of Amsterdam
  Project leader: Prof R.A. Coutinho

- Department of Retrovirology of the Academic Medical Center, University of Amsterdam
  Project leader: Prof J. Goudsmit

- Department of Internal Medicine of the Academic Medical Center, University of Amsterdam
  Project leader: Prof J.M.A. Lange

- Department of Clinical Viro-Immunology of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam
  Project leader: Prof F. Miedema

Coordinator
1984 - 1995: Prof J. van der Noordaa
1995 - : Prof R.A. Coutinho

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c/o Nieuwe Achtergracht 100
1018 WT Amsterdam
The Netherlands
tel. (31) (20) 555 52 86
fax (31) (20) 555 55 33

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Results 1984 - 1995

The investigators are greatly indebted to the homosexual men who were willing to participate in these studies for so many years. We thank all those who contributed to the Amsterdam Cohort Study of HIV infection and AIDS among homosexual men. We especially acknowledge the continuous help of Nel Albrecht, Margreet Bakker and Marijke Roos. We are greatly indebted to Prof. Jan van der Noordaa who was the coordinator of the Amsterdam AIDS Cohort Studies until October 1995 and who wrote the summary of the results until 1993. The social scientific studies have been done by the Department of Social and Organizational Psychology, University of Utrecht (projectleader: Prof. W. Stroebe).

introduction

The Amsterdam cohort study of Human Immunodeficiency Virus (HIV) infection and AIDS among homosexual men (HM) started in 1984, shortly after the first cases of Acquired Immuno Deficiency Syndrome (AIDS) had been diagnosed in The Netherlands.

A multidisciplinary approach encompassing epidemiology, social science, virology, immunology and clinical medicine has significantly contributed to our knowledge and understanding of the various aspects of HIV-1 infection. Over the past years the Amsterdam study among HM has been especially directed toward the following topics:

- prevalence and incidence of HIV-1 infection and AIDS
- risk factors for transmission and changes in sexual behavior
- natural history of HIV-1 infection
- intervention in HIV-1 infection

Studies of HIV-1 prevalence and incidence have provided insights into the introduction and spread of the virus among homosexual men in The Netherlands and other industrialized countries. The studies on risk factors for transmission and changes in sexual behavior have provided a basis for primary prevention.

The results of the natural history studies have led to the recognition of a number of pathogenetic events that appear to be essential for (non)-progression to AIDS. The long-term follow-up of HIV-1 infected persons has enabled us to define several prognostic markers for progression to disease. A better understanding of the natural history of HIV-1 infection has also resulted in a more rational approach to the development of vaccines and antiviral treatment.

And finally, investigation of early zidovudine treatment of asymptomatic individuals laid the groundwork for subsequent controlled trials with anti-retroviral drugs and for studies of the emergence of resistance to zidovudine.

methods

The study population consists of homosexual men living mainly in and around the city of Amsterdam, the Netherlands. Men could enter the study if they had no complaints or symptoms other than lymphadenopathy associated with HIV-1 infection and had had sexual contact with other men in the 6 months preceding entry. The men were recruited through announcements in the gay press, advertisements and by word of mouth. The study started in October 1984 and until April 1985 men were enrolled independent of their HIV-1 antibody status. Between October 1984 and April 1985 748 men were enrolled of whom about one third was HIV-1 positive. Between April 1985 and February 1988 only seronegative men could enter the study as we considered studying seroconverters for HIV one of the most important aims of the study. Since 1988 both seropositive and seronegative men could enter but no extra effort for recruitment was made and only a limited number entered the study. In June 1995 we started a special recruitment campaign among young (≤ 30) homosexual men as we felt that insufficient data on HIV prevalence, incidence and risk behavior were available on this group.

Seropositives and seroconverters are seen every 3 months. Clinical, epidemiological and social scientific data are collected with standardized questionnaires and by physical examination. Blood is taken for virological and immunological tests and for storage (both serum and viable cells). Seronegatives are seen by a nurse every 6 months (every 3 months until 1 October 1988) and similar data are collected but no immunological tests are done nor are cells stored.

If patients are diagnosed with AIDS they are referred to the Academic Medical Center where they are seen by one of the study physicians. About 20% of participants with AIDS are going to other hospitals in town. Data on morbidity, treatment, secondary diagnoses, virology and immunology after AIDS diagnosis are collected through treating physicians and are presently being validated. Cases of AIDS are also ascertained through cross-linking with the Amsterdam AIDS registry.

prevalence, incidence and risk factors for HIV infection and AIDS

One of the first goals of the Amsterdam cohort study among HM was to gain insight into the spread of HIV-1 among homosexual men in Amsterdam. Data concerning the introduction and spread of HIV-1 prior to the start of the present cohort study in October 1984 came from sera obtained in a previous cohort study (1980-1982) of men with homosexual contacts, in whom the efficacy of a hepatitis B vaccine had been tested.[1] Five of 685 participants (0.7%) showed antibodies against HIV-1 on entry into the study, after their stored serum samples collected during the period November 1980 to December 1981 were retrospectively tested. In 1981, three new cases of infection with HIV-1 appeared and during 1982, 12 seroconversions were detected. From these data it may be deduced that HIV-1 was introduced among homosexual men in Amsterdam in the late seventies.

The further spread of HIV-1 in Amsterdam was studied in the cohort of 748 homosexual men between the ages of 18 and 65 years who entered the study between October 1984 and April 1985. In 233 (31.4%) participants, antibodies against HIV-1 could be demonstrated, which indicated an important increase in the number of infections in 1983 and 1984.[2,3,8] The further spread of HIV-1 among study participants can be seen from the annual number of seroconversions which took place among seronegatives after October 1984. Over a period of eleven years, 123 new infections (seroconversions) were registered among seronegative men (table 1 and figure 1).

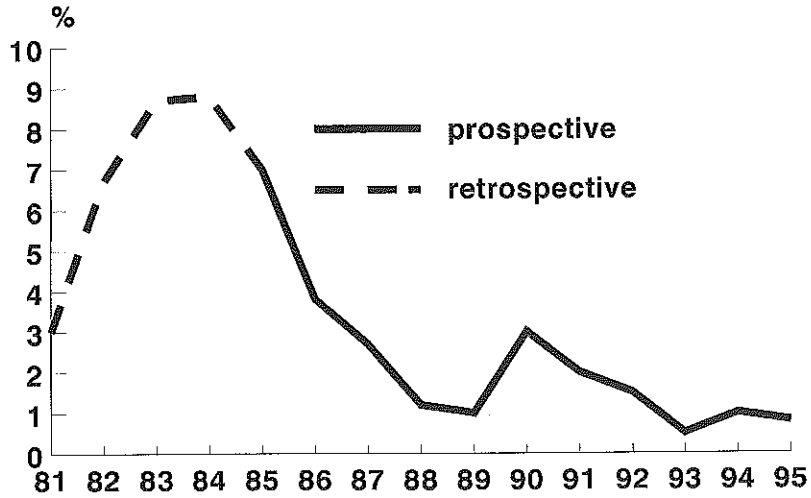
A marked decline was observed in the annual incidence of new HIV-1 infections, from 7.4% in 1985 to 1.0 % in 1989. It should be noted that the annual incidence of 1.0 % in 1989 was followed by a rise to 2.9% in 1990 indicating a trend toward unsafe sexual behavior.[69,90] This trend was not limited to participants in the cohort study, as reflected by an increase in the number of new cases of (rectal) gonorrhoea and syphilis reported among homosexual and bisexual men (including young men[131]) who visited the Amsterdam STD-clinics.[61,62] This relapse towards unsafe sexual behavior appeared to be temporary since the incidence stabilized after 1992 at around 1% per year.

Table 1
Annual incidence of HIV infection in the Amsterdam cohort study among homosexual men

Period	Person years at risk	Number of seroconversions	Incidence (cases per 100 per person years)
1985	487	36	7.4
1986	574	22	3.8
1987	543	14	2.6
1988	509	6	1.2
1989	497	5	1.0
1990	477	14	2.9
1991	463	9	1.6
1992	454	6	1.3
1993	436	1	0.2
1994	420	4	1.0
1995[1]	370	3	0.8

[1] Preliminary figures

Yearly HIV incidence among homosexual men in the Amsterdam Cohort Study, 1981-1995



Many of the participants of the above mentioned Hepatitis B vaccine study which took place in 1980-1982 consented to follow-up testing for HIV-1 or participated in the HIV cohort study which started in 1984. The cumulative HIV-1 incidence in this Amsterdam cohort was compared with the cumulative incidence (CI) in similar Hepatitis B vaccine study cohorts in New York (started in 1978) and in San Francisco (started in 1978).[133]

In 1980 the CI was 1% in Amsterdam (A'dam), 14% in New York City (NYC) and 14% in San Francisco (SF). Over the years the CI gradually increased in A'dam, while the CI rose dramatically through 1985 in SF and NYC. In 1990, the end of this study, the CI was 17% in A'dam, 38% in NYC and 52% in SF. After adjustment for age, number of sexual partners and Hepatitis B virus (HBV) incidence, the HIV-1 infection curves for NYC and SF became similar, while the curve for A'dam remained significantly different. Although this difference might be partly caused by differences in sexual activity of the men in the three cities, these data indicate that the later introduction of HIV-1 in Amsterdam, which made it possible to start intervention measures early, has had a profound impact on the spread of HIV-1 among HM in this city.

Through 1995, a total of 175 cases of AIDS have been diagnosed among participants in the cohort study. Among subjects who were HIV-1 seropositive at entry, the cumulative AIDS incidence after 10,5 years was 59.2%. Similarly, 58.3% of those who seroconverted during the study have been diagnosed with AIDS after ten years. This indicates that most persons who were seropositive upon entry into the study, had seroconverted in the preceding one to two years.

The treatment-free incubation period distribution was estimated from data of 269 men who were HIV-positive when entering the study and 79 men who seroconverted during follow-up.[118] Time since the start of zidovudine treatment was excluded and no cohort member received PCP prophylaxis by February 1990 (end of this study) by which date 69 men had developed AIDS. The incubation period distribution was estimated by direct Kaplan-Meier analysis and by using Weibull and gamma distributions. We found that both the Weibull and the gamma distribution provide equally good empirical descriptions of the incubation period distribution for up to 7 years post seroconversion, but the estimated gamma distribution (median 9.2; mean 10.2; percentage AIDS at 7 years 33%) should be preferred beyond that time due to a slowing of the hazard rate. The progression of HIV-1 infection from the moment of seroconversion to the development of AIDS and death was also studied among 362 HIV positive HM who participated in HBV vaccine studies, which were performed in the period 1978-1982 enabling long follow-up.[133] The median time to the development of AIDS was 10.2 years and the median time from AIDS to death 20 months.[142] Younger age, an initial diagnosis of Kaposi Sarcoma and more recent calendar time were associated with slower progression, while older age was associated with faster progression from seroconversion to death.

In the multicenter Tricontinental seroconverter study in which data from 403 seropositive HM with a known date of seroconversion originating from five different cohort studies (San Francisco (2 studies), Sydney, Vancouver and Amsterdam) are merged, a shorter incubation period was found. In this study which is coordinated in Amsterdam the median time from seroconversion to AIDS was found to be 8.9 years and from AIDS to death 17 months; no significant differences could be demonstrated by geographical location.[162] Younger age and the use of PCP prophylaxis were related to slower progression from seroconversion to death, while the rate of disease progression was not found to be related to sexual behavior, a history of STDs and the use of recreational drugs. Interestingly, in this study we found a slower progression among early seroconverters which caused an interesting debate in scientific journals and initiated further studies by several other groups.

In an effort to estimate the prevalence of HIV-1 seropositivity among homosexual men in Amsterdam, we compared the ratio of HIV-1 prevalence to AIDS incidence in the cohort with back-projections from the yearly number of new AIDS cases in Amsterdam. The number of HIV-1 infected homosexual men in Amsterdam was estimated to be between 1800 and 3500 and the estimates using the ratio and back-calculation method fell within the same range.[93] These estimates were confirmed by estimating the magnitude of the HIV epidemic among HM in Amsterdam by the utilization of survey data in predictive models.[135] With this method the number of HIV infected HM in Amsterdam was estimated to be $\pm$ 3500.

In 1993 the Centers for Disease Control (CDC) in Atlanta, USA, included all HIV infected people with a single CD4+ T-cell count <200/mm^3 in the AIDS case definition. As there is evidence that CD4+ T-cell counts vary within and between individuals, we evaluated the decline in CD4+ T-cell counts in relation to the incidence of AIDS among 403 HIV positive men with a known date of seroconversion who participated in five different cohort studies in the Tricontinental seroconverter study.[136] We found no differences in the incidence of AIDS (1987 definition) between the five cohorts, but there were considerable differences in CD4+ T-cell depletion, probably because of laboratory differences. This means that PCP prophylaxis might start too late in some centers and too early in others. The difference in decline of CD4+ T-cells by site will also influence the comparison of studies and will lead to considerable variation in observed incubation time to AIDS if the CD4+ T-cell count <200/mm^3 is a criterium for AIDS.

As a decline in the proportion of Kaposi Sarcoma among AIDS cases had been reported, while at the same time others reported that the incidence of KS remained stable, we investigated this discrepancy in more depth in the Tricontinental seroconverter study.[188] The percentage of KS among incident AIDS cases decreased over the years following seroconversion but not over calendar time. This demonstrates that there is no evidence that the hypothesized KS cofactor - now identified as HHV-8 - is declining over calendar time.

As some studies reported that herpes zoster (HZ) in HIV positives is associated with more rapid progression, we studied the incidence of HZ in our cohort.[187] The incidence of HZ was 3.3 per 1000 person years (pyrs) in HIV-negatives and 51.5 per 1000 pyrs in HIV positives. The incidence of HZ was found to increase with decreasing CD4 counts and T-cell function. The hazard ratio for AIDS after HZ in positives was increased (1.6) but was not independent from the CD4 cell counts.

