AIDS Reviews 2006;8:51-9

The Role of Neutralizing Antibodies in HIV Infection

Michael Humbert[1,2] and Ursula Dietrich[1]
[1]Georg-Speyer-Haus, Institute for Biomedical Research, Frankfurt, Germany; [2]Dana-Farber Cancer Institute, Boston, MA, USA

Abstract

Neutralizing antibodies against HIV-1 play an important role in preventing viral infections. Less clear is their role in the containment of viral replication in infected individuals. However, evidence is accumulating that neutralizing antibodies may help the cellular arm of the immune response to prevent or delay the progression to AIDS. Detection of neutralizing antibodies depends on the* in vitro *neutralization assays used, and standardization of the assays is essential in order to be able to compare the magnitude and quality of a neutralizing antibody response in sera or other fluids from HIV-infected patients, uninfected HIV-1 exposed persons, or vaccinated animals/persons. Viral mechanisms to prevent neutralization include high variability and extensive glycosylation of the Envelope proteins, Envelope trimerization and shedding as well as late exposure of functionally important entry domains by conformational changes induced upon CD4 binding. These are also the difficulties encountered in the design of immunogens able to induce neutralizing antibodies upon vaccination. **(AIDS Reviews 2006;8:51-9)**
Corresponding author Ursula Dietrich, ursula.dietrich@em.uni-frankfurt.de

Key words

HIV. Neutralizing antibodies. Immunogens. Vaccination.

Neutralizing antibodies during the course of HIV-1 infection

Generally, antibodies against HIV-1 appear about 12 weeks after infection, which is when sera from HIV-infected persons first are detected positive by diagnostic tests like ELISA or Western blot (Fig. 1). This immune response is clearly mounted by the gradual increase in virus load in the patients and follows the peak of viremia observed early during primary infection.

Neutralizing antibodies (NAb, i.e. antibodies able to interfere with the infection of target cells), however, are usually detected at much later time points towards the end of the first year after infection[1-4]. From these observations it was deduced that NAb do not play a major protective role once the infection has occurred. Rather, the containment of viral replication in the early asymptomatic phase of the infection was mostly attributed to the cellular arm of the immune system, especially since the appearance of a strong cytotoxic T-lymphocyte (CTL) response timely precedes the strong decay of primary viremia. However, in recent years evidence is accumulating that NAb may also contribute – besides CTL – to the control of virus replication in infected persons or experimentally infected animals. Before reporting on these data, we will discuss some important technical issues concerning NAb, i.e. their detection by *in vitro* neutralization assays as well as mechanisms of neutralization and escape.

Correspondence to:
Ursula Dietrich
Georg-Speyer-Haus
Paul-Ehrlich-Str. 42-44
60596 Frankfurt, Germany
E-mail: ursula.dietrich@em.uni-frankfurt.de

Detection of neutralizing antibodies by *in vitro* neutralization assays

Antibody mediated neutralization is measured by *in vitro* neutralization assays. A variety of different neutralization assays is being used in the laboratories, which complicates comparison of the data obtained by different labs. Experts in the field promulgate standardization of the procedures in order to be able to compare results[5].

