HIV-1 vaccine design: Learning from natural infection
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Chapter 1

General introduction
**Introduction**

Since the first cases of the acquired immunodeficiency syndrome (AIDS) were identified in 1981, more than 34 million individuals have died from this disease [1, 2] (WHO, 2014). Currently around 37 million people are infected with the human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, and an estimated 2 million people were newly infected in 2014. Sub-Saharan Africa is the most affected region, where almost 26 million people are living with HIV-1. There is no safe and effective vaccine that can protect against HIV-1 infection, neither is there a cure. However, treatment with antiretroviral therapy (ART) can suppress viral replication and halt disease progression, turning HIV-1 infection from a progressive into a chronic disease. However, access to treatment is still limited and even in 2014 only 15 million people were receiving ART. This still leaves HIV-1 infection a devastating disease and a major public health threat, especially in poorly developed countries. The need for a safe and effective vaccine to stop the AIDS epidemic is therefore urgent.

**Origin of HIV**

In 1983 HIV-1 was discovered as the causative agent of AIDS [3], and soon after a simian immunodeficiency virus (SIV) with striking similarities to HIV-1 was isolated from captive rhesus macaques with similar clinical human AIDS symptoms [4, 5]. In 1986 HIV-2 was isolated and characterized as HIV-1’s relative, although it is less pathogenic [6]. HIV-1 and HIV-2 entered the human population through multiple cross-species transmissions of SIVs from non-human primates (NHP), probably in West-Central Africa [7]. The most likely transmission routes were exposure to infected blood or tissue, when hunting and butchering infected NHPs for bush meat, and bites or other injuries caused by infected NHPs [8, 9]. There are four HIV-1 groups which are all the result of independent cross-species transmissions; group M and N most closely related to SIV originated from chimpanzees, and group O and P were most likely transmitted via gorillas [10]. HIV-1 group M, which can be further divided into 9 subtypes (A-D, F-H, J and K) and sub-subtypes (A1-A4, F1 and F2), is the only group that has spread in humans worldwide, and is responsible for more than 99% of all HIV-1 infections. Subtype C is mainly restricted to Africa and India, whereas subtype B predominates in Europe, the Americas, and Australia [11]. In addition, circulating recombinant forms (CRFs), derived from recombination between viruses from different subtypes, are becoming more common. HIV-2 is most closely related to SIV from sooty mangabeyes and can be divided into nine groups (A-I). Retrospective studies identified HIV-1 in serum from 1959 and biopsies from 1960 from patients living in Kinshasa (Democratic Republic of the Congo) [12, 13]. Relaxed molecular clock dating techniques estimated that
SIV has given rise to transmissible HIV lineages throughout the twentieth century, and for group M already very early during this century [14].

**HIV-1 transmission and disease progression**

HIV-1 transmission can occur via sexual contact across mucosal surfaces, by percutaneous inoculation, and from an HIV-1 infected mother to her child during pregnancy, delivery and breast feeding [15]. The virus mainly infects CD4+ T-cells, resulting in a progressive depletion of these cells, the hallmark of AIDS, although other cells, such as dendritic cells (DCs), monocytes and macrophages, can also be infected [16]. Especially DCs, which are one of the first cells to encounter HIV-1, have an important role early after (submucosal) transmission [17, 18]. DCs are a subset of the antigen presenting cells (APCs) and are therefore in constant interaction with numerous cell types. Upon pathogen encounter, DCs undergo maturation and migrate from the mucosal sites towards secondary lymphoid organs. There the DCs can easily transfer the virus to the residing CD4+ T-cells via cell-to-cell transmission (trans-infection) or by productively replicating and thus producing new infectious particles (cis-infection) [19-22].

