

The Amsterdam Cohort Studies on HIV infection and AIDS

A summary of the results 2001-2009



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Colophon

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PROLOGUE

This is the fourth overview of the Amsterdam Cohort Studies (ACS) on HIV infection and AIDS, this time covering the period between 2001 and 2009. Chapter One describes the framework of the ACS, including the collaborating institutes, daily routines and various sub-studies. In addition to the original cohorts of men who have sex with men and drug users, the ACS now also encompass two new cohorts: the HIV-infected and HIV-exposed children and the primary HIV infection cohort, each a valuable addition to the studies. Moreover, the ACS OPEN project is making the multidisciplinary ACS data more accessible for the scientific community. Highlights of the results obtained within the ACS framework by the collaborating institutes are summarized in Chapter Two, and Chapters Three and Four list the 260 scientific publications and 44 PhD theses covering these results. Finally, Chapter Five outlines the future plans for the studies.

This compilation of the research results of the years 2001 to 2009 could not have been done without the help of many of the collaborators at the participating institutions, including Will Maruanaya, Margreet Bakker, Ben Berkhout, Marion Cornelissen, Bill Paxton, Debbie van Baarle, José Borghans, Ingrid Schellens, Taco Kuijpers, Henriëtte Scherpbier, Peter Reiss, Miriam Casula, Jan Prins, Marlous Grijzen, Frank de Wolf, Rosalind Beard, Evelien Bunnik, and Neeltje Kootstra. Special thanks go to Anneke Krol and Ineke Stolte for their major contribution to chapter one, to Ellen Kwak for excellent assistance and to Maria Prins, Lia van der Hoek and Hanneke Schuitemaker for critical reading and valuable input on all chapters.

As the studies originally started in 1984, this booklet also marks the 25th anniversary of the ACS. The overview presented here again illustrates the power of the ACS multidisciplinary approach to provide valuable new insights and the continued value of the samples and data collected over the past 25 years.

(ABW, October 2009)

chapter one

Cohorts

1



INTRODUCTION TO THE AMSTERDAM COHORT STUDIES

The Amsterdam Cohort Studies (ACS) on HIV infection and AIDS started shortly after the first cases of AIDS had been diagnosed in the Netherlands. In October 1984 men who have sex with men (MSM) were enrolled in a prospective cohort study. Approximately 10% of the male population in Amsterdam is homosexual and due to earlier field studies and prevention activities concerning sexually transmitted infections (STI), good relationships had been established with these men, facilitating recruitment. A second cohort among drug users (DU) was initiated in 1985. Enrolment and follow-up of DU is possible due to the well organized healthcare system for DU in Amsterdam. This system enables access to the majority of city's opiate dependent DU. In 2009, the cohorts have reached 25 years of follow-up. Over 4000 men and women have participated in the ACS. The ACS have been conducted in accordance with the ethical principles set out in the declaration of Helsinki, and ACS participation is voluntary: written informed consent (most recent version approved by the AMC Medical Ethics Committee in 2007 for the MSM cohort and in 2009 for the DU cohort) is obtained for every participant.

ACS FRAMEWORK

Within the ACS, different institutes collaborate to bring together the data and biological sample collections. These are the Public Health Service of Amsterdam (PHSA) (Cluster Infectious Diseases, Department of Research), the Academic Medical Center (AMC) of the University of Amsterdam (Departments of Medical Microbiology, Experimental Immunology, and Internal Medicine, and the International Antiviral Therapy Evaluation Center) and the Jan van Goyen Medical Center (Department of Internal Medicine). Until 2007, collection of blood cells was performed at the Sanquin Blood Supply Foundation, but this activity has moved to the AMC, to the Department of Experimental Immunology. However, the Sanquin Blood Supply Foundation is still affiliated with the ACS. From the beginning, research in the ACS has had a multidisciplinary approach (epidemiology,

social science, virology, immunology and clinical medicine). This unique collaboration has been very productive, significantly contributing to the knowledge and understanding of many different aspects of HIV-1 infection (see also collaborating institutes below). This expertise has contributed directly to advances in prevention, diagnosis and management of HIV infection. There are also many collaborations between the ACS and other research groups both within and outside of the Netherlands.

The initial aim of the ACS was to investigate the prevalence, incidence and risk factors of HIV-1 infection and AIDS, the natural history and pathogenesis of HIV-1 infection, and the effects of interventions. Over the past 25 years, these aims have remained mostly the same although the emphasis of the studies has changed. Early on, the primary focus was to elucidate the epidemiology of HIV-1 infection, while later on more in-depth virological and immunological studies were performed to investigate the pathogenesis of HIV-1 infection. In recent years, focus has also shifted to include the epidemiology and the natural history of other blood-borne and sexually transmitted infections among the participants of the ACS.

Previously, three overviews were published of the results of the ACS in the periods 1984-1992 (separate overviews for MSM and DU), 1984-1996 and 1997-2000. The booklet presented here, which was compiled by A.B. van 't Wout with input from all collaborating institutes, gives an overview of the most important research outcomes over the subsequent period (2001-2009), along with a listing of all publications and PhD theses over these years. It also commemorates the 25th anniversary of the Amsterdam Cohort Studies on HIV infection and AIDS.

ACS AMONG MSM

The study population consists of MSM living mainly in and around the city of Amsterdam, The Netherlands. **TABLE 1** shows the numbers of participants in the ACS among MSM and its sub-studies. The first wave of enrolment took place between October 1984 and April 1985 (Protocol 1). Included were



symptomatic MSM aged 18-65 with at least two sexual partners in the six months prior to intake. These men were recruited through announcements in the gay press, advertisements and by word of mouth. Between April 1985 and February 1988 only seronegative men could enter the study (Protocol 2). Enrolment was re-opened to HIV-1 infected individuals from February 1988 until December 1998 (6000 numbers). Some of these participants entered the ACS because they were found to be HIV positive while participating in another study of the PHSA or to start with antiretroviral treatment (early zidovudine trials). In February 1996, the follow-up of the 'old' HIV seronegative participants was terminated. In June 1995, a special recruitment campaign was started among young (≤ 30 years) MSM (JOHO). From April 2005 MSM of all ages are invited to participate. This study is still ongoing and in 2007 the research protocol has been updated and new informed consents were obtained, also including provisions for genetic research. A few participants entered the ACS but could not be classified in any of the abovementioned studies (9000 numbers) or were allowed to start their anti-retroviral treatment within the ACS from February 1997 onwards (7000 numbers).

In February 1999, follow-up and treatment of all HIV-infected participants was transferred to the Jan van Goyen Medical Center as part of the Netherlands HIV Monitoring Foundation (SHM, formerly National Athena Monitoring Project). In October 2003, the ACS initiated the HIV positive protocol (HOP, HIV study among recent HIV-positive MSM) for MSM who seroconverted or were HIV-positive at study entry in the cohort of young MSM after 1999. From June 2006 specific individuals with a self reported high or low risk for HIV-1 infection, as well as HIV-positive steady partners of HIV-negative participants and all steady partners of HIV-positive participants are invited to participate in the ACS. On average, 90% of MSM who visited the ACS in a given calendar year, returned the next year as well. **TABLE 2** shows the current number of MSM in follow-up.

Daily routine MSM

HIV-positive cohort participants are seen every three months. Clinical,

epidemiological and social scientific data are collected with standardized questionnaires (six monthly) and by physical examination. Blood is taken for virological and immunological tests and for storage (**TABLES 3 AND 4**). HIV-negatives are seen by a study nurse every six months and similar data are collected, but immunological tests and cell storage is only performed for selected groups of individuals: specific individuals with a self reported high or low risk for HIV-1 infection (n=20 reporting high risk and n=10 reporting low risk) and HIV-negative partners of HIV-infected cohort participants (n=25). In October 2008, 6-monthly screening of STI (Chlamydia, Syphilis, Gonorrhea and Hepatitis C Virus (HCV) - the latter in HIV positives only) of all current ACS participants started in close collaboration with the Sexually Transmitted Diseases (STD) clinic of the PHSA.

In the period before 1999, HIV-infected MSM who participated in the ACS and who developed an AIDS event during follow-up were referred to the AMC for treatment and much effort has been put into aligning the AMC and the ACS registry regarding events (clinical follow-up). Until 2000, AIDS cases were also ascertained through cross-linking with the Amsterdam AIDS registry. Once a year information on survival status is obtained through active follow-up and matching the ACS data against the local population registries and the SHM. The cause of death is obtained from the Amsterdam AIDS surveillance registry (until 2000), hospital records and from next of kin.

ACS AMONG DU

Participants are recruited at methadone outposts, the weekly STD-clinic for drug-using prostitutes, and by word of mouth. HIV-negative and asymptomatic HIV-positive injecting and non-injecting DU (IDU and non-IDU) using hard drugs (i.e. heroin, cocaine, and methadone) at least three times per week are invited to participate. **TABLE 5** shows the number of participants in the ACS among DU and its sub-studies. The first wave of enrolment took place between December 1985 and September 1990 when inclusion stopped until August 1991. Enrolment was then re-opened and recruitment is still continuing. In 1998, a special recruitment campaign was



started among young DU (≤ 30 years). Although this was a cross-sectional study design, a quarter is being followed in the DU cohort. Again, in June 2000, much effort was put into recruiting young DU (JODAM study). The research protocol has been updated in 2009 and new informed consents are currently being obtained, including provisions for genetic research (Protocol 3). From July 2009, young DU (≤ 30 years) and recent injecting DU of all ages are recruited and invited to participate in the ACS.

Daily routine DU

Regardless of HIV status, until 2003 all participants were seen every four months, thereafter every six months, but many return more irregularly. Clinical, epidemiological and drug use related information is collected at each occasion by interviewing participants using a standardized questionnaire. In April 1989 this questionnaire was thoroughly revised and all participants were physically examined by a physician at each visit. In January 1999 this examination was terminated for the HIV-negatives. Blood is taken for virological tests and cryopreservation and from April 1989 immunological tests are part of the daily routine in HIV-negative as well as HIV-positive participants. Since 1995 a limited number of tests are performed in a small subset of the HIV-negatives.

Data on hospitalization are collected at each visit from the participants, independently and through the clinics of the Cluster for Mental Health of the PHSA. As of 1997 much effort has been put into aligning hospital and ACS event registration for all seroconverters and DU participants using highly active antiretroviral therapy (HAART). Clinical data are also ascertained through cross-linking with the Amsterdam AIDS registry (until 2000) and the SHM. After AIDS diagnosis DU can still participate. Yearly, deaths and causes of death are identified by determining participants' status at the register of population in their city of residence and through locating and examining coroners' reports and medical records from the Cluster for Mental Health of the PHSA, hospitals, and general practitioners. On average, 90% of DU that visited the ACS in a given calendar year, returned the next year as well. **TABLE 6** shows the current number of DU in follow-up.

INTERVENTION, VACCINATION, SUB-STUDIES

Starting in 1987, with a preliminary study of zidovudine in asymptomatic HIV-infected participants, ACS has participated in several early multi-centered trials of antiretrovirals. As of 1999, the SHM monitors all HIV-infected patients, including ACS participants, attending their treating physician regularly in one of the 24 HIV treatment hospitals throughout the country. As per mid 2009, data obtained during those on average 3 monthly visits are collected from in total more than 15,000 patients, with more than 12,000 of them in actual follow-up. Using the data from this so-called ATHENA observational cohort, SHM performs studies on the effect of antiretroviral treatment on the HIV infection and on the HIV epidemic in the Netherlands. It includes studies on viral and host co-factors, HIV resistance, changes in HIV transmission potential and changes in risk behavior in both homosexual and heterosexual risk groups.

In addition to studies on anti-HIV treatment, a specialized multidisciplinary unit for the treatment of chronic HCV infections in DU was established at the PHSA in 2005. Since this approach was proven to be successful for ACS participants, in 2007 HCV care and treatment is offered to all HCV-infected DU in Amsterdam.

ACS has also participated as a study-site in three vaccination studies. Recruitment of the P24-HIV and rgp120 study among HIV positives started between March 1993 and March 1994. The Vaxgen (multi-centered) double-blind placebo-controlled study among high risk HIV-uninfected MSM men started in 1999 and ended in 2002.

Several sub-studies have been done within the ACS. Specific subgroups of participants were tested for evidence of infection with other viruses (Epstein Barr Virus (EBV), GB Virus C (GBV-C), Hepatitis A Virus (HAV), Human Herpes Virus 8 (HHV8), Human T-Lymphotropic Virus Type 1 and 2 (HTLV-1 and -2). In 2003, a large back-testing effort was initiated for evidence of infection with HCV, Hepatitis B Virus (HBV), and Herpes Simplex Virus type



1 and 2 (HSV-1 and -2): All ACS participants in the MSM and DU cohorts with at least two visits between the start of the ACS and 2002 were tested retrospectively using the first and/or last sample available in each case. On finding seroconversion (defined as the presence of virus-specific antibodies in a previously seronegative individual), samples taken between these two visits were tested to determine the seroconversion interval. In addition to these virological sub-studies, also in-depth interviews were held with specific subgroups (e.g. qualitative research to investigate the factors facilitating initiation of cocaine and heroin among young DU).

PRIMO-SHM STUDY

In addition to the cohorts mentioned above, the ACS are now also including patients who present with primary HIV-1 infection at the outpatient clinic of the AMC in the so called primo-SHM study. Some of these patients are seronegative men who seroconverted in the MSM cohort at the PHSA. Some of them are still also followed in the HOP protocol of the ACS at the PHSA. The primo-SHM study is a randomized study on the effect of early quadruple antiviral therapy as compared to no therapy. As of September 2009, 270 primary HIV infection patients have been recruited for this study, of whom 157 patients have been included in the randomized study. Inclusion is still ongoing. Sampling is more frequent early after entry into the study. Follow-up of individuals randomized to the no-treatment arm is discontinued 1 year after the start of HAART caused by a CD4+ T cell decline to <350 cells/µl blood. Similarly, follow-up is discontinued 1 year after re-initiation of HAART for individuals who have to reinitiate therapy because of a CD4 decline to <350 cells/µl blood after scheduled interruption of the first HAART regimen that started during the primary infection phase.

