chapter 1

Introduction
Introduction

Interactions between pathogens and their vertebrate targets have brought us, humans, both progress and continuous problems (1). Pathogens that can replicate in the presence of an immune response but which are not immediately lethal to the host, are in general most persistent. The human immunodeficiency virus (HIV), discovered in 1983 (2) as the etiologic agent of the acquired immunodeficiency syndrome (AIDS), is the cause of a major world wide pandemic. HIV-1, but also HIV-2, combines the characteristics of a well adapted pathogen. The virus is transmitted by sexual contacts, blood-blood contact and vertically from mother to child. In the absence of antiretroviral treatment, HIV infected patients in general develop AIDS within 8 to 10 years after infection. The disease is characterized by profound immunodeficiency, ultimately leading to death from opportunistic infections and malignancies. By now over 36 million people are infected worldwide with 15,000 new infections every day. The cumulative death number is now estimated to be more than 20 million (3). The virus does not distinguish between people, but most cases are found in developing countries, specifically the African continent and with rising numbers in Asian countries, China and Russia.

Origin of HIV-1
HIV-1, a retrovirus, belongs to the subfamily of lentiviruses, which also includes HIV-2, feline immunodeficiency virus, equine infectious anemia virus and simian immunodeficiency viruses (SIV). The genome of retroviruses consists of two single strands of RNA which contain at least three open reading frames (ORFs), encoding the structural proteins gag, pol and env. HIV-1 does also contain seven other ORFs for regulatory and accessory genes called vpr, vpu, nef, tat, rev and vif. The virus has two long terminal repeats (LTRs), that are important for transcriptional regulation and integration (4). Various simian immunodeficiency viruses are found in African chimpanzees (Pan troglodytes), sooty mangabeys (Cercocebus atys) and African green monkeys (Cercopithecus aethiops) (the later two are also defined as old world monkeys) and were therefore suggested to be the reservoir for HIV-1. Indeed, HIV-1 clustered in phylogenetic analyses with SIV derived from chimpanzee and HIV-2 with SIV obtained from old world monkeys (5-9). Although the first detection of HIV-1 disease in the human population dates back to 1959 (10), phylogenetic analyses indicate that the species barrier must have been crossed by the virus already before 1940 (11). From a pathogenetic point of view it is intriguing that in contrast to humans, chimpanzees and old world monkeys do not develop disease (5;6;12;13). However, SIV infection of new world monkeys, including pig-tailed and rhesus macaques (Macaca nemestrina and Macaca mulatta, respectively) does induce disease and these animals are therefore considered to be useful as experimental models for AIDS in humans (5;8;14-18).

HIV tropism and pathogenesis
HIV infection of cells is a multi-step process that depends on the formation of a protein fusion complex involving CD4 and a coreceptor on the target cell, and the HIV envelope glycoproteins (gp) on the virion (19-25). The HIV envelope is synthesized as a gp160 precursor protein which is subsequently cleaved in two noncovalently associated subunits, gp120 and gp41. Gp41 trimerizes and associates with three gp120 proteins to form oligomeric complexes (26-33). The coreceptors for HIV are 7-transmembrane domain, G protein coupled receptors of the chemokine receptor family of which CCR5 (R5) and CXCR4 (X4) are most important as HIV-1 receptors (34-40). Cells that express all necessary components for HIV infection and that can therefore be infected in vivo include human T cells, thymocytes, monocytes, macrophages, microglia, dendritic cells and Langerhans cells.
Figure 1
Structure and orientation of the HIV-1 gp120 core. The gp120 core was crystallized in ternary complex with two-domain soluble CD4 and the Fab fragment of the 17b antibody. Here only the gp120 core is shown, seen from the perspective of CD4. At the indicated N- and C-terminus of the protein, gp41 is proposed to interact, and hence the viral membrane is located towards the top of the diagram. This would position the target membrane at the bottom. The inner gp120 domain shown on the left and the outer domain on the right, the bridging sheet is at the bottom between the inner and outer domain. The position of the CD4-binding cavity in gp120 is indicated by an asterisk. The residues of gp120 that are involved in CD4 binding are shown spacefilled, image was made with RASMOL (184) and adapted from Kwong (77), Wyatt (185) and Rizzuto (87).