To study the relation between the course of the HIV infection and genetic factors, we typed 106 seroconverters from our cohort for HLA. We found a significant association between HLA-B35 and progression to CD4+ T-cells <200/mm^3, while HLA-DR1 was related to the occurrence of Kaposi Sarcoma.[146]

risk factors for transmission of HIV and changes in sexual behavior

Considerable attention has been paid in the Amsterdam Cohort Study among HM to risk factors that influence the transmission of HIV. Anal receptive sexual practices emerged as the most important risk factor for HIV-1 transmission among study participants. The number of partners proved not to contribute as much as was previously supposed. The use of certain drugs such as cannabis and nitrite also appeared to increase the risk of HIV-1 infection.[7,13]

The possibility of HIV-1 transmission by orogenital sex has been investigated in 102 study participants for whom HIV-1 antibody seroconversion dates were known.[95] In questionnaires and face-to-face interviews, nine of these men denied to have receptive anogenital intercourse in the six to nine months prior to seroconversion, which suggests transmission by other routes, e.g. orogenital. However, psychological barriers to reporting the practice of anogenital receptive intercourse might lead to overestimation of transmission by other routes.

The role of genital ulcerative infections caused by herpes simplex virus type II and Treponema pallidum in the acquisition of HIV-1 infection has also been studied. Genital ulcerative disease was not found to be an important independent risk factor for the acquisition of HIV-1 among HM in Amsterdam.[47] With regard to changes in sexual behavior, seropositive subjects showed the greatest decrease in the number of sexual partners over time, but did not abandon anogenital sex. On the other hand, continuation of anogenital practices has also been noted among seronegative subjects and among persons who would not agree to be tested for HIV-1 antibodies.[14,23]

Study of the impact of knowledge of HIV-1 status on high-risk sexual behavior with steady and non-steady sexual partners has demonstrated a differential effect.[39] Over time, seronegative study participants and persons who were not tested decreased their practice of receptive anogenital intercourse with non-steady partners but not with steady partners. In contrast, it appeared that the percentage of seropositive subjects who performed anogenital insertive intercourse with non-steady partners remained constant. In addition to serologic status, age has also been identified as a determinant of safe or unsafe behavior in the cohort study. Younger men in particular were shown to be engaging in intercourse without using condoms.[67] The decline in HIV-1 infection (see table 1 and figure 1) has been assumed to be linked to changes in sexual behavior. The number of seropositive men who practiced

anopenetrative intercourse fell from a mean of 10.6 to 1.4 for those positive for HIV-1 antibody, while the number of seronegative who practiced anoreceptive intercourse fell from a mean of 3.7 to 0.5.[38,40] Serial cross-sectional and longitudinal assessments of changes in sexual behavior showed significant increases in the proportion of men who refrained from anogenital contacts as well as in the proportion of men who consistently used condoms.[89]

A longitudinal survey of 84 men who seroconverted during the study suggested a peak in risky behavior prior to seroconversion. The "decline" often observed immediately after seroconversion is, in fact, an indication of a return to previous sexual activity.[48,49] In a further analysis of the sexual risk behavior of the men under study, the hypothesis was formulated that sexual role differentiation could be an important factor in the spread of HIV.[109] In a mathematical approach four groups were defined on the basis of their risk behavior: men who practiced no anogenital intercourse, men who practiced anogenital insertive sex only, men who practiced anogenital receptive sex only, and men who practiced both types of behavior. Differences in HIV-1 prevalence and in the transmission probabilities of the sexual behaviors among the four subgroups suggested that the group structure and the migrational process (individuals moving from one group to another) are important factors limiting or accelerating the spread of HIV-1 among homosexual men.[108]

To assess the potential role of bisexual men in the transmission of HIV to women, we asked 679 HM participating in our cohort study questions on sexual behavior with women. In the last 6 months only 3% reported heterosexual contacts and the HIV positives always used condoms with their female partners.[147] This indicates that at least the HM in our study do not play a significant role in the heterosexual spread of HIV.

Little is known about the relationship between psychological reactions to the threat of AIDS and changes in sexual behavior. In the Amsterdam study it appeared that men who did not seek social support were more likely to continue having anogenital contacts with casual partners. However, other coping styles could not be related to sexual behavior.[68] Data on longitudinal behavior patterns established that there is a substantial group of men with variable behavior, in addition to groups that exhibit consistent no-risk, change to no-risk and consistent risk behavior. It is important to assess which differences in psychological processes underlie these behavioral distinctions and to take into account any observed differences when designing health promotion programs. Qualitative interviews, focusing on the perspective of the men, showed that whether or not HM consistently protected themselves from HIV infection and the strategy they adopted depended on three major factors: motivation and intention, significance of anal sex and risk perception without specific relationships.[138]

Since it was unclear to what extent the cohort is representative of the male homosexual community in Amsterdam, a comparison was made between the incidence of HIV-1 infection in the cohort and the incidence of hepatitis B, syphilis and rectal gonorrhoea among homosexual men in the Amsterdam in the period 1981-1987.[37] A sharp decrease was observed in all registered STDs among homosexual men. The strong similarity between the incidence of these infections and the incidence of HIV-1 infection in the cohort showed that the process of sexual behavior change among homosexual men in the Amsterdam Cohort study was representative for the homosexual community in Amsterdam. It appeared that the incidence of syphilis tends to reflect behavioral changes sooner than the incidence of hepatitis B.[46]

The rise in the incidence of HIV-1 infection observed in 1990 (table 1 and figure 1) prompted us to investigate whether this was caused by a rebound in unsafe sexual behavior. It appeared that recently infected men as compared with men who had remained seronegative had increased both their frequency of anogenital receptive intercourse and their number of partners. No significant changes over time in the probability of infection per partner were found, indicating that the increase in the HIV-1 incidence cannot attribute to a raised infectivity of the virus nor to a higher prevalence of HIV-1 in the community.[69,90] These findings underline the continuous need for educational efforts to promote and to maintain safe sexual techniques by homosexual men. Future research into the determinants of risk behavior was considered to give important clues to minimize the group of men who does not or cannot change its behavior.[26] Therefore, in the second half of 1990 as well as in the second half of 1991 data were collected regarding sexual intercourse with steady and casual partners. Although comparison of these data showed no significant increase in the proportion of men practicing anogenital intercourse with steady partners (24.6% in 1990 and 27.7% in 1991), the percentage of men practicing unprotected anogenital intercourse with casual partners had increased significantly from 13.1% in 1990 to 24.0% in 1991.[112,113] The relapse into unprotected anogenital intercourse with casual partners occurred more often among men who had a less positive attitude towards condom use and who were not involved in a primary relationship. Lower personal efficacy with respect to using condoms with casual partners and less strong motivation to avoid anogenital intercourse with casual partners were also noted.[116] In another study about relapse into unsafe sex, we analyzed time from safe to unsafe sexual behavior using survival methods.[139] HM from the cohort study were included if they ever reported an episode of safer sexual behavior (no unprotected anogenital intercourse) for at least 12 months. After 88 months of follow-up the cumulative incidence of unsafe sexual behavior was 88% among the 402 participants who met the inclusion criterium. Younger age, HIV seropositivity and the use of poppers were related to a shorter time to unsafe

sexual behavior. These data can be useful in targeted prevention programs for specific groups of HM.

We also analyzed the process of risk behavior change in our cohort using time series data to form transition probability matrices.[166] The results indicate that behavior change may be viewed as a homogeneous Markov process and not as a static pattern. The most important property of a Markovian process is that the past is irrelevant and that only current data are necessary to predict the future. This method may also be applied to other risk groups and risk behaviors.

In a separate study the effectiveness of condom use among HM was investigated in relation to type of condom and lubricant used.[114,115] Although there was a considerable decrease in the failure rate of condoms, it appeared that there were still men who failed to use the right types of condom and lubricant. Health education interventions should especially address the use of adequate lubricant.

As over time the participants of the cohort study were aging and only limited knowledge was available on young homosexual men, we investigated sexual behavior and the HIV prevalence in a group of HM $\leq$30.[120] Unsafe behavior was relatively common among the 154 HM $\leq$30 recruited through advertisements, gay bars and the STD clinic, but the HIV prevalence (5%) was relatively low. In this group of young HM safe and unsafe sexual behavior could satisfactory be explained by psychosocial factors.[168] The affective value attached to anal sex was a strong determinant of practicing anal sex despite the AIDS risk. Consistent condom use appeared to be specifically furthered by self efficacy in practicing safe sex.

In consequence to the published hypothesis that insertive oro-anal contact (active rimming) might be associated with Kaposi's Sarcoma (KS), baseline and follow-up behavioral data among individuals diagnosed with AIDS with and without KS were studied.[132] We found that the number of sexual partners with whom active rimming had been practiced did not differ significantly at any time between the AIDS patients with and without KS.

natural history of HIV infection

In addition to the research described above on risk factors for the transmission of HIV-1 and changes in sexual behavior, considerable attention has been given to the following aspects of the natural history of the HIV-1 infection in relation to the progression to AIDS:

- the primary infection
- HIV antibody response and serum antigenaemia: prognostic relevance
- decline of CD4+ T-cell numbers
- loss of T-cell functions: prognostic relevance
- mechanism of T-cell dysfunction
- HIV-1 phenotype: biology and prognostic relevance
- molecular biology of HIV-1 phenotype
- neutralization
- antigenic variation
- viral load and disease progression
- long term asymptomatics, viro-immunologic characteristics

Primary infection

In order to study primary HIV-1 infection, 97 men who were initially HIV-1 antibody seronegative and who reported a febrile episode lasting at least three days, were tested serologically for representative viruses.[18] In 60 of these men serologic evidence of a viral infection was found. The most common causes of infection were influenza A or B (17 men) and HIV-1 (14 men). Influenza-like symptoms such as headache, sore throat and myalgia occurred as frequently in HIV-1 infection as in influenza. Primary HIV-1 infection should therefore be included in the differential diagnosis of febrile influenza-like illness in individuals at risk of HIV-1 infection.

To assess the diagnostic value of IgM antibodies in early HIV-1 infection sequential serum samples from 55 men with primary HIV-1 infection were tested for the presence of IgM antibodies to HIV. IgM antibodies were found in only five cases, indicating the limited value of IgM antibody detection in early diagnosis of HIV-1 infection.[20]

Changes in CD4+ T-lymphocyte numbers were studied in relation to HIV-1 antibody seroconversion as well as to the presence of HIV-1 antigen (p24) in serum.[16] A decline in CD4+ T-cell lymphocyte numbers was noted

three to six months prior to HIV-1 antibody seroconversion, independent of the presence or absence of HIV-1 antigen. Following antibody seroconversion however, subjects without antigenemia had higher CD4+ T-cell numbers than subjects who were antigen-positive. During a 21-month follow-up period CD4+ lymphocyte numbers declined steadily in men who were HIV-antigen-positive but remained stable in those who were antigen-negative.

A subsequent study revealed that both syncytium-inducing (SI) and non-syncytium-inducing (NSI) virus variants[22] can in principle be transmitted and that if SI variants are transmitted this is associated with rapidly falling CD4+ T-cell counts. Persons with pronounced clinical signs showed marked increase in numbers of activated CD8+ T-cells.[104] In general, however, it was observed that preferentially NSI macrophage tropic viruses were present at the start of infection and established persistence.[106,161]

Impairment of the in vitro immune response could be shown to have occurred within three months of HIV-1 seroconversion, as reflected by the inability to mount a secondary immune response in vitro to certain viral and bacterial antigens.[58]

In investigating the period of infectivity before seroconversion, we used the very sensitive and highly specific polymerase chain reaction (PCR) to determine whether HIV-1 sequences can be detected in blood samples of HIV-infected individuals before seroconversion.[86] Only two of 15 samples collected three months before HIV-1 antibody seroconversion were found to be HIV-1 PCR positive, indicating that a latent infection for longer than six months is rare. No HIV-1 sequences were found in 42 seronegative subjects at high risk of HIV-1 infection or in 19 seronegative partners of seropositive men.

With regard to viral transmission, we studied the characteristics of HIV-1 genomic RNA populations in serum in five transmitter-recipient pairs with the aim of determining which viruses initiate a new infection. We found a loss of sequence heterogeneity following transmission and observed consensus sequence similarities in this group of HIV-1 transmitter-recipient pairs. On the basis of these data, we concluded that HIV-1 transmission results in the selection of a limited number of genomes carrying on the infection in the new host, but does not generally lead to a shift in the sequence population.[110]

One hundred and eight men who seroconverted for HIV-1 during follow-up were studied for predictors of rapid progression to AIDS by analysis of the clinical presentation of the primary infection and serological and immunological characteristics.[119] Symptomatic primary infection with fever and skin rash, absence of anti-HIV core and transient HIV p24 antigenaemia at the time of seroconversion were independent predictors of progression to AIDS. The CD4+ T-cell count after seroconversion was a predictor for subsequent progression to a CD4+ T-cell count $<200 \times 10^6/l$, but not for rapid progression to AIDS. Later studies about the relationship between genetic factors and clinical disease, showed an increased frequency of HLA-B62 (RR 6) among subjects with fever and skin rash during primary infection.[146]

HIV antibody response and serum antigenaemia: prognostic relevance

Longitudinal studies of the presence of antibodies against the different viral proteins demonstrated that the disappearance of the antibodies against certain core proteins (p17, p24) of the virus were predictive of the development of AIDS.[5,10] In connection with this finding, it appeared that the prolonged presence of HIV-1 antigen in serum indicated an enhanced risk of developing AIDS.[4,6] In a number of cases, a transient antigenaemia preceded the development of antibodies against the p24 core protein (anti HIV-1 core IgG) and the envelope proteins (anti HIV-1 envelope IgG) of the virus. Reappearance of HIV-1 antigen later during infection coincided with a decrease in antibodies against p24 core protein. The formation of immune complexes appeared to play an important part.[11,12] Antibodies against the envelope usually remained detectable for prolonged periods.