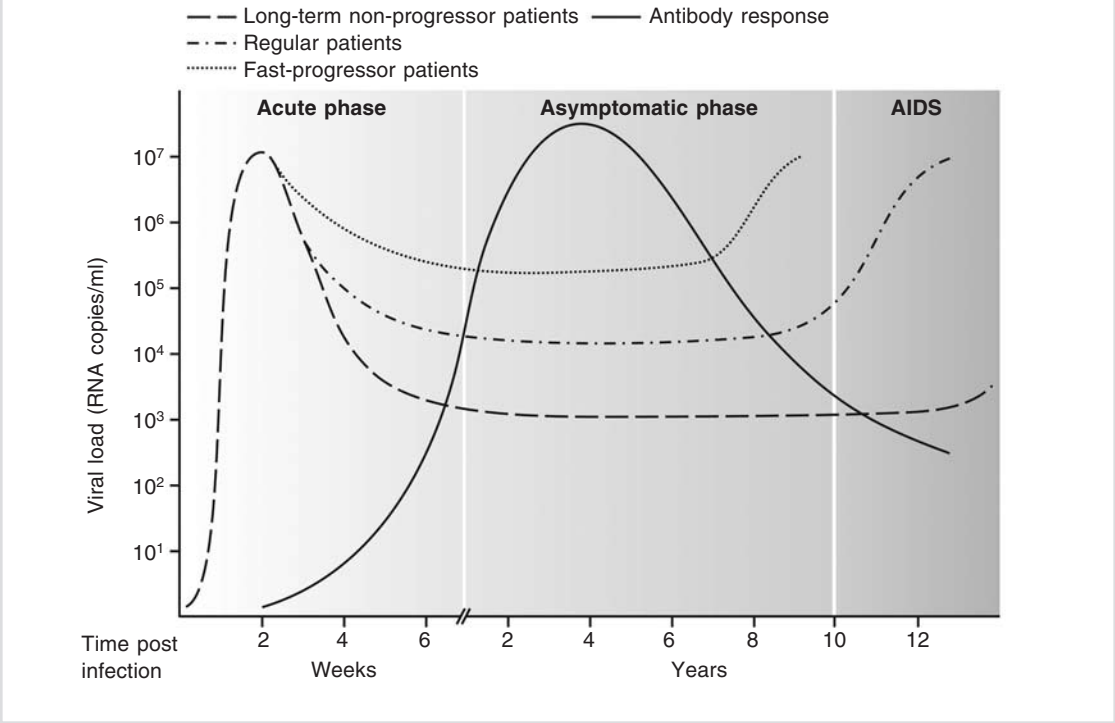

Figure 1. *Antibodies against HIV-1 are first detected a few weeks after infection (solid line) and decline upon the onset of AIDS after several years. NAb may contribute to maintain viremia at low levels (dashed line) during the asymptomatic phase of the infection, resulting in delayed disease progression as compared to patients with higher viral load after seroconversion (dotted and dot-dash lines).*

Hopefully one day, neutralization assays will be routinely used to monitor NAb induced by vaccines, and the only way to compare such immune responses is by standardized neutralization assays. Neutralization assays measure the reduction of infectivity of a virus added to target cells after pre-incubation with the neutralizing agent, in this case NAb. Virus production is then monitored in comparison to a control without the neutralizing agent or an unspecific agent added and the antibody or serum dilution giving 50% neutralization is determined.

Today it is known that primary HIV-1 viruses (isolated from patients) differ from cell line adapted viruses in the amount and the structure of the Env spikes they present at the surface[6-9]. Furthermore, primary target cells like lymphocytes and macrophages also differ from established cell lines in the proteins on their surface, including proteins that are responsible for the primary attachment of HIV-1 to cells. Consequently, results from neutralization assays performed with primary viruses on primary cells differ from those obtained with clonal HIV-1 isolates on cell lines. Generally, primary viruses are more difficult to neutralize than cell line-adapted viruses. Whereas the primary assays are much closer to the natural situation of HIV-1 infection, the more artificial cell line-based neutralization assays are much easier to standardize. Various setups including the readout of reporter genes (luciferase, green fluorescent protein) or measurement of p24 production are available to detect a reduction in viral infectious titer. Recombinant reporter viruses (replication-competent viruses) carrying the reporter gene can be cloned and generated by transfection of 293T cells with the recombinant plasmids. Alternatively, pseudoviruses carrying the *env* protein from the HIV-1 isolate to be analyzed on an *env*-deleted virus particle, which has not necessarily to be derived from HIV-1, can be produced by co-transfection of an *env*-deleted viral backbone genome with the marker gene and a plasmid carrying the respective *env* gene into packaging cells (Fig. 2). The generated pseudoviruses are not replication competent, but they can enter into target cells for HIV-1 in single-round infection assays. Virus entry and neutralization are then quantitated according to the reporter-gene expression within the cells.