HIV-INFECTED AND HIV-EXPOSED CHILDREN

At the Emma Children's Hospital in the AMC, both HIV-infected and HIV-exposed children are in follow up (**TABLE 7**). Data from both groups are collected by the SHM and collaborations with the Departments of OB/GYN

and Experimental Immunology at the AMC exist to study factors involved in neonatal HIV-1 transmission. Of the 59 HIV-infected children currently in follow up, 58 were infected with HIV-1 and 1 with HIV-2. Two patients were co-infected with HBV. The HIV-1 infected children are included in the Pediatric Amsterdam Cohort on HIV-1 (PEACH). The HIV-exposed children are studied in the context of the European Collaborative Study on Mother-to-Child Transmission (MTCT) of HIV (ECS), an ongoing birth cohort study recently merged with the Pediatric European Network for Treatment of AIDS (PENTA).

ACS OPEN PROJECT

Over the past 25 years vast amounts of data on social-scientific, demographic, clinical, and biomedical information have been obtained from the participants of the ACS by the different collaborating institutes. In 2005, the “ACS Open” project group, composed of data-managers and scientists from all participating research groups, started to connect these data sets and built an easily accessible multidisciplinary database that comprises all longitudinally obtained epidemiological, social-scientific and biomedical information and contains data regarding the availability of stored samples in the repositories. In 2010 these data sets will be available for scientists in the collaborating institutes and their collaborators.

The ACS data are also very suitable for universities and research institutes to teach students in epidemiology, biomedicine, and social science how to analyze longitudinal data sets. Therefore, a multidisciplinary data set containing limited information has been made available for general use and launched on the Internet (www.amsterdamcohortstudies.org).

COLLABORATING INSTITUTES

Department of Research, Cluster Infectious Diseases, Public Health Service of Amsterdam

Project leader Dr. M. Prins

The PHSA research focus is mainly on the prevalence, incidence and determinants of HIV infection and related risk behavior in MSM and DU. Also

psychosocial scientific studies concentrating on understanding behavioral trends and determinants of sexual behavior are done, as well as studies on the clinical course of HIV infection. In the past 10 year the research line has been extended with research on other sexually-transmitted and/or blood-borne infections with a main interest in HCV infections, focusing on prevention, (molecular) epidemiology, clinical outcomes and the interaction with HIV.

Department of Medical Microbiology, Academic Medical Center, University of Amsterdam

Project leader Prof. Dr. B. Berkhout

The Virology Laboratories of the Department of Medical Microbiology focus their studies on the HIV-1 and HCV characteristics that are related to immune evasion, drug-resistance, viral fitness and pathogenesis. In particular, the onset of the HIV-1 infection and the benefit of early short HAART regimens, frequency and effect of HIV-1 dual-infections, characteristics of HIV-1 latency, HCV infection within the context of HIV-1 infection, discovery of unknown pathogens, and early markers for therapy failure are under investigation.

Department of Experimental-Immunology and Sanquin Landsteiner Laboratory, Academic Medical Center, University of Amsterdam

Project leader Prof. Dr. H. Schuitemaker (Coordinator ACS)

Research in the Department of Experimental Immunology and the Sanquin Landsteiner Laboratory at the AMC related to the Amsterdam Cohort Studies focus on HIV-1 evolution in relation to host mediated selection pressure and additionally on the role of HIV-1 phenotype variation and host genetic factors in AIDS pathogenesis. In the past 5 years, in depth studies on HIV-1 specific humoral immunity have been initiated, results of which will be relevant for vaccine design.

Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam

Project leader Prof. Dr. J.M. Prins

The Department of Internal Medicine coordinates the Primo-SHM study, a multicenter, open-label, randomized, semi-factorial clinical trial that compares a course of early HAART (24 or 60 weeks) with no treatment. Patients are recruited in the Dutch HIV treatment centers. Analyses are directed at the effects of early treatment on viral load set point, rate of CD4+ T cell decline, and time that patients can remain off-treatment after early treatment, and immunological and virological explanations for differences between treated and untreated groups.

HIV Treatment Center, Emma Children's Hospital, Academic Medical Center, University of Amsterdam

Project leader Prof. Dr. T.W. Kuijpers

At the Emma Children's Hospital in the AMC, both HIV-infected and HIV-exposed children are in follow up. Research has a focus on the effect of antiviral therapy in children and on the biology of vertical transmission.

Section Clinical Viro-Immunology, Department of Immunology, University Medical Center Utrecht

Project leader Dr. D. van Baarle

Two main research lines are carried out at the Department of Immunology. One line focuses on T-cell dynamics and determinants of CD4+ T-cell depletion in both HIV-1 and HIV-2 infection. The other line focuses on determinants of protective HIV-specific T-cell immunity and the role of HLA background in T-cell efficacy. More recently, studies have been initiated into virus-host interactions in HCV infections.

HIV Monitoring Foundation

Project leader Prof. Dr. F. de Wolf

The HIV Monitoring Foundation (SHM) monitors all HIV-infected patients attending their treating physician regularly in one of the 24 HIV treatment hospitals throughout the Netherlands, including ACS participants who are tested positive for HIV. ACS and SHM perform collaborative studies analyzing and modeling HIV incidence and prevalence in the pre- and post-HAART era.



FOUNDERS

The ACS were originally founded as a collaboration between the PHSA (formerly known as the Municipal Health Center, projectleader Prof. Dr. R.A. Coutinho), the Sanquin Blood Supply Foundation (formerly known as the Central Laboratory of the Red Cross Blood Transfusion Service, projectleader Prof. Dr. F. Miedema), and the AMC Departments of Medical Microbiology (projectleader Prof. Dr. J. Goudsmit) and Internal Medicine (projectleader Prof. Dr. J.M.A. Lange), under coordinating leadership of Prof. Dr J. van der Noordaa. We are all greatly indebted to their vision.

chapter two

The results

2

THE HIV-EPIDEMIC

HIV in MSM

As of 1999, ACS participants are monitored by SHM when tested positive for HIV and both ACS and SHM perform studies analyzing and modeling HIV incidence and prevalence in the pre- and post-HAART era. A drastic change in the HIV-1 epidemic among MSM occurred with the availability of HAART in high income countries around the mid-nineties. HIV-related morbidity and mortality in these countries strongly decreased. However, unintended effects of HAART were that risk behavior and STI increased among both HIV-1 negative and positive MSM (inter)nationally (175), and also among MSM in the ACS (51, 178, 179, 218). Among HIV-infected MSM these increases were found to be associated with immunological and virological improvements due to HAART (52), safer sex burnout (176), and positive perceptions of the viral load (178), while among HIV-negative MSM HAART-related optimism predicted a change from safe to unsafe sexual behavior (48, 179). Despite increases in sexual risk behavior and STI, the HIV-incidence among MSM in the ACS remained relatively stable, around 1.06/100 person years (PY), from 1991 till 1997, but more recently slowly increased to 2.1/100 PY in 2008 (**FIGURE 1, TABLE 8**). This is in line with mathematical modeling conducted with ACS and SHM data, showing a resurgent epidemic among MSM, most likely predominantly caused by increasing sexual risk behavior (10).

In the early years after the introduction of HAART, most new HIV-1 infections among MSM in Amsterdam occurred within steady relationships (44, 257), steady partners therefore being an important target group. To address this, in the last years the psychosocial studies of the ACS have investigated high risk sexual behavior in steady relationships, its determinants and how to successfully reduce it (45, 46). The important role of primary infections in ongoing HIV transmission (258) stresses the need to increase prevention efforts to identify HIV-1 infections at an earlier stage.

HIV in DU

The trend in HIV incidence among DU in the ACS differed from that observed among MSM: HIV incidence has substantially declined to 0/100 PY in most recent years, accompanied by a reduction in injecting drug use and needle sharing (**FIGURE 2, TABLE 9**). This decline has occurred despite continued sexual risk behavior. The last seroconversions that did occur were mainly related to unprotected heterosexuals contacts (112). In contrast with MSM, there was no evidence among DU for increased injecting and/or sexual risk behavior after HAART initiation (169). However, potential HAART initiation was based on limited drug use.

Phylogenetic analyses among IDU from the European Seroconverter Study demonstrated migration of HIV strains across European borders and the increasingly heterogeneous virus populations, particularly among IDU in the Netherlands, Austria and Switzerland, which reflect the international character and travel behavior of these IDU populations (134).

With the harm reduction approach, the Amsterdam methadone programs have reached an estimated 2,700 of the 3,500 to 4,000 opiate users in Amsterdam. Full participation in a harm reduction program was associated with lower incidence of both HIV and HCV infection in ever-injecting DU, indicating that combined prevention measures might contribute to the reduction of spread of these infections (212). Specifically the provision of methadone in such a program was strongly related to decreased mortality from natural causes and from overdose (111). Among DU still alive, the estimated prevalence of abstinence for at least 4 months from drugs and methadone was only 27% at 20 years since initiation (184).

To increase knowledge on risk behavior, HIV-incidence and -prevalence among young DU, a longitudinal study among 210 young DU (<30 years) was initiated in 2000 (JODAM). Although injecting behavior in this group has strongly decreased over time, risk behavior was still considerable and HIV-prevalence was widespread among those who did inject (249). Moreover, although the prevalence and incidence of injecting were relatively low, it



was still an option for opiate users, especially for those who have a history of injecting drug use (28).

In-depth interviews among young DU participating in the ACS were conducted to investigate self-reported factors facilitating initiation of cocaine and heroin use (253), self-reported motives for and against injecting (255), and the unmet needs and perceived barriers to health care use (254). These studies revealed new insights which are hardly addressed in prevention and should inform new prevention initiatives and programs.

HIV clinical course

Pooled data from 22 cohorts - including the ACS among MSM and DU - showed that in the HAART era - compared to the pre-HAART era - the cumulative incidence for all AIDS-related causes of death decreased, but that AIDS-related opportunistic infections remained the most common cause of death, suggesting that AIDS-related events will continue to be important in the future (168). The decline in AIDS-related mortality was the result of both HAART and a decline in the HIV incidence (167).

The risk for progression to AIDS or natural death is similar across Western Europe, although IDU across Europe differ in other factors, such as the risk of non-natural death and timing of HAART initiation (198). Initial HAART response in DU was similar to MSM in the ACS, but DU started HAART at lower CD4+ T cell counts and higher viral loads and DU HAART response never reached the levels of MSM. Therefore, it is likely that HAART was less effective in the long term (169).

MARKERS OF DISEASE PROGRESSION

T cell counts and viral load

Although lower levels of CD4+ T cells and CD4+/CD8+ ratios were found during summer and spring, exposure to ultraviolet radiation does not seem to have a direct suppressive effect on immune parameters in HIV-infected persons (182). A study among a large cohort of European HIV-infected

women (discontinued in 2001) including DU women from ACS suggested that postmenopausal women have lower CD4+ T cells than premenopausal women, perhaps because of changes in the level of reproductive hormones. Pregnancy had no statistically significant effect on CD4+ T cell counts (206). Although women appear to have lower HIV RNA levels and higher CD4+ T cell counts shortly after HIV-infection compared to men, no substantial sex difference in the benefit of antiretroviral therapy was found (152). A collaborative study using data from 23 HIV seroconverter cohort studies including ACS, found that sex differences in HIV disease progression have become larger in the era of HAART (90). The effects of age and polymorphisms in the HIV coreceptor genes CCR5 and CCR2 were primarily mediated through CD4+ T cell count and viral load (64).

CD4+ T cell counts of HIV-infected subjects followed in a cohort in Ethiopia (Ethio-Netherlands AIDS Research Project (ENARP); discontinued in 2005) were remarkably lower than those of the ACS seroconverters in the Netherlands and this difference persisted during follow-up. HIV RNA levels were lower in Ethiopian seroconverters shortly after seroconversion, but subsequently increased to similar levels (158).

CD4+ T cell depletion and turnover

HIV-infection is characterized by chronic CD8+ and CD4+ T cell activation. There is accumulating evidence that high levels of immune activation in HIV infection are detrimental. A prospective cohort study found increased levels of CD4+ or CD8+ T cell activation or division to be independent predictors of progression to AIDS (75). Even signs of an activated immune system prior to HIV infection turned out to be related to an increased risk of development of AIDS (75) or rapid CD4+ T cell depletion (194) after HIV-1 seroconversion.

Despite low CD4+ T cell counts, no evidence was found for a faster progression to AIDS among Ethiopians compared to MSM from the ACS, presumably because of a slower decline in CD4+ T cell counts (120). Interestingly, subsequent research into immune activation levels in HIV-



infected Ethiopians revealed lower percentages of proliferating Ki67+ cells within the naïve and memory CD4+ and CD8+ T cell subsets compared to Dutch HIV-infected patients matched for CD4+ T cell count. Thus, the slower CD4+ T cell decline in HIV-infected Ethiopians may be explained by lower levels of immune activation.

Because high HIV viral loads often concur with high levels of immune activation, a small group of atypical long-term asymptomatic HIV-infected individuals (LTA) with high viral loads was studied. HIV-1 isolates from these LTA turned out to be as pathogenic as viruses from HIV-infected patients with normal rates of disease progression, but these LTA had lower levels of activated T cells than normal progressors (36), suggesting that the lack of immune activation prevented disease progression. In analogy, in a group of patients who initially responded well to HAART, but subsequently experienced increases in viral load, maintenance of CD4+ T cells was associated with low levels of immune activation (77). Collectively, these data support the hypothesis that persistent hyper-activation of the immune system during HIV infection causes erosion of the naïve T cell pool resulting in CD4+ T cell depletion.