It was subsequently shown that the decline of anti-core antibodies in the later stage of HIV-1 infection was indeed at least partly a consequence of the increased antigen load[60] and that the selective loss of B cells producing core antibodies also played a significant role in the loss of serum core antibodies.[17]

Thereafter, it became apparent that the intrinsic ability to mount an immune responses and immune complex formation also contributed to measurable antibody levels in the early phases of HIV-1 infection.[75]

The predictive value of the long-term presence of HIV-1 antigen in the blood for the development of AIDS was analyzed after an average study period of 19.3 months when a total of 15 cases of AIDS had developed in the cohort of seropositive men (consisting of 198 men who were upon entry seropositive and 58 who seroconverted). AIDS was diagnosed in 24% of the HIV-1 antigen seropositive participants in contrast to 1% of antigen-negative participants.[9] Our initial conclusion that the presence of HIV-1 antigen predicts the development of AIDS was confirmed after a study period of 30 months. In addition to the presence of HIV-1 antigen, the course of anti-core antibodies and changes in the numbers of CD4+ T-cells were monitored.[17] After an average study period of 30 months, there were 29 cases of AIDS among the total group of 306 seropositive participants (238 who were seropositive upon entrance and 68 who seroconverted), which corresponds to an endpoint attack-rate of 16.8%. The majority of AIDS cases occurred in participants with long-term HIV-1 antigenaemia (endpoint attack-rate 43.9%), while the number of cases in the group without demonstrable HIV-1 antigen

was significantly lower (endpoint attack-rate 6.8%). In addition, the absence of antibodies against p24 as well as low numbers (< 0.5 x 10^9/l) of CD4+ T-cells were significantly associated with the development of AIDS.

After a follow-up period of 39 months[27] the number of AIDS cases had increased to 38, corresponding to an AIDS attack-rate of 20.8%. The mean period of HIV-1 antibody seropositivity before diagnosis of AIDS was shorter in subjects who were core-antibody-negative, antigen positive or had low CD4+ lymphocyte counts (< 0.5x 10^9/l) than in those who were core-antibody-positive, antigen negative or had normal CD4+ lymphocyte counts. It became apparent however, that differences in AIDS attack-rates among the HIV-antigen-positive and negative groups became less pronounced after longer follow-up. For men who were antigen-negative the AIDS attack-rates at 39 and 30 months were 13.3% and 6.9%, respectively. These results during prolonged follow-up suggested two patterns of progression to AIDS:

a. development of AIDS within a relatively short time after HIV-1 infection, often associated with antigenaemia and core-antibody negativity; and
b. development of AIDS at a later time, associated with the absence of antigenaemia and core-antibody positivity.

For both patterns low CD4+ numbers remained highly predictive of progression.

Although p24 antigenaemia in asymptomatic HIV-infected subjects could be associated with an increased risk of rapid disease progression to AIDS it remained to be determined whether antigenaemia predicted a more severe course of disease once the diagnosis of AIDS had been made. Prior to the introduction of zidovudine the median time of survival was 12 months in p24 positive AIDS patients and 13 months in p24 negative patients. It thus became apparent that p24 antigenaemia at the time of diagnosis is not a predictor of survival.[52]

We also studied another viral protein (p17) that might be related to protective immunity, since a decline in antibodies had earlier been shown to be related to disease progression.[43] A synthetic peptide representing the conserved region of p17 elicited neutralizing antibodies in rabbits. Sera of 9% (7/76) of AIDS patients and 18% (40/223) of HIV-1 seropositive asymptomatic homosexuals were positive for antibodies against this synthetic peptide indicating the minor importance of this epitope.

In the search for additional prognostic serologic markers it was found that most HIV-infected individuals had HIV-1 protease antibodies, as measured by ELISA using a recombinant form of protease.[24] The absence of antibodies to protease or a decline in antibody levels appeared to be strongly associated with an unfavorable clinical course in both children and adults.

Antibody response to a synthetic peptide covering a LAV-1/HTLV-IIIB neutralization epitope were not related to disease progression.[25] The presence or absence of antibodies to accessory gene encoded proteins such as nef, rev, tat, vpu and vpr, appeared only partly related to disease progression.[30-32,56] Absent, transient or intermittent levels of antibody to the nef protein were significantly associated with low antibody titres to HIV-1 core proteins, with the appearance and persistence of p24 antigenaemia, and with the presence of low circulating CD4+ numbers. However, the numbers of cases of AIDS and AIDS-related diseases in the nef-specific antibody-negative group did not differ significantly from those in the nef-specific antibody-positive group. It appeared that low antibody reactivity to the accessory gene products nef, rev and tat was associated with AIDS relatively rapid after infection with HIV.[55] When used in combination with persistent p24 antigenaemia and low CD4 counts, negative antibody profiles to nef and protease, respectively, were as sensitive and specific in predicting progression to AIDS as was low anti-core reactivity.[78]

In addition to serological markers, CD4+ lymphocyte counts, and serum B2 microglobulin levels,[28] we studied dehydroepiandrosterone (DHEA) serum levels as a predictor of progression to AIDS.[103] The steroid hormone DHEA has been reported to be a modest inhibitor of in vitro HIV-1 infection. DHEA has been shown in animal models to protect against certain viral infections, presumably by enhancing interleukin-2 synthesis by activated T-cells. In our longitudinal study a relationship was demonstrated between low DHEA serum levels in HIV-1 infected men and progression to AIDS. During follow-up, a decline in DHEA levels could be shown only in those subjects who progressed to AIDS. We also studied whether soluble tumor necrosis factor-α receptors (sTNFαR) are predictors of disease progression in HIV infection.[171] Serum concentrations of sTNFαR types I and II were determined at entry and 3-5 months before AIDS diagnosis in 20 HIV positives and compared with the levels in HIV negatives and HIV positives who remained asymptomatic. Both at baseline and 5 months before AIDS diagnosis, sTNFαR type II appeared to be a strong and early predictor of disease progression.

To prepare for the specific monitoring of HIV-2 infections in the cohort studies, we developed and evaluated the diagnostic value of a set of HIV-2 envelope peptides. We showed that sera from individuals participating in the cohort studies only rarely cross-reacted with these HIV-2 peptides, while sera from African HIV-2 infected individuals were highly reactive.[70] These results indicated that HIV-2 has not yet been introduced within the cohort group.

Decline of CD4+ T-cell numbers

To gain insight into the kinetics of CD4+ T-cell loss during the progression from the asymptomatic to the symptomatic stage, rates of decline of CD4+ were compared in progressors and non-progressors in the cohort. In seropositive participants who remained asymptomatic during the follow-up period, CD4+ T-cells declined slowly and continuously. In subjects who developed AIDS, a biphasic decline was observed. Until 18 months before AIDS was diagnosed CD4+ T-cells declined slowly and steadily (5.6 x 10^6 cells/l/month), at a rate similar to that in the non-progressor group. Thereafter, a three to five times faster decrease in CD4+ T-cells was observed.[105]

In view of the recently proposed recommendation by the US Centers for Disease Control (CDC) that the AIDS case definition include all HIV-1 infected persons with one or more CD4 counts below 200/µl, we studied the incidence of AIDS in 161 seropositive men with at least one CD4 T-cell count below 200/µl.[80] The median interval between the first low CD4 count and the development of AIDS was 651 days (95% confidence interval 429 to 865 days). Our data show that a considerable proportion of HIV-infected persons with a CD4 count below 200/µl would be prematurely labelled as AIDS patients with the attendant negative psychological and social consequences.

Loss of T-cell function: prognostic relevance

After finding that declining and low CD4 T-cell numbers predicted transition to AIDS independent of antigenemia or anti-core antibody titres[21], we investigated T-cell reactivity in vitro as a measure of immune function in vivo. It appeared that before CD4 cell numbers declined, the responsiveness of T-cells to stimulation with anti-CD3 MAb was decreased.[21,34] Immediately after seroconversion, T-cell reactivity to anti-CD3 MAb dropped to approximately 60% of the normal response, while the response to antilymphocyte serum (ALS) and the lectin phytohaemagglutinin (PHA) after a transient decline returned to nearly normal values. Thus, low anti-CD3 responsiveness preceded the decline in CD4 cell numbers and the decline of reactivity to PHA and ALS.[33] To compare T-cell function in groups with and without progression, and thereby determine whether low T-cell reactivity predicted with progression to AIDS, we developed and validated an assay that applies 100 µl whole blood in a four day culture protocol stimulated with anti-CD3 Mab. With this assay, T-cell function can be routinely measured.[57] In individuals who progressed to AIDS, PHA and ALS responses were decreased approximately one year before diagnosis whereas low anti-CD3 responsiveness (< 10% of the normal response) was already demonstrable at least two to three years before onset of disease. A disease free survival analysis showed that low anti-CD3 reactivity predicted the development of AIDS. Thirty-five percent of subjects in the low T-cell responder group developed AIDS within 21 months, as compared with only 10% in the responder group. The prognostic relevance of measuring lymphocyte function in vitro was confirmed in a longitudinal study of 122 seropositive HM over 4.5 years.[184] Low T-cell reactivity as determined by whole blood lymphocyte culture in which cells were stimulated with monoclonal antibodies to CD3 was found to be a strong predictor for progression to AIDS independent of CD4 count and the presence of SI variants. This study showed that measuring T-cell function in vitro is of value for staging HIV infection and may be useful for monitoring therapy. The usefulness of measuring T-cell function was also shown in a study about predictive markers of survival after AIDS in HM diagnosed with AIDS in our cohort. T-cell reactivity at the moment of the AIDS diagnosis as measured with stimulation with PHA and monoclonal antibodies to CD3 were found to be independent predictors for survival (as well as CD4 cell count).[165]

In a longitudinal study[73] of six subjects who rapidly progressed to AIDS, low anti-CD3-induced T-cell responsiveness could already be demonstrated in two subjects at the time of seroconversion. A further decrease in T-cell function appeared to coincide with the emergence of more virulent HIV-1 variants as determined by replication rate and syncytium induction capacity of the isolates.

Mechanism of T-cell dysfunction

T-cell unresponsiveness in asymptomatic HIV-infected men was analyzed in well-defined T-cell activation systems.[64] It appeared that the selective unresponsiveness could not be explained by a defect in the early intracellular signalling pathways but that a selective depletion of CD29+ memory T-cells especially early in the infection, contributed to differential loss of T-cell functions. The diminished proliferative response to anti-CD3 was significantly lower in AIDS patients and was shown to be due to decreased interleukin 2 production indicating intrinsic activation defects in T-cell functions[45]. As HIV-1 infection progresses, an increase in T-cell dysfunction occurs which cannot be explained by loss of memory cells. This intrinsic activation defect, also demonstrable in CD8+ cells, may be due to the outgrowth of immature T-cells, among others.[45,72] In a later study we investigated in detail whether functional loss of T-cells is preferentially observed for memory T-cells or whether both naive and memory T-cells are affected.[150] We studied the proliferative response of CD4+ cells from HIV

infected men to alloantigens to which normally both naive and memory T-cells respond. The decreased proliferative response to alloantigens was associated with a decreased precursor frequency of alloreactive T-cells. The frequency was decreased in both the naive and memory subset of CD4+ cells indicating that in later stages of HIV infection both subsets are affected. We also investigated whether given the impaired responsiveness of T-cells from HIV infected individuals to signal one (T-cell receptor dependent), co-stimulation through CD28 and CD27 after interaction with their natural ligands CD80 and CD70 is intact[182]. T-cell proliferative responses to signal one were decreased in a large fraction of asymptomatic HIV infected men, but co-stimulation with CD80 or CD70 remained intact and was even sometimes increased compared with controls.

In a series of studies[101], we obtained evidence for the involvement of Programmed Cell Death (PCD) or apoptosis in HIV-1 infection. Both CD4+ and CD8+ T-cells died from PCD after overnight culture. PCD was enhanced significantly by in vitro activation. PCD was observed in clinically stable asymptomatic subjects, but correlated not with CD4+ counts. Interestingly, in almost all patients tested the fraction of cells dying through apoptosis was highest in the CD8+ T-cells. Since PCD was not overcome by IL-2 or by co-stimulation with anti-CD28, which could restore T-cell activation, it cannot fully explain the observed diminution in T-cell functional activities. It may, however, be a reflection of increased turnover of T-cells, and especially of memory cells. In later studies we found that in individuals with a primary infection the number of T-cells dying due to apoptosis was much higher than in the asymptomatic phase of infection and parallelled transiently increased numbers of CD8+ cells[153]. Death of T-cells was not quantitatively correlated with CD4+ cell numbers or appearance of SI variants and cell death was not confined to a specific T-cell subset nor correlated with expression of certain T-cell activation markers. These results imply that the extent of programmed cell death of T-cells in HIV infection does not correlate with progression to disease, but can be interpreted as reflecting persistent high level immune activation.[153,183]

Taken together, these studies indicate the relevance of early immune abnormalities to the pathogenesis and clinical course of AIDS.[102] One explanation could be that the different T-cell abnormalities which are found in asymptomatic HIV-infected individuals - apoptosis, non-responsiveness failure to produce IL-2 - are the result of effects of HIV on antigen presenting cells (APCs). Alteration of the functions of the antigen-presenting cell may program T-cells for activation induced death and may induce anergy in IL-2 and interferon-gamma secreting Th1 cells. This may result in the predominance of Th2 allergic responses instead of cellular immunity dependent on Th1 cells in HIV infected persons with failing immunity.[124,151]

Indeed T-cell clones isolated from HIV infected individuals from our cohort consistently showed increased IL-4 production, often parallelled by increased IL-5 and decreased IFN-gamma production compared with T-cell clones from HIV negative controls.[152] In two individuals from whom cells were available before and after infection, an increase in Th2 cytokine secreting T-cell clones was observed after HIV infection.

