Markers of HIV-1 infection and its pathogenesis

Maas, J.J.

Citation for published version (APA):
Chapter 8

General discussion
There can be little doubt that the HIV epidemic is one of the most devastating pandemics of this century. In some parts of Africa, AIDS has reduced life expectancy by more than 17 years. Until now, at least 11 million Africans have died and currently another 22 million are estimated to be HIV-1 infected, thereby jeopardising the economic development of Africa. While the number of new infections there is still increasing, in Europe and the USA, the AIDS epidemic is more or less under control. Based on the epidemiological developments in their own regions, and the emergence of promising new treatment alternatives as HAART, western governments have reduced or re-allocated much of their financial contribution to AIDS research. Money spent on the prevention of HIV-1 infection no longer holds pace with the number of people affected by the virus; much of the funding formerly used of studying the natural history of HIV-1 infection, is now used of studying treatment possibilities that can only be implemented in Western countries. This severely hampers the swift development and introduction of effective vaccines and/or affordable treatment for AIDS.

Reductions in government funding have also limited the possibilities of the Amsterdam Cohort Study on HIV-1 infection and AIDS among homosexual men (ACS). Using an unique multidisciplinary approach, including: epidemiology, social science, virology, immunology and clinical medicine, the ACS has published approximately 440 articles in scientific magazines and also 50 PhD theses. Thus, the ACS has contributed substantially to the knowledge of HIV and AIDS. The introductory Chapter 2 provides a full 'historic' overview of the history, study groups and logistics of the Amsterdam Cohort Study on HIV/AIDS among homosexual men. The other chapters in this thesis are focussed on the use of various markers of progression and their pathogenesis.

The purpose of the study presented in chapter 3 was twofold. The first aim was to validate the previously described concept of blind T cell homeostasis in the ACS, using the same algorithm applied in the Multicenter AIDS
Genera l discussion

Cohort Study (MACS), Baltimore, USA. The second aim was to investigate whether there was an association between failure of T cell homeostasis, which is denoted as the inflection point (IP), and the NSI/SI switch. This NSI/SI switch has been studied on many occasions in the ACS.

In accordance with the previously described homeostasis concept, a biphasic trajectory was observed with a relatively stable number of peripheral CD3+ T cells in the first phase, followed by a steep CD3+ T cell decline in the last and second phase prior to AIDS. Amongst subjects who developed AIDS, failure of homeostasis was often preceded by a NSI/SI switch. The median post-IP CD3+ T cell decline observed among SI carriers was significantly steeper than that of the NSI carriers.

Similar patterns had previously been found amongst participants of the ACS with regard to the relationship of NSI/SI switch and CD4+ T cells. Schellekens et al. and Spijkerman at al. 11,12 found a three to fivefold greater CD4+ T cell decrease among SI carriers when compared with subjects carrying the NSI phenotype. Likewise, also the HIV RNA load increased exponentially in the time preceding both the IP and the SI switch, but levelled-off afterwards 13,14.

The study described in chapter 3 may possibly provide a further explanation of the pathogenesis of the previously described findings. We speculate that a likely explanation for the acute inflection of the total T cells may be an interference of T cell renewal 11,15, due to the broadening of the target cell population by CXCR4 bearing cells as naive and precursor cells 16-19.

In chapter 4, a study is presented in which the in vivo Delayed-Type Hypersensitivity (DTH) skin testing is compared with the various in vitro assays used to assess the extent of immunological deterioration of HIV-1 infected individuals. In this study, an association was found between the presence of skin test anergy and a suppressed T cell reactivity after stimulation with CD3 antibodies and CD2 and CD28 antibodies simultaneously. This is consistent with the findings of Tomar et al. and
Markers of HIV-1-infection and its pathogenesis

Kerby et al., who both found a relationship between DTH skin reactivity and in vitro lymphocyte transformation after stimulation with various recall antigens \(^{20,21}\). Furthermore, antigen-specific lympho-proliferative responses (LPRs) to certain recall antigens such as Candida and tetanus toxoid \(^{22,23,24}\) also standardised T cell reactivity as mentioned above \(^{25-27}\), have both been shown to be strongly associated with the degree of immune deterioration and stage of HIV-1 infection.