Because of the correlation between CD4+ T cell depletion and CD4+ T cell division, it has alternatively been proposed that increased T cell production in HIV infection could reflect a homeostatic response to the progressive loss of CD4+ T cells. Using in vivo 2H₂O labeling, it was shown that newly-produced naïve CD4+ and CD8+ T cells in HIV-infected individuals are preferentially lost from the naïve T cell pool. Even in the memory T cell compartment, recently-produced T cells tended to be lost. These data suggest that the increased levels of T cell production during HIV infection are not a homeostatic response to the loss of CD4+ T cells; they seem to be a cause rather than a consequence of the chronic loss of CD4+ T cells.

VIRAL FACTORS

A new HIV-1 variant

A virus discovery program identified a novel HIV-1 variant distinct from the known subtypes (219). This virus was discovered in a Dutch patient from the ACS who encountered the virus before 1989, most probably via heterosexual contact in Africa. This new subtype X variant belongs to the major (M) group and has limited similarity with subtype K (FIGURE 3). Subtype X represents one of the many branches of the viral phylogenetic tree, apparently a branch that did not expand in the epidemic, possibly because of sub-optimal replication fitness.

Dual HIV-1 infections

The incidence of HIV-1 dual infections is generally thought to be low, but since dual infections are associated with accelerated disease progression, their recognition is clinically important. Several contributions were made in this relatively novel field over the years (220). First, a major breakthrough was the recognition that generally available HIV-1 genotyping data can be used to screen for double infections (40). The HIV-1 genotype test is routinely requested by physicians to assess the presence of drug resistance mutations in patients with failing antiretroviral therapy. ACS participants are genotyped upon HIV-1 seroconversion. Standard genotyping was reported to have an additional use in detecting dual infections, which can create a mixed population sequence that yields a large number of ambiguous nucleotide positions. A second possibility is that super-infections can be recognized by a sudden rise of the plasma viral load (FIGURE 4). For this, 14 patients were studied who experienced such “blips” and two super-infection cases were identified (91). These results indicate that a sudden rise of viral load is infrequently associated with HIV-1 super-infection. Therapy failure is usually associated with the evolution of a drug-resistant virus variant, but super-infection with a drug-resistant variant cannot be easily excluded. To study this, viral sequences were analyzed from 101 patients who failed on therapy (12). No evidence for super-infection with a resistant HIV-1 strain was obtained, which is probably related to the low prevalence of drug resistance variants in the current epidemic (11).

Increasing viral fitness

Fitness is a parameter describing the capacity of a virus to replicate in a particular environment. Recent in vitro studies of HIV-1 have shown that the replication fitness of its subtypes correlates with their prevalence in the human population. The replication fitness of primary HIV-1 isolates obtained at the beginning of the AIDS epidemic in Amsterdam (1986) were compared with that of more current viruses (1996-2004). The virus collection of the ACS of MSM is homogeneous with regard to virus type (primarily subtype B) and transmission route (MSM) and is thus ideally suited for such an evolutionary analysis. A robust trend of increasing viral fitness over time was documented (60). This result strongly argues against natural attenuation of HIV-1 due to serial genetic bottlenecks during transmission. Instead, increased viral fitness is likely due to adaptation to the specific host cell environment.

In individual cases, viral fitness can be significantly reduced by naturally occurring mutations in the viral genes. The first such mutation was reported in a non-coding regulatory RNA element of the HIV-1 genome (81). This mutation affected the RNA dimerization initiation signal, as verified by in vitro RNA dimerization experiments. Interestingly, virus isolates from this patient acquired additional changes in the flanking sequences over time, which coincided with an increase in viral load. Such changes improve RNA dimerization through an effect on the overall folding of this RNA domain.

Viral coreceptor use

HIV-1 infection generally starts off with CCR5-using HIV-1 variants (R5 variants) which probably relates to their macrophage-tropism. Indeed, transmission of CXCR4-using HIV-1 variants (X4 variants) is rare but transmission of macrophage-tropic X4 variants has been observed (96). In the natural course of infection, 50% of HIV infected individuals progress to AIDS in the presence of solely R5 variants whereas in the other 50% of individuals, X4 variants evolve from R5 variants, preceding accelerated CD4+ T cell decline and more rapid disease progression (57). After the appearance

of X4 variants, they co-exist with R5 variants resulting in frequent genetic recombination events (240). Through the study of mutagenized gp120 envelopes based around viral sequences generated from samples from ACS participants, whose HIV-1 had switched coreceptor usage, specific alterations in the V1V2 and V3 regions were shown to lead to altered coreceptor usage profiles, specifically N-linked glycosylation patterns (146). These same alterations influenced the extent to which CC- and CXC-chemokines and neutralizing antibodies could inhibit HIV-1 (128) and how effectively HIV-1 could interact with the DC-SIGN molecule expressed on dendritic cells (129). In Ethiopian individuals infected with HIV-1 subtype C, whose HIV-1 had switched coreceptor usage, similar gp120 amino acid changes were involved as were seen with the subtype B viruses from ACS participants (145).

Extensive analysis of the viral quasispecies residing in the naïve, central and effector memory CD4⁺ T cell subsets for several different HIV-1 subtypes showed a more diverse viral population in the predominantly infected subset - the central memory subset - and furthermore a lack of viral compartmentalization among all subsets (79). Emergence of CXCR4-using HIV-1 was accompanied by a pronounced increase in the infection level of the naïve subset, confirming previous findings in the ACS.

Early viral load and CD4⁺ T cell counts, but not coreceptor expression levels were found predictive for the development of X4 HIV-1 (236). Early after their first appearance, X4 variants were more sensitive to neutralization by CD4 binding site directed agents (25), which may explain their absence early in infection, before deterioration of host immunity has occurred. After X4 emergence, both X4 and R5 variants continued to evolve. Over time, R5 viruses developed an increased cytopathicity (108) and resistance to inhibition by RANTES, the natural ligand of CCR5, and small molecule CCR5 inhibitors (93, 94). Similarly, late stage X4 variants were relatively resistant to AMD3100, a CXCR4 antagonist (172). X4 variants had higher cytopathicity than R5 variants, which could be attributed solely to the coreceptor usage of these groups of viruses (35, 104, 109).

Tropism testing

The recent availability of CCR5 antagonists as anti-HIV therapeutics has highlighted the need to accurately identify CXCR4-using variants in patient samples. The Trofile assay (Enhanced Sensitivity version, Monogram Biosciences) has become the most widely used method to define tropism in the clinic prior to use of a CCR5 antagonist. By comparison, the MT-2 assay has been used since early in the HIV epidemic to define tropism in clinical specimens, especially in the ACS. In a comparative study using samples from the ACS it was shown that either assay may be appropriate methodology to define tropism in patient specimens (38).

HIV in dendritic cells

Dendritic cells (DCs) in the sub-epithelium are among the first cells encountered by HIV-1 during transmission. These cells can capture and subsequently degrade the virus, but a significant fraction of the viruses is in fact transmitted to CD4+ T lymphocytes via an immunological synapse. Intriguingly, antibody-neutralized HIV-1 can be reactivated by passage through DCs, which apparently strips off the antibody as a novel immune escape action (234). In a follow-up study, HIV-1 transmission by DCs to T cells was found to be significantly higher for X4 than R5 strains (235). Furthermore, antibodies were able to inhibit R5 strains, but no impact on the transmission of X4 viruses was measured. Taken together, these results illustrate that DCs transmit X4-using viruses more efficiently than R5 strains, which could explain the eventual change in coreceptor usage in some patients.

Novel virus detection assays

The HIV-1 RNA load in plasma usually becomes undetectable under successful therapy, so much so that other methods are needed to monitor ongoing low level virus replication and evolution. Sensitive methods were designed for the quantitation of unspliced and multiply spliced HIV-1 RNA and proviral DNA in PBMC (136). A novel semi-nested real-time reverse transcription-PCR (RT-PCR) was developed that combines the accuracy and precision of real-time PCR with the sensitivity of nested PCR. This method

is superior to the conventional single-step RT-PCR in sensitivity (four copies per reaction), accuracy, dynamic range (six log₁₀), and the power of quantitative detection of HIV-1 RNA and DNA in clinical samples. The novel method will be an important asset for future ACS studies.

HOST FACTORS

High risk seronegatives

Some individuals in the ACS have remained seronegative despite multiple self-reported high-risk sexual contacts (high risk seronegatives; HRSN). The ability to isolate HIV-1 DNA from HRSN was considered evidence for their exposure to the virus (97). Interestingly, cells from HRSN were also relatively resistant to HIV-1 infection in vitro which turned out to be due to high production of RANTES by these cells (92). In addition, HRSN had lower activation of CD4+ T cells as compared to cellular activation levels prior to seroconversion in individuals who did become infected which may have resulted in too few potential HIV-1 target cells in HRSN to support the establishment of infection (95).

Secreted inhibitory factors

A number of host factors present in bodily secretions, both in human milk and seminal plasma, have been identified to potentially bind to DC-SIGN and prevent viral capture by dendritic cells and transfer to CD4+ T lymphocytes (127). These molecules are expected to play an important role in preventing HIV-1 transmission or disease progression and are currently under investigation to dissect the mechanism of action and to evaluate whether these molecules can instruct the design of novel drug leads.

Non-adaptive host control

Host genetic factors can influence the course of HIV-1 infection albeit that some of the reported associations could not be confirmed in the ACS (106, 107). The influence of a host human leukocyte antigen (HLA) B57 typing or the effect of heterozygosity for a 32 base pair deletion in the gene encoding the CCR5 coreceptor for HIV-1 on the clinical course of HIV-1 infection is

now generally accepted (82, 126, 232, 233), albeit that a CCR5 $\Delta 32$ heterozygous genotype does not influence therapy outcome (250). In the ACS, an effect on HIV-1 susceptibility could be demonstrated for a polymorphism in the Cyclophilin A gene (159), whereas an effect on disease course was demonstrated for polymorphisms in TRIM5 α , a factor with innate antiviral activity (233). Interestingly, TRIM5 α escape variants of HIV-1 emerged in late stage disease, indicating that this host cellular innate immune factor is indeed influencing the course of infection (98). In order to identify additional host genetic factors that may influence HIV-1 disease course, a so called genome-wide association study (GWAS) was performed in the ACS for which more than 300,000 single nucleotide polymorphisms (SNPs) were typed and associated with the clinical course of infection. Currently, the interesting associations between SNP-genotypes and disease course are being validated using other cohorts. In addition, associations between SNPs and disease course found in other cohorts could be confirmed with this data set (232).

Immune control by CTL

Already in the acute phase of infection, cytotoxic T lymphocytes (CTL) are considered to contribute to the control of HIV-1 replication (132) and recognize their peptide epitopes in the context of HLA molecules. However, functional defects in HIV-specific CD8⁺ T cells observed during the natural course of HIV-1 infection (100) have prompted in depth studies into the role of HIV-specific CD4⁺ and CD8⁺ T cells in HIV-disease progression. Unexpectedly, no protective role for HIV-specific cytokine-producing CD4⁺ T or CD8⁺ T cells was found in a prospective study involving 96 individuals of the ACS with a known date of seroconversion. This suggests that other features of the T cell response may be associated with protection against disease progression. Host factors, such as the HLA background, which as mentioned earlier is strongly associated with time to HIV disease progression, may determine the efficacy of the T cell response.

Interestingly, CTL responses restricted by the non-protective HLA A2 allele were generally less well-preserved during disease progression than CTL

responses restricted by protective HLA alleles, in individuals expressing either one of these alleles. In contrast, in individuals co-expressing a protective HLA allele in combination with a non-protective HLA allele, CTL responses restricted by protective HLA alleles were found to be lost at least as fast as CTL responses restricted by the non-protective HLA allele. HLA B57-peptide complexes had a stronger interaction with the TCR than HLA A2-peptide complexes (85) and HLA B27-peptide complexes (162), which may contribute to the dominance of HLA B57-restricted T cell responses in HIV infection. These data suggest a different mechanism of protection for HLA B27 and HLA B57.

Mutations in CTL epitopes can interfere with antigen presentation and CTL recognition, allowing the virus to escape from cellular immune pressure. However, CTL escape mutations may coincide with viral attenuation, which can be concluded from the rapid reversion of CTL escape mutations upon transmission to an HLA discordant recipient (FIGURE 5) (130). Viral attenuation due to CTL escape mutations will result in low viral load despite evasion from immune control. HLA B57 is overrepresented among HIV-1 infected long-term non-progressors (LTNPs) which has been attributed to strong CTL responses against epitopes in the viral Gag protein. HLA B57+ HIV-1 infected progressors and LTNP from the ACS were compared for viral sequence variation in 4 dominant epitopes in Gag, and for their ability to generate CTL responses against these epitopes and their escape variants (131). Prevalence and appearance of escape mutations in Gag epitopes and potential compensatory mutations were similar in HLA B57 LTNP and progressors, as was the magnitude of CD8+ IFN- γ responses directed against the wild-type or autologous escape mutant Gag epitopes. Interestingly, HIV-1 variants from HLA B57 LTNP had much lower replication capacity than viruses from HLA B57 progressors which did not correlate with specific mutations in Gag. These data implied that the different clinical course of HLA B57 LTNP and progressors was not associated with differences in CTL escape mutations or CTL activity against epitopes in Gag but rather with differences in HIV-1 replication capacity.

HIV-specific immunity and HIV evolution at a population level

As discussed, HIV is known to escape from CTL responses, while escape mutations may revert upon transmission to an HLA-discordant partner. However, not all escape mutations seem to revert and therefore the viral accumulation of mutations leading to escape from the HLA alleles of the human population was investigated. CTL epitopes in HIV strains isolated early after seroconversion from individuals who seroconverted either in 1985 or in 2005/06 were analyzed by sequencing (**FIGURE 6**). This revealed that over the past 20 years, HIV has adapted to the human immune system by decreasing the number of CTL epitopes that can be presented via HLA B molecules that are associated with a low relative hazard of disease progression. This finding is counterintuitive, because the protective HLA alleles are not very common in the human population, and HIV-epitopes restricted by protective HLA alleles are expected to revert upon transmission to a new HLA-discordant host. However, these reversions may be hampered by compensatory mutations.

