Neutralizing antibodies to the HIV-1 envelope glycoproteins
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Summary

The human body is constantly exposed to potential pathogens, like bacteria, fungi, parasites and viruses. The immune system provides the body with specific defenses against these pathogens in the form of antibodies and cellular immune-responses (CD4+ and CD8+ T cells). Some pathogens, such as the human immunodeficiency virus type 1 (HIV-1), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), thwart the immune system by attacking key regulator cells or molecules. For HIV-1, it are the CD4+ T cells, which play a coordinating role in the development of the immune response, that become infected and affected in their function. Thus, HIV-1 sabotages the immune system and the body is unable to control the HIV-1 infection.

To prevent HIV-1 infection by vaccination it is envisioned that the vaccine-induced neutralizing antibody response should be sufficiently strong to blunt the initial virus replication, so as to buy the cellular immune-response enough time to develop. In HIV-1 vaccine development, however, the induction of an antibody response with significantly potent neutralizing antibody titers has not been achieved and progress has been slow. Understanding neutralization of the HIV-1 virus at the molecular level is thought to be critical for a knowledge-based approach for the development of a vaccine against HIV-1.

In this thesis, which focuses on the neutralizing antibody response against the HIV-1 envelope glycoproteins (the primary targets of neutralizing antibodies in natural infection), we have utilized the so-called "phage-display" technology, whose ability to capture and dissect antibody responses of infected individuals in great detail, offers a powerful tool for identifying and isolating neutralizing antibodies. The interaction of these antibodies with potential vaccine-candidates can thus be studied. In Chapter 1, the origin of the phage-display technology is described in the context of a short historical overview of antibody research in general. Furthermore, the technical aspects of phage-display technology and relevant developments described in the recent literature are reviewed.

As stated, the primary targets of neutralizing antibodies are the HIV-1 envelope glycoproteins. There are two glycoproteins, gp120 and gp41, which form complexes consisting of three copies of each glycoprotein, i.e. (gp120-gp41). These envelope glycoprotein (Env) complexes bind sequentially to two receptor proteins on the membrane-surface of host cells, namely CD4 and a co-receptor. This interaction ultimately leads to the entry of the virus into the host-cell via the mechanism of membrane-fusion. It is the initial interaction between the Env complex and the host cell receptor proteins that is blocked by most neutralizing antibodies. In Chapter 2 a literature-overview is given of the relevant research addressing antibody-mediated neutralization of HIV-1, prior to the research presented in this thesis.

One of the key aspects in antibody-mediated neutralization of HIV-1 is that neutralization correlates with antibody-binding to the functional Env complex as present on infective virus particles. Approaches to mimic this functional Env conformation have sofar been unsuccessful and as a result antibodies elicited by these Env mimics seldom display neutralizing activity against primary HIV-1 virus isolates. In Chapters 3 and 4 an approach for the isolation of functional Env complexes from infected cells was evaluated, involving the solubilization with a mild non-ionic detergent in combination with size-exclusion chromatography. This approach resulted in a preparation that most likely contains a mixture of functional and non-functional Env complexes in a membrane background. This Env preparation was not able to isolate neutralizing antibodies via the phage-display technology, nor could it induce a neutralizing antibody response. Strategies to improve the preparation are discussed.

Instead of trying to design functional Env mimics and assessing whether these can induce neutralizing antibodies, another approach is to study neutralizing antibodies and determine the structural requirements for Env mimics to be able to induce these kinds of neutralizing antibodies. Prior to the research presented in this thesis, only three neutralizing antibodies had been isolated world-wide, that were considered broadly neutralizing. Two of these, termed b12 and 2G12, are directed against binding-sites (epitopes) on the gp120 glycoprotein and one, termed 2F5, is directed against an epitope on the gp41 subunit. In this thesis two novel neutralizing antibodies are described against two additional neutralizing epitopes, which were isolated via the phage-display technology.

The first new neutralizing antibody, termed X5, is described in Chapters 5, 6 and 7 and is directed against an epitope on gp20, that is exposed after the interaction of the Env complex with CD4 at the cell-surface and which overlaps the binding-site for the co-receptor. Although X5 has broadly neutralizing activity when tested as an antibody fragment (a smaller part of the whole antibody), it is less effective as a whole antibody molecule. In Chapter 6 it is argued that this is because after binding to CD4 the available space between virus and host-cell surface is not enough to accommodate a whole antibody
molecule. Strategies to design vaccines that aim at eliciting antibodies against the co-receptor binding-site are thus likely to be ineffective. It does however suggest that this site could be used as a target for so-called small-molecule entry inhibitors. The crystal structure of X5, described in Chapter 7, may guide the design of such small-molecule inhibitors.

The second novel neutralizing antibody, termed Z13 is described in Chapter 8 together with another antibody termed 4E10. Both antibodies recognize an epitope on gp41, adjacent to the epitope recognized by the 2F5 antibody and may thus act in the same way. Unlike most neutralizing antibodies, 2F5 does not interfere with binding of the Env complex to the host-cell receptors, suggesting another neutralization mechanism. The epitopes recognized by these antibodies may serve as a template for an effective vaccine against HIV-1.

Finally, in Chapter 9 the implications of the studies described in this thesis are discussed in the light of relevant developments described in the recent literature addressing antibody-mediated neutralization of HIV-1. With the completion of this thesis progress has been made in defining broadly neutralizing epitopes on the HIV-1 envelope glycoprotein. Significantly, five broadly neutralizing and thus conserved epitopes have been defined on the surface of the highly variable virus. Four of the sites defined are accessible by antibody on the native virus and antibodies against these epitopes should therefore be elicited by a vaccine. The fifth epitope, defined by antibody X5, may be a potent target for a small molecule drug. In summary, novel leads for the development of a vaccine have been defined.