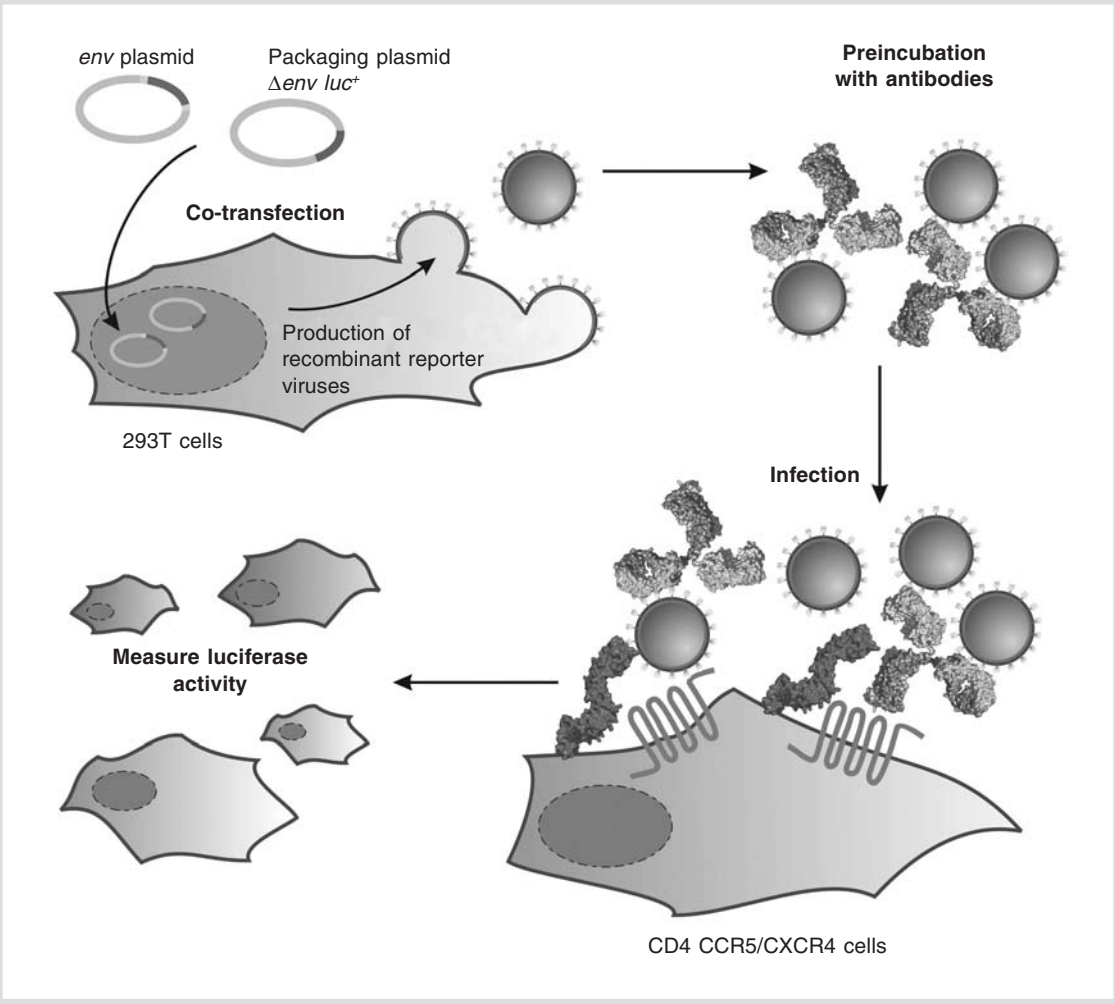

Figure 2. In vitro *neutralization assay based on the production of replication-incompetent HIV-1 pseudoviruses carrying a luciferase reporter gene. Reporter pseudoviruses carrying the env gene from patient viruses are generated upon co-transfection of an env-deleted, packagable, reporter plasmid and a plasmid encoding the patient's virus env gene. Pseudovirions are then used for infection of HIV-1 target cells after pre-incubation with the antibodies to be tested for neutralization. Neutralization will result in reduced infection of the target cells as compared to a control without antibodies that can be quantified by measuring the luciferase activity in the cell lysate.*