In the Netherlands, the protective HLA B27 allele is moderately prevalent (approximately 8-16 % of HLA B alleles). A stable HIV-1 strain, carrying HLA B27 CTL escape mutations, was identified among participants of the ACS (39). This indicated that vaccines targeted at inducing a CTL response might easily be circumvented by the virus. Also, patients carrying protective HLA alleles might no longer be protected from disease progression in the future.

Humoral immunity against HIV

After the failure of the STEP trial in which vaccine elicited HIV-1 specific CTL activity did not protect from infection or disease progression, HIV-1 specific neutralizing humoral immunity received renewed interest. HIV-1 can easily escape from neutralizing antibodies with type specific activity (24). This is assumed to be different for cross-reactive neutralizing antibodies that can neutralize multiple unrelated HIV-1 variants, which is considered to relate to the conserved nature of their epitopes. Therefore, cross-reactive neutralizing humoral immunity is the aim in vaccine

research and the epitopes of some well defined broadly neutralizing antibodies are now being identified to serve as an immunogen. However, among recently transmitted HIV-1 variants in the ACS a relatively high prevalence of resistance against these broadly neutralizing antibodies was observed (153), which indicates that more epitope specificities are needed to get a broadly protective vaccine. Viruses isolated early during the course of infection were mostly sensitive to HIVIg (purified immunoglobulin preparation from pooled plasmas of HIV-infected individuals), indicating that certain antibody specificities or combinations of specificities could protect against infection when present at the moment of exposure (26).

In LTNP and progressors, a similar prevalence of cross-neutralizing humoral immunity was observed, suggesting that neutralizing humoral immunity does not protect from disease progression (226). Indeed, the presence of cross-reactive neutralizing activity in serum was not associated with a prolonged AIDS-free survival (56). Interestingly, in the sera of ACS participants, the neutralizing activity against multiple unrelated subtype B variants was much higher than against viruses from other subtypes. This may suggest that subtype specific vaccines should be considered (225). Moreover, a significant increase in the breadth of serum neutralizing activity was observed with duration of infection implicating the importance of prolonged antigen exposure for the maturation of the HIV-1 specific B cell response (226).

INTERVENTION

Tolerability and toxicity of HAART

With the ever-expanding spectrum of (broadly speaking) equally efficacious HAART regimens to choose from, it is increasingly important to understand the pathogenesis of different antiretroviral drug toxicities to assist in the selection of safer regimens and to identify rational approaches for counteracting specific toxicities. Mitochondrial toxicity is thought to underlie a fairly broad range of clinical drug toxicities (e.g. lipoatrophy, lactic acidosis, myopathy), especially early in the HAART era. In particular,



thymidine analog reverse transcriptase inhibitors were identified as driving mitochondrial toxicity by way of inhibiting mitochondrial DNA (mtDNA) polymerase gamma and thereby mtDNA replication. At the same time however, evidence emerged that HIV infection itself, in the absence of antiretroviral therapy, might also affect mitochondria. In order to detect mitochondrial toxicity prior to the emergence of clinical symptoms and to determine whether this is drug- and/or virus-related, the quantification of mtDNA as a biomarker of mitochondrial toxicity was evaluated. First, the levels of mtDNA were determined in cryopreserved PBMC obtained from 36 HAART naïve ACS participants prior to seroconversion, at one year and at five years after seroconversion (31). mtDNA content decreased one year after seroconversion, suggesting an effect of HIV infection itself on mtDNA levels. Analogous results from previous cross-sectional studies supported the hypothesis that patients starting antiretroviral therapy containing nucleoside analog reverse transcriptase inhibitors (NRTI) might already be predisposed to developing mitochondria-related clinical drug toxicities due to the effect of HIV infection itself on mtDNA. On the other hand, the suppression of HIV infection by antiretroviral therapy might also be expected to exert a restorative effect on mtDNA. This latter hypothesis was further supported by an open-label, pilot study in which patients naïve to protease inhibitors, non-nucleoside reverse transcriptase inhibitors and the NRTI stavudine, were randomized either to receive an NRTI-sparing regimen containing efavirenz and ritonavir boosted indinavir, or the same regimen plus stavudine (33). Irrespective of the regimen, mtDNA quantification in PBMC revealed an increase over the course of 48 weeks of therapy, indicating a possible restorative effect on mtDNA as a result of suppression of HIV infection. This, however, does not rule out that longer exposure to mitochondrially toxic drugs such as stavudine might over the longer term negatively tip the balance towards reduced mtDNA content. To investigate mitochondrial toxicity in different blood cell subsets, PBMC from HAART-naïve ACS seroconverters, taken prior to seroconversion, at one year and at five years after seroconversion (32) were sorted into CD4+ and CD8+ T cell subsets and analyzed for mtDNA content. mtDNA levels decreased in the CD8+, but not CD4+ T cell compartment, in particular

five years after seroconversion, suggesting an effect due to chronic HIV infection. As the CD8+ T cell compartment is highly activated during chronic HIV infection, a subsequent cross-sectional analysis was carried out in five outpatient clinic patients to determine mtDNA content in both activated and non-activated CD4+ and CD8+ T lymphocyte fractions. mtDNA levels were lower in the activated compared to the non-activated CD8+ T cell fraction, with a similar trend in the CD4+ cell fraction.

Transmission of drug-resistant HIV strains

A total of 100 primary HIV-1 infections (32 at the AMC and 68 in the ACS) were identified from 1994 to 2002. Transmission of drug-resistant mutations decreased over calendar time, with 20% drug-resistant mutations transmitted before 1998 versus only 6% after 1998 (11). Interestingly, in the first years after seroconversion CD4+ T cell decline was slower in persons carrying primary drug-resistant viruses that showed evolution at the resistance-associated positions than in those who didn't, exemplifying the decreased fitness of viruses carrying primary drug resistance mutations (9).

Fitness of drug-resistant HIV strains

Previous research on drug-resistance mutations indicated that such adaptations in the HIV-1 genome can have a severe impact on the viral replication capacity. ACS samples were used to study diverse aspects of drug-resistance, including the first description of a drug-dependent virus (2), the identification of novel drug-resistance mutations (8) and transmission of drug-resistant HIV-1 variants (9, 11, 47, 70).

Sero-reversion

Sero-reversion is defined as the loss of antibody reactivity and is rare in HIV-infected patients, even when treated with antiretrovirals (69). This was confirmed in 80 patients who were treated with HAART during chronic HIV-1 infection and whose HIV-1 plasma viral load was undetectable for at least 5 years, which argues for ongoing virus replication (below the detection level) under successful therapy (41).



Primo-SHM Study

The Primo-SHM study was initiated to address whether short-term HAART during primary HIV infection can affect the viral set-point. It is a multicenter, open-label, randomized, semi-factorial clinical trial that compares short-course early HAART (24 or 60 weeks) with no treatment. Patients are recruited in the Dutch HIV treatment centers (ATHENA Observational Cohort, mentioned above). In the semi-factorial design, participants are randomized over three study arms (no treatment, 24 or 60 weeks of HAART), or only the two treatment arms if treatment is clinically indicated and the physician or patient insists on starting HAART. Patients with a primary HIV infection who do not want to be randomized are also prospectively followed, if they agree to regular follow-up visits including storage of plasma and cells.

By September 2009, 270 patients have been enrolled in the Primo-SHM cohort, of whom 157 were randomized over the three study arms. A first analysis demonstrated that early HAART initiated during primary HIV infection lowers the viral load set-point established after treatment interruption, irrespective of treatment duration (24 versus 60 weeks)(174). A lower viral load set-point has been shown by others to correlate with a slower disease progression and with less failure of antiretroviral therapy.

Vaccine trials

The ACS participated in the VaxGen's AIDSVAX Study, the first phase III HIV vaccine trial. Results were disappointing and revealed no effect on prevention of HIV infection. In addition, therapeutic vaccination with p24-VLP was not related to slower HIV-1 disease progression (114) and vaccination with pneumovax had no protective effect against all-cause pneumonia among DU who were at increased risk for pneumonia (113).

HIV-INFECTED AND HIV-EXPOSED CHILDREN

Within the context of PEACH, the efficacy, pharmacokinetics and metabolic side-effects of HAART, co-morbidity, the neurological and social development, and multiple vaccine responses were investigated (3-7, 42, 124, 125, 137, 163,

164, 181, 227). To date, 54 children were treated with HAART resulting in undetectable viral HIV RNA load (less than 40 copies per ml) in 49 children (163, 164). One patient died from liver failure (165). Two patients experienced liver fibrosis and osteoporosis during HAART, while one patient experienced temporary renal disease and osteoporosis during tenofovir treatment.

Follow up of 39 HIV-1 infected children, who were naïve to protease inhibitors and were treated with nelfinavir and 2 NRTI, showed increasing percentages of children achieving undetectable viral loads over time, resulting in 54% with undetectable viral loads after 240 weeks of treatment. Young age was strongly associated with virological failure, but even in children with virological failure, immunologic parameters and clinical improvement were sustained up to 7 years, albeit with increasing lipodystrophy (163), which clearly contrasted with some of the findings at 2 years of follow-up (27, 241, 242, 244).

In order to improve compliance and virological suppression, the feasibility and effectiveness of a once-daily regimen of efavirenz in combination with 3 NRTI as first and second line combination antiviral therapy was assessed. In an observational, prospective, single-center study of 36 HIV-1 infected children, 76% and 67% of the children still had undetectable viral loads at 48 and 96 weeks, respectively. No significant difference was found in efficacy between first and second line HAART. Again, all children showed a sustained immunological and clinical improvement, irrespective of their virological response. In 14 children study medication was stopped, mostly because of adverse effects, although no lipid abnormalities or abacavir-related hypersensitivity reactions were observed. Based on these observations it was concluded that once-daily combination antiviral therapy is safe, convenient and effective in children with HIV-1 infection (164).

The HIV-exposed children are studied in the context of ECS and PENTA, in which HIV-1 infected pregnant women are enrolled and their infants prospectively followed according to a standard protocol (23, 137). The ECS was established in 1985 in Western Europe to estimate the rate of and risk



factors for MTCT and currently involves over 9000 mother-child pairs. At the AMC only 3 cases of transmission have been identified. These could be attributed to unforeseen incidents during pregnancy. The current measures of antiretroviral treatment of the pregnant mother combined with standard delivery and postnatal HAART in the HIV-exposed children have successfully prevented MTCT in the last decade.

CO-INFECTIONS

The ACS have expanded in recent years to include studies of other pathogenic viruses (CMV, EBV, HAV, HBV, HCV, HHV8, HSV-1 and -2, HTLV-1 and -2, and GBV-C), opening up new avenues for further research.

HCV

HCV in DU

In 2007, the retrospective testing for HCV was completed for 1276 DU and 1846 MSM with at least 2 cohort visits. The most important mode of HCV transmission is through exposure to infected blood. As expected, among ever-injecting ACS DU, the prevalence of HCV antibodies was 85% at study entry and 31% were co-infected with HIV. The yearly HCV-incidence dropped from 28/100 PY in the 1980s to 2/100 PY in recent years, most likely due to a decrease in injecting risk behavior. As shown in Figure 7 the HCV-incidence in ever-injecting DU was on average 4.4 times the HIV-incidence, a pattern seen over the entire study period (214). Among self-declared never-injecting DU the HCV antibody prevalence at ACS entry was 6.3%. HCV strains that circulate among never-injectors phylogenetically cluster with those circulating among their injecting counterparts. Although this is all suggestive for underreporting of past injecting behavior, household or sexual transmission of HCV from injectors to non-injectors cannot be ruled out. This does, however, stress the need for HCV-testing among DU who report never injecting (215).

HCV in MSM

Retrospective HCV screening of ACS MSM participants in the period 1985-2003 showed an overall HCV-incidence of 0.18/100 PY among HIV-positive MSM, but was 0/100 PY among HIV-negative MSM. After 2000, HCV incidence among HIV-positive MSM increased 10-fold. HCV appears to be emerging as an STI, although sexual transmission is considered to be inefficient. Phylogenetic analysis of HCV strains circulating among HIV-positive MSM revealed distinct MSM-specific clusters of independently co-circulating HCV lineages. After 2000, outbreaks of acute HCV among HIV-positive MSM were also reported from other Western European cities. A large European MSM specific transmission network was found, linking the independently reported outbreaks. Evolutionary analysis of HCV lineages circulating among European MSM demonstrated that the start of this outbreak can be traced back to the period 1996-2000, the same timeframe in which HAART was introduced and a rise in sexual risk behavior among MSM was observed. The role of HIV itself remains unclear (209, 210).

HCV clinical course

A study among HIV/HCV co-infected HIV seroconverters showed that HIV disease progression is faster in individuals with more than one HCV genotype (196). HCV/HIV co-infected DU remain at increased risk of dying from hepatitis/liver-related death in the era of HAART compared to HCV-mono-infected DU, suggesting that HIV continues to accelerate HCV disease progression (171). The rate of spontaneous viral clearance among DU from the ACS was 33% following acute infection, and higher in women, DU without HIV and without a chronic HBV infection. Multiple HCV infections were observed in 10 of 24 HCV-seroconverters with spontaneous viral clearance (11 re-infections; 3 super-infections) and in 13 of 35 HCV-seroconverters without viral clearance (20 super-infections). Actually, the incidence of HCV re-infection was at least similar to that of initial HCV infection. Although partial immunity cannot be excluded, this will further complicate vaccine development. Harm reduction will remain dependent on precautionary measures preventing the further spread of HCV, and treatment of those chronically infected (209).



In 2005, within the DU cohort, a feasibility study was started to evaluate the possibility of HCV testing and treatment combined with methadone programs (Dutch C). By July 2009, 59 DU have started HCV treatment. With this approach excellent uptake and successful treatment outcomes have been achieved.