The association that was observed in this study also provides a rational for the potential use of skin testing to assess the extent and durability of immune reconstitution in persons who receive HAART. In a recent study amongst HIV-1 infected individuals from the ACS, a group was selected of persons who, having started treatment with HAART, had responded well in respect of both an increased CD4\(^+\) T cell count and a HIV RNA load decline below the threshold of quantification. In this group, no difference in DTH scores before and after initiation of HAART (median duration HAART 2.1 year) could be determined (Figure 1) \(^{28}\). However, three months after starting HAART a temporary improvement of the skin test reactivity was observed (Figure 2). A similar DTH improvement was also recognised by Wendland et al. \(^{29}\). Unfortunately, because the follow-up in this study was limited to only six months of HAART, no statements could be made regarding the possible long-term
General discussion

improvement of the *in vivo* DTH. In this study, parallel to the enhanced DTH reactivity, also an increase of the *in vitro* antigen-specific T cell proliferation response was seen.²⁹

**Figure 2. DTH scores, measured at starting HAART and three months later (n=4, p=0.06).**

However, if we compare these long-term *in vivo* DTH results with other clinical-immunological *in vitro* studies, a discrepancy between the *in vivo* and *in vitro* results becomes apparent. In several studies, T cell reactivity has shown a rapid increase during the first weeks of HAART, followed by a gradual increase later on.³⁰-³⁴ This is in contrast to the observed lack of DTH skin test reactivity, two years after starting HAART. Although the precise reason for this *in vivo/vitro* gap is still unknown and because the DTH reactivity seen among treated HIV-1 infected individuals was much lower than the DTH reactivity seen among healthy HIV seronegative individuals, it is evident that it must be a consequence of the partial immune restoration. DTH skin testing can therefore be useful as an additional tool in monitoring the extent of immune recovery in HIV-1 infected individuals on HAART.

In chapter 5 we found that a mutation in the gene encoding for Mannose-Binding Lectin (MBL)³⁵ was associated with a delayed progression of the disease. Most notably, this mutation appeared to be associated with a delayed onset of Kaposi’s sarcoma (KS) and, to a lesser extent, to the onset of other AIDS defining events. Mannose-Binding Lectin (MBL) is a serum protein which plays a central role in the opsonisation and phagocytosis of several yeasts, bacteria such
As *N meningitidis* 37, *Myobacterium tuberculosis* 38 and *leprae*, *Pneumocystis carinii* 39, and HIV 40. In addition, serum MBL levels are also associated with systemic lupus erythematosus and chronic hepatitis B infection 41-43. The MBL mutation is known to be associated with lower MBL serum levels 35. Because serum MBL has a close resemblance to C1q, the first factor of the complement cascade, it is able to activate both the classical and the alternative complement pathways 44-46. Serum MBL is, furthermore, capable of *in vitro* neutralisation of HIV-1 by binding to gp120 or gp41.

A protective effect of the MBL mutation was previously found in a French seroconverters study. The French investigators found an association between the MBL mutation and delayed CD4⁺ T cell loss 47. Another important consequence of the variance in MBL alleles is that carriers of the MBL variant allele are at an increased risk of becoming infected with HIV 45,48,49. It was demonstrated that carriers of the MBL variant alleles were more susceptible to HIV-1 infection than MBL wild type carriers.

From these studies, it can thus be postulated that carriers of the MBL variant allele may have a disadvantage at their first encounter with HIV. However, once infected, it may turn out to be beneficial to be carrying the MBL variant allele 50. To explain this advantageous effect, we postulated that HIV-1, besides direct binding of HIV-gp120/gp41 51-55 to the cellular CD4 receptor, is also capable of infecting host cells through the pathway of (mainly antibody-dependent) complement activation 56-58. This is in accordance with the great variability seen in incubation times amongst individuals with solely the non-syncytium-inducing virus phenotype, which cannot be explained by an expanded usage of known co-receptors such as CCR1, CCR2b, CCR3, CCR5 and CXCR4 16.