Recently it was shown that pseudoviruses and replication-competent viruses generated after transfection into cell lines exhibit similar neutralization sensitivity[6]. However, passage of the replication-competent viruses on peripheral blood mononuclear cells (PBMC), led to decreased sensitivity to NAb. As for primary viruses, this loss in neutralization capacity was due to the acquisition of increasing amounts of envelope glycoproteins upon passage on PBMC. This example again underlines that the setup of the neutralization assays directly influences the outcome and detection of NAb. For the required standardization, pseudoviruses or recombinant reporter viruses with their advantage in simplicity and practicability as well as cell lines as targets are good to start with, but one should keep in mind that PBMC-derived viruses behave differently and are less sensitive to neutralization[6]. But also, on a given cell line and for a given neutralizing agent (antibody or serum), reporter viruses carrying different Env differ in their sensitivity to neutralization. This is due to the intrinsic variability of the *env* gene of HIV-1, which can be more than 40% between Env derived from different subtypes. Therefore, standardization of neutralization assays also requires a well established set of standard viruses derived from different HIV-1 subtypes circulating in the world. Re-

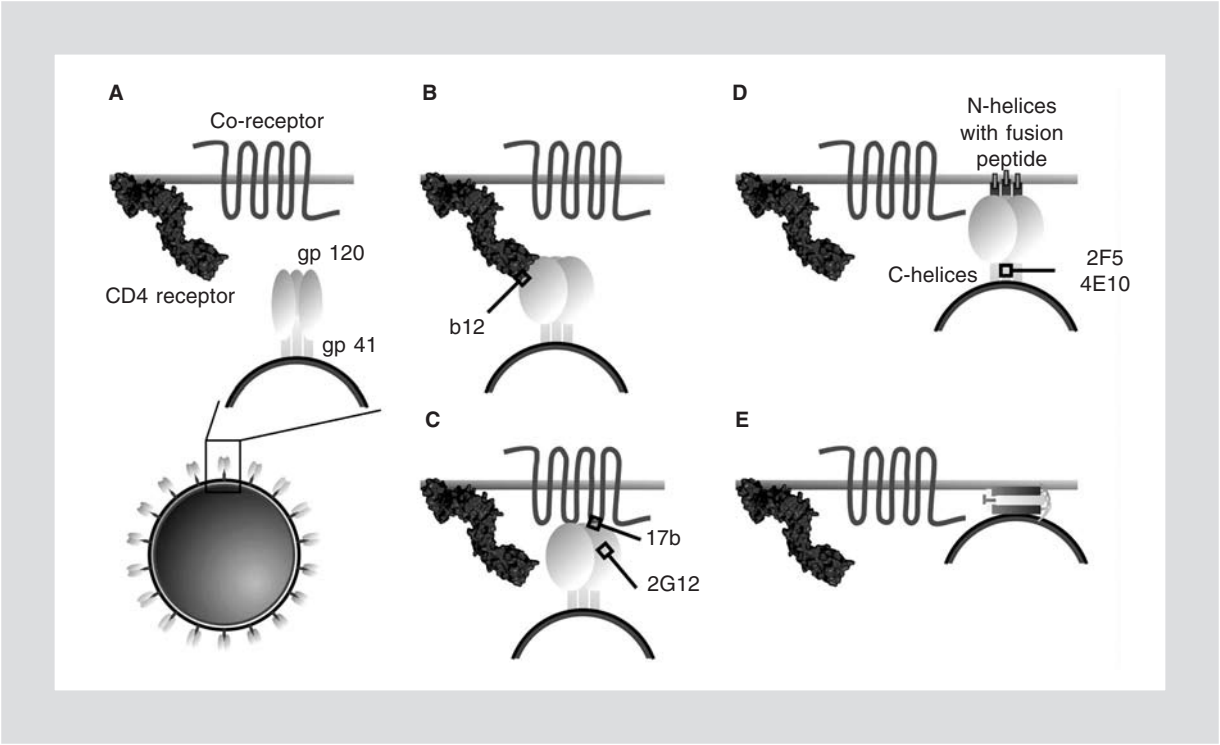

Figure 3. *The multi-step HIV-1 entry process is sensitive to neutralization by very rare monoclonal NAb. B12 targets the viral gp120 and interferes with the CD4-binding site, 2G12 is directed against a glycosylated epitope on gp120, 17b binds to coreceptor binding domains in gp120 that become exposed after CD4-binding, and 2F5 as well as 4E10 neutralize virus entry at the late stage of membrane fusion between virus and cell by binding to gp41.*

cently, such sets of standard isolates have been proposed for neutralization studies[5].