HCV immunology

The specificity of the CD4+ memory T cell responses was most predictive of HCV clearance following acute infection, as CD4+ T cell responses targeting non-structural proteins were associated with resolved infection. In chronic carriers, HCV-specific T cell responses are much more focused on core protein, and also after HIV-infection T cells respond more to core protein. Analysis of both persistent exposure (continuous risk behavior) and persistence of viral RNA as potential factors influencing the height and specificity of the T cell response revealed that continuous exposure leads to boosting of the immune response. Conversely, continuous presence of viral RNA affected the specificity of the T cell response and lead to more core protein-specific T cells. After IFNalpha/ribavirin therapy, both height and breadth of the HCV-specific T cell responses declined parallel to the decline in viral load, suggesting that enhancement of HCV-specific T cell responses did not play a major role in forced viral clearance (160, 213).

HBV

Between 1984 and 2003, also sera of MSM and DU in the ACS with at least two visits were retrospectively screened for anti-HBc antibodies. After 2003, most MSM and DU participating in this study were vaccinated against HBV, making further testing redundant. The sera of 1268 DU, both injecting and non-injecting, were screened for anti-HBc antibodies. Of the 598 participants who were anti-HBc negative at entry, 83 subsequently seroconverted for anti-HBc antibodies. The incidence of HBV declined from 5.9/100 PY between 1985 and 1993 to 0/100 PY in 2002. Of the acutely-infected injecting and non-injecting DU, 88% were infected with the same genotype D, serotype ayw3 strain. Current injecting was the most important risk factor for an HBV infection. The decline in the incidence

of HBV among DU in Amsterdam was probably caused by a decline in injecting behavior (112). Injecting and non-injecting DU were infected with the same strain, indicating that DU infect one another, regardless of their risk behavior. No reports of new cases among DU and the disappearance of the specific genotype D strain suggest that DU may no longer be a high-risk group for HBV infection in Amsterdam. However, trends in drug use need to be monitored, in case injecting drug use regains popularity in the Netherlands thereby increasing HBV transmission risk among DU (231).

After screening the sera of 1862 MSM for anti-HBc antibodies, 1042 MSM proved to be negative for anti-HBc antibodies at entry, of whom 64 subsequently seroconverted during follow-up at a median age of 32. At the moment of seroconversion, 31 MSM were HIV-positive. HBV incidence declined dramatically in the first years and then remained stable throughout the study period. Although HBV is generally considered more infectious than HIV, this study shows that the trend and magnitude in HBV and HIV incidence among MSM are similar. With the exception of 3 MSM, all were infected with an identical genotype A strain. This strain has been circulating not only among MSM of the ACS, but also among the general MSM population in the Netherlands, for at least 2 decades (228, 229).

HSV-1 and -2

Between 1984 and 2003 seroprevalence of HSV-1 and HSV-2 was determined amongst HIV-positive and HIV-negative MSM. Of these men, 65% were HSV-1 antibody positive, whilst 41% were HSV-2 antibody-positive. Of the total group, 30% were positive for both. HSV-1 and HSV-2 prevalence decreased over calendar time among HIV-negative MSM, but remained stable in those who were HIV-positive. The association between HIV infection and HSV-2 became stronger over time (170). HSV-2 testing for incident infections will be completed enabling study of the relationship between HSV-2 and HIV infections.

HHV8

In contrast to the high prevalence and incidence of HHV8 among MSM in the ACS (respectively 21% and 3.6/100PY) (53), the prevalence of infection



among DU was only 2.5% at entry in the ACS (154). Contrary to HIV-1, the prevalence and incidence of HHV8 were relatively stable over time. As no association with needle sharing was found and the incidence of HHV8 was also low, HHV8 transmission through injecting drug use is rare (154).

Patients with Kaposi's sarcoma (KS) had both latent and lytic viral HHV8 RNA present in both their KS lesions and in their PBMC, although expression was much higher in the lesions and this tended to be associated with disease stage (148, 149). Similarly, HHV8 DNA was detected more often in patients progressing to KS than patients who do not develop the disease, suggesting that the level of viremia might be associated with KS and disease progression. However, no correlation was found between viral load and progression to KS or disease stage in longitudinal samples from 19 ACS participants who progressed to KS (149, 150). In addition to HHV8 itself, host genetic factors influence KS, such as IL-8 as an autocrine growth factor. Indeed, an IL-8 promotor polymorphism (-251 A/T) known to be associated with IL-8 production, was also associated with disease severity of KS (222).

GBV-C

Several studies found that infection with GBV-C – a virus which is related to HCV – delays HIV disease progression. This finding could not be confirmed in the ACS. In contrast, the presence of GBV-C RNA was shown to be dependent on the presence of sufficient CD4+ T cells, rather than contributing to preservation of CD4+ T cell numbers during HIV infection, which provided new insights into the pathogenesis of GBV-C (216).

EBV

EBV, a common gamma-herpesvirus, persists for life in the B cells of the human host. In healthy individuals, EBV-infected B cells are tightly controlled by CD8+ T cells. In HIV-infected individuals, EBV is frequently associated with AIDS-related diffuse large cell non-Hodgkin Lymphomas (AIDS-NHL). However, EBV viral load levels in the blood did not predict the occurrence of these AIDS-NHL (200) and were often already elevated early in HIV-infection. The level of EBV load increased after HIV-seroconversion,

which was paralleled by an increase in lytic-cycle specific T cells (143). Furthermore, this increase in EBV load was associated with an increase in immune activation markers. Most interestingly, there was strong correlation between EBV load before and after HIV-seroconversion and before and after treatment by HAART, supporting the idea that chronic immune activation of the immune system can lead to an increase in the number of EBV-infected B cells, without altering inter-individual differences in EBV set-point (142).

In parallel to these virologic studies, EBV-specific T cells were studied. A loss of IFN γ -producing EBV-specific CD8+ T cells was observed in HIV-infected individuals progressing to AIDS-NHL, which correlated with total CD4+ T cell numbers. This suggested functional exhaustion due to lack of EBV-specific CD4+ T cell help (200). Indeed, when analyzing EBV-specific CD4+ T cell responses, a decline of EBV-specific memory CD4+ and CD8+ T cell responses was observed during HIV-infection. However, whereas latent antigen EBNA-1 specific CD4+ T cells were lost well before diagnosis in all subjects who developed AIDS-NHL, these cells were better preserved in progressors to non-EBV related disease and slow progressors. Therefore, EBNA-1 specific CD4+ T cells appear to be important in the maintenance of control over EBV-infection. Interestingly, T cells recognizing the lytic EBV antigen BZLF-1 were not lost in all progressors to AIDS-NHL, suggesting a different function of these cells in the surveillance of EBV-infected B cells (141). Moreover, effective treatment with HAART led to a restoration of EBNA-1 specific T cells to levels comparable to healthy individuals. Despite this, EBV load remained elevated indicative of a definite alteration of the EBV set-point by HIV-infection. In contrast, T cells recognizing BZLF-1 decreased, suggestive of a decreased EBV-reactivation rate (140).

CMV

CMV, a common beta-herpesvirus, is associated with CMV-end organ disease in HIV-infected individuals. In AIDS patients, activation of HHV8 and CMV is observed frequently and can result in KS and CMV-related diseases, respectively. To test whether the presence of a detectable CMV



DNA load in PBMC is also correlated with the disease process in AIDS-related KS, both the HHV8 and the CMV DNA load was determined by real-time PCR. While HHV8 DNA levels were correlated with KS, and CMV DNA levels were correlated with CMV-related disease, neither CMV prevalence nor CMV viral load were related to KS (221). CMV viral loads increased only shortly before development of CMV-disease and similar to EBV-specific T cells, CMV-specific T cells directed against the pp65 antigen decrease towards the development of CMV-disease (20). HAART was shown to restore pp65-specific T cells (87).

Tables & figures

TABLE 1

Total number of MSM ever included in a study until 31-Dec-2008.

Each participant can participate in as many as 6 studies over calendar time.

Category	Total	Death	HIV antibody status		
			Negative	Positive	Seroconverter
At least 1 visit at PHSA	2383	335	1588	585	210
Protocol 1	748	215	415	238	95
Protocol 2	265	24	225	1	39
Young MSM (JOHO)	872	1	775	29	68
6000 numbers	196	58		196	
7000 numbers	26	7		26	
9000 numbers	28	4	1	27	
Jan van Goyen Medical Center ¹	176	24		132	44
Open AZT	26	23		19	7
Double-blind AZT	56	41		51	5
Early antiretroviral treatment	186	66		141	45
Combination Study	12	11		9	3
Delta Study	10	8		6	4
Triple Study	6	1		4	2
AZT/3TC/D4T	46	2		36	10
Native	12	3		10	2
Atlantic	9	3		8	1
RGP120 Vaccine	16	4		3	13
P24 Vaccine	21	6		20	1
Vaxgen ²	14		11		3
HOP ³	95	1		36	59
Intake from 1-Apr-2005	214		199	3	12
Inclusion of partners	28		12	11	5

¹ Initially, 227 were eligible for follow-up at the Jan van Goyen Medical Center, however 51 decided to go to another hospital or had other reasons for refusing further follow-up

² These men are also participants of the young MSM study

³ HIV study among recent HIV-positive MSM (of which 15 also participate in Primo-SHM study)

MSM: men who have sex with men, AZT: zidovudine, 3TC: lamivudine, d4T: stavudine.

TABLE 2

Total number of MSM in follow-up in ongoing studies: participants who had a visit at the PHSA on or after 1-Jan-2008 or within SHM/Jan van Goyen Medical Center on or after 1-Jan-2008.

Site of last visit	Total	Follow-up visit	Newly recruited	HIV antibody status		
				Negative	Positive	Seroconverter
PHSA	532	504	28	481	13	38
SHM/Jan van Goyen Medical Center	208	208			140	68

TABLE 3

Overview of immunological assays performed in the ACS.

Period	PHA		ALS		ACD3		CD228		CD328		CD2		CD3		CD4		CD8		MT-2	
	MSM	DU	MSM	DU	MSM	DU	MSM	DU	MSM	DU	MSM	DU	MSM	DU	MSM	DU	MSM	DU	MSM	DU
1984		X		X		X		X		X		X		X		X		X		X
1985																				
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2008																				

Proliferation assays measure T cell function as the ability to respond to different stimuli: **PHA**: phytohemagglutinin (before 1994, with human pooled serum (HPS), after 1994, without HPS; PHA responses with and without HPS are not comparable), **ALS**: anti-lymphocyte stimulation test, **ACD3**: monoclonal antibodies (mAb) against the CD3 receptor, **CD228**: CD2 and CD28 mAb, **CD328**: CD3 and CD28 mAb.

T cell subsets CD2, CD3, CD4 and CD8: Before 1988, single indirect immune-fluorescence staining on isolated peripheral blood mononuclear cells (PBMC) with CD2, CD3, CD4 and CD8 mAb using the dual platform method. Between 1989 and 1994, double direct immune-fluorescence staining on isolated PBMC. After 1994, direct staining on whole blood. From 2003 onwards, CD4/CD8 T cell subsets for participants in follow-up at the Jan van Goyen Medical Center determined at the Onze Lieve Vrouwe Gasthuis. From 2007 onwards, T cell subsets for HIV-positives seen at the PHSA generated using the single platform method.

MT-2: Determination of viral use of the co-receptor CXCR4. Although the MT-2 assay is not an immunological assay, it is presented here because it is developed and routinely determined in the same department that runs the immunological assays (Experimental Immunology).

MSM: Men who have sex with men, **DU**: Drug users, **X**: ACS among DU started in 1985

Medium-grey box: performed at every visit, **light-grey box**: not performed on a routine basis, **dark-grey box**: performed once a year.

TABLE 4

Overview of virological assays performed in the ACS.

Virological assays	Company	Period MSM / DU
HIV screenings assays		
HTLV-III screening test	Homemade	1984-1985
HTLV-III EIA	Abbott	1985-1989
Vironostika anti-HTLV-III ELISA	Organon International	1985-1987
Wellcozyme anti-HTLV-III	Wellcome	1986
recombinant HIV-1/HIV-2 EIA	Abbott	1989-1993
recombinant HIV-1/HIV-2 3rd generation	Abbott	1993-1995
HIV-1/HIV-2 3rd generation plus EIA	Abbott	1995-1998
HIV IMX (MEIA system)	Abbott	1998-2001
VIDAS HIV DUO	Biomerieux	2001-2003
AxSYM HIV Ag/Ab Combo	Abbott	Since 2003
Other HIV-antibody assays		
HIV-1 anti-CORE EIA	Abbott	1986-1992
ENVACOR HIV-1 EIA	Abbott	
Detection of CORE		1989-1992
Detection of ENV		1987-1990
HIVAb p24 (rDNA)	Abbott	1992-1997
HIV-antigen assays		
HTLV-III Antigen EIA	Abbott	1986-1992
HIVAg-1 EIA polyclonal	Abbott	1990-1998
Confirmation assays		
HTLV-III Western Blot	Homemade	1984-1985
LiaTek HIV-1/HIV-2	Organon Teknika	1986
HIV Blot version 2.2 (HIV-1 en HIV-2)	Genelab Diagnostics	Since 1986
HIV-2 Blot version 1.2	Genelab Diagnostics	1995-2005
INNO-LIA HIV-1 HIV-2 assay	Innogenetics	Since 2005



Virological assays	Company	Period MSM / DU
HIV RNA assays		
NASBA HIV-1 QT	Organon Teknika	1996-997
NucliSens HIV-1 QT	Organon Teknika	1997-2006
bDNA	Chiron	1999-2007 (MSM)
		2003-2006 (HOP)
M2000rt	Abbott	Since 2006
Genotypic resistance assay		
Viroseq Protease en Reverse Transcriptase	Abbot	Since 2004
Other virus-antibody assays¹		
HBV AxSYM Core anti-HBc	Abbott	1984-2002
HBV AxSYM HBsAg	Abbott	retrospective
HCV AxSYM HCV version 3.0	Abbott	1984-2003 and since 2007 1985-2005 (DU)
Deciscan HCV plus immunoblot	Biorad	1985-2004 (DU)
HSV-1 and 2 HerpeSelect	FOCUS Technologies	1984-2002 (MSM)
HSV-1 and 2 Western Blot	Homemade, ICPMR	1984-2002 (MSM)

HTLV-III: human T lymphotropic virus III (old name for HIV), **HIV:** human immunodeficiency virus, **HBV:** hepatitis B virus, **HCV:** hepatitis C virus, **HSV-1 and 2:** herpes simplex virus type 1 and type 2, **EIA:** enzyme immuno assay, **ELISA:** enzyme linked immuno-sorbent assay, **LiaTek:** line immuno assay technique, **MEIA:** microparticle enzyme immuno assay, **NASBA:** nucleic acid sequence-based amplification, **bDNA:** branched DNA assay, **ICPMR:** Institute for Pathology and Medical Research, Sydney, NSW, Australia, **MSM:** men who have sex with men, **DU:** drug users, **HOP:** HIV among recent HIV-positives.