HIV-1 phenotype: biology and prognostic relevance

In addition to the markers described above we studied the role of HIV-1 in vitro cytopathic effect in progression to AIDS. In a transsectional study it was noted that HIV-1 isolates differed in their syncytium-inducing capacity and in vitro growth patterns and replication kinetics. The frequent isolation of syncytium-inducing (SI) isolates from patients with AIDS or AIDS related complex (ARC) in comparison to asymptomatic HIV-1 seropositive individuals suggested that this type of isolates might influence the course of infection[22]. In a subsequent study sequential isolates from 20 initially asymptomatic HIV-1 seropositive men were studied for differences in replication rate, SI capacity and host range.[35] A significant correlation was found between the mean replication rate of HIV-1 isolates and the rate of CD4+ T-cell decrease. In individuals with low-replicating NSI HIV-1 isolates no significant CD4+ loss was found, while recovery of high-replicating SI isolates was associated with subsequent rapid CD4+ T-cell decline and development of ARC or AIDS. How differences in risk of progression to AIDS and AIDS mortality are related to the biological properties of HIV-1 isolates was further analyzed.[36] The relation between in vitro properties of their sequential HIV-1 isolates and clinical course before and after the development of AIDS was studied longitudinally in 49 HIV-Ab seropositive individuals. On the basis of differences in syncytium-inducing capacity, replication rate and host range of their HIV-1 isolates, the individuals could be divided into three groups. The most rapid progression to AIDS (median, 15 months) and the lowest survival rate following AIDS diagnosis (median survival, 12.5 months) was observed in individuals with high-replicating, SI HIV-1 isolates, followed by individuals with high-replicating, NSI isolates. In contrast, most individuals with low-replicating, NSI HIV-1 isolates remained asymptomatic during the study period (median follow-up until AIDS diagnosis > 42 months). The few individuals from this group who developed AIDS were still surviving at the end of the study period (median survival > 34 months).

In a study on long term infected individuals SI variants were shown to

emerge generally only in the course of HIV-1 infection and can only rarely be detected at seroconversion.[97,106] Also in these persons, appearance of SI isolates was strongly associated with progression to AIDS.[97] Longitudinal data from 178 HIV-1 positive subjects over a 30 months period showed that 66.6% of the persons with SI variants at entry progressed to AIDS, compared with 15.9% of persons without SI variants at entry.[122] The emergence of SI variants coincided with the onset of an accelerated rate of CD4+ cell decline. These data indicated that the SI phenotype of HIV-1 isolates may be a valuable prognostic marker for the early identification of a subgroup of asymptomatic individuals who are at high risk for rapid progression to AIDS.[129]

A separate epidemiological study[63] demonstrated that seropositive subjects who had sexual intercourse with a person with AIDS had a more rapid disease progression. This observation also points to a possible role of HIV-1 virulence in progression to AIDS. In this epidemiological analysis no other risk factors for progression to AIDS were found.

Studies of the biological properties of non-syncytium HIV-1 variants revealed an enhanced tropism for monocytes.[79] The prevalence and biological phenotypes of monocytotropic variants were investigated in different stages of HIV-1 infection in asymptomatic and symptomatic subjects. By co-cultivation of peripheral blood mononuclear cell samples with monocyte-derived macrophages monocytotropic HIV-1 variants, mostly of an NSI phenotype, could be isolated in all stages of infection. Analyses of HIV-1 phenotypes at the clonal level, employing limiting-dilution procedures, demonstrated a shift from monocytotropic to T-cell tropic viruses in individuals progressing to AIDS. Moreover, HIV-1 isolated from bronchoalveolar lavage fluid revealed more monocytotropic characteristics as compared with peripheral blood HIV-1 isolates. These findings indicated that monocytotropic isolates are responsible for the persistence of HIV-1 infection.[106,127] The macrophage-tropic HIV-1 variants also initiate infection as during transmission there usually is a selection for NSI macrophage tropic HIV variants reflecting advantage of such strains over the more virulent SI variants. This was shown in both sexual and parenteral transmission. In three primary infection cases homogeneous virus populations (macrophage tropic, NSI) were present prior to seroconversion thus excluding humoral immunity in the recipient as the selective pressure in favor of macrophage tropic variants.[159,161] It could however be that humoral immunity in the donor plays a role and that virions inherently more resistant to neutralization by donor antibodies have a greater chance of initiating infection than virions more sensitive to neutralization[160].

The observation that these virus variants are mainly in tissue macrophages and not in blood monocytes can be explained by our finding that monocytes become infected only during differentiation to macrophages.[107] Macrophages are often considered to be non-proliferating and as retroviruses establish productive infection only in proliferating cells and macrophages are susceptible to HIV-1 infection, we studied this contradiction in more depth. From our experiments we could conclude that the productive infection of macrophages is restricted to the cell fraction of monocyte-derived-macrophages (MDM) with proliferative capacity.[158] This means that the cellular requirements for productive HIV-1 infection in primary macrophages does not differ from the requirements in T-cells, as reported by other workers before.

In another study we tested the ability of 19 primary virus isolates - obtained from HIV-positive participants of the cohort study - to infect monocyte-derived macrophages (MDM) from donors who were negative for HIV (and HTLV, CMV and hepatitis viruses).[141] Two HIV-1 isolates were able to establish a productive infection in MDM from all donors, eight completed lacked this capacity and the other primary isolates established an infection in some but not all donors (intermediate isolates). PCR analysis demonstrated that the capacity to replicate in MDM was determined at the level of virus entry. However for intermediate macrophage tropic isolates replication was abrogated at the level of reverse transcription.

In a subsequent study we were able to show that the changes within the V3 domain associated with a shift in virus phenotype occurred not only in the genome of cultured viruses, but also in the PBMC prior to culture amplification. Although the V3 genome transition lagged three to nine months behind the virus isolates, these results indicate that the in vitro results obtained with isolates are relevant to the in vivo situation.[99]

From the different studies that we and others did, it is clear that macrophage-tropic HIV-1 variants play an important role during all stages of HIV infection. Therefore, the inhibitory effect of IL-10 on HIV-1 replication in macrophages suggest this cytokine as a candidate for use in therapeutic trials. However, as IL-10 also has an important role in cross regulation of Th phenotypes and the impaired immunity associated with Th2 expansion argues against the use of IL-10 in HIV infected individuals.[156]

Molecular biology of HIV-1 phenotype

In view of the possible importance of biological variability of HIV-1 isolates in the pathogenesis of AIDS, a panel of phenotypically distinct yet genetically highly homologous infectious molecular clones was studied for SI capacity and T-cell line tropism.[71] These clones were derived from HIV-1 isolates, mostly recovered by direct clonal isolation, from a single individual in whom a transition from NSI to SI isolates had been identified over time. In most cases the phenotypes of the molecular clones matched those of their

parental isolates very well, which demonstrates that biological variability of HIV-1 isolates can be attributed to single molecular clones. Further analysis showed evidence that T-cell tropism is not caused by differences in the level of HIV-1 expression but might be caused by restriction at the level of virus entry and might therefore be related to differences in the viral envelope. Subsequent studies indeed revealed that phenotypic differences are related to the highly variable V3 domain of the viral envelope protein gp120.[87,88,91] The observed relation between phenotypic differences in HIV-1 and the highly variable V3 domain was further analyzed by single amino acid substitution.[87] This study demonstrated that introduction of positively charged amino acids at position 306 or 320, previously[91] shown to be strongly associated with the SI phenotype in field isolates, was minimally required for production of SI viruses. In addition, it was shown that naturally occurring mutations at position 324 could also modulate the virus phenotype. The strong association between the SI capacity of HIV-1 and the presence of positively charged amino acid residues at positions 306 and/or 320 in V3 region of gp120 could be confirmed in another study in 97% of 402 primary HIV-1 isolates, indicating the usefulness of the V3 genotype for the prediction of the viral phenotype.[169] Four SI specific oligonucleotides were designed for selective amplification of V3 from SI but not from non-SI HIV-1 isolates and this PCR analysis may be a simple tool for the monitoring of the viral phenotype in HIV-1 infected individuals.

To further elucidate the contribution of the Long Terminal Repeats (LTR) and cell specific signaling to differences in cytotropism, we analyzed the events in the replication cycle of the four infectious HIV-1 molecular clones with distinct biological characteristics obtained from an HIV positive HM in the cohort.[126] After transfection of four full length infectious molecular clones into monocyte derived macrophages (MDMs) competent virus could be rescued from the MDMs by coculture with PHA-stimulated PBLs. Proviral DNA could be detected only in monocytes exposed to clones that were shown to establish productive infection. No differences in responsiveness to cell type specific signals were demonstrated if LTR-CAT constructs were transiently transfected in stimulated T-cells. From these experiments we could conclude that cell type-specific signaling of the HIV-1 LTR does not contribute to HIV tropism, but that host range restriction is located at an early step in the viral replication cycle.

Subsequent studies on phenotype evolution of HIV-1 showed that combinations of changes in V2 and V3 were related to the SI phenotype.[117] It appeared that SI viruses had gone through additional changes in V2 that might be necessary for the emergence of the subsequent changes in V3. The configuration of a hypervariable locus in the V2 domain, as studied in isolates of four individuals over a time period of 18-36 months, appeared to be predictive for conversion of NSI to SI phenotypes, and could sometimes be detected as early as three years before phenotype conversion. The identified hypervariable V2 locus was considered to allow early discrimination between stable NSI variants and NSI variants that have the potential to convert to the SI phenotype. This distinction could be useful for clinical monitoring. Later studies showed however that the use of a PCR detecting V2 length polymorphism as a predictive marker for SI phenotype evolution is limited.[170] The reason could be that changes in V2 are only transiently required to allow SI phenotype evolution,[185] also explaining the absence of elongated V2 domains in SI variants relatively late after SI seroconversion.[167]

Laboratory HIV-1 isolates are neutralized by soluble CD4 (sCD4) whereas primary HIV-1 variants are resistant to sCD4 neutralization. To determine possible differences in sCD4 neutralization sensitivity between phenotypically distinct primary HIV-1 variants, we studied a panel of NSI and SI variants isolated from participants of the cohort study.[172] Primary SI and NSI variants appeared to be equally resistant to sCD4 neutralization, indicating that the specific properties of the viral envelope reflected in adaptation to T-cell lines, influence sCD4 neutralization sensitivity.

The findings of variation in virus phenotype in association with changes in immune functions has led to the development of an integrated model for the pathogenesis of AIDS.[50,51,59] In this model the immune system is crucial in maintaining the asymptomatic state. Once the immune system has been weakened below a certain level, more virulent (SI) variants may emerge and CD4+ T-cells decline rapidly, resulting in rapid progression to AIDS. The failure of immunity allows for overt virus replication and increase of viral load as is seen in AIDS.[154]

Neutralization

Since neutralizing antibodies may play a role in protective immunity it was of considerable interest to define epitopes on the viral envelope that elicited neutralizing antibodies. The major neutralizing epitope on HIV-1 was identified in the third variable region of the envelope glycoprotein (gp120).[19,65] With an eye towards obtaining a better understanding of the natural history of the HIV-1 infection as well as information for future vaccine composition, antigenic variation and immunodominance of the principal neutralization domain of HIV-1 were investigated.[82]

Cross-reactivity of experimental and natural sera with recombinant proteins containing the V3 region of four HIV-1 variants showed that a group of viruses including HIV-1 MN and a Dutch isolate (168-1) had antigenically similar V3 regions, while the laboratory strain HTLV-IIIB and the RF strain represented distinct serotypes. As shown in antibody binding studies, the antigenic similarity of the V3 region from isolate 168-1 and strain MN could be assigned to the central area of the V3 region (residues 307-317).

A serologic survey of 397 Dutch sera, 214 of which were derived from homosexual men indicated that viruses from the MN and 168-1 group predominate in the Netherlands, which might have important implications for future vaccine composition.[65] On the basis of the V3 variations observed among the viruses circulating among the participants in the cohort studies, we were able to show that V3 peptide cocktails of limited size with the potential to cover a large proportion of V3 sequence variation may be feasible vaccine candidates.[74] One should, however, be aware that, in different geographical areas, there are variants circulating with V3 regions that are antigenically different from the 168/MN group.[83] For example in Tanzania, we found that the diversity of the HIV-1 population, as measured by serological methods and with sequence analysis of the V3 region is much greater than in the Netherlands.[137] This was studied by comparing 55 sera from Tanzanians with symptomatic HIV infection with sera from Dutch AIDS patients.

Finally, HIV-1 has a great capacity to escape from immune pressure. Therefore, vaccine candidates should contain other neutralization epitopes, preferably group-specific, in addition to the V3 domains of circulating viruses. However, recent information indicates that a prophylactic vaccine should not only induce an antibody response but should also elicit cellular immunity against HIV-1 infection.[125]

After the identification of V3-binding antibodies, a second population of neutralizing envelope antibodies was detected in human sera.[41] In contrast to the antibody population that neutralizes virus through binding to the V3 domain, this second antibody population inhibited the attachment of gp160 to the cellular receptor CD4- and neutralized distinct HIV-1 variants.[44] The binding site for these antibodies was shown to be discontinuous and apparently conserved between isolates.