Not every exposure to HIV-1 eventually leads to an established infection. Some studies have shown that even multiple high risk HIV-1 exposures did not lead to infection in all individuals (highly-exposed seronegative individuals), probably due to immunologic and/or genetic preposition [23]. The per-act risk of HIV-1 acquisition is the highest for blood transfusion (risk of 9250 per 10000 exposures), followed by mother-to-child transmission (risk 2260 per 10000 exposures), sexual intercourse and percutaneous inoculation [24-26]. The risk of infection via sexual contact is the highest for men who have sex with men (MSM), especially for men practicing receptive anal intercourse with a seropositive partner (risk of 138 per 10000 exposures). In contrast, HIV-1 infection via heterosexual transmission is more than 10 times lower, the highest for receptive penile-vaginal intercourse (8 per 10000 exposures) [24]. The risk of HIV-1 infection through needle-sharing injecting drug use lies between the numbers for homo- and heterosexual transmission (63 per 10000 exposures) [24, 27]. Most of the established systemic infections are the result of only one single viral variant, the transmitted-founder (T/F) virus, which is in heterosexual transmission around 80%, and is a consequence of multiple bottlenecks the virus has to overcome, in both donor and recipient [28]. The most severe bottleneck is in the recipient, where the ability of the recipient’s target cells (e.g. expression of the appropriate receptors) to become infected is of great importance. In addition to this, viral characteristics have been proposed to play an important role [29].

However, the most important factor that is associated with the risk of HIV-1 transmission is the number of viral copies per ml plasma (viral load); every log increase in viral load, increases the risk of transmission with 2-3 fold [30]. High viral loads are usually observed during the early and late stages of infection, thereby emphasizing the importance of the
donor’s disease stage in transmission risk [31-33]. A peak increase in viral load is typically observed with superinfections, when an infected individual is infected with a new distinct HIV viral strain, although it is unclear whether this increases the risk of transmission [34]. Furthermore, other sexual transmitted infections that cause ulcers or inflammation, whereby the transmitted HIV-1 positive individual becomes more infectious and/or the negative individual becomes more susceptible, increase the risk of transmission [35]. In contrast to these biological factors that increase the risk of HIV-1 infection, behavioral interruptions, such as condom use, male circumcision and intake of pre-exposure prophylaxis (PrEP) can significantly reduce this risk [36-38].

During the acute phase of infection there is a rapid increase in viral replication and a progressive depletion of CD4⁺ T-cells [39], associated with the impairment of cellular immunity, and increased susceptibility to opportunistic infections [40]. The rapid depletion of CD4⁺ T-cells is mainly observed in the gut-associated lymphoid tissue (GALT), where these cells are 10 times more frequently infected than in the blood and secondary lymph nodes [39, 41]. Depletion in the lymph nodes is mainly attributable to depletion of T-follicular helper (Tfh) cells [42]. Overall the depletion of CD4⁺ T-cells can be counteracted by immune responses that decrease viral load to a steady state (viral load set-point) [43], and the regeneration of CD4⁺ T-cells or redistribution of resting CD4⁺ T-cells that had been sequestered in the lymph nodes, eventually preserving (partially) the pool of uninfected CD4⁺ T-cells [44]. However, this process does not restore all CD4⁺ T-cells populations, is not stable over time and eventually results in immunodeficiency and death by opportunistic infections and malignancies. The asymptomatic steady state period between acute HIV-1 infection and AIDS varies between individuals, but in the absence of therapy this takes generally 7-11 years [45, 46].

**HIV-1 virology and replication cycle**

HIV belongs to the genus of Lentiviruses in the family Retroviridae. Retroviruses are enveloped RNA viruses that replicate in the host cell via the process of reverse transcription. HIV consists of two copies of single-stranded positive sense RNA molecules (viral genome), surrounded by a capsid. The genome, around 9.5 kb in length, encodes for the structural proteins Gag, Pol and Env, that are further processed into core proteins (capsid (CA), nucleocapsid (NC) and matrix (MA)), viral enzymes (reverse transcriptase (RT), integrase (IN) and protease (PR)), and the envelope glycoproteins (Env; gp120 and gp41), respectively. In addition to the structural proteins, the regulatory proteins Tat and Rev and the accessory proteins Nef, Vif, Vpr and Vpu are also encoded in the genome [47].