Note: To compare test results, some assays have been used simultaneously.

1 Most of this testing occurred retrospectively, as many other virus-antibody assays weren't available at the start of the ACS.

TABLE 5

Total number of DU ever included in a study up to 31-Dec-2008.
Each participant can participate in more than one study over calendar time.

	Total	Death	HIV antibody status at entry		
			Negative	Positive	Seroconverter
At least 1 visit at the PHSA	1647	411	1229	322	96
DU cohort	1488		1075	318	95
Clinical follow-up	96 ¹			30	66
'98 young cross-sectional study	293 ²		261	21	
JODAM ³	210		199	6	3
Abstinent cohort	177		122	9	8
MATE ⁴	170		150	12	8
Entry for HCV treatment	18		17	1	

1 Clinical data missing from 14 of the originally included 110 participants.
2 Inclusion criteria not met for all original 452 participants. Of the remaining 293, 11 provided too little saliva to determine HIV status. From this cross-sectional study, 88 participants are currently in follow-up in the DU cohort.
3 JODAM: Follow-up study among young DU.
4 MATE: measuring addiction for triage and evaluation (interviews).

DU: drugs users, HCV: Hepatitis C Virus.

TABLE 6

Total number of DU ever included in a study up to 31-Dec-2008.
Each participant can participate in more than one study over calendar time.

Study	Total in follow-up	HIV antibody status at entry		
		Negative	Positive	Seroconverter
DU cohort	335	284	34	17
JODAM	55	52	1	2

TABLE 7

Number and origin of HIV-infected children in follow-up in 2009 and HIV-exposed children in care between 1999 and 2009 at the Emma Children's Hospital.

Origin	HIV-infected (Mix)	HIV-exposed (Mix)
Africa	41 (4)	164 (21)
Surinam	8 (0)	53 (7)
British Guyana	1 (0)	0 (0)
Netherlands Antilles	2 (0)	1 (0)
Asia	3 (2)	5 (4)
Netherlands	2 (0)	33 (0)
Other	2 (1)	10 (3)
Total	59 (7)	270 (35)

Note: number in brackets denotes the number of children of mixed origin.

TABLE 8

MSM number of HIV-positives, number of person years, and yearly HIV incidence per calendar year according to age at entry.

Year	MSM <30 years at study entry			MSM >30 years at study entry			All homosexual men		
	No. HIV-positives	Person years	Incidence	No. HIV-positives	Person years	Incidence	No. HIV-positives	Person years	Incidence
1985	11	149.41	7.36	26	337.91	7.69	37	487.32	7.59
1986	9	177.12	5.08	13	397.90	3.27	22	575.02	3.83
1987	7	164.73	4.25	7	378.57	1.85	14	543.29	2.58
1988	3	151.56	1.98	5	357.54	1.40	8	509.10	1.57
1989	3	145.03	2.07	2	350.97	0.57	5	496.00	1.01
1990	5	137.75	3.63	9	339.73	2.65	14	477.48	2.93
1991	5	129.85	3.85	4	335.28	1.19	9	465.13	1.93
1992	3	124.30	2.41	3	330.98	0.91	6	455.28	1.32
1993	1	115.42	0.87	0	322.26	0.00	1	437.68	0.23
1994	1	109.93	0.91	3	312.50	0.96	4	422.43	0.95
1995	1	150.32	0.67	2	299.58	0.67	3	449.90	0.67
1996	5	327.16	1.53	3	265.24	1.13	8	592.40	1.35
1997	5	294.17	1.70	2	60.70	3.29	7	354.88	1.97
1998	1	300.21	0.33	0	45.03	0.00	1	345.24	0.29
1999	6	358.45	1.67	0	34.58	0.00	6	393.03	1.53
2000	2	367.00	0.54	0	36.05	0.00	2	403.05	0.50
2001	4	359.38	1.11	1	35.37	2.83	5	394.75	1.27
2002	5	374.38	1.34	1	35.23	2.84	6	409.61	1.46
2003	6	386.32	1.55	2	44.94	4.45	8	431.26	1.86
2004	6	373.16	1.61	0	50.94	0.00	6	424.10	1.41
2005	6	368.97	1.63	1	71.44	1.40	7	440.41	1.59
2006	9	361.68	2.49	3	94.53	3.17	12	456.21	2.63
2007	6	355.62	1.69	2	111.08	1.80	8	466.70	1.71
2008	5	253.45	1.97	2	94.43	2.12	7	347.88	2.01

**TABLE 9**

DU number of HIV-positives, number of person years, and yearly HIV incidence per calendar year according to injecting status at entry.

Year	Injecting DU only			AI DU		
	No. HIV-positives	Person years	Incidence	No. HIV-positives	Person years	Incidence
1986	5	58.05	8.61	5	74.05	6.75
1987	5	139.69	3.58	6	182.49	3.29
1988	9	206.91	4.35	10	266.74	3.75
1989	7	240.78	2.91	8	314.00	2.55
1990	12	267.37	4.49	13	352.52	3.69
1991	4	253.91	1.58	5	341.02	1.47
1992	5	272.84	1.83	7	373.15	1.88
1993	5	293.25	1.71	5	397.42	1.26
1994	5	305.61	1.64	7	416.31	1.68
1995	8	316.37	2.53	9	439.83	2.05
1996	7	313.26	2.23	7	449.17	1.56
1997	3	307.84	0.97	4	446.56	0.90
1998	4	310.84	1.29	4	458.79	0.87
1999	0	319.82	0.00	1	500.76	0.20
2000	0	307.11	0.00	0	491.74	0.00
2001	0	321.07	0.00	1	549.84	0.18
2002	0	297.92	0.00	1	519.89	0.19
2003	0	275.25	0.00	0	486.40	0.00
2004	0	259.06	0.00	0	459.81	0.00
2005	0	237.04	0.84	3	420.74	0.71
2006	2	220.07	0.00	0	389.18	0.00
2007	0	198.67	0.00	0	351.27	0.00
2008	0	128.56	0.00	0	228.23	0.00

FIGURE 1

Yearly HIV-incidence of all MSM ACS participants between 1985 and 2008.

Lines show HIV incidence (expressed as cases per 100 person years (PY)) in the MSM ACS participants ≤ 30 or >30 years at study entry for the respective year.

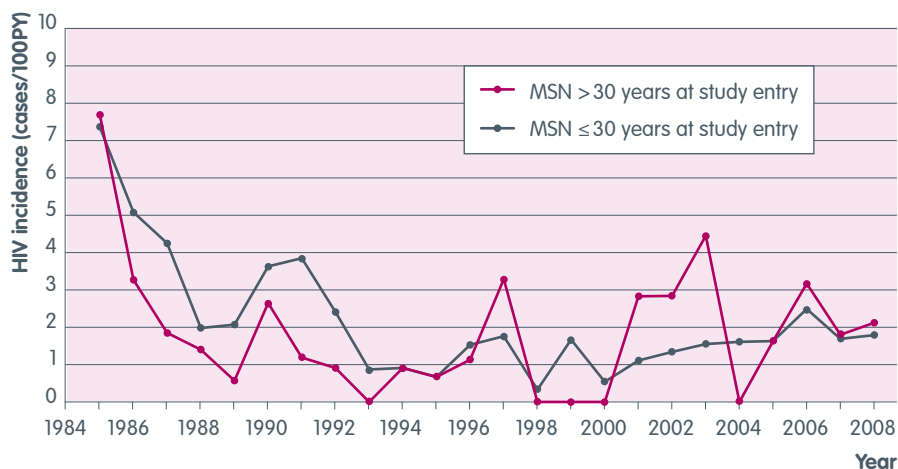


FIGURE 2

Yearly HIV-incidence of all DU ACS participants between 1986 and 2008.

Lines show HIV incidence (expressed as cases per 100 person years (PY)) in the DU ACS participants only injecting or all DU for the respective year.

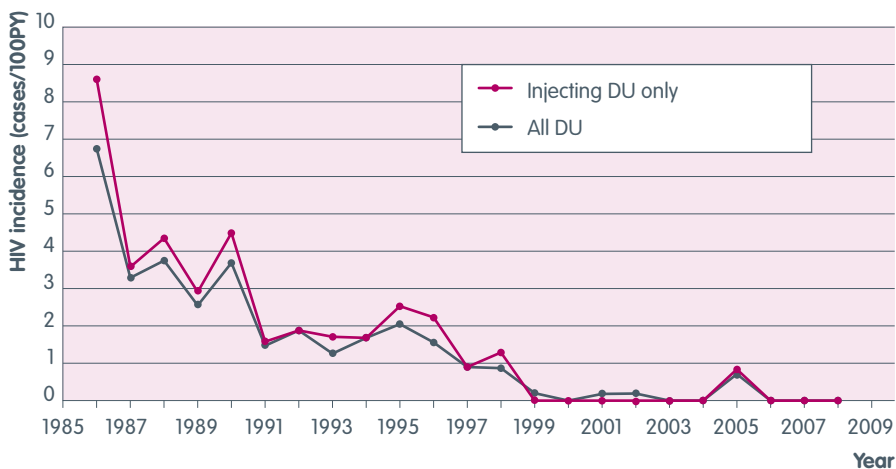


FIGURE 3

Characterization of an HIV-1 group M variant that is distinct from the known subtypes.

Phylogenetic analysis of the H10986 isolate. H10986 sequences are indicated in bold. Two early (95) and two late (01) complete genome sequences were included. The bootstrap values are shown only for the branches containing the subtype K and H10986 sequences (219).

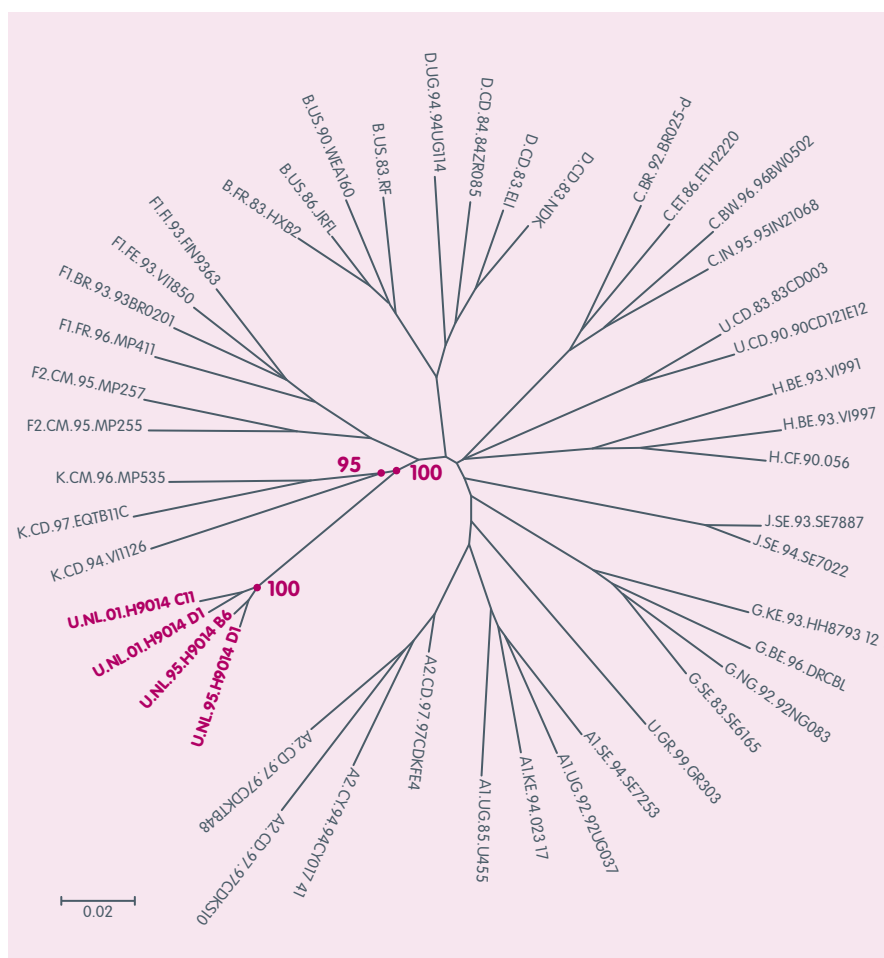


FIGURE 4**HIV-1 plasma viral load at different clinical stages.**

HIV infection is characterized by an acute phase with a high viral load, which decreases as specific immunity develops (solid line). After seroconversion (SC), the chronic phase of the infection starts, lasting several years. The chronic phase of the infection is traditionally followed by the AIDS phase, but is now increasingly replaced by the start of antiretroviral therapy (ART) in many parts of the world.