To further delineate this antibody population, envelope antigens were used that were deleted in each or combinations of all variable regions of the HIV-1 envelope.[66] These antigens were probed in an enzyme-linked immunoassay with sera of 75 seroconverters and 200 seropositive subjects. Two distinct antibody response patterns could be observed. First, there were individuals who did *not* show reactivity to constant regions of the envelope (V3-restricted responders). Second, there were individuals who *did* show reactivity to both constant and variable envelope regions. The non-responders to constant envelope regions had a higher frequency of HIV-1 core antigen positivity and HIV-1 core antibody negativity. This findings suggested that host antibody responses to the envelope are associated with HIV-1 expression and possibly disease progression.

To assess the role of group-specific neutralizing antibodies in immunity, the induction of such antibodies was studied after seroconversion of seven Dutch HM and four British hemophiliacs.[100] Group-specific neutralizing antibody varied between individuals and neutralizing antibodies to laboratory adapted HIV-1 strains (e.g. IIIB) could be detected in only five of 11 individuals within 32 weeks of seroconversion. These findings suggested that the neutralizing target for HIV-1 was poorly immunogenic in vivo.

The type-specific humoral response to HIV-1 infection was further studied by investigating the specificity of the antibody response to the major neutralizing domain.[111] Therefore the relation between V3 sequences of the HIV-1 variant circulating in the host and the antibody specificity of the host was analyzed. The relation between the V3 sequence of the virus population and serum specificity, as measured by reactivity to a panel of V3 peptides, was investigated both early after infection and at later stages of infection when new variants had emerged. The specificity (best recognized peptide) of the antibody response early after seroconversion accurately reflected the virus population around the time of seroconversion. Changes in serum specificity at later stages of infection were rare and the early response appeared to be preserved despite the change of V3 sequences and the broadening of serum recognition of V3 peptides. The specificity of the antibody response to the V3 domain was investigated in serum obtained early after seroconversion from 129 HIV-1 positive Dutch men. The majority of the serum samples showed their highest reactivity to a peptide sequence homologous to the US/European consensus sequence. The accurate reflection in early sera and the preservation of specificity at later stages of infection indicated that probing serum reactivity for V3 peptides could be a reliable way to survey the circulation of HIV-1 variants with particular V3 domains.[92] The temporal development of HIV-1 neutralizing activity and antibodies to the gp120-V3 neutralization

domain were studied longitudinally in sera from 20 seroconverters.[164] We found early (within 10 months) and frequent (90%) V3 mediated cross neutralization of HIV-1 MN demonstrating a close relationship between MN and the HIV-1 variants circulating in the Netherlands. HIV-1 IIIB appeared to be more divergent and neutralizing activity to this strain developed later and was predominantly mediated through epitopes other than V3.

To investigate the influence of V3 loops of naturally occurring viruses on the neutralization sensitivity of a molecular cloned virus, we inserted SI and NSI V3 loops of an HIV infected man (H594) and two laboratory SI strains in an infectious molecular clone of HIV-1 LAI.[174] High sensitivity of the chimeric viruses containing the laboratory V3 regions to neutralization by sequential sera from H594 and to a heterologous serum pool was found. In contrast insertion of primary isolate NSI and SI V3 loops reduced the neutralization by autologous serum but not by the heterologous pool. These results indicate that the neutralization sensitivity of the viruses depends on the capacity of the V3 region to influence the conformation of the virus envelope. These V3 dependent conformational changes partially explain the neutralization sensitivity of lab strains and the relative resistance of primary strains.

In another study we investigated whether the length of the disease free period was related to the level of antibodies to V3 and to recombinant gp120 in serum.[163] By comparing fast progressors and slow progressors we demonstrated that levels of IgG antibodies to the envelope epitopes are poor predictors of rapid progression and suggest that the role of V3 directed neutralizing antibodies in preventing subversion of the immune system is not decisive in natural HIV-1 infection. In a longitudinal study of the immune response of six HIV infected individuals, we tested the sequential sera of each individual by Pepscan analysis, which showed amino acids in the V3 domain that contributed to antibody binding.[180] The position and the number of the mutations that occurred during infection corresponded with the position and number of aminoacids in the V3 domain that were important for binding to anti-V3 antibodies in early infection.[180] The specificity of the antibody binding hardly changed during infection.

Antibodies recognizing a conformation dependent antigenic site overlapping with the CD4 binding site on gp120 are responsible for a major part of the virus neutralizing cross reactivity in human sera. To investigate the nature of this antigenic site in more detail, we generated three virus neutralizing human monoclonal antibodies produced by Epstein-Barr virus transformation of peripheral blood mononuclear cells from two HIV positive HM participating the cohort study.[128] Two of these monoclonal antibodies neutralized a broad range of HIV-1 strains whereas one exhibited a more restricted pattern of neutralizing activity.

Antigenic variation

The antigenic variation of the V3 region itself was further analyzed in two seroconverted homosexual men over a period of five years.[81] Analysis of the genomic RNA coding for the major neutralization domain of the V3 region revealed that the genomic diversity increased during infection, resulting in antigenic variation and subsequent differences in antibody binding specificity. In one of the two subjects it could be shown that substitution of an amino acid at position 308 of the glycoprotein (gp120) elicited a new antibody population. The other patient showed a decline in V3-specific antibodies simultaneous with an increase in genomic RNA levels and progression to AIDS. At that time a new variant with major changes in the major neutralization domain had emerged. It could be shown in this study that naturally occurring mutations in genomic RNA lead to antigenic variation followed by an immunological response in the host. On the basis of these studies we have been able to develop a mathematical model and compare it to the available data. This model of the dynamic interaction between viral diversity and the immune system postulates the existence of an antigen diversity threshold, below which the immune system is able to regulate viral population growth but above which the virus population induces a collapse of the CD4+ lymphocyte population.[77] This model has been subsequently extended to include specific host components (an immunological threshold) as cause of the failure of the functional antibody response to HIV-1 in the course of infection.[98]

One may hypothesize that increasing variation due to the high error rate of reverse transcriptase and negative selection pressure against genomes less fit for replication is positively correlated with disease progression. To test this hypothesis the predominant genomic RNA sequences encoding the HIV-1 gp120 V3 region present at seroconversion and 5 years later were studied in sera from progressors and non-progressors in our cohort.[181] The rate of evolution of the V3 region in the host was not found to be related to disease progression as measured by CD4 decline, p24 production, HIV-1 RNA load and the biological phenotype, but to the length of the immunocompetent period. These results plead against an antigen diversity threshold playing a role in the pathogenesis of HIV.

Population wide variation in genomic RNA of the viral envelope region encompassing the V3 loop of HIV-1 was studied using serum samples of 74 newly infected individuals from three Dutch cohorts: 30 HM, 32 injecting drug users (IDU) and 12 hemophiliacs. [123,149] During acute infection, HIV-1 RNA sequences present in serum are relatively homogeneous, which makes direct sequencing feasible. This offered an opportunity to study the infecting virus variants before mutations had accumulated in the host. The sampling

dates ranged from 1980 to 1991, thus spanning the entire AIDS epidemic in the Netherlands. The diversity in the sequenced region increased over time in both the HM and the IDU risk groups. Furthermore, this increase was associated with an increase in antigenic variation, as witnessed by serum reactivity to a V3 peptide panel. Despite this diversification, some 1990 sequences still closely resembled the earliest 1980 sequence, making ancestral inferences problematic. No evidence was found of a change in the master sequence of the virus quasispecies over time. At the amino acid level, no risk group associated variation was found, but at the nucleotide level, the IDU and HM/hemophiliac sequences could be distinguished on the basis of a single silent nucleotide change in the tip of the V3 loop. Hemophiliac sequences could not be distinguished from those of HM. In spite of the large and increasing genetic variability, all sequences were similar to the European/American HIV consensus sequence than to that of non-Western strains.

The increasing viral quasispecies diversity in and between individuals over time can also be studied with a relatively simple technique called the heteroduplex mobility assay (HMA) as was shown in a collaborative study using longitudinal samples from individuals in our and other cohorts.[140]

The silent mutation which distinguished the IDUs in our study from HM - the second glycine residue at the tip of the V3 loop being coded by GGC in stead of GGA or GGT - was also found in HIV infected IDUs from Germany and Edinburgh, Scotland, but not in HM.[148] These data show that 'molecular epidemiology' is a very valuable tool for tracing the spread of HIV.

In an attempt to uncover variations in V3 sequences associated with AIDS Dementia Complex (ADC) we compared V3 sequences obtained from serum and cerebrospinal fluid samples of four AIDS patients with and eight without ADC.[179] We found a significant ADC associated difference occurring at several amino acid positions which may indicate that the virus variant found in ADC and non-ADC has different biological properties.

Using a simple method for the detection of HIV-1 RNA in feces - by reverse transcription followed by a nested PCR encompassing the V3 region - we showed that HIV RNA could be found in the majority HIV infected symptomatic and asymptomatic individuals.[186] In one patient a remarkable difference in the HIV sequences between isolates in feces and serum was observed.

Viral load and disease progression

The discovery of the prognostic significance of p24 antigenemia heralded the importance of the amount of circulating virus and the number of infected cells for the rate of disease progression. Initially the level of HIV-1 DNA in peripheral blood cells, most likely reflecting the number of infected cells, was quantified using the PCR-aided template titration assay (PATTY).[94] It was shown in 1992 that long-term HIV-1 infected individuals of whom 19 were tested had HIV-1 copy numbers ranging from 0.8-100 per 10^3 PBMC. Significantly higher copy numbers were found among p24 positive individuals than among p24 negative individuals, indicating that the number of HIV-1 infected cells was related to prognosis. In addition it was shown that HIV-1 DNA copy numbers peaked at the moment of antibody seroconversion following the p24 peak, that occurs prior to seroconversion, indicating that HIV-1 DNA cellular loads may lag behind virus loads themselves produced by cells. HIV-1 RNA is transcribed in the cytoplasm into linear DNA, transported into the nucleus and in the nucleus integration occurs. As side product of integration circular DNA (cDNA) accumulates in the nucleus. Therefore it was decided to make a quantitative cDNA assay as measure of newly infected cells.[175] It was shown that indeed cDNA levels were higher in progressors than in slow or non progressors, but still cDNA levels did not discriminate sufficienctly. Therefore a competitive isothermal quantitative RNA assay (NASBA) was subsequently used to establish its prognostic value. 20 seroconverters who developed AIDS (12 without SI virus and 8 with SI virus) were studied as well as 21 seroconverters that remained symptom-free during the same follow-up period.[143] At seroconversion no difference between progressors and non-progressors was observed in the height of viral RNA levels in contrast to the first time point thereafter (3 years post-seroconversion). In contrast to the progressors, HIV-1 RNA copies in sera of non-progressors had declined significantly. This difference was especially noted between NSI progressors and non-progressors. These results indicated that a significant decrease in the number of HIV-1 RNA copies in the early phase of infection postponed the development of AIDS.

Following these leads long-term asymptomatic individuals were compared to slow and rapid progressors among the group of HIV-1 seropositives and seroconverters.[173] Early infection, before immunological markers or clinical manifestations allowed group discrimination, subjects who were later classified as LTAs had significantly less serum viral RNA than progressors. No significant increase in viral load was found in progressors, indicating that the initial viral load defines clinical outcome.

Finally the hypothesis was tested that decreasing the viral load might benefit the HIV-1 infected individual. Serum RNA levels were measured in 28 seropositive asymptomatic individuals participating in a trial on the efficacy of zidovudine. Sixteen individuals remained asymptomatic until 4 years after the onset of the trial, whereas 12 individuals were diagnosed with an AIDS-defining event.[176] The decline of HIV-1 RNA copy number was much stronger

in the non-progressors compared to the progressors, suggesting that early RNA responses to antiretroviral therapy might predict clinical outcome.

Long term asymptomatics, viro-immunologic characteristics

Early studies often focused on determinants and predictors of rapid progression. It is however important to study HIV infected persons who remain asymptomatic for long periods. Such studies may identify viral and host factors that correlate with such slow or non progression. In our cohort we compared 61 HIV infected men who remained asymptomatic for >7 years with 142 men who progressed to symptomatic HIV infection within 7 years.[144] Real non progression appeared to be rare: in only 2/61 the CD4 T-cell count had not declined during follow-up. Slow progression was not found to be associated with sexual behavior or the use of recreational drugs. Slow progressors were characterized by stable and relatively preserved T-cell function (independent of the T- cell count) and by seropositivity for antibodies to HIV core.

We also studied HIV infected individuals with low CD4 T-cell counts to determine the rate of non-progression in this group. For this purpose we did a case-control study comparing 58 men who remained AIDS free for more than 2 years after their CD4 T-cell count dropped <200/mm^3 with 63 men who progressed to AIDS within 2 years after their CD4 count T-cell declined <200/mm^3.[145] Presence of SI HIV variants, p24 antigen and a low T-cell response after stimulation with PHA were found to be predictors for progression to AIDS. Eight out of 58 men remained AIDS free for more than 4 years; all eight were p24 antigen negative and 7/8 had only NSI variants.

HIV-1 specific CD8 cytotoxic T lymphocytes (CTL) are generally believed to play a crucial role in controlling disease progression and to be important for vaccine development.[50,125] We studied the HIV-1 gag CTL epitopes and the HLA restriction of their recognition and tried to determine precursor frequencies of HIV-1 gag specific CTL in the blood of seropositive men.[130] We found that B-lymphoblastoid cell lines infected with recombinant vaccinia viruses containing a gene coding for HIV-1 gag or other viral proteins can be used as stimulator cells to determine CTL precursor frequencies in peripheral blood cells of patients. This method will allow the longitudinal analysis of HIV-1 specific CTL responses in HIV infected and vaccinated individuals.

