Cross-reactive neutralizing humoral immunity in HIV-1 disease: dynamics of host-pathogen interactions
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Chapter 1

General introduction
INTRODUCTION TO THE HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1)

In 1981, a new disease appeared in the human population that was characterized by a deficiency of the immune system. This acquired immune deficiency syndrome (AIDS) was marked by a reduction in CD4+ T-cell numbers and the presentation of unusual infections and cancers. Two years after the recognition of AIDS, the causative agent, an at the time new human retrovirus belonging to the lentivirus family, was identified and named the human immunodeficiency virus type 1 (HIV-1). HIV-1 has been introduced into the human population by cross-species transmissions of the simian immunodeficiency virus (SIV) from non-human primates in West-Central Africa in the beginning of the twentieth century.

Although important progress has been made in the prevention of new HIV-1 infections, and the reduction of the annual number of AIDS related deaths through anti-retroviral therapy, the number of people living with HIV-1 continues to increase and in 2009 approximately 33 million people were infected globally. AIDS-related illnesses remain one of the leading causes of death and are projected to continue as a significant global cause of premature mortality, particularly in developing countries. Despite major advances in the development of antiretroviral treatments and in our understanding of the pathogenesis of HIV-1, the development of a cure, or a vaccine to prevent HIV-1 infection remain enormous scientific challenges.

HIV-1 INFECTION AND DISEASE COURSE

HIV-1 spreads through unprotected sexual intercourse, blood-blood contact, or from mother to child during pregnancy, childbirth and breastfeeding. HIV-1 can infect a broad range of immune cells, nevertheless HIV-1 mainly infects CD4+ T-cells, through a multi-step process. In addition to the binding of the CD4 receptor, HIV-1 requires binding to a co-receptor to enter the cell. Chemokine receptors CCR5 and CXCR4 are the most important co-receptors for HIV-1 entry. After entry, HIV-1 integrates into the host-cell DNA, ensuring the replication of HIV-1.

During primary infection high viral load levels can be observed, reaching a peak which is mirrored by a severe loss of CD4+ T-cells from the peripheral blood. Hereafter a decline in viremia can be seen that subsequently settles at a generally lower steady level, the viral setpoint. This decline may be a consequence of an effective immune response and/or due to the limitation of target cells. In the absence of therapy, HIV-1 infected individuals generally develop AIDS within 7-11 years after infection, however the clinical course of HIV-1 infection can be highly variable. Approximately 10-15% of infected individuals are rapid progressors who have a fast CD4+ T-cell decline and who develop AIDS within 3-4 years after infection. Approximately 5-10% of HIV-1 infected individuals are long-term non-progressors (LTNP) who can remain healthy without antiretroviral therapy for more than 10 years. In addition, a small group of individuals known as elite controllers remain to
have low to undetectable viral loads for at least one year\textsuperscript{19}. Both host (for example HLA-B57 and CCR5Δ32) as well as viral factors (for example HIVΔ nef) have been associated with slower HIV-1 disease progression\textsuperscript{20,21}.

**HIV-1 Envelope Structure and Function**

Entry of HIV-1 is mediated by the viral envelope glycoprotein (gp) on the surface of the virion. The HIV-1 envelope glycoprotein is synthesized as a gp160 precursor protein, which is subsequently cleaved into two subunits; surface protein gp120 and transmembrane protein gp41. Three subunits of gp120 bind non-covalently to three subunits of gp41 to form a trimer on the outside of the virion\textsuperscript{22}.

Gp120 is composed of five conserved regions (C1-C5) that are interspersed with 5 variable regions (V1-V5)\textsuperscript{23}. The conserved regions form a central core consisting of an inner domain, which interacts with gp41 and is important for trimer formation, and an outer domain, which interacts with the (co)receptors. The variable regions can be highly diverse between patients as well as within patients, and form flexible loop structures on the outer domain of gp120\textsuperscript{24}. When gp120 binds to the CD4 receptor, conformational changes occur in the protein, which reveals the co-receptor binding site that was occluded before CD4 receptor binding\textsuperscript{25,26}. After sequential binding of gp120 to the co-receptor, gp41 mediates membrane fusion and insertion of viral genomic material into the cell\textsuperscript{25,27}.