As mentioned before, complement and Fc receptors on the surface of target cells may act as receptors for HIV-1 60-62. This is nicely illustrated by the positive relation which was found between the extent of antibody-dependent complement-mediated antibody enhancement and HIV-1 RNA viral load 54,55,63 and stage of
General discussion

disease\textsuperscript{64}. Furthermore, an inverse relationship was found between MBL serum levels and CD4\textsuperscript{+} T cell count and the stage of HIV-1 infection\textsuperscript{65}. In general, the higher the serum MBL levels the lower the CD4\textsuperscript{+} T cell counts and vice versa.

Taking into account that serum MBL is capable of initiating the complement system, MBL variant allele carriers may - due to an impaired first-line defence - be less likely to induce a vigorous immune response during primary infection, than wild type carriers. It can thus be speculated that the set point of HIV-1 infection is not only influenced by the genetic host factors of the adaptive immunity such as CCR5 and CCR2 but also by the MBL-complement-mediated pathway.

How can we explain the observed protective effect of MBL variant alleles on the development of KS? Here the following is hypothesised. It has been shown that KS is one of the few vascular-proliferations in humans who expresses Mannose-receptors on their endothelial \textsuperscript{66}. Low MBL serum levels, as seen amongst MBL variant allele carriers, may therefore inhibit the proliferation of the KS-associated herpes \textsuperscript{8,67} infected precursor spindle cells \textsuperscript{68,69}, thereby preventing the development of KS \textsuperscript{53}.

The aim of the study presented in chapter 6 was to determine the occurrence of early pre-AIDS weight loss among HIV-1 asymptomatic homosexual individuals, and to establish its predictive value for getting AIDS \textsuperscript{70,71}. In those who developed AIDS, a biphasic weight pattern was found showing a relatively stable period followed by a steep weight decline that started around 6 months prior to the subsequent diagnosis of AIDS. To rule out that this decline was caused by a not yet diagnosed AIDS-defining illness such as an opportunistic infection, we also examined the weight patterns per diagnosis separately.

Surprisingly, this biphasic pattern could also be recognised amongst individuals diagnosed with localised KS, an affliction that is not considered to have a big impact on metabolism in general. The observed weight loss is therefore likely to be, at least in part, a
Markers of HIV-1-infection and its pathogenesis

In the last study of this thesis, we studied the possible suppressive effect of sun light exposure on cellular immunity. This is particularly relevant because Kaposi’s sarcoma can be treated nowadays using photo-therapy. The relation between exposure to sunlight and cellular immunity was studied, using the following assays: CD4+ T cell count, CD4/CD8 ratio and T cell reactivity after stimulation with antibodies targeting the CD3 receptor. By means of a questionnaire, the exposure to ultraviolet-B radiation (UVB) in the previous two years was assessed. Subsequently, the outcomes of those questionnaires were compared with the immunological assessments performed within that same period. In this study no indications could be found of a UVB induced immunological interaction. This is in agreement with the few available clinical UVB studies among HIV-1 infected individuals, which were also unable to detect any clinical association.

In the last study of this thesis, we studied the possible suppressive effect of sun light exposure on cellular immunity. This is particularly relevant because Kaposi’s sarcoma can be treated nowadays using photo-therapy. The relation between exposure to sunlight and cellular immunity was studied, using the following assays: CD4+ T cell count, CD4/CD8 ratio and T cell reactivity after stimulation with antibodies targeting the CD3 receptor. By means of a questionnaire, the exposure to ultraviolet-B radiation (UVB) in the previous two years was assessed. Subsequently, the outcomes of those questionnaires were compared with the immunological assessments performed within that same period. In this study no indications could be found of a UVB induced immunological interaction. This is in agreement with the few available clinical UVB studies among HIV-1 infected individuals, which were also unable to detect any clinical association.

In large parts of the world, the use of elaborated laboratory tests such as CD4+ T cell count and HIV RNA assays are limited or even unavailable. In a non-western patient population therefore, body weight assessments may provide additional information to CD4+ T cell measurements.
**General discussion**

**Final remarks**

The aim of this thesis was to investigate the validity, relevance and predictive value of several markers of progression of HIV-1 infection (both established and not-established) and subsequently to speculate about their pathogenesis.