Cell tropism is determined by specificity of the envelope glycoprotein for either the CCR5 or CXCR4 chemokine receptor (48-54). This specificity can explain the property of syncytium inducing (SI), CXCR4 dependent HIV-1 variants to infect immortalized T cell lines in vitro, and memory and naive T cells in vivo (55-58). Emergence of these variants occurs in about 50% of HIV-1 infected patients and is associated with more rapid disease progression (34,35,59). Non-syncytium inducing (NSI), CCR5 dependent HIV variants establish primary...
infection in humans, using CCR5 to enter macrophages and memory T cells (43;60-65). Apart from appropriate coreceptor expression, optimal conformation and membrane organization of CD4 and the coreceptor is critical for infection. Macrophages, monocytes and T cells all express CD4, CXCR4 and CCR5. Still, X4 utilizing HIV isolates seem unable to establish infection in macrophages, which has been explained by the different membrane organization and biochemical properties of CD4 and CXCR4 on these cells (66-70). A successful interaction with the chemokine coreceptor is believed to be determined by the presence or absence of the receptor in complex with, or close proximity to, CD4 (71-73).

**HIV envelope structure and function**

The five variable domains of gp120 (V1 to V5) can be highly diverse between patients and even within one patient (74-76), whereas the five constant (C1 to C5) domains of gp120 provide the basis for the protein structure. The crystal structure of the gp120 core derived from the HIV variant HXB2c, lacking the V1/V2 and V3 variable loops has been revealed (77). The gp120

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More information on available Abs can be obtained from the database HIV Molecular Immunology Database 1999, edited by Bette Korber. Los Alamos National Library.
crystals were obtained in complex with two-domain soluble CD4 (D1D2 sCD4) and the Fab fragment of 17b, a monoclonal antibody (MAb) that blocks the chemokine-receptor binding of gp120. Gp120 could be divided into three core structures of which the inner domain, which contains the conserved N- and C-terminus, is assumed to interact with gp41. The outer domain is highly glycosylated and relatively variable, and the inner and outer domain are linked by a bridging domain which consists of four antiparallel β-sheets (78-80). In addition, gp120 contains a relatively deep depression formed at the interface of the three domains, in which CD4 can bind (Figure 1).

Since the gp120 crystal was obtained with CD4 and the 17b MAb bound to it, the structure of the native protein remains unclear. However, crystallization of the IgG1b12 MAb, which mimics CD4 in its binding to gp120 (81-83) revealed an extraordinary complementarity determining region (CDR) H3 loop that fitted the CD4 binding cavity. This suggests that the CD4 binding cavity is present in the native structure as well. The conformation dependent interaction between the D1D2 domain of CD4 to the CD4-binding site (CD4-bs) of gp120 induces a conformational change between the inner, the outer and the V1/V2 and V3 domains. It thereby stabilizes the formation of a previously shielded coreceptor binding epitope, which now becomes solvent accessible (25;77;84-86). This newly formed structure is highly conserved among HIV-1, HIV-2 and SIV isolates and its interaction with the coreceptor is mainly determined by the charge of the residues and the V3-loop (19;20;26;87). Formation of this pre-fusion complex activates gp41 (88;89), resulting in insertion of the hydrophobic fusion peptide into the target membrane, bringing both membranes together, allowing fusion (27;90-93). This process is efficiently inhibited by peptides that recognize the surface of the pre-fusion trimeric coiled coil within gp41 (94-97).