Entry of target cells is initiated by the binding of Env on the viral membrane to the main receptor CD4. This leads to conformational rearrangement in Env and subsequent binding to the coreceptors, CCR5 or CXCR4, followed by fusion of cell and viral membranes [48-51]. When both membranes are fused, the capsid is released into the cytoplasm where it is
uncoated. After uncoating, the viral genome is released into the cytoplasm, together with the accessory proteins. Once inside the cell, the viral reverse transcriptase transcribes the single stranded RNA genome into linear double-stranded DNA [52, 53]. During this process the virion must travel towards the nucleus and cross the nuclear membrane, which is facilitated by the pre-integration complex (PIC; consisting of viral proteins, host proteins and viral DNA) [54]. Inside the nucleus, the dsDNA integrates into the host chromosome, mediated by the integrase enzyme. To generate new viruses, integrated proviral DNA must be transcribed into RNA, which is driven from the 5’ long terminal repeat that is recognized as promotor by the RNA polymerase II. The transcribed viral mRNA is then further processed (spliced) and translated into 15 proteins, using the host’s ribosomes. The new proteins are then directed to the site of assembly and once there, all necessary proteins, as well as two copies of the viral genome, are incorporated into a new virion, followed by budding from the membrane [53]. Once budded, the new virion consists of a capsid surrounded by the viral envelope and is ready to infect new target cells [55, 56].

Envelope glycoprotein complex and viral variation

The HIV-1 envelope glycoprotein complex (Env) on the viral surface is composed of a trimer of heterodimers of the surface glycoprotein gp120, non-covalently linked to the transmembrane glycoprotein gp41. Env is synthesized as a polyprotein precursor, termed gp160, and glycosylated during folding in the endoplasmic reticulum, after which oligomerization takes place to form trimers [57]. The oligomerized gp160 trimer is then transported to the Golgi network where it is cleaved into the gp120 and gp41 subunits [58], and there some N-linked glycans acquire complex modifications. Each monomer of gp120 consists of five conserved regions (C1-C5) that are interspersed with five variable regions (V1-V5) [59]. The constant regions form the core, whereas the variable regions form flexible loop structures on the outside of Env that mask the conserved regions. Gp120 normally contains 18 cysteine residues that can covalently link with each other creating nine disulphide bridges, which are considered crucial for the formation of tertiary structures [60]. These cysteine residues are highly conserved across all isolates of HIV-1, and a subset define the boundaries of the variable regions. Roughly half of the molecular mass of gp120 can be contributed to N-linked glycans [61], which can be attached to the 20-35 potential N-linked glycosylation sites (PNGS) in gp120 and 3-5 PNGS in gp41. These N-linked glycans contribute to Env folding, help the virus to bind to host cell surfaces and, most importantly, mask Env from immune recognition [62, 63].

Gp41 is a transmembrane glycoprotein which is organized into three domains, namely the ectodomain, the transmembrane and the cytoplasmic tail. The ectodomain contains an N-terminal hydrophobic region (fusion peptide), a polar region, two heptad-repeat regions (HR1 and HR2) and the membrane-proximal external region (MPER). The transmembrane domain is the part that anchors Env into the lipid membrane. The C-terminal cytoplasmic
tail of gp41 has multiple biologic functions and influences multiple properties of Env, such as viral infectivity and Env incorporation [64]. Gp41 plays an important role after initial binding of Env with its main receptor and subsequent coreceptors. The stored energy in gp41 that is released after the disentanglement of gp120 and gp41, drives the fusion of the cellular and viral membranes [65].