In several long-term natural history studies on HIV/AIDS, biphasic patterns are recognised regarding CD4+ CD8+ T cells, memory/naive cells and HIV RNA Load. In the third study of this thesis, a strong association was found between the moment of NSI/SI switch and failure of blind T cell homeostasis. However, here an inconsistency arises. Currently, as summarised above, there have been many other adequate explanations for the observed homeostatic failure. Despite this inconsistency regarding the "blindness" of the concept, estimations of the moment of homeostatic failure have proven to have an additional predictive value, instead of using the NSI/SI switch alone. Finally it could be said in this time of rapidly evolving knowledge of the pathogenic mechanisms of HIV-1 infection that the adjective ‘blind’, when referring to the blind T cell homeostasis, has become somewhat redundant.

Although both antigen-specific and non-specific in vitro T cell reactivity recover significantly during long-term potent HAART, the degree of in vivo DTH skin test reactivity, despite a temporary increase shortly after starting HAART, hardly improves. This discrepancy is most likely due to the partial character of the HAART induced immune reconstitution. However, because it can be postulated that this gap will eventually become smaller if we are able to restore cellular immunity sufficiently by long-term viral suppression, standardised DTH skin testing should always be implemented in modern clinical trials.

In this thesis it was shown that the morbidity and mortality of HIV-1 individuals are influenced by a mutation in the gene encoding for MBL, which is part of both the innate immunity and the complement system. To obtain conclusive evidence of the role of MBL, adequately sized case-control studies have to be designed. Furthermore, although it is clear from
numerous publications that the extent of complement activation directly affects the level of HIV RNA and survival, the clinical relevance has still to be determined.

Although in the Western world, severe weight loss is hardly ever seen anymore, in third world countries systematic weight assessments may still be useful. Finally, no harmful effects could be found on the cellular immunity of asymptomatic HIV-1 infected individuals following exposure to UVB, which is very good news for HIV-1 infected sun lovers.
General discussion

Reference List

1. Piot P, Madavo C, and Samba E: UN leaders call AIDS in Africa a massive development catastrophe. Officials brief reporters on social and economic impact and needs for significant increase in resources to Fight the epidemic. 1999. UNAIDS press release.


Markers of HIV-1-infection and its pathogenesis


14. Spijkerman I, de Wolf F, Langendam M, Schuitemaker H, and Coutinho RA: Emergence of Syncytium-inducing Human Immunodeficiency Virus type 1 variants coincides with a transient increase in viral RNA level and is an independent predictor for progression to AIDS. *Journal of Infectious Diseases* 1998;178: 397-403.


18. Blaak H, van 't Wout AB, Brouwer M, Hooibrink B, Hovenkamp E, and Schuitemaker H: In vivo HIV-1 infection of CD4RA+ CD4+ T cells is established primarily by syncytium-inducing variants and correlates with the rate of CD4+ T cell decline, 1999;(submitted)


General discussion


22. Quinti I, Palma C, Guerra EC, et al: Proliferation and cytotoxic responses to mannoproteins of 
   *Candida albicans* by peripheral blood lymphocytes of HIV- infected subjects. 


25. Miedema F, Roos MTL, Gruters R, and Schellekens PTA: Low T cell responsiveness to activation 
   via CD3/TCR: A prognostic marker for AIDS Useful for monitoring antiviral Therapy, in 
   Janossy G, Miedema F (eds): *Immunodeficiency in HIV-1 infection and AIDS.* Karger; 

26. Roos MTL, Miedema F, Meinesz AAP, de Leeuw NASM, Pakker NG, and Lange JMA: Low T cell 
   reactivity to combined CD3 plus CD28 stimulation is predictive for progression to AIDS: 

27. Schellekens PTA, Koot M, Roos MTL, Tersmette M, and Miedema F: Immunologic and virologic 
   1995;10 (suppl 2):S62-S66

28. Maas JJ, Coutinho RA: Reliability of PPD skin testing and DTH skin test reactivity in HIV-infected 
   individuals on HAART. 7th European conference on clinical aspects and treatment of 
   *HIV-infection* 1999;344:(Abstract)

   antigen-specific CD4 T cell responses in vitro is correlated with CD4 memory T cell 
   reconstitution, whereas improvement in delayed type hypersensitivity is related to a decrease 

   potent antiretroviral therapy in previously untreated human immunodeficiency virus type 1 
Markers of HIV-1-infection and its pathogenesis


General discussion


Markers of HIV-1-infection and its pathogenesis


Markers of HIV-1-infection and its pathogenesis