**HIV neutralization**

In contrast to a small panel of MAbs that have the potential to neutralize primary HIV-1 isolates (Table 1), most MAbs directed against gp120 and gp41 do not interfere with binding of primary viruses to their target cell (82;98;99). This was initially not recognized since most patient-derived isolates were maintained on immortalized T cell lines, like H9, which we now know confers a neutralization sensitive HIV-1 phenotype (100-106). These T cell line adapted (TCLA) isolates are in general sensitive to neutralization by MAbs directed against the V3- and V2-loop and the CD4-binding domain (107-111). Such high affinity MAbs are elicited through gp120, gp140 or gp160 vaccination, and in natural HIV infection of humans and chimpanzees (112-115). It is assumed that these immunogenic epitopes are exposed on non-oligomeric primary envelopes and TCLA isolates, but not on viruses that are derived from, and maintained on, primary PBMC (116-117). This dichotomy in sensitivity to neutralization has been ascribed to a wide variety of characteristics of the HIV envelope protein including glycosylation (118-121), temperature dependent shedding of gp120 from gp41 (122-130), the interaction between gp120 and gp41 (63;79;131), number of envelope spikes on the virion membrane (132;133), and most importantly affinity of gp120 for CD4 (134-138). A correlation between the efficiency to use CD4 and the sensitivity to neutralization of X4 isolates has been demonstrated, but only in the context of the oligomeric envelope complex (71;123;134;139-144). Primary isolates depend on a relatively high expression level of CD4, for infection while TCLA isolates can infect cells expressing only low levels of CD4. This difference in CD4 binding affinity correlated with elevated binding of env directed Abs to the oligomeric envelope complex of TCLA variants compared to primary HIV variants (61;145-147).
**In vivo HIV neutralization and vaccine development**

The presence of predominantly neutralization-resistant HIV variants *in vivo* is considered to be a major obstacle for the success of a potential HIV vaccine. A prophylactic and therapeutic vaccine should prevent entry of the virus by inducing protective humoral and cellular immunity. Antibodies, cytotoxic T lymphocytes (CTL) and natural killer (NK) cells elicited by such a vaccine should be directed against cell-free and cell-associated virus. CTL can interfere with virus spread by recognition and killing of infected cells (148), and high CTL responses indeed correlated with long-term nonprogression (149-151). Sterile immunity can only be achieved by vaccines that elicit not only an adequate CTL response, but also Abs that fully block binding of oligomeric gp120 to CD4 or the coreceptor. These Abs should be able to react with different envelope conformations as present in different subtypes, to prevent the development of escape variants. Ongoing viral escape from neutralizing Abs *in vivo* has been illustrated by the lack of neutralization of autologous virus by serum obtained at the same time point, while neutralization against earlier isolates can be observed (152-161). However, protection against virus transmission via breast milk, oral, mucosal or parenteral routes has been shown (162-169).

Antibodies that neutralize TCLA isolates are induced by vaccination with monomeric gp120, gp140 or gp160 and can be isolated from naturally infected patients (112;113). These Ab responses are dominated by Abs against the NH$_2$- and COOH-termini of gp120, the highly glycosylated outer domain and the variable loops V2 and V3, and against epitopes surrounding the CD4-binding site (110;170-172). However, these epitopes are better exposed on TCLA isolates (and monomeric gp120) than on the oligomeric envelope structure of primary isolates and thus not relevant for neutralization of these isolates (112;173;174).

**Scope of this thesis**

In this thesis, biology and biochemistry of virus neutralization sensitivity of HIV-1 was studied. First, we analyzed which conformations of gp120/gp41 are recognized by antibodies present in sera obtained from patients at time points around seroconversion and later in the course of infection. We compared these recognition patterns with the capacity of these Abs neutralize autologous and heterologous HIV isolates (chapter 2). In chapter 3, we studied a laboratory worker who was accidentally infected in 1986 with the TCLA molecular HIV-1 clone HXB2, and who developed AIDS in 1992 (175). Viruses were isolated from this patient three and seven years after infection and studied for their sensitivity to neutralization. A more detailed analyses of the virus isolated seven years after infection is described in chapter 4. We studied the sensitivity to neutralization of different biological virus clones and HIV envelope chimeras by agents directed against the CD4 binding site. To study the absence of disease development in HIV-infected chimpanzees, we tested the sensitivity to neutralization of viruses obtained from either a chronically TCLA IIIB infected chimpanzee and a chimpanzee that was inoculated with a primary HIV-1 isolate (chapter 5). Adaptation to growth in H9 cells of four primary isolates was studied in chapter 6.

**References**

chapter 1


introduction


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