Infection with HIV-1 is characterized by the large number of different viral variants that can be found. This is mainly attributable to the variability in Env, and is a consequence of at least three features. The enzyme reverse transcriptase is highly error-prone due to the lack of proofreading activity, resulting in the introduction of on average one substitution per genome per replication round [66, 67]. The replication cycle is very rapid, resulting in a large number of new virus variants per day [68]. And, within an infected individual two or more HIV variants can undergo recombination events, leading to new recombinant forms. Eventually, numerous closely related but genetically non-identical viral variants can be observed in an infected individual, where variation can be as high as 10% [69]. In addition, all these variants are also constantly subjected to competition, selection and immune pressure [70]. Together this eventually leads to a high genetic variation of 15-20% observed within subtypes, and up to 25-35% between subtypes.

**Immune responses against HIV-1**

To survive and respond to invading pathogens (such as bacterial, parasitic or viral), living organisms have developed multiple mechanisms and strategies. Classically, these can be divided into two main groups, namely the innate and adaptive (cellular and humoral) immune responses.

The innate immune response is the first line of defence and recognizes certain features (pathogen-associated molecular patterns (PAMPs)) common to many pathogens. This response is fast and does not, in contrast to the adaptive immune response, rely on the clonal expansion of effector cells. CD4+ T-cells, macrophages and dendritic cells (DCs) are cells that harbour pattern recognition receptors (PRRs), and thereby can recognize PAMPs. Normally during viral infection, this leads to the induction of interferon production and eventually to the transcription of interferon stimulated genes, creating an antiviral state that supresses the infection [71]. However, HIV-1 successfully evades the innate immune response either by avoiding recognition or by resisting the antiviral defence mechanism, resulting in the impairment of a robust interferon response, especially in macrophages and DCs that are rich in PRRs. For example, the viral RNA genome is concealed by capsid proteins, and with the help of some host factors it is unable to be recognized by PRRs [72, 73]. Similarly, the efficient export to the nucleus (by PIC), where integration into the host genome takes place, is also partially facilitated by a host factor that minimizes exposure to the PRRs [72, 73]. There are also indications that sensing of HIV-1 by the immune system is
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prevented by host factors that keep viral nucleic acids below the detection limit of PRRs [74].

Although HIV-1 can effectively evade the innate immune response, there still can be sensing of the virus. One of the examples is the recognition of Env by mucosal epithelial cells, which themselves are not targeted by HIV-1 infection [75]. Recognition by these cells triggers pro-inflammatory cytokines and chemokine production and activates and recruits nearby innate immune cells, such as natural-killer (NK) cells. NK cells represent an innate subset of antiviral effector cells with immune regulatory and cytotoxic functions, thereby important for recognition and direct killing of infected cells [76]. Killing is mediated by killer-immunoglobulin-like receptors (KIRs) on NKs that can recognize short epitopes, derived from viral proteins, presented by the human leukocyte antigen (HLA) class 1 molecules on infected cells, eventually resulting in the release of and lysis by perforin and granzyme B. Although, the immune pressure exerted by NK cells can eventually result in viral escape variants that are able to evade this innate response, the mechanism by which remains unclear [77]. NK cells have, in addition, also a critical role in immune regulation and adaptive immune responses.

Early after HIV-1 infection, specific cellular immune responses emerge that are initially directed against epitopes of Nef and Gag. These CD8+ T-cell (or cytotoxic T-cell; CTL) responses appear rapidly after infection, already before the peak of viral load, and play an important role in the initial viral control. CTLs harbour specific T-cell receptors by which they can recognize HLA-presented viral epitopes on infected cells, similar to recognition by NK cells. Recognition of the infected cells results in release of, and lysis by, perforin and granzyme B. In HIV-1 infection the early CTL responses are usually narrowly focussed on a few immunodominant epitopes, a pressure which eventually leads to viral escape [78]. These escape mutants do come with a cost in viral replication capacity, but which can be overcome by compensatory mutations [79, 80]. Interestingly, some HIV-1 infected individuals do not progress towards AIDS in the absence of therapy, termed “elite-controllers” or “long-term non-progressors” (LTNPs) [81]. The most common feature among LTNPs is the expression of a select group of HLA-B molecules, by which these individuals can present a greater range of viral epitopes, thereby controlling viraemia levels [82, 83].