An HIV-1 dual infection during the acute phase is called a co-infection, after seroconversion it is referred to as a superinfection. HIV-1 superinfections often result in an increase, sometimes temporary, of the viral load (dotted line) and an earlier start of therapy.

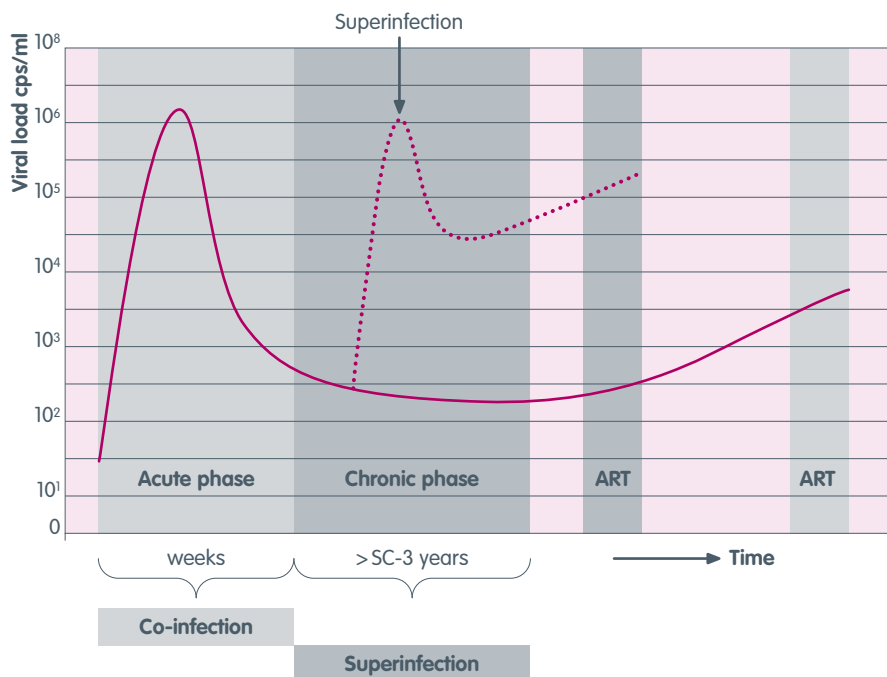


FIGURE 5

CTL escape and viral fitness.

Targeting of wild-type epitopes (WT) by cytotoxic T lymphocytes (CTL) may lead to positive selection of viruses with escape mutations (MT) in these epitopes which will not be presented to or recognized by CTL. Each selected escape mutation may come at a potential replicative fitness cost (increasing shades of grey to indicate increasing fitness cost, † for mutations that are incompatible with viral replication). Upon transmission to a host that shares HLA alleles with the donor (HLA-identical host) mutations initially may revert in the new host because of the absence of HIV-specific CTL. When HIV-specific CTL arise there can be positive selection of the same escape mutations. Upon transmission to a host that does not share HLA alleles with the donor (HLA-disparate host) reversion of mutations to the WT epitope will be driven by the gain of fitness associated with the WT. t: time point

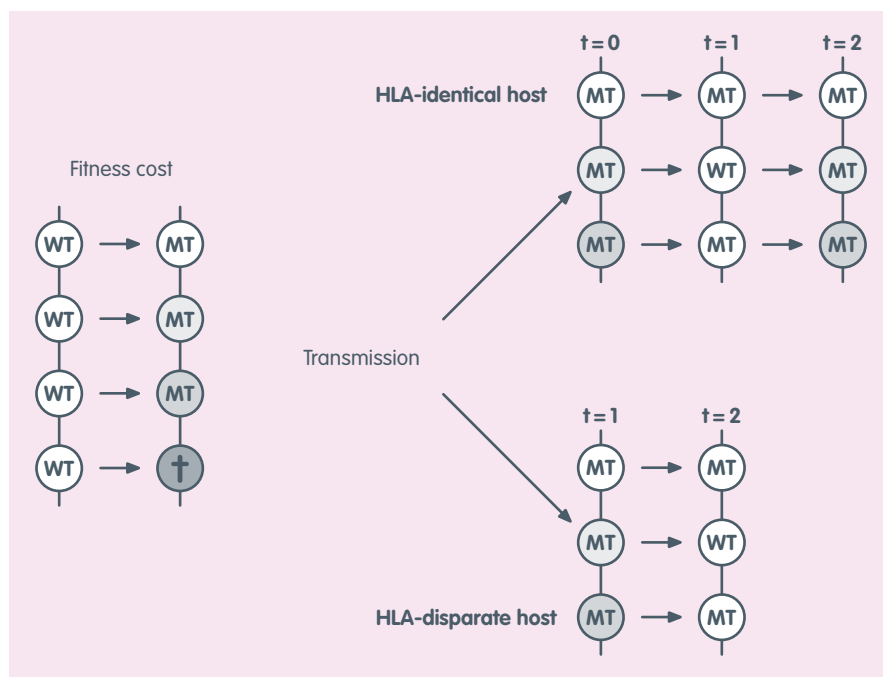


FIGURE 6.**Adaptation of HIV to the human immune system: evidence from the ACS.**

Early in the epidemic (1985) many different fragments of HIV were still recognized by T cells of the immune system. After 20 years of HIV evolution (2005), some of these fragments have disappeared, leaving the corresponding T cells non-functional.

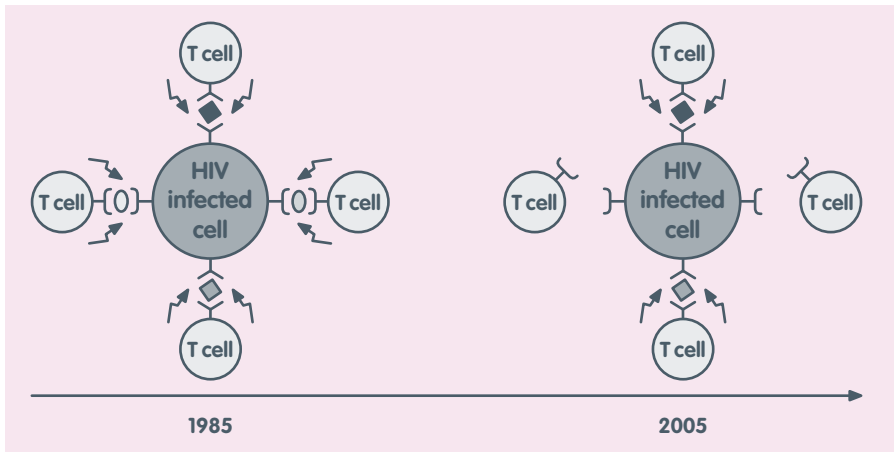
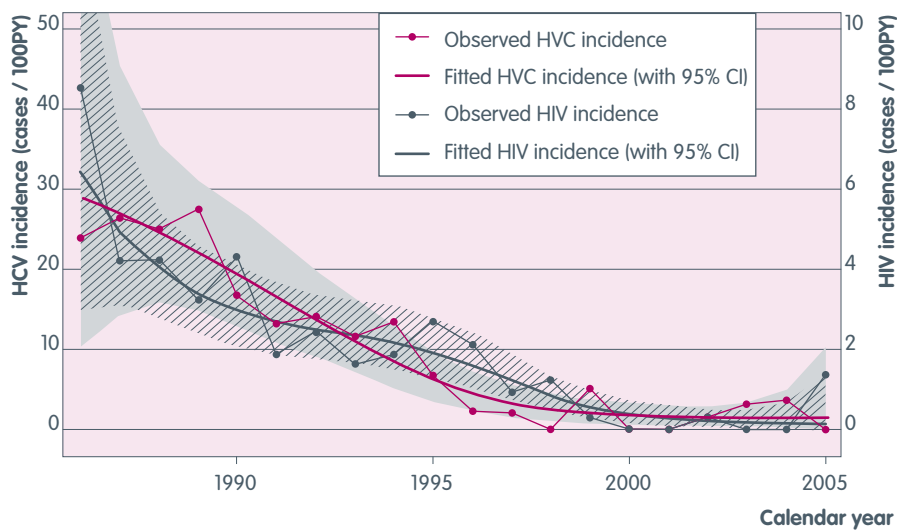


FIGURE 7

HCV incidence in ever-injecting DU. Shown are the observed and fitted (with 95% CI) HCV (left y-axis) and HIV (right y-axis) incidence curves among ever injecting DU in the ACS (1985–2005).



chapter three

Publications

3

PUBLICATIONS

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chapter four

Theses

4

THESES

2001

E.L. op de Coul (May 9). Epidemiological trends of HIV-1 shown through phylogenetic trees.

S. Kostense (Jun 26). Mechanisms of decreasing HIV-1 specific CD8+ cell activity during progression to AIDS.

R.M. van Praag (Sep 5). Anatomical and cellular reservoirs for HIV-1 during potent antiretroviral therapy.

B.H.B. van Benthem (Sep 18). Epidemiological studies of HIV infection in women.

N. Renwick (Dec 18). Human herpesvirus 8 and Kaposi's sarcoma in the Amsterdam Cohort Studies: disease association, transmission and natural history.

2002

R.P. van Rij (Mar 21). Chemokine receptors in HIV-1 infection and AIDS pathogenesis.

T. Beaumont (Jun 14). HIV-1 sensitivity to neutralization: biological and molecular studies.

M.D. Hazenberg (Sep 13). T-cell turnover and thymic function in HIV-1 infection.

N.H.T.M. Dukers (Sep 20). Epidemiology of HIV-1, HHV-8 & HSV among homosexual men.

S.C. Yates (Oct 17). HBV load in treated and untreated individuals.

M. Penning (Nov 8). HBV RNA as a new marker of virus replication.

D. Kwa (Dec 12). Host and viral factors in AIDS pathogenesis.

2003

H. Huthoff (Dec 16). Higher order structure of the HIV-1 leader RNA: A case for RNA switches that regulate virus replication.

2004

A.M. Polstra (Jun 15). Human herpesvirus 8: virology and disease.

J.A. Bogaards (Jun 30). Modeling AIDS control strategies.

F.A. Koning (Nov 26). Determinants of host HIV-1 susceptibility and R5 HIV-1 evolution.

A. Tsegaye (Dec 15). T cell dynamics and HIV specific CTL responses in Ethiopians.

2005

W. Ayele (Mar 24). Diagnostics tailored to HIV-1 subtype C: the Ethiopian experience.

C. Bronke (Mar 24). Cytomegalovirus-specific T-cell dynamics in HIV infection.

E.R. Piriou (Mar 31). Viro-immunology of EBV-HIV coinfection.

C.A. Jansen (May 13). Analysis of HIV-specific T-cell immunity in AIDS pathogenesis.

L.C.H.I. van Asten (Sep 22). Epidemiological studies among injecting drug users infected with HIV. Highly active antiretroviral therapy. Tuberculosis. Hepatitis C Immunology.

I.G. Stolte (Dec 16). The impact of highly active antiretroviral therapy on sexual behaviour among homosexual men.

2006

U. Davidovich (Feb 24). Liaisons dangereuses - HIV risk behavior and prevention in steady gay relationships.

V. Bekker (Jun 21). Pediatric HIV-1-infection: perspectives on vaccination strategies and immune reconstitution during long-term antiretroviral therapy.

M.J. Geels (Sep 5). Sequence analysis as a tool to determine viral evolution and escape from host immune responses in HIV-1-infected individuals.

H.J. Scherpbier (Oct 18). HAART in HIV-1-infected children: 10 years of clinical experience.

2007

A.K. van der Bij (Jan 9). Epidemiology of re-emerging sexually transmitted infections.

M. Casula (Jan 25). Mitochondrial toxicity of HIV-1 infection and its treatment.

N. Vrisekoop (Mar 1). T-cell dynamics in healthy and HIV-infected individuals.

M.A. Naarding (Mar 9). Inhibition of mother to child transmission of HIV-1 during breastfeeding.

E.D. Quakkelaar (Mar 23). Antibody neutralization of HIV-1.

C. Smit (Apr 10). 25 years of HIV: Trends in mortality, HIV coinfections, and HIV-related risk behaviour.

B. Tegbaru (Jul 10). Immunological consequences of Mycobacterium tuberculosis and human immunodeficiency virus coinfection in Ethiopia.

2008

A. Buchholz. Health-related quality of life and psychosocial functioning in problem drug users.

E. Witteveen (Sep 26). Knowledge gained through experience in young problem drug users. Reflections on interventions and change.

T.J. van de Laar (Nov 28). Molecular epidemiology of hepatitis C virus.

2009

I.M. Schellens (Feb 26). Impact of HLA class I-restricted T cells on HIV-1 disease progression.

M. Navis (Mar 6). Cellular immunity driving HIV-1 evolution.

R. van Gent (Mar 31). Lymphocyte dynamics in health and disease.

J. Scherrenburg (Apr 23). T-cell immunity to herpes viruses in immune disorders.

M. Rits (Jun 23). Cellular factors involved in HIV-1 replication.

R. van Houdt (Jun 24). Molecular epidemiology of hepatitis B in the Netherlands.

C.H.S.B. van den Berg (Jun 26). Hepatitis C virus: epidemiology and immunology.

T. van Montfoort (Sep 1). Interaction of HIV-1 with dendritic cells; implications for pathogenesis.

D. Bezemer (Sep 3). Impact of antiretroviral therapy on HIV-1 transmission dynamics.

chapter five

Future studies

5



THE HIV EPIDEMIC, RISK BEHAVIOR AND HARM REDUCTION

Studies on the HIV epidemic in both MSM and DU will be continued to observe trends in the incidence of HIV infection and other sexually transmitted diseases and blood-borne viral infections. In addition, studies on risk behavior, both in HIV negative and positive individuals and before and after seroconversion will be continued, with for MSM an emphasis on harm reduction strategies such as conscious risk management strategies, premeditated risk versus reoccurring incidental risk, the role of condom induced erectile dysfunctions (COINED) in sexual risk behavior, sexual risk behavior within different types of casual partners and the change of the safe sex norm over time. In MSM, molecular typing techniques for STI will be combined with the modeling of data obtained by questionnaires to study networks of STI spread. Furthermore, the uptake for self-test for STI will be investigated.