To gain more insight into the role of HIV-1 specific CTL in non- and slow-progression, we investigated temporal relations between gag specific precursor CTL (CTLp), CD4 T- cell counts and T-cell function in 6 HIV infected men who remained asymptomatic for >8 years and 6 subjects who progressed to AIDS within 5 years after seroconversion.[177] In the 6 long term asymptomatics persistent CTL responses coincided with normal and stable CD4 cell counts and preserved T-cell function. This may indicate that HIV-1 gag specific CTL contribute to the maintenance of the asymptomatic state by effectively controlling HIV replication. However, in four out of 6 progressors a rise of gag specific CTLp frequencies early in infection was parallelled by increasing numbers of HIV-1 infected CD4 cells. During subsequent clinical progression loss of gag specific CTLp coincided with precipitating CD4 counts and severe deterioration of T-cell function.

In another study comparing HIV positives who remained asymptomatic > 7 years (LTAs) with progressors. Early in infection before immunologic markers or clinical manifestations allowed group discrimination subjects who were later classified as LTAs had significantly less serum viral RNA than progressors.

Our studies and those from others to date indicate that long term asymptomatics are a heterogeneous group with the majority of individuals showing slow to very slow disease progression, while a very small number may survive HIV infection permanently. Further studies are necessary to elucidate the biological determinants of slow and non progression.[178]

After we had shown that the presence of HIV-antigen in a strong predictor for faster progression to AIDS,[4,6,9] a preliminary study of AZT dosing and toxicity was performed in 24 symptom-free seropositive participants with long-term HIV-1 antigenaemia.[15,29,53] This was the first time that AZT was used in asymptomatic HIV-positive subjects. After a follow-up period of 92 weeks disease progression occurred in four subjects, despite sustained reduction of serum HIV-1 p24 antigen levels: Pneumocystis carinii pneumonia was diagnosed after 60, 80, 90, and 93 weeks, respectively. The median CD4+ cell count of these four men was 0.2x 10^9/L at entry into the study, and had declined to 0.07 x 10^9/L at the moment AIDS was diagnosed. In 20 subjects no disease progression occurred. The median CD4+ cell count of these 20 men was 0.4 x 10^9/L at entry into the study and was 0.45 x 10^9/L at the end of the study period. AZT treatment was better tolerated in asymptomatic seropositive subjects than in patients with AIDS or ARC. Anaemia caused symptoms in three of 24 men, but prolonged leucopenia or neutropenia did not occur. None of the men developed clinical or convincing biochemical evidence of zidovudine-associated myopathy. Median serum HIV-1 p24 antigen levels in these 20 subjects were 42% lower at the end of the study period than at entry into the study. In five of the 20 men, an initial decline in antigen levels, was followed by a rise to above pretreatment values.

A subsequent study indicated that the number of subjects with rising and declining serum p24 antigen levels differed significantly ($P < 0.001$) between an untreated group and a zidovudine-treated group. Triton pretreatment of serum samples, which presumably enables detection of virion-associated p24, did not lead to substantial increases in p24 levels.[54] Serum B2-microglobulin levels, known to be a marker for disease progression, were studied during long-term zidovudine treatment.[76] A decline in B2-microglobulin levels was found to parallel a decline in p24 antigen levels during the early phase of zidovudine treatment (48 weeks). After prolonged treatment (2.5 years) rising B2-microglobulin levels, in contrast to p24 antigen levels, were shown to have predictive value for disease progression.

In addition to p24 antigen and B2-microglobulin levels as prognostic markers during the first two years long-term zidovudine-treatment, immunological markers such as CD4+ T-cell numbers and anti-CD3-induced T-cell responsiveness were studied.[72] Nine treated asymptomatic men, five of whom progressed to AIDS, were compared with 10 untreated asymptomatic men, five of whom progressed to AIDS. At intake into the cohort study, one year before the start of treatment, CD4+ T-cell numbers in the two groups were not significantly different. However, progressors already exhibited an extremely low anti-CD3-induced T-cell responsiveness as compared with non-progressors. T-cell responsiveness and the number of CD4+ T-cells improved six months after the start of zidovudine treatment, but only for a short period. It appeared that differences in clinical course in zidovudine-treated asymptomatic HIV-infected men were associated with T-cell function at intake.

Zidovudine sensitivity of HIV-1 was studied[42] in 18 subjects by virus isolation from cryopreserved peripheral blood mononuclear cells at three time points (before treatment, 16 to 32 weeks and 48 to 112 weeks). The polymerase chain reaction was used to detect mutations at codon 215 of the reverse transcriptase gene, a mutation associated with reduced drug sensitivity during treatment of AIDS patients. After two years of treatment, 16 of 18 isolates were mutant which correlated with the limited in vitro sensitivity data.

A mutation at codon 70 was first shown to occur during zidovudine treatment of asymptomatic individuals. This mutation appeared only transiently and was replaced by the mutation at codon 215. Additional mutations (codons 67, 70 and 219) which coincide with highly resistant virus were observed only after progression to disease.[84] A fifth mutation (codon 41) could be identified relatively early in the development of zidovudine resistance. This mutation was detected only after the appearance of the codon 215 change in the reverse transcriptase coding sequence.[96]

A subsequent study on HIV-1 biological phenotype and the development of zidovudine resistance in relation to disease progression showed no significant difference between SI and NSI isolates in the frequency of five mutations causing zidovudine resistance.[85] Among 24 zidovudine treated asymptomatic men[42] progression to AIDS was more rapid in those who either harbored SI viruses or showed conversion from NSI to SI. Conversion from NSI to SI was apparently not prevented by zidovudine treatment.

To evaluate the efficacy of zidovudine in asymptomatic HIV-positives, we participated in a multicenter placebo controlled trial in HIV-1 positives with CD4 counts between 200 and 400 or >400 with p24 antigenaemia.[155] In the first of the two study years the rate of progression to AIDS or severe ARC was significantly higher in the placebo than in the zidovudine group. However, at the end of the study no difference in progression rate was found, indicating that zidovudine is delaying progression in high risk asymptomatic HIV positives, but that this effect is temporary.

The effect of zidovudine on disease progression in 52 asymptomatic HIV-1 infected men in relation to CD4 T-cell numbers, T-cell reactivity and biological phenotype was studied in the Amsterdam participants of this double

blind randomized trial over 2 years.[121] Zidovudine did not prevent the emergence of SI variants and clinical progression was observed in persons with SI variants despite zidovudine treatment. In this study zidovudine treated men who did not develop SI variants did not progress to AIDS. This indicated that the beneficial effect of zidovudine during the asymptomatic phase may be mainly limited to persons who did not develop SI variants in the course of HIV-1 infection. This means that the HIV phenotype is not only important as a prognostic marker but also to predict the expected efficacy of zidovudine treatment.[157]

In the participants of this zidovudine study we also showed that HIV-1 RNA load in serum can be used to monitor the response to antiviral therapy in p24 positive as well as -negative individuals. Posttreatment changes in p24 antigen levels were not indicative for clinical outcome whereas RNA copy numbers were.[176]

Primary prophylaxis against Pneumocystis Carinii Pneumonia is highly effective and recommended for HIV positives with a CD4 count <200/ul. By adding CD4 cells <20% of the total circulating lymphocytes to the eligibility criteria - as was originally the case - the data from our cohort study suggest that the number of men at risk for PCP can be reduced.[134] Out of 513 HIV positive men 150 progressed to AIDS by January 1993 and 49/150 had PCP as the primary diagnosis. In 11/49 men the number of CD4 cells never dropped <200 up to 14 days before PCP diagnosis, while in 7 out of these 11 the percentage did decrease below 20% before PCP was diagnosed. These 7 cases would have received primary PCP prophylaxis if the percentage was part of the eligibility criteria

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dissertations

1988 **J.M.A. Lange**
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F. de Wolf
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1989 **G.J.P. van Griensven**
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M. Tersmette
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1990 **P. Reiss**
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R. Gruters
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J.J. de Jong
The V3 domain of HIV-1: role in syncytium formation, replication and antigenicity

G. Zwart
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S.E.C. Koken
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E.M.M. de Vroome
AIDS-voorlichting onder homoseksuele mannen; diffusie van veilig vrijen in Nederland (1986-1989)

1995 **L. Meyaard**
Mechanisms of T cell dysfunction in HIV infection

R.A.M. Fouchier
Molecular determinants of HIV-1 phenotype

C.L. Kuiken
Evolution of the HIV-1 V3 region: epidemiological and functional influences

S. Jurriaans
Virus load in HIV-1 infection

I.P.M. Keet
HIV-1 seroconversion and its aftermath among homosexual men. Studies on acquisition of HIV-1 and natural history of HIV-1 infection

II THE AMSTERDAM COHORT STUDY ON HIV INFECTION AND AIDS AMONG DRUG USERS

Results 1984 - 1995

The researchers thank the drug users for their participation in the various studies.

The studies were done in collaboration with many different Institutes. We especially are indebted to the Drugs Department of the Municipal Health Service of Amsterdam and the Department of Social Psychology, University of Amsterdam.

We thank Dr J.A.R. van den Hoek, who coordinated this study through 1995.

introduction

The Amsterdam cohort study on HIV infection and AIDS among drug users was set up at the end of 1985, one year after the start of the cohort study among homosexual men. At that time only one drug user with AIDS had been reported in the Netherlands, but studies from the United States had shown that HIV infection among this group could spread very fast, mainly through the sharing of needles and syringes. An important reason for starting a study among drug users in Amsterdam was that the helping system for drug users here operates in a different way than in most other countries and enables contact to be established with the majority of drug users living in Amsterdam. For this reason, a longitudinal study was considered to be feasible.

The **aims** of the study are

a. to study the prevalence and incidence of HIV infection and AIDS in relation to (changes in) drug use and sexual behavior
b. to study the prevalence and incidence of other blood and sexually transmitted infections
c. to evaluate the impact of various HIV-prevention programs for drug users
d. to study determinants of risky injecting and sexual behavior
e. to study the natural history of HIV infection and AIDS

Studies on the HIV prevalence, - incidence and risk behavior helped to elucidate the epidemiology of HIV infection among drug users in The Netherlands and elsewhere and have provided a basis for primary prevention. The evaluation of intervention measures helped to delineate the role and importance of such measures especially within the context of the Amsterdam harm reduction approach. The natural history studies were aimed at identifying differences with homosexual men e.g. in pre-AIDS mortality and indicative diseases.

methods

Participants are recruited at methadone outposts, the weekly STD-clinic for drug-using prostitutes and by word of mouth. Both injecting and non-injecting drug users (IDU and non-IDU) are invited to participate. Participation is voluntary and, after extensive information has been given, informed consent is obtained. Blood samples for serology, virology and immunology (after 1988) are taken and participants are interviewed using a standardized questionnaire which includes questions concerning clinical symptoms, medical history, lifestyle, use of oral and intravenous drugs (methadone included), and prostitution. Blood samples of each visit (both serum and cells) are stored. Participants are asked to return for a follow-up visit every four months, but many return more irregularly. On each occasion, the same interview is conducted and blood samples are collected. Twenty-five Dutch guilders are paid per follow-up visit to encourage continued participation. Since April 1989, all participants (both the HIV-positives and -negatives) are physically examined by a physician at each visit. Depending on certain substudies additional questions on specific psychosocial or behavioral issues are added to the basic questionnaire.

This is an open cohort study and new participants can enter at any time. Data on hospitalization are collected at each visit from the participants and also independently through the Drug Department of the Municipal Health Service. Cases of AIDS are ascertained through crosslinking with the Amsterdam AIDS registry.

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.

results

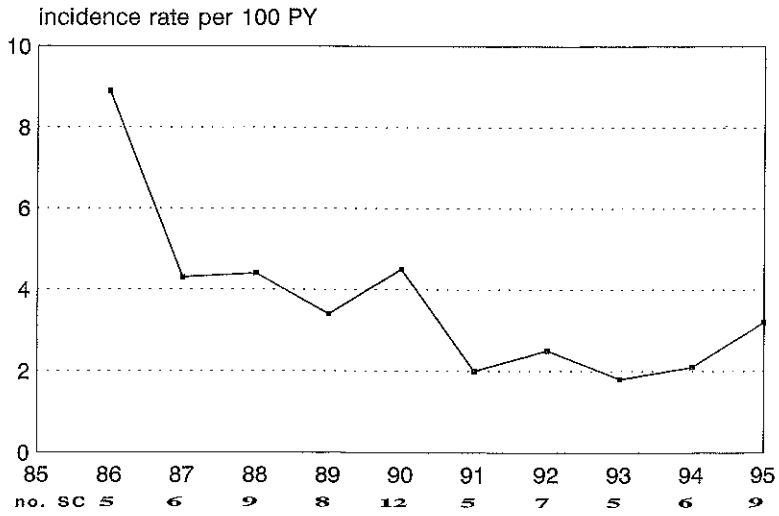
Prevalence and incidence of HIV infections and AIDS

Between December 1985 and January 1996 1177 drug users (all without AIDS-related disease) entered the study. The recruitment of new participants was interrupted for one year between September 1990 and September 1991. In the first year of the study (1986), the HIV prevalence among drug users with a history of injecting drug use was approximately 30%[3,4] and remained more or less stable among new intakes in this group in the following years[19] (see table 1). The HIV incidence rate per 100 person years (pyrs) among HIV-negative injecting drug users in the cohort was very high in the first year of the study (1986: 9 per 100 pyrs) and gradually declined afterwards coinciding with a decline in (injecting) risk behavior. In the last years the annual incidence is rather stable at 2-3 per 100 pyrs (figure 1).[19,28] By January 1996, a total of 75 seroconversions had occurred among the participants who were HIV negative at entry. From an additional 33 drug users who were HIV positive at entry the date of seroconversion can be estimated as earlier blood samples - collected for reasons unrelated to HIV - were available at different laboratories. Among the HIV positive participants and the seroconverters 83 cases of AIDS had been observed by January 1996.