Inducing a protective antibody response that can neutralize diverse HIV-1 strains is high on the wish-list for the prevention of HIV-1. Therefore, humoral immune responses, or antibody mediated immune responses, have been a main focus of interest since the discovery of HIV-1. In the majority of infected individuals an anti-Env antibody response is elicited, already early during infection. Although, these antibodies mainly target gp41 and V3, they do not react with native Env trimers and do not affect viral load or select for escape variants, hence they are non-neutralizing [84-87]. Neutralizing antibodies can be detected around three months after infection, but due to the immune pressure exerted by these antibodies, the virus escapes. This means that neutralizing antibodies and their target viruses rarely coincide in an infected individual, and therefore eventually these antibodies
Factors involved in antibody development

Factors influencing the development of bNAbs can be divided into host and viral factors. Until now, host factors that show an association with the development of bNAbs are the MHC class 1 genes, coding for HLA molecules, low number of CD4+ T cells (even before infection) and high numbers of circulating Tfh cells [88, 89, 101, 102]. The latter two seem to be counterintuitive and the mechanism by which these Tfh cells stay preserved is unknown, although it is suggested that in individuals who did not develop bNAbs the CTL responses could be more effective in killing circulating Tfh cells or the replenishment of these cells was lower [102]. A genome wide association study among individuals who were infected with HIV-1 showed several single-nucleotide polymorphisms in the MHC class 1 genes, that were associated with the development of bNAbs [101]. In this study associations were also observed between the protective HLA-B*57 alleles with the absence of bNAbs, and the enrichment of the HLA-B*07 alleles with the presence of bNAbs. The latter allele is associated with a faster disease progression, confirming the notion that bNAbs do not protect against disease progression. Well known viral factors that are associated with the development of bNAbs are high viral load at set-point and large Env diversity early during infection, both suggesting the importance of sufficient antigen stimulation [93, 103]. In addition, a superinfection with a second virus from a different subtype is thought to increase bNAb responses, although this remains somewhat controversial [34, 104-106].
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An important factor in shaping bNAb responses, is the interplay between the viral evolution and the humoral immune response within an infected individual [107, 108]. During a typical HIV-1 infection neutralizing antibodies arise that target the epitopes of the autologous virus. These antibodies exercise selective pressure on the virus and rapidly select for escape variants by specific mutations in the variable regions of Env and changes in the number and positions of PNGS on Env (thereby shielding the targeted epitope), or in the targeted epitope itself (mutating the specific epitope) [109-112]. Multiple recent studies have revealed different mechanisms whereby viral evolution shapes the development of bNAb responses. Two studies showed that the escape from strain-specific neutralizing responses created a conserved epitope that became the later target of bNAb [113, 114]. This implies that the exposure of an occluded epitope is probably a result of a trade-off between viral fitness and antibody escape. Several other studies have suggested that bNAb mature from earlier binding or strain-specific neutralizing antibodies, where increased breadth is acquired in response to the serial emergence of escape mutations within the epitope [107, 108, 115, 116]. Defining the virological factors that are associated with the developmental pathway of bNAb, could provide opportunities for Env based immunogens.

HIV-1 vaccines

Although many people have access to antiretroviral therapy, every year the number of new infections exceeds the number of new people on treatment, urging the need for a protective vaccine. Based on the success of prior vaccines against many infectious diseases, many of which work through inducing neutralizing antibodies, an Env-based vaccine which elicits bNAb that can prevent HIV-1 infection, would probably be the most effective weapon in controlling the global pandemic. The value of pre-existing neutralizing antibodies was demonstrated by the protection against HIV/SHIV when bNAb, even in low doses, were passively administered to non-human primates [117-122]. In addition, the observation that HIV-1 infected individuals can develop bNAb, indicates that there are no fundamental barriers for the induction of bNAb by Env in humans. However, to date, efforts to design an HIV-1 vaccine with methods used in the development of other successful viral vaccines have not succeeded.