Mechanism of neutralization

Several mechanisms of neutralization are known today. The most common mechanism of antibody mediated neutralization is interference with HIV-1 entry into target cells: NAb have been well characterized that block infection by binding to different structural intermediates exposed during the HIV-1 entry process[10,11]. These antibodies include b12 against the CD4-binding site in gp120, 2G12 directed against carbohydrates in gp120, 17b, 48d or X5 that bind the CD4-induced coreceptor binding sites in gp120, as well as 2F5 and 4E10 targeting gp41 during the very last step of entry, membrane fusion (Fig. 3). Generally these antibodies block infection by binding to distinct epitopes on the viral Envelope that are functionally important for infection. Consequently, HIV-NAb are also known that bind to cellular receptor sites implicated in HIV-1 entry[12]. Moreover, mapping the epitopes of such antibodies by the phage display technology served to identify cellular HIV-1 entry epitopes[13,14].

The stoichiometry of the neutralization reaction is still not very clear. Some studies describe that covering the virus with antibodies over a critical density prevents interaction of the gp120 protein with the CD4 receptor[15-17]. One study showed increased neutralization of HIV-1 particles if covered beyond a critical density[18]. In contrast, a recent study by Sodroski, et al. contradicts this theory[19]. The binding of one single antibody molecule to one gp120/gp41 trimer is sufficient to inactivate its function. The efficiency of antibody binding to the envelope trimer therefore determines the potency and susceptibility to neutralization[19]. Of note is also that viral stocks contain a majority of deficient particles[20,21]. These defective virions may contain a low amount of intact envelope trimers[20,22,23], lose their gp120 content by shedding[24-26], or exhibit potential envelope heterogeneity[7,27-30]. Recent studies showed that the amount of processed gp120/gp41 envelope trimers varies on the surface of the virions and usually is between 7-19[6,7,22], depending also on the cell type the viruses have been generated from[6]. This again underlines the importance of standardization, not only for the neutralization assays *per se*, but also for the production of the virus stocks to be used. Antibodies can also exert their neutralizing

effect by simply cross-linking virus particles, leading to high molecular aggregates that can be eliminated by phagocytosis. Alternatively, they can activate the complement cascade or lead to antibody directed, cell-mediated cytotoxicity, both leading to killing of infected cells, rather than simply neutralizing cell-free virions[31,32]. It should also be mentioned that occasionally antibodies might also have enhancing effects, especially after immunization with whole virus particles[33].

Viral escape and immune evasion

The envelope glycoproteins, especially the surface of gp120, are the major virus-specific targets accessible to NAb. During natural HIV-1 infection, the NAb titer is generally low and directed against the autologous virus isolate of the patient[1,34-36]. Only occasionally, broadly neutralizing antibodies that also neutralize heterologous HIV-1 strains have been described[34]. HIV-1 uses a variety of strategies to escape the NAb directed against it by the host. First, conserved structures and domains of gp120 important for binding to the receptors and for viral entry are covered by highly variable loops, designated V1 to V5, and thus protected from antibody attack[37,38]. Although NAb can also be directed against these variable structures (in particular against V3) there is rapid escape due to point mutations, insertions or deletions in the variable loops, as well as a shift in glycosylation sites[39]. Several studies identified three different domains in gp120 important for immunogenicity: the neutralizing face, the non-neutralizing face, and the silent face. Most NAb are directed against the neutralizing face; the silent and the non-neutralizing face are not accessible due to heavy glycosylation or intermolecular interactions in the gp160 trimer[38,40,41]. Preferential targets for broadly neutralizing antibodies are the conserved regions of gp120 building the receptor binding sites[41-45]. Epitopes for antibodies against the CD4 binding site or coreceptor binding sites in gp120 are conformational (i.e. discontinuous and highly conserved) as all natural HIV-1 isolates, despite their high variability, generally infect cells via CD4 and CCR5 or CXCR4. The CD4bs and CCR5bs are masked by the V1/V2 and V3 loops[41] and hardly accessible in the native, functional trimer. Binding to the CD4 receptor changes the location of these loops exposing the CD4i epitopes, amino acids forming the coreceptor binding site (Fig. 3). At this intermediate stage of infection, however, the virus particle is already close to the cellular membrane limiting the space for accession of NAb. In line with this, it has been shown that smaller single-chain Fv or Fab antibodies can neutralize much more efficiently at this step than the large, complete antibody molecules[46].