For DU, it is planned to conduct a modeling study to investigate whether next to harm reduction programs, demographic processes (e.g. differential mortality, limited number of new entries in the IDU population) contribute to the decline in HIV, HBV, and HCV infections. In addition, investigation of mortality trends in HIV negative and positive DU will be continued.

MARKERS OF DISEASE PROGRESSION

T-cell production is the major determinant of restoration of the CD4⁺ T-cell pool during HAART. (Naïve) T-cell generation may derive from thymic output and/or homeostatic T-cell proliferation. Moreover, failure of thymic output may be at play during T-cell loss in HIV infection. Using in vivo DNA labeling the role of immune activation in T-cell loss will be further investigated in studies comparing naïve T-cell turnover rates in HIV-1 and HIV-2 infection. HIV-2 infection is characterized by slower progression to disease, despite similar levels of HIV-2 induced immune activation. In patients on HAART it will be tested whether poor production or rapid loss of naïve T cells causes poor T-cell reconstitution, and whether high levels

of T-cell proliferation late during HAART are due to residual activation by the virus and hamper T-cell reconstitution, or homeostatically regulated and contribute to T-cell reconstitution.

VIRAL FACTORS

As AIDS patients are likely to carry additional infectious pathogens because of their immune-compromised state, the search for new HIV variants/subtypes and possibly novel viruses in ACS participants will continue. Newly identified viruses may then fuel the research on co-infections.

The analysis of HIV-1 dual-infections will continue apace in diverse directions, including the occurrence of viral recombination and its impact on viral fitness. A rather unique collection of more than 20 HIV-1 dual-infection cases have been identified. For this, novel sensitive technology (e.g. deep sequencing) will be incorporated to unravel complex molecular interactions.

Studies on the role of viral characteristics, including genome defects, immune evasion mutations, drug-resistance mutations and viral fitness, in the course of infection, will be continued, both in individual patients and at the population level. Follow-up studies may allow the description of novel molecular mechanisms of virus replication and virus-host interaction.

Studies on HIV evolution with respect to coreceptor usage will be continued as these have gained renewed interest with the availability of CCR5 inhibitors as a new drug regimen. Especially the application of novel technologies, such as massive parallel pyrosequencing, may allow a more sensitive detection and more detailed characterization of virus variants during transition of coreceptor usage, thus furthering our understanding of this process. Insights obtained in ACS participants during the natural course of HIV infection are expected to help understand and predict changes in coreceptor usage during CCR5 inhibitor treatment.



Studies on the natural history of HIV infection and the pathogenesis of AIDS have recently shifted their focus to the initial stages of infection, when the viral attack on the host is prominent. This research will address issues related to viral transmission such as the selection of the viral envelope. Moreover, the dynamics of HIV escape from host immunity will be studied immediately after a new infection has been established. Analysis of HIV sequences in feces from patients with primary HIV infection will allow investigation of the earliest immune escape events that may occur locally at the site of massive viral replication in this phase of the infection. The susceptibility of a broad array of immune-cell subpopulations and the effect the virus has on their dynamics during the early moments of the infections and the consequences on disease progression will be investigated as well. A key aspect of the focus on the onset of the infection is the investigation of the benefit of early short-course HAART regimens (see also below).

The precise role dendritic cells play in HIV infection in terms of both viral transmission and disease progression will be further investigated. Two new natural molecules have been identified that can bind DC-SIGN and can block HIV transfer to CD4 cells. Bile-salt stimulated lipase (BSSL) is a molecule found in the gut and human milk and MUC6 is a glycoprotein with similar activity found in semen, both of which are variable at the genotypic as well as phenotypic level. Polymorphisms in these two glycoproteins will be studied for their association with risk of HIV transmission and with disease progression in the case of BSSL since the molecule is found in plasma. Genetic differences will be linked to markers of HIV disease progression. The role of dendritic cells in selection of viral variants during disease progression will be further analyzed through longitudinal monitoring of individuals from the ACS and by determining whether escape from neutralization by specific antibodies or selection of viral variants based on interaction with dendritic cells does indeed occur.

Preferential infection/replication of HIV in specific cell types in terms of cytokine/chemokine production has not been fully delineated, nor have the implications of this for disease progression. Understanding differences

in cell type specific HIV replication could aid in the development of new strategies aimed at stimulating clinically beneficial responses. In addition, the effect of co-infections on cell type specific HIV-1 replication and specific CD4 T cell responses will be evaluated. This has been initiated for HIV and tuberculosis co-infection, where it has been found that cells producing different profiles of CC-chemokines and cytokines are indeed infected at different levels. These studies will be expanded to patient groups that allow monitoring such co-infections as malaria and helminthes.

HOST FACTORS

Host factors determining the special phenotype of HRSN will be investigated by combining in vitro susceptibility studies with genome-wide screening for host polymorphisms. In addition, the molecular mechanisms of host factors identified in previous in vivo and in vitro GWAS will be validated using ACS samples. As individual GWAS are usually only powered to reveal the host polymorphisms with the strongest clinical effects, the results of the existing ACS GWAS will be combined with other HIV/AIDS GWAS in international meta-analyses powered to identify host polymorphisms with significant but less strong effects, thus more fully utilizing the results of these screens.

The role of innate restriction factors like TRIM5 α , APOBEC3G/F and BST-2, in HIV pathogenesis will be further analyzed using samples from the HIV positive participants of the ACS. Expression levels of the innate restriction factors in the different CD4 subpopulations will be analyzed during the course of infection. Furthermore, the sensitivity of primary HIV variants to the inhibitory effect of the restriction factors will be studied during the course of infection and the molecular mechanism of viral escape will be identified.

In view of the predominantly disappointing outcomes of HIV vaccine trials, it is crucial to better understand the efficacy of HIV-specific CD8⁺ T-cell responses. This efficacy is thought to be largely dependent on qualitative



parameters of the specific T-cell population. The hypothesis will be tested that the maintenance of a diverse TCR repertoire including high affinity T-cell clones against HIV may confer protection against progression to AIDS and that this distinguishes CD8+ T cells restricted through protective HLA-alleles, like HLA-B57 and B27, from those restricted to other alleles. To this end, TCR-diversity will be studied of epitope-specific T cells directed to different HIV proteins and restricted through different HLA-molecules in the natural course of HIV-infection and after early treatment interruption in relation to virus variation and T-cell functional profile and avidity. These detailed analyses of the clonotype composition of HIV-specific T cells will lead to more insight in the induction and maintenance of effective antigen-specific T-cells, the cells that should ultimately be induced by an HIV-vaccine.

The absence of a correlation between potent neutralizing activity in serum and the clinical course of infection indicates rapid escape of HIV even from strong neutralizing autologous humoral immunity. The epitopes at which potentially neutralizing activity is directed will be identified and may benefit the design of a novel vaccine.

The observation that only 30% of individuals has a potentially neutralizing immune response may imply that their genetic make-up is different or that they were infected with virus variants on which critical epitopes capable of eliciting these humoral immune responses are better exposed. Both options will be studied by combining genome wide SNP data with data on neutralizing activity in serum and by phylogenetic analysis of gp160 envelope sequences which will be generated from the viruses from all cohort participants.

INTERVENTIONS

With the aging of the epidemic, unprecedented and complex challenges need to be addressed in order to maximize care of the older HIV-infected population. The compound effects of HIV infection, its treatment and

increasing age on the incidence and prevalence of cardiovascular disease, malignancies, cognitive impairment, depression and frailty are of increasing concern. Future studies will be directed at unraveling the underlying mechanisms for these co-morbidities.

Recent research suggests that HIV and/or its associated effects on the immune system may detrimentally affect a wide range of organ systems, resulting in an increased risk of developing what have been called non-AIDS clinical events. These include but are not limited to events such as myocardial infarction, chronic renal and liver disease, osteoporosis and bone fractures, and malignancies previously not traditionally associated with HIV infection. The pathogenesis of these events is undoubtedly multi-factorial and apart from the effects of HIV also includes traditional risk factors such as increasing age, lifestyle related factors and long-term toxicities of drugs used for the treatment of HIV infection. In order to unravel the effect of HIV and the immune system on the various mentioned organ systems, investigation of relevant biomarkers in samples obtained before and after HIV seroconversion prior to the start of antiretroviral therapy as well in persistently HIV seronegative matched controls may yield further insight.

The lowered set-point observed after short-course early HAART warrants further investigation. Firstly, it still needs confirmation whether early HAART during primary HIV infection postpones the time until restart of HAART long enough for patients to enjoy a longer total time off-therapy. In addition, fundamental research needs to establish the immunological and virological explanations for the lowered set-point.

HIV-INFECTED AND HIV-EXPOSED CHILDREN

Pediatric HIV research will focus on two important aspects. First, long-term side-effects of HAART medication in children and teenagers will be evaluated. In addition to lipodystrophy, it is expected that HAART-related late puberty, osteoporosis, and vascular changes may complicate

treatment and affect adherence. Both the antiretroviral- and side-effects of the HAART combinations used thus far in Amsterdam are being studied prospectively in a long-term follow-up program and will be evaluated in due time. Second, neonatal transmission and the mechanism of HIV entry in a naïve CCR5-negative T cell system will be studied. Both cord blood and mucosal tissues of neonates are being investigated in detail, including the impact of inflammatory factors on the efficiency of HIV transmission in case of neonatal T cells. The Global Child Health program and cohort studies in Africa will provide the necessary perspective for field studies on mother-to-child-transmission in daily practice.

CO-INFECTIONS

Research on viral and other co-infections will be intensified. The retrospective testing on co-infections should provide a wealth of information for further studies including the field of epidemiology, disease progression, virus interactions and poly-microbial disease. Work has been initiated to study HCV infection within the context of HIV-1 infection. Future studies are aimed at completing HCV screening among MSM in the ACS until 2008 to see whether the HCV incidence continues to rise after 2003. Furthermore, recently all HBV, HCV, and HIV seroconverters have been identified enabling the combination of hepatitis and HIV research lines. The order of acquisition of these infections will be investigated in MSM and DU. Also the effect of HBV and HIV co-infection on the natural course of HCV infection (clearance, viral load trajectory, morbidity and mortality) will be studied in HCV seroconverters. Studies on the effect of viral hepatitis on HIV outcomes and studies on HCV re- and super infection will be continued.

The new cohort of HIV-positive MSM with acute HCV infection was started to 1) elucidate risk factors for sexual transmission of HCV; 2) investigate treatment results of acute HCV in the presence of HIV; 3) determine the rate of HCV re-infection and superinfection. In 2005, within the DU cohort, a feasibility study was started to evaluate the possibility of HCV

testing and treatment combined with methadone programs (Dutch C). It is planned to investigate trends in quality of life, drug abuse, co-medication and psychiatric symptoms before and after HCV treatment. A qualitative study of the reasons for DU to initiate and finish HCV treatment is currently undertaken. A cost effectiveness analyses will be done, as well as long-term follow-up relapse and re-infection studies.

HCV molecular evolution will be studied, e.g. envelope protein characteristics that are associated with HCV disease progression, in particular within the context of the cellular and humoral immune responses. Specifically, the role of specific T-cell immunity in progression to liver disease and adaptation of HCV to the host immune response will be investigated. Future studies are also aimed at HCV-specific immunity in relation to re-infections with HCV, which occur frequently. In addition, the consequences of HIV/HCV co-infections for general and specific immunity will be studied.

For HBV, future studies are aimed at mapping occult infections within these high risk populations of the ACS. It will be assessed what part of new HBV infections is caused by either acute or chronic infected individuals, by using differences in viral load, full length sequencing, and mathematical modeling. New studies on HPV are aimed at the type-specific prevalence, incidence, risk factors, and clearance of HPV among MSM. For HSV-2, the incidence in DU and MSM and the role of HSV-2 in HIV acquisition will be investigated. Finally, ongoing studies are aimed at unraveling the role of T cells directed against multiple EBV and CMV-antigens to see if these responses are protein-specific or reflect more general kinetics of latent and lytic (early) antigen-specific responses during natural course of infection and after antiretroviral therapy.



EPILOGUE

With this overview, four overviews (regarding the periods of 1984-1992, 1984-1995, 1996-2000 and 2001-2009) now cover the first 25 years of the ACS. This multidisciplinary collaboration has resulted in a rich research output. Indeed, we expect the 100th PhD thesis resulting from research related to the ACS to be defended by the end of the year. The results of studies conducted in the context of the ACS have enormously enriched our understanding of one of the biggest public health challenges of our time, the HIV/AIDS pandemic. Our results have strengthened evidence-based practices and fostered decisions to be based on sound scientific insights.

But continued vigilance is warranted. Indeed, although the availability of effective antiretroviral drugs has dramatically improved the perspective for people with HIV, the side effects of antiretroviral therapy and co-morbidities of HIV infection pose new challenges for patients, physicians and scientists. Current prevention measures have been unable to stop the spread of HIV on a global level. In addition, despite huge research efforts, there are still no effective HIV vaccines.

The physicians and scientists who started the ACS when the HIV/AIDS pandemic just started 25 years ago could not have foreseen that their initiative would result in an extremely valuable resource allowing excellent research then, now, and hopefully in the future. This was only possible with the continuous collaboration of all men and women who were willing over many years to pay regular visits to the Public Health Service of Amsterdam, the Jan van Goyen Medical Center, or the Academic Medical Center, to donate their blood and to answer questionnaires, and in that way, to support research. For many HIV-infected participants in the cohort, the effective antiretroviral drugs came too late and we could do little to help them. However, these men and women fully supported the studies because new results could still benefit others.

We are indebted to all cohort participants for their invaluable contribution to science and the understanding of the HIV/AIDS pandemic.

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