Ideally an effective HIV-1 vaccine should induce a humoral and cellular immune response, preventing both infection of uninfected exposed individuals and reduce replication and lower viral load in infected individuals. Until now more than 250 trials have been conducted to test HIV-1 vaccine candidates, where only six have reached clinical efficacy. In 1998 and 1999 the VAX004 and VAX003 trials were the first Phase III vaccine candidates, were two gp120 proteins were used to induce neutralizing antibodies. VAX003 was a mixture of subtype B and E gp120s and was tested in intravenous drug users, and VAX004 consisted of two subtype B gp120s tested in MSM and women at high risk for infection via heterosexual intercourse [123-125]. Although the vaccines were safe and immunogenic, neutralizing
antibodies were induced only against laboratory-adapted easy-to-neutralize (Tier-1) viruses, and not against primary neutralization resistant (Tier-2) viruses, and no vaccine mediated protection against infection was observed [126, 127]. These results skewed the vaccine field towards T cell vaccines. In 2004 the STEP trial was conducted to evaluate whether a vaccine could induce cellular immunity that could protect against infection or reduce viral load. A replication-defective recombinant adenovirus 5 (rAd5) expressing clade B gag, pol and nef was tested in a high-risk population [128-130]. The study failed to demonstrate protection against infection or reduction in clinical markers of disease progression. Actually this study was stopped completion as a subset of the vaccinees were even at higher risk of infection [131]. Analysis showed that this subset of vaccinees were MSM who were uncircumcised and had high levels of pre-existing Ad5 antibodies. The Phambili trial, a sister of the STEP trial using the same regimen in heterosexual males and females, was terminated early after start in 2007 when results from the STEP trial were analysed [132]. The last two initiated studies were conducted with a live vector vaccine or DNA prime (RV144 and HVTN505, respectively), and a protein subunit boost to induce both cellular and humoral responses. HVTN505 was started in 2009 and tested in circumcised MSM without pre-existing Ad5 antibodies. The DNA prime consisted of env from clades A, B and C, plus gag, pol and nef from clade B. The Ad5 boost encoded env from 3 clades and clade B gag and pol [133]. This trial was stopped prematurely in 2013 due to lack of efficacy in preventing infection or lowering viral loads in the infected vaccinees [134]. The RV144 trial was conducted in Thailand in 2003 and enrolled more than 16,000 men and women with increased risk of infection [135]. The vaccinees were primed with a non-replicating recombinant canarypox viral vector encoding CRF01-AE env and clade B gag and pol. The boost consisted of the recombinant virus along with clade B and CRF01_AE gp120s. A modest efficacy of 31.2% in protection against infection was observed at 42 months of follow-up, although the vaccine effect was only transient. Analysis on the correlates of protection suggested that V2 antibodies were induced that could bind to the surface of infected CD4+ T-cells and mediate antibody dependent cellular cytotoxicity (ADCC) of these infected cells [136, 137]. In addition, these antibodies were shown to neutralize easy-to-neutralize viruses and capture low-levels of virus [136, 138]. In vaccinees who were infected a decrease in viral load set point was not observed [139]. Efficacy trials using a similar vaccine concept to attempt to reproduce the RV144 trial results are ongoing in a high-risk heterosexual populations (southern Africa) and in MSM (Thailand).