The chemokine receptors CCR5 and CXCR4 can be used as co-receptor by R5 and X4 HIV-1 strains, respectively\textsuperscript{28-30}. The envelope glycoprotein has developed multiple mechanisms to evade the host humoral immune response, including trimeric exclusion, occluded (co)receptor binding sites,\textsuperscript{31-36} and the shielding of conserved epitopes by the highly variable flexible loops and the presence of many glycans on the outer domain, which reduce the immunogenicity of the envelope glycoprotein\textsuperscript{36} (Figure 1.1).

![Figure 1.1: The HIV-1 envelope glycoprotein](image)

Schematic representation of the HIV-1 envelope glycoprotein in its trimer structure with the variable loops and glycans protecting the surface of the envelope glycoprotein against neutralizing antibodies.
THE GENETIC DIVERSITY AND EVOLUTION OF HIV-1

One of the characteristics of HIV-1 is its enormous sequence diversity. During infection, each day between $10^8$ and $10^{10}$ viral particles are being produced and eliminated. The error-prone viral reverse-transcriptase enzyme and the lack of proofreading mechanisms during reverse transcription of the viral RNA result in frequent mutations in the viral genome. The large turnover of virus in combination with this high mutation rate results in a mixed population of related but distinct HIV-1 variants, also termed the viral quasispecies. Viral variants within a quasispecies are continuously competing, and the dominant sequence reflects the most fit variant at that time point. After accidental introduction of beneficial mutations in the viral genome or due to changing environmental factors, such as the introduction of antiretroviral agents or the emergence of effective HIV-1 specific immune responses, an initially minor virus population may become dominant, after which a new, so-called population equilibrium is established.

All viral genes are prone to mutation and the proteins they encode are subject to variation. However, large sequence variation is not allowed in each viral genomic region as this may interfere with viral fitness. For example, the gag and pol regions are relatively conserved as viruses with mutations in those regions, which generally come at a fitness cost, are outcompeted by coexisting viruses that lack this mutation. Only when the positive selection pressure on such mutations is higher than the fitness cost associated with it, the mutant virus will be outcompeted by the wild type variants.

The envelope glycoprotein of HIV-1 is highly variable, creating an enormous sequence variation which may be as high as 10% within the viral quasispecies in a single individual. Apparently, the regions in which this huge sequence variation occurs are not critical to the viral replication process.

Despite the high diversity, some viruses are more closely related to each other which has led to a classification of HIV-1 variants into clades, also called subtypes. The main group (M-group) is subdivided into subtypes A to K and different circulating recombinant forms (CRFs), which have different geographic distributions. Subtype B for instance predominates in Europe, the Americas, and Australia, whereas subtype C predominates in Sub-Saharan Africa and the Indian subcontinent. The prevalence of intersubtype recombinant strains is increasing and creates even more HIV-1 genetic diversity. The viral envelope glycoprotein currently already differs by up to 35% between subtypes and up to 20% within subtypes, with the variable regions and also the third constant region (C3) being the most diverse between subtypes.

THE HUMORAL IMMUNE RESPONSE AGAINST HIV-1 IN NATURAL INFECTION

The majority of HIV-1-infected individuals mount an HIV-1-specific neutralizing humoral immune response within weeks to months after primary infection. This response is considered to be strain-specific as neutralizing activity is generally restricted to the
autologous virus variant and mainly directed against the variable regions of the envelope glycoprotein. These antibodies rapidly select for escape variants of HIV-1 that have become resistant to neutralization as a result of amino acid substitutions, insertions and/or deletions in the variable regions, and/or changes in the glycan shield. Escape from neutralizing antibodies may be mediated by mutations in the epitope as a consequence of which the antibody is no longer able to bind, or by changes in other regions of the envelope that prevent access of the antibody to the neutralizing epitope. In response to neutralizing antibody pressure, the envelope glycoprotein can evolve to escape from neutralizing antibodies through variations in the variable loops, including large insertions and deletions, and changes in the number of potential N-linked glycosylation sites (PNGS). In particular, length and glycosylation characteristics of the V1V2 loop seem to play a role in resistance against neutralizing antibodies, possibly by shielding underlying regions of the envelope glycoprotein from antibody recognition. Irrespective of the mechanism, such viral escape variants will rapidly be selected by the humoral immune pressure and will replace the neutralization sensitive virus variants (Figure 1.2).