Table 1
HIV-prevalence among injecting drug users who entered the Amsterdam AIDS Cohort Study by year

Year	Number entering	Injectors (ever)	HIV-positive at entry (injectors only)	HIV-prevalence among injectors (%)
1985/1986	286	236	78	33
1987	214	179	54	30
1988	158	134	37	28
1989	114	91	29	32
1990	81	65	18	28
1991	40	32	13	41
1992	84	64	14	22
1993	79	67	17	25
1994	63	48	10	21
1995	58	35	6	17
Total	1177	951	276	29

Annual HIV incidence rates among HIV negative drug users who ever injected



Risk factors for HIV infection in IDU who were positive at entry were: a higher frequency of borrowing used needles and syringes, date of first injecting drug use longer ago, recent injecting drug use, relatively prolonged time living in Amsterdam, German nationality and injecting of heroin and cocaine together.[3,4,22] Risk factors for *new (incident)* HIV infection were duration of time living in Amsterdam and recent onset of injecting. Injecting mainly at home was related to a decreased incidence[28]. Among drug users who never injected, only a very limited number of HIV infections have been found to date and most of these infections occurred among males who had homosexual contacts.[3,4] So far, the heterosexual spread of HIV among the population of drug users in Amsterdam appears to be limited, although there is some evidence for spread among non-injecting drug users from Surinam and the Netherlands Antilles.[35]

To assess the generalizability of the HIV prevalence in our study group, we conducted other prevalence studies among drug users. One study was part of a multi-center (EC) study conducted in 1990 among IDU in twelve European countries. IDU who had injected drugs recently were recruited 'on the streets'. In Amsterdam an HIV prevalence was found of 37%, which did not differ significantly from the HIV prevalence among current injectors in our cohort. Risk factors for HIV infection were also similar to the risk factors found in the cohort study indicating that the cohort participants are reasonably representative for IDUs in Amsterdam.[34] In 1991, among clients of two methadone buses and one neighborhood methadone post of the drugs department of the Municipal Health Service in Amsterdam, we did an anonymous and voluntary HIV prevalence study by means of a saliva test.[20] This test, developed by the National Institute of Public Health and Environmental Hygiene (Bilthoven, The Netherlands), appeared highly suitable for anonymous HIV-testing among high-risk groups.[29] In the study among methadone users the HIV prevalence was 24% among IDU and 3% among drug users who had never injected. In the cohort study, these percentages were 30 and 4 respectively.[20]

In 1993 we also studied the HIV prevalence among Surinamese and Antillean DU in Amsterdam as this group was underrepresented in our cohort study.[35] The HIV prevalence among this group - recruited on the street - was 17% among the 29 male injectors and 4.5% among the 156 male non-injectors. This study showed a higher prevalence among non-injecting heterosexuals from Surinam and the Netherlands Antilles than had been found among non-injectors in the cohort study (mostly Caucasians). However, underreporting of injecting and homosexual contacts in the Surinamese/Antillean group cannot be excluded as an explanation for this difference.

In The Hague - only 60 kilometers away from Amsterdam - the prevalence of HIV infection and risky injecting behavior was studied among drug users entering treatment in 1988. Only one of the 101 drug users in The Hague was infected with HIV, while among a group of 241 drug users who were studied in the same period in Amsterdam the prevalence was 27%. However, with regard to risky injecting, no differences in frequency of borrowing or lending of used needles and syringes were found between the two groups.[13]

By combining data collected in the cohort study (HIV prevalence, HIV incidence, incidence of AIDS and the mortality rate) with data on the number of notified cases of AIDS, we estimated the cumulative number of HIV-infected IDU in Amsterdam. As of July 1 1991, the estimated cumulative incidence of HIV infection among IDU in Amsterdam was about 1050. Ninety of these cases had been diagnosed with AIDS at July 1 1991, while an estimated 150-200 HIV-infected IDU died before AIDS had been diagnosed. The number of HIV-infected IDU residing in Amsterdam at that date who were still alive and free of AIDS was estimated to be about 750-800. The prevalence of HIV-infected IDU (being alive and free of AIDS) living elsewhere in the Netherlands was roughly estimated at about 500.[32]

There is some overlap between the risk group of drug users and the risk group of homosexual men. By retrospectively determining the serostatus of IDU taking part in the cohort and comparing these data with the available seroprevalence estimates in the cohort of homosexual men, we found indications that HIV was introduced later among IDU than among homosexual men in Amsterdam. AIDS surveillance data confirm this difference: the first case of AIDS in Amsterdam among homosexual men was diagnosed in 1982, while the first case among IDU was not diagnosed until 1985, by which time 50 cases had already been diagnosed among homosexual men. From these data we concluded that HIV was probably introduced among IDU by IDU who had homosexual contact, although spread by IDU infected in other countries could not be ruled out.[31] Molecular epidemiological studies which were performed later indicated that the last explanation is probably correct. We found that HIV-1 variants in IDU were distinct from those found in homosexual men (and hemophiliacs).[37,43] The main difference was a silent mutation in a codon at the tip of the V3 loop of the envelope protein gp120. As this mutation is also found in other HIV infected IDU in Europe and the US it seems likely that the introduction of HIV-1 in the Amsterdam IDU occurred through sharing equipment with drug users from other countries.[42]

Prevalence and incidence of other infections

Infections with Hepatitis C Virus (HCV) appeared to be very common and were predominantly found in drug users with a history of injecting drug use. Seventy-four percent of the IDU had antibodies to HCV versus 10% in the non-IDU. Continuous and daily i.v. drug use in the previous five years were independent risk factors. Remarkable was that the annual incidence was quite stable at a very high level over the years - about 8% per year - , while the HIV incidence in our cohort tended to decrease. The behavior change described earlier, seemed to be insufficient to lower the incidence of HCV infection, which may be due to the higher prevalence of HCV among the injecting drug-using community.[9] Hepatitis B Virus (HBV) infections are also very common among drug users: 68% of the participants were found to be infected at entry and the incidence among the susceptibles was 3-5% per year. A study on risk factors for new infections indicated that HBV appears to be more heterosexually transmitted, while HCV and HIV are associated with injection behavior.[40] This observation, together with the possibility that HCV is more readily transmitted than HIV, indicates that the incidence of HCV infection is a highly sensitive, surrogate marker for the occurrence of risky injecting behavior. However, its value as a surrogate marker for the incidence of HIV infection is limited.[9] Unlike infections with HCV, infections with HTLV-1 and HTLV-2 were very rare: out of the 346 drug users tested, only 2 participants were infected with HTLV-1 and one subject (a male non-IDU from Surinam) with HTLV-2.[17]

Among 25 drug users who originated from HTLV-I endemic regions (e.g. Surinam) we studied the prevalence of HTLV-I (the cause of adult T-cell leukemia and tropical spastic paraparesis) and HTLV-II. No infections were found using serology and PCR in peripheral cells.[33]

We also studied the incidence of Sexually Transmitted Diseases (STD) and gynecologic disorders in prostitutes participating in our cohort study comparing HIV positive and HIV negative women.[54] HIV positive prostitutes were found to have a strong and significantly increased risk for primary genital herpes, recurrent genital herpes and recurrent genital warts; a moderately increased risk for gonorrhoea, trichomoniasis, vaginal candidiasis and genital ulcers of unknown etiology. Of these HIV related outcomes, the risk for recurrent genital herpes and genital warts were strongly associated with decreased CD4 counts. The excess morbidity of STD and gynecologic disorders in HIV infected women is clearly demonstrated in this study, emphasizing the need for easy accessible medical care for this group.

Sexual behavior

Studies on the heterosexual behavior of IDU with regard to their private (steady and non-steady) and commercial partners[6,10] showed that more than three-quarters of the study group was heterosexually active, especially the women, who apart from having private partner(s), often work as prostitutes. In the beginning of our studies condoms were infrequently used with private partners: only 20-30% of the IDU said they (almost) always used condoms with their private partners. But condom use by prostitute women with their commercial partners was much more common: 86% of the prostitute women reported (almost) always using condoms in vaginal contact. Despite frequent condom use, the majority of the prostitutes (84/104) contracted one or more STD in the period during which frequent condom use was reported.[6] A history of STD was common not only among drug users with a history of prostitution, but also among IDU without such a history. A history of gonorrhea was reported by 29% of the non-prostitute men, syphilis by 6% and genital herpes by 10%. For non-prostitute women these percentages were 33%, 11% and 11% respectively.[10] IDU who knew that they were infected with HIV tended to have fewer commercial partners and to use condoms more frequently with private partners than IDU who were negative or not yet tested for HIV. Despite this, approximately half the infected IDU having private partners used condoms rarely or not at all in vaginal intercourse with these partners.[10] We conclude from our studies on sexual behavior that limited changes in sexual behavior occur, that over time a large turnover is found in IDU who report having steady, casual or commercial partners and that the potential for HIV transmission from IDU to drug-using and non-drug using partners is clearly present.[10,11,30,59] As STD are frequently diagnosed among drug users and STD facilitate the transmission of HIV, drug users should be regularly checked for STD especially the women since STD in this group are often asymptomatic.[60]

A history of prostitution was frequent not only in female drug users in Amsterdam[6] but also in male drug users[18]; 20% of the male participants reported having had sex with men in exchange for money. No differences were found between injecting and non-injecting drug users. Younger age, German nationality and previous private homosexual contacts were independent predictors of a history of male prostitution. No evidence was found to suggest that male prostitution in itself contributed to the risk of HIV infection. Previous, predominantly homosexual *private* sex contacts, frequency of needle sharing and prolonged injecting drug use were the independent predictors of HIV infection.[18]

We evaluated whether under the influence of the AIDS/HIV epidemic the sexual risk behavior of drug using prostitutes and the incidence of sexually

transmitted diseases (STD) in this group had changed over the years. In a group of 281 drug using prostitutes participating in our cohort study, the number of commercial contacts declined over time, always using condoms increased (as measured at intake) and the reported STD incidence declined. From these data we concluded that drug using prostitutes have reduced their sexual risk behavior.[48] One of the drawbacks of this study was that it was based on self report of STD and this may be subject to bias for several reasons. To validate the decrease in reported STD among drug using prostitutes, we compared self reported STD with clinic diagnoses obtained from our STD clinic.[55] This study showed that self report of STD is indeed unreliable and both underreporting and overreporting was frequently found. Therefore in future studies preferably diagnosed STD should be used to monitor the trend in sexual behavior in risk groups like prostitutes.

Risk reduction and evaluation of prevention programs

During follow-up a strong reduction in borrowing and lending of used needles and syringes was observed and this behavioral change was not dependent on being informed of HIV serostatus.[7] Over time, the use of the needle and syringe exchange program increased. However, reduction in needle sharing was less seen among new entrants to the study. Therefore, we concluded that the risk reduction observed during follow-up was mainly an effect of the study, with the exchange program only having a limited impact. Despite this risk reduction, the number of new HIV infections declined less than among homosexual men.[19,28] This relatively high incidence of new HIV infections reflects the fact that, despite the previously described risk reduction, risky injecting behavior among seronegative drug users is still highly prevalent. Approximately 30% of the HIV-seronegative current IDU participating in the study in 1989-1990 reported having injected drugs in the past 4-6 months at least once with a needle previously used by someone else.[24]

A substantial decline in injecting risk behavior (borrowing and lending of used equipment and re-using needles and syringes) was observed among the participants of our cohort study at intake over the years 1986-1992.[47] Indications were found for a protective effect of voluntary HIV testing and counselling on all above mentioned high risk behaviors and from the needle and syringe exchange program on re-using injection equipment. It appeared that attenders of methadone or exchange programs had reduced borrowing and lending to the same extent as non-attenders. Therefore, methodologically, evaluation of specific measures remains difficult. The combination of preventive measures in Amsterdam - and elsewhere[59] - is likely to be responsible for the observed decline in injecting risk behavior[49]. However, the HIV incidence is still rather high (see above), indicating that new and innovative approaches to prevention among IDU are badly needed.[36]

We and others found evidence that testing and counselling had a beneficial effect on risk behavior among IDU. As concern was raised about possible adverse effects of HIV testing and counselling, we investigated whether notifying IDU of their positive HIV serostatus contributes to suicide and overdose mortality risk. Although the suicide/overdose mortality rate was higher among HIV positive IDU than among HIV negative IDU, notification of a positive test result did not lead to a sudden and temporary rise in suicide/overdose mortality.[51] The difference between positives and negatives in suicide/overdose rate is likely to result from other factors and therefore we concluded that, provided that appropriate counselling is offered, there is no reason to discourage voluntary HIV test result notification. In order to learn which subgroups of IDU underwent testing and counselling we studied the characteristics of IDU who reported a previous test result at enrolment in our cohort study.[56] Over the years the proportion of IDU previously tested increased. IDU at high risk for HIV infection - e.g. foreign IDUs, prostitutes, heroine users, IDU with a positive partner, IDU with a history of pneumonia - appeared to be more often previously tested. Assuming that testing and counselling has a protective effect on risk behavior, voluntary testing of IDU should be more actively promoted taking into account the characteristics identified for testing and non-testing.

