Cross-reactive neutralizing humoral immunity in HIV-1 disease: dynamics of host-pathogen interactions
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The need for an effective vaccine to prevent the global spread of human immunodeficiency virus type 1 (HIV-1) is well recognized. It is assumed that a protective vaccine should ideally elicit cross-reactive neutralizing humoral immunity in combination with a cellular immune response. Unfortunately, to date HIV-1 envelope-based immunogens have been unable to elicit HIV-1 specific potent and cross-reactive neutralizing humoral immune responses. The design of an immunogen that is capable of eliciting neutralizing antibodies is complicated as HIV-1 has developed multiple mechanisms to evade neutralizing antibodies, including the inaccessibility of relevant epitopes due to the trimeric structure of the HIV-1 envelope protein, the density of glycosylation, and the occluding variable loops on the outer domain of the envelope spike. A better understanding of the evasive mechanisms of HIV-1 from humoral immunity and of the mechanisms driving the development of broadly neutralizing antibodies may be crucial for vaccine immunogen design.

During 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. The rapid escape of HIV-1 from autologous type-specific neutralizing antibodies seems to be the underlying explanation for the absent correlation between autologous humoral immunity and HIV-1 disease course. Broadly neutralizing antibodies may bypass the viral defense mechanisms as they have the ability to neutralize HIV-1 variants from different subtypes. The epitopes targeted by these broadly neutralizing antibodies are the conserved domains on the envelope trimer, located at the CD4-binding site, glycan shield, conserved regions of the V1, V2 and V3 region, and the membrane proximal external region (MPER) of gp41.

In this thesis, the prevalence, development and characteristics of cross-reactive neutralizing humoral immunity in HIV-1 infected individuals was studied. In chapter 2 we uncovered the presence of subtype-specific neutralizing activity in many HIV-1 infected individuals and observed a positive correlation between the titer and breadth of neutralizing activity in patient sera. The development of a potent cross-reactive neutralizing humoral immune response takes at least 2 to 3 years (chapter 3), albeit not in all HIV-1 infected individuals. It has become apparent that about one-third of HIV-1 infected individuals develop cross-reactive neutralizing activity in serum during the first 3 years (chapter 4), suggesting that the B cell repertoire in humans should indeed be sufficient to generate potently neutralizing antibodies in response to a vaccine. However we also observed that cross-reactive neutralizing activity in serum does not seem to have an impact on the clinical course of HIV-1 infection. Possibly cytotoxic CD8+ T-cells rather than neutralizing antibodies may contribute to the
control of already established infections while neutralizing antibodies may be essential for protection from infection.

Subtype-specific humoral immunity has been suggested as an interesting alternative to deal with the huge sequence variation that is challenging HIV-1 vaccine development. As reviewed in chapter 5, subtype-specific humoral immunity may provide new leads on the way to a potent HIV-1 vaccine. However, developing and administering multiple HIV-1 vaccines is far less ideal than having a single vaccine that would cover all circulating HIV-1 variants. Moreover, the fact that truly broadly neutralizing antibodies exist, implicates that a single protective antibody-based vaccine against HIV-1 may be an achievable goal.

To further investigate the effect of cross-reactive neutralizing activity on disease progression and the adaptation of HIV-1 to the humoral immune response, we studied HIV-1 evolution in several patients using replication competent clonal HIV-1 variants. The major advantage of working with replication competent clonal HIV-1 variants is that biological properties of the virus can be studied in the context of the original genetic background and the complete viral genome, which obviously is not the case with cloned viral gene fragments from plasma in the background of a molecular HIV-1 clone. Chapter 6 shows that replication competent clonal HIV-1 variants isolated from PBMC may equally represent the viral quasispecies in blood as sequences obtained from serum and PBMC proviral DNA. By using replication competent clonal HIV-1 variants that were isolated over the course of HIV-1 infection from 6 patients, we could demonstrate a rapidly evolving resistance to neutralization by autologous sera, explaining the absent role for neutralizing antibodies, even of those with cross-reactive neutralizing activity, on the clinical course of HIV-1 infection. Despite the fact that cross-reactive neutralizing antibodies are most likely directed against epitopes that are conserved among HIV-1 variants even of different subtypes, the escape of HIV-1 did not coincide with a loss of viral fitness (chapter 7). We observed that the escape of HIV-1 to the humoral immune response was correlated with an increase in length and number of potential N-linked glycosylation sites of the envelope glycoprotein. Another example of the interaction between HIV-1 and its host is described in chapter 8. We observed that over the course of infection in most individuals viruses emerged that were resistant to one or more broadly neutralizing antibodies. For instance b12-resistant virus variants emerged late in infection in a substantial proportion of HIV-1-infected individuals and interestingly this could occur in the absence of both humoral and cellular immunity (chapter 9). For vaccine design, it will be important to understand which mechanisms drive the selection of broadly neutralizing antibody resistant virus variants.

In addition, certain selective forces may drive differential evolution of the cell-free and cell-associated virus pool, in which case, sequences from both sources would be ideally required to obtain a more complete picture of the interactions between HIV-1 and its host. In chapter 10 longitudinally obtained HIV-1 envelope glycoprotein sequences from serum RNA, PBMC proviral DNA and replication competent clonal variants from a single
individual with reported cross-reactive neutralizing activity in serum were analyzed in detail. The results suggest a role for neutralizing antibody pressure on HIV-1 evolution as HIV-1 variants that were unable to persist in peripheral blood were more sensitive to autologous serum neutralization, had shorter envelopes with fewer potential N-linked glycosylation sites and showed lower replication kinetics than successfully evolving HIV-1 variants. The adaptation of HIV-1 to neutralizing antibodies, through the increase in length and glycosylation of the envelope glycoprotein, was further demonstrated in chapter 11. The increase in length and/or glycosylation of the V1V2 region of the HIV-1 envelope glycoprotein was shown to be directly responsible for the protection of HIV-1 against gp120-directed neutralizing antibodies, possibly by shielding underlying epitopes in the envelope glycoprotein from antibody recognition.

This thesis describes the dynamic interactions between HIV-1 and its host humoral immune responses. Although neutralizing antibodies may not be able to influence HIV-1 disease course, neutralizing antibodies do have an impact on HIV-1 evolution. New insights in these interactions have revealed the importance of the accessibility of the vulnerable epitopes on the HIV-1 envelope glycoprotein in a vaccine immunogen and the need to achieve sterilizing immunity with a neutralizing antibody based vaccine.