**HIV-1 Env as a vaccine immunogen**

The trials conducted in the past have shown that inducing cellular and humoral immune responses is possible, but the induction of a protective neutralizing antibody response is rather difficult. As neutralizing antibodies are in many cases the correlate of vaccine induced
immune protection against viral infections, one major remaining aim is the induction of bNAbs against HIV-1 by Env based antigens. However, there are some big hurdles which have to be overcome. Where some infected individuals can develop bNAbs, this generally takes years and is the result of a complex interplay between virus evolution and immune responses. In addition, it has been shown that these bNAbs have very unusual features, such as long heavy chain third complementarity-determining regions and high levels of somatic mutations. This suggests the need for an antigen that stimulate appropriate germline B-cells followed by sequential antigens that drive the antibody response towards full bNAb development, thus simulating what is observed in some HIV-1 infected individuals who developed bNAbs. Whether this is possible is unknown, although promising results in light of this B-cell-lineage immunogen design have been achieved by two studies in mice [140, 141]. The antigens needed to induce these bNAbs should be a stable antigenic mimic of the native trimeric envelope, which is supported by the findings that monomeric gp120 proteins (used in VAX003 and VAX004) were not able to induce bNAbs. BG505 SOSIP.664, a clade A Env that forms stable trimers and is isolated from an infant that later during infection developed bNAbs responses, is therefore a hopeful and interesting immunogen [142, 143]. The soluble protein is truncated at position 664 and is stabilized by multiple modifications. A disulphide bond between gp120 and gp41 and an Ile to a Pro mutation in gp41 at position 559 (together termed SOSIP.664) [144, 145]. The natural occurring cleavage site, REKR, has been optimized to RRRRRR [146], and a PNGS was introduced at position 332. The trimer closely resembles the native viral spike when visualized by negative stain electron microscopy (NS-EM), cryo-EM and crystallography [147-149]. In addition, multiple epitopes for bNAbs but not non-bNAbs are displayed on these trimers [147]. Rabbit immunizations with BG505 SOSIP.664 trimers induced, for the first time, strong and consistent antibody responses against an (autologous) Tier-2 virus, whereas previous studies only elicited antibodies against Tier-1 viruses [150]. Tier-2 viruses are moderately sensitive to antibody mediated neutralization, however most commonly transmitted viruses have a Tier-2 phenotype, therefore induction of bNAbs against Tier-2 viruses is a prerequisite. Whether BG505 SOSIP.664 trimers are also able to induce bNAbs in individuals remains to be determined, but the induction of autologous Tier-2 NAbs in rabbits is a promising step towards the ultimate goal of vaccine induced immune protection against HIV-1.
General introduction

Scope of this thesis

The induction of sterilizing immunity against HIV-1 infection probably requires an immunogen that can elicit a protective bNAb response targeting the viral Env spike. In this thesis, we studied the NAb responses in MSM and IDU HIV-1 infected individuals in combination with evolution of the viral Env protein. Furthermore, we used this information to design new Env-based immunogens.

First, we established the prevalence and potency of broadly neutralizing activity (bNAC) in the serum of MSM and IDU infected participants of the ACS (Chapter 2). We measured these responses around 3 years after infection, as their development normally takes relatively long. However, when studying elite neutralizers (one MSM and two IDU infected individuals) longitudinally, we observed that bNAC responses in these individuals is much faster, and can already be observed around the first year after infection (Chapter 3). Next, in Chapter 4 we focused on the autologous and heterologous antibody response in combination with viral escape, in six individuals who did, and three individuals who did not develop bNAC. Viral diversity and evolution were found to be major contributors to the maturation of the immune response. In Chapter 5 we investigated the contribution of superinfection and in Chapter 6 the role of early viral characteristics in the development of bNAC responses. Next, we studied the interplay between the virus and the immune responses in one IDU infected elite neutralizer, who developed a bNAC response against the glycan at position N332 (Chapter 7). Furthermore, we report on the role of the second position in the N332 glycan motif in shaping the Ab responses, both within infected individuals and at a population level (Chapter 8). In Chapter 9 and Chapter 10 we generated native-like Env (SOSIP) trimer immunogens, based on early viral Env sequences from ACS participants, and reported their capability to induce NAb responses in rabbits. Finally, the results and implications of these findings are discussed in Chapter 11.
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Reference List

include the CASCADE EU Concerted Action. Concerted Action on SeroConversion to AIDS and Death in Europe. 


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