Thus, the multi-step process of infection involving subsequent binding of two different receptors – binding to the second being dependent on binding to the first – is a very sophisticated mechanism to protect epitopes functionally important for infection from NAb.

The evidence for a protective role of neutralizing antibodies

Although the humoral immune response clearly contributes to prevent infections with HIV-1, its role in controlling established HIV-infections is still unclear and often the basis for discussion. Whereas the control of established infections was mainly attributed to the cellular arm of the immune response[10,47], the humoral arm is coming into focus again, as several publications show that antibodies – induced by the infection or administered passively – can indeed help to control virus replication[48]. In a study by Ruppach, et al., NAb could be detected in patients with known time point of infection as early as eight weeks after infection when using primary macrophages as target cells in the neutralization assays[49]. Furthermore, the authors found an inverse association between the neutralizing titers of the patient sera and the viral load in the respective persons. This may indicate a potential role of NAb in early virus control. Richman, et al. demonstrated the presence of NAb after six months in a longitudinal study in patients followed after primary infection using HIV-1 reporter viruses and a cell line neutralization assay[50]. However, these antibodies only neutralized the autologous, contemporaneous virus isolate and selected for rapid evolution of Env mutants that were neutralization-resistant. Recent publications proved the presence of NAb in fluids of highly exposed, persistently seronegative persons (HEPS), or infected patients able to control or delay HIV-induced disease to some extent[34,36,50-58]. Since 90% of the worldwide infections with HIV-1 are transmitted via mucosal surfaces, a vigorous mucosal immune response would be beneficial to prevent the establishment of HIV-infection. Both a strong cellular immune response, but also HIV-NAb, have been detected in highly exposed uninfected sexual partners from HIV-infected persons[52,59,60]. Nothing is known so far about the epitopes recognized by these antibodies, however. NAb – if present in the bloodstream – can reduce the infectivity of the virus, lower the risk of transmission[61-63], or be beneficial in containing the infection[48]. A few monoclonal antibodies