Cross-reactive neutralizing humoral immunity, which can neutralize viruses from different subtypes, may bypass these viral defense mechanisms targeting the more conserved regions on the envelope glycoprotein. However only a few so called broadly neutralizing antibodies, that can neutralize HIV-1 variants from different subtypes, have been isolated from HIV-1 infected individuals. The epitopes of the broadly neutralizing antibodies are conserved domains on the envelope trimer, such as the CD4 binding site, and the membrane proximal external region (MPER) of gp41. These broadly neutralizing antibodies, either alone or in combination, have been shown to give protection from infection after passive transfer in several macaque models. These results together with the high potency of the broadly neutralizing antibodies give hope for a protective vaccine against HIV-1 infection.

Figure 1.2: Escape of HIV-1 from neutralizing antibodies
Neutralizing antibodies are elicited by the viruses present early after infection and rapidly select for antibody escape variants. The emergence of escape variants causes the development of new neutralizing antibodies leading to successive cycles of antibody production and viral escape.
**General introduction**

**HIV-1 vaccine development**

It is generally assumed that an HIV-1 vaccine should elicit both humoral and cellular immune responses. In combination, these responses ideally can protect against acquisition of infection or second best, against disease progression by reducing viral load which will also have an impact on the spread of HIV-1 in the population. Broadly neutralizing antibodies are likely to be a key component of protective vaccine-elicited immunity against HIV-1, however, to date, no immunogens have been developed that elicit such broadly neutralizing antibodies.

The design of an immunogen that is capable of eliciting broadly neutralizing antibodies is complicated as the recombinant envelope glycoprotein, even in trimeric form, and vector-expressed HIV-1 envelope glycoproteins do not seem to expose the relevant epitopes. In addition, vaccine-elicited antibodies will have a tough job as HIV-1 seems to be relatively resistant to neutralizing antibodies and is able to rapidly escape from antibody neutralization. Another major obstacle in the development of an effective HIV-1 vaccine is the large sequence diversity, especially of the viral envelope glycoprotein. The nature of neutralizing antibody responses in natural HIV-1 infection may offer new clues for vaccine design. One of the current approaches is the characterization of the epitopes of the very potent broadly neutralizing antibodies that are known to date and to use these epitopes as immunogens to elicit HIV-1 specific neutralizing antibodies with similar potency and breadth.

**Scope of the thesis**

In this thesis, the prevalence, development and characteristics of cross-reactive neutralizing humoral immunity in HIV-1 infected individuals is studied. First, the prevalence of subtype-specific (chapter 2) and cross-reactive neutralizing activity (chapter 3) in serum was studied in 35 participants from the Amsterdam Cohort Studies. Subsequently the impact of cross-reactive neutralizing activity on HIV-1 disease progression was studied in chapter 4. Whether subtype-specific and cross-reactive neutralizing activity are relevant for vaccine development is reviewed in chapter 5.

In chapter 6, the genetic composition of replication competent clonal HIV-1 variants isolated from peripheral blood mononuclear cells (PBMC), HIV-1 proviral DNA from PBMC and HIV-1 RNA in serum is compared at different stages in the course of HIV-1 infection. In chapter 7 the autologous neutralizing antibody response and the escape of HIV-1 from neutralizing antibodies in patients with cross-reactive neutralizing activity is reported.

To further investigate the interaction between HIV-1 and its host we describe the changes in sensitivity to broadly neutralizing monoclonal antibodies b12, 2G12, 2F5 and 4E10 during the course of infection in chapter 8, while chapter 9 focuses in more detail on the changes in sensitivity to b12 neutralization during viral evolution in a patient.
The impact of cross-reactive neutralizing serum activity on viral evolution in a patient is described in chapter 10. Subsequently the adaptation of HIV-1 to humoral immunity, with a focus on the role of the V1V2 loop in the envelope glycoprotein of HIV-1 in the resistance to neutralizing antibodies, is reported in chapter 11.

Finally, in chapter 12 the main results and implications of this thesis are summarized and discussed in the context of current knowledge and HIV-1 vaccine development.

REFERENCES


General introduction