In the USA, there is an increase in the risk of HIV infection due to increasing cocaine use. Cocaine injecting has been shown to be related to risky injecting behavior and to HIV seropositivity. Smoking or inhaling cocaine appears to be related to risky sexual behavior and to HIV infection.[12] It seemed, therefore, relevant to monitor drug use trends, especially with regard to cocaine use. From 1985 - 1989 we found that heroin smoking and cocaine freebasing had increased in later intake groups of IDU, while there were no significant changes in injecting variables. No relationship between sexually risky behavior and cocaine freebasing was found. HIV seropositivity was less among cocaine freebasers (19%) than non- freebasers (32%). The increase in cocaine freebasing among these IDU, all primary opiate users, was not found to lead to an increase in sexually risky behavior, while it may diminish the spread of HIV through less sharing of needles.[14]

In Amsterdam a special approach towards drug users, called "harm reduction", has been developed.[8] The goal of harm reduction is "to create a situation that greatly reduces the risk of addicts harming themselves or their environment". As part of this approach "low-threshold" methadone programs and a large-scale needle/syringe exchange program have been implemented.[53] Therefore, we were interested to see if we could find evidence that

participation in the low-threshold methadone programs and the exchange program reduces the spread of HIV among injecting drug users. Long-term, regular participants in low-threshold methadone programs (LTM-users) were compared to short-term and/or irregular participants. After controlling for possible confounders (demographic and drug use variables), LTM-users had a slightly increased risk of HIV infection, which was not statistically significant. With regard to current use, LTM-users are as likely as non-LTM-users to inject daily and share needles as often.[22]

This finding was confirmed in a study assessing risk factors for seroconversion to HIV.[28] No evidence was found that receiving daily methadone at methadone posts reduced the chance of becoming infected with HIV. Neither did this study find evidence of a protective effect of obtaining needles and syringes via the exchange program. However, data suggested that exchanging needles and syringes may have been protective at the start of the program. This latter finding is in agreement with an earlier study on the impact of the needle and syringe exchange program in Amsterdam on injecting behavior.[2,5]

In general we can conclude that the HIV/AIDS epidemic has had a considerable impact on the risk behavior of injecting drug users over time. In several studies we tried to evaluate whether this risk reduction is linked to specific intervention measures. It should be taken into account that the best way to evaluate such intervention programs, is a true experiment with random allocation of drug users to participate or not participate in such programs. This allocation is not feasible in Amsterdam because of the easy accessibility of intervention programs in line with the harm reduction approach. Therefore, in our studies we usually compared self-selected attenders with non-attenders, which has several methodological drawbacks, making it sometimes difficult to reach definite conclusions on the value of specific intervention programs.[50] However, we conclude that the combination of the various intervention programs results in a considerable risk reduction.[47,59]

Determinants of risky injecting and sexual behavior

In 1989-1990, current needle sharing did not differ significantly between exchangers and non-exchangers. Factors related to needle sharing were previous needle sharing, relatively prolonged moderate-to-heavy alcohol use, current cocaine injecting and not having steady housing.[24]

In another study on determinants of risky injecting behavior[23], it was found that psychopathology or stress among HIV-positive IDU was not associated with lending of used needles to others. Among HIV-negative IDU, psychopathology, but not stress, was associated with an increased HIV-risk.[15] Even if the measured psychopathology is secondary to injecting, this suggests that HIV risk reduction programs should consider more seriously the fact that psychopathology may hamper risk reduction. We also assessed beliefs, attitudes and intentions with regard to HIV risk behavior among HIV-positive drug users.[21] Over a period of approximately four months, 20% of the HIV-positives put others at risk of HIV infection, mainly through unsafe sex. Forty-nine percent think they might infect someone with HIV in the future, again mainly through unsafe sex. Most unsafe sex was reported by female prostitutes (either with commercial or private drug users) and by non-Dutch drug users. Although the majority of these HIV-positives intend to use condoms, self-efficacy and response efficacy is low; i.e. many do not think they are able to use condoms consistently, and many have limited confidence in the efficacy of condoms in preventing HIV transmission.

High risk behavior among IDU has been reduced under the influence of the HIV/AIDS epidemic, but a total elimination of risk behavior has not been achieved. A more fundamental preventive measure may be to keep DU from starting to inject at all. In our cohort we studied the rates of transition from non-injecting to injecting drug use and the risk factors for this transition.[46] Among DU who had never injected drugs 30% began injecting within 5 years and among those who had had their last injection 1-5 years before entering our cohort, 70% started injecting again within this period. Risk factors for an increasing risk to start injecting were: previous injecting history, ethnicity other than Surinamese/Antillean, regular long term use of cocaine, current use of heroin and a current steady relationship with a partner who injected. Given the high and stable incidence of initiation of injection among DU and the fact that the risk factors identified in this study cannot be directly translated into practical prevention strategies, the prevention of the transition from non-injecting to injecting drug use appears to be difficult.

Natural history of infection

To investigate whether drug use affected immunological parameters, we conducted a cross-sectional study. Absolute numbers of CD4+ lymphocytes and T-cell reactivity were lower in HIV-positive than in HIV-negative people. The functional capacity of the T-cell system as measured after stimulation with a monoclonal antibody directed against CD3 was found to be strongly associated with the frequency of injecting, while no relationship was found between the frequency of injecting and the total number of lymphocytes or T-cell subsets. HIV-negative and HIV-positive drug users who injected a mean of three times a day in the preceding months had a T-cell reactivity which was

40-50% lower than that of seronegative and seropositive drug users who had not injected in the preceding months.[16]

The clinical symptoms associated with seroconversion for HIV-1 were studied among eighteen IDU who seroconverted.[38] Five out of 18 (28%) were admitted to hospital for bacterial pneumonia while one subject suffered from esophageal candidiasis. For comparison none of the 27 homosexual men who seroconverted for HIV, three out of 177 (2%) drug users negative for HIV and 10 of 112 (9%) drug users positive for HIV reported bacterial pneumonia. Other clinical symptoms did not differ between drug users who seroconverted and those who remained negative for HIV. We concluded from this study that drug users who are admitted to hospital for bacterial pneumonia should be tested to detect primary HIV-1 infection.

Some studies have reported antibody silent periods of several years after the moment of infection. As such a long window period would have serious consequences e.g. for the detection of HIV infected blood donors, we analyzed samples from 23 HIV negative IDU who seroconverted during follow-up.[41] Polymerase Chain reaction (PCR) was used to detect early HIV infection. In five of the 23 seroconverters the PCR was positive (in plasma, cells or both) in the sample preceding the first antibody positive serum. In 3/5 the p24 antigen test was also positive. In all five individuals the second last antibody negative sample (148-266 days before antibody seroconversion) was PCR negative. None of 40 seronegative IDUs at high risk of infection were found PCR positive. We concluded from this study that silent HIV infection for a limited period is observed in IDUs and has to be taken into account. In a separate study in a smaller group of seroconverters we found that antibodies to the regulatory gene product nef could only rarely be detected preceding antibody detection to structural proteins.[1]

In a study on progression of HIV infection among 126 IDU progression seemed to be slower among IDU who borrowed injecting equipment more often.[39] The relative hazard for an IDU who had borrowed more than 100 times was 0.16 compared to an IDU who had borrowed less than 10 times, corrected for the CD4 count, p24 status and age. A low CD4 count at baseline, p24 positivity or core antibody negativity and age over 30 were associated with faster progression. From this study it seemed that progression among IDU is slower than the progression among homosexual men (HM). A study about the prevalence, incidence and predictive value for progression to AIDS of the HIV-1 syncytium-inducing (SI) phenotype in HIV infected IDU and HM also implicated a difference in pathogenesis and natural history of HIV infection linked to transmission group.[58] In this study we found a remarkable lower prevalence and incidence of the SI phenotype among IDU compared to HM, although in both risk groups the SI phenotype was found to be associated with accelerated CD4 decline and progression to AIDS.

The reported high prevalence of thrombocytopenia among HIV infected DU could be confirmed.[27] The prevalence of thrombocytopenia among HIV positive DU was 34% compared with 9% in HIV negative DU. Among HIV positive homosexual men the prevalence of thrombocytopenia was 16% and in HIV negative HM 3%. Independently associated with thrombocytopenia were: HIV seropositivity, a history of injecting drugs, an increased number of lymphocytes and neutrophils and a larger mean platelet volume. Thrombocytopenia is sometimes treated with splenectomy and we found that in HIV infected IDU splenectomy results in an elevation of both the CD4 and the CD8 count.[44] Therefore in splenectomized HIV infected patients the CD4 percentage and not the absolute count should be used to monitor progression of the HIV infection.

Since CD4 counts and T cell function are employed as markers for progression we studied the diurnal variation of these markers among DU.[25,45] CD4 counts increased by 37% in eight hours while the T-cell reactivity increased by 93%. The diurnal variation among DU appeared to be higher than reported among other HIV risk groups. Therefore sampling time should be registered and taken into account in the analysis of these markers.

We examined the influence of HIV infection on morbidity and non-AIDS mortality in IDU without AIDS participating in our cohort study. A high and rising incidence of bacterial pneumonia was found without a consequential rise in non-AIDS mortality. This contrasts with reports of studies conducted in New York City, where bacterial pneumonia-related mortality has been found to increase markedly, coincident with the AIDS epidemic. Early detection of bacterial pneumonia and easy access to both inpatient and outpatient medical care as it exists for drug users in Amsterdam may be important factors in preventing early death due to common bacterial pathogens in IDU without AIDS.[26]

Several studies indicate that mortality without AIDS diagnosis (pre-AIDS death) is quite common among HIV infected IDU. We quantified pre-AIDS death among IDU and compared it with that among homosexual men.[52] After 6.5 years of follow-up an estimated 44% of 455 HIV infected HM participating in the cohort study among HM had been diagnosed with AIDS and 1% had died without an AIDS diagnosis, while 33% of the 279 HIV infected IDU participating in the cohort study among IDU had been diagnosed with AIDS and 20% had died without an AIDS diagnosis. The high pre-AIDS mortality among IDU has important consequences for HIV infection epidemic modellers and will limit the number of recorded cases of AIDS in this risk group. If not taken into account in the models the extent of the HIV epidemic among DU will be underestimated.

Since 1993 recurrent pneumonia and pulmonary tuberculosis are included in the European/US AIDS case definition. The impact of this change

was studied in a cohort of 153 HIV infected IDUs and 502 HIV infected HM.[57] As expected this changed definition will strongly increase the number of persons diagnosed with AIDS among IDU but will hardly influence the AIDS incidence among HM.

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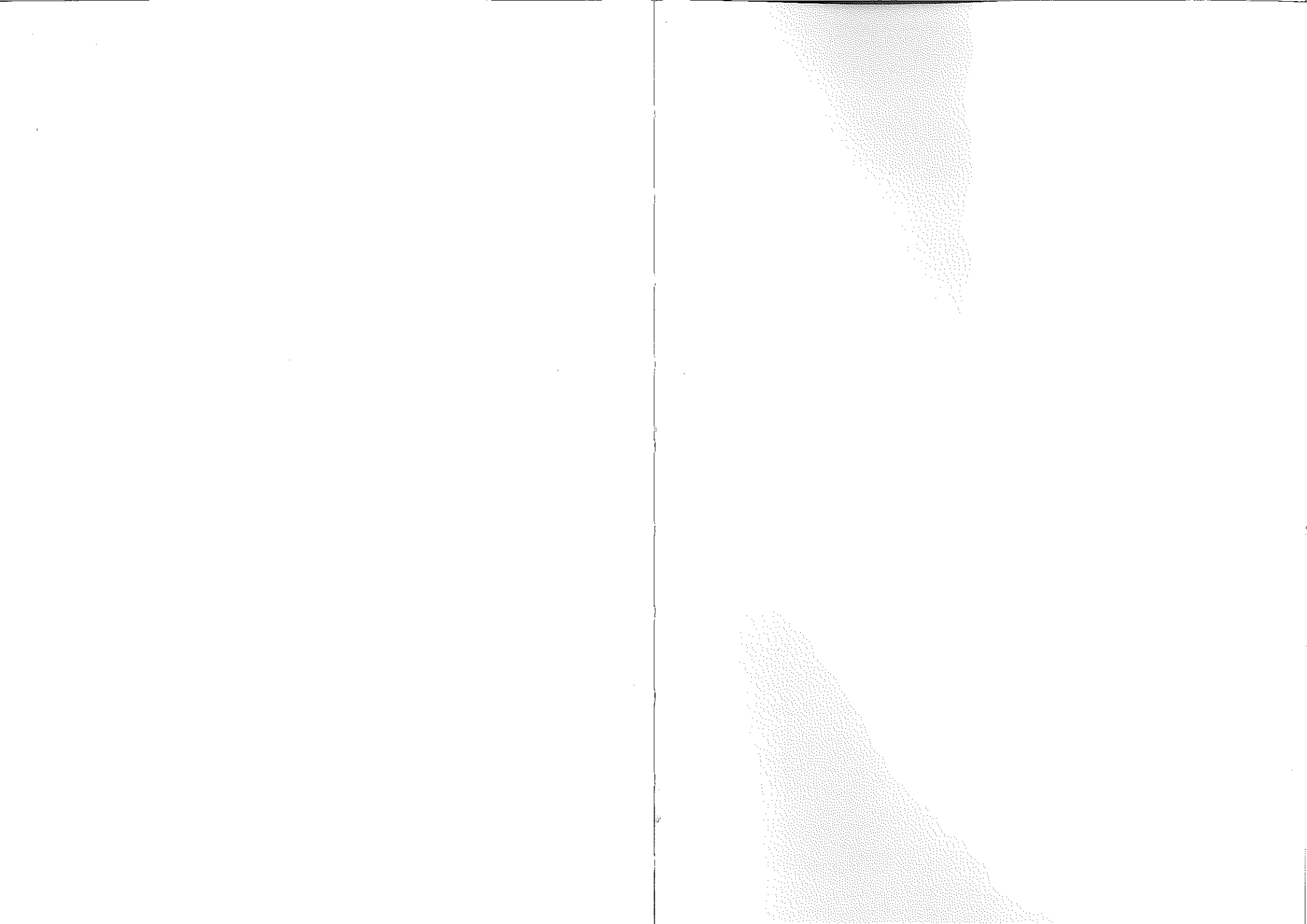

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