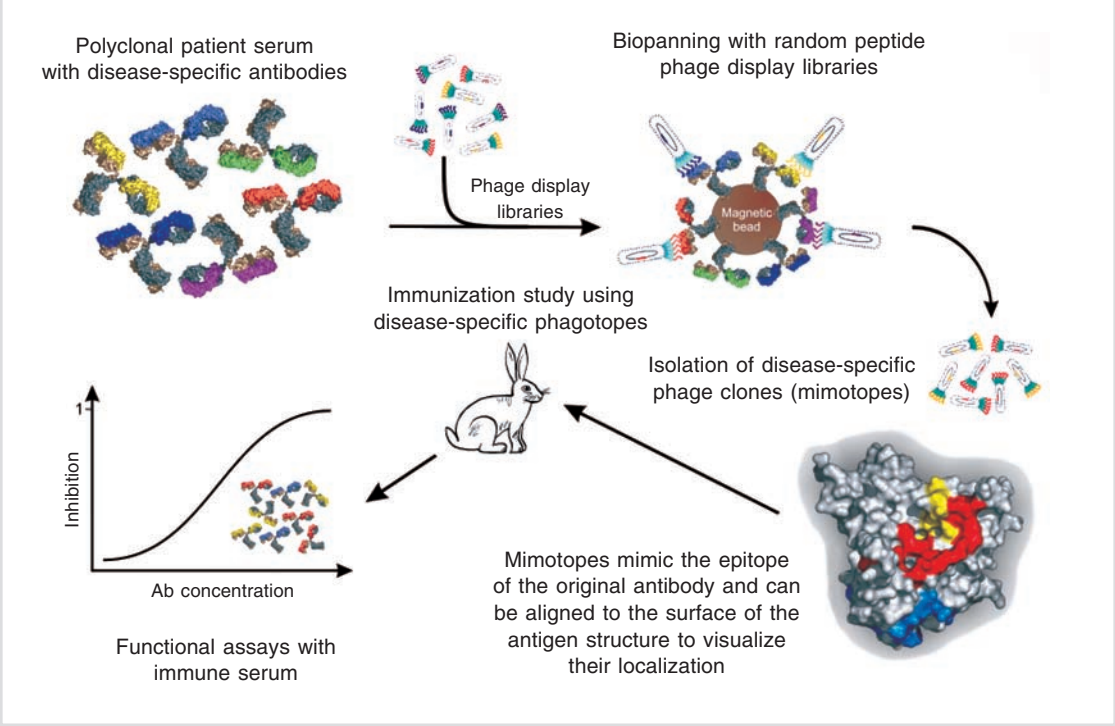

Figure 4. *Reverse vaccinology approach for the identification of epitopes for HIV- NAb in LTNP sera. Patient antibodies immobilized on magnetic beads are incubated with phages displaying random peptide sequences on their surface (biopanning). After several rounds of positive and negative selection, disease-specific phage clones are enriched. The peptide sequences of these clones are analyzed with respect to homology to the linear and the three-dimensional structure of the gp120 protein using a special software[82]. Phages with peptide sequences that can be aligned as conformational epitopes on the surface of gp120 are used for the immunization of animals to analyze their potential to induce HIV- NAb. If these are detected with* in vitro *neutralization assays, the selected mimotope indeed mimics a conformational epitope for HIV-NAb in the original patient serum.*

against HIV-1 with exceptionally broad neutralizing capacity (b12, 2G12, 2F5, 4E10) generated from HIV-1 positive individuals were intensively studied to examine the role of such antibodies for protection. These antibodies, originally raised against HIV-1 clade B, have potent cross-neutralizing capacity *in vitro* as single agents and/or in combination against primary HIV-1 clade A, B, C, and D isolates[64-66]. When used as postexposure prophylaxis in monkeys, two of four rhesus neonates were protected after oral challenge, and the two infected animals were protected from disease progression[67]. The treatment with a combination of broadly neutralizing antibodies could therefore be a promising approach to prevent postnatal HIV transmission. A combination of various such NAb was protective against intravenous[68,69], intravaginal[70,71], and oral[69,72] challenges in monkeys[47,73]. The studies document the valid assumption that NAb, if used in combination and therefore potent enough, are indeed able to prevent HIV infection or to markedly delay disease progression. A recent investigation studying the impact of monoclonal NAb in combination in humans supports the positive effects with respect to protection and delayed disease progression[48]. However, it was also shown that the neutralizing response needs to be "exceptionally broad and potent to be effective"[48].

The presence of NAb in HIV-infected individuals who contain the infection for decades without any antiviral therapy may also indicate that HIV is vulnerable to the humoral immune response in these persons. Several studies identified potent cross- NAb in the sera of such individuals, known as long-term nonprogressors (LTNP)[2,56,74-76]. However, multiple factors are known to cause non-progressive disease, including host genetic factors[77,78], deletions in viral regulatory genes[79], and a strong CTL response[80,81]. A recent study clearly demonstrates the presence of broadly neutralizing antibodies in LTNP sera and applied a reverse vaccinology approach to identify the epitopes of those antibodies, as these should in turn be able to induce NAb upon vaccination (Humbert, et al., submitted).

Immunogens and preliminary vaccines

The development of an HIV vaccine, able to induce NAb to prevent the primary infection and/or to support CTL for containing an established infection, is an extraordinary challenge. A vaccine-induced immune response generating NAb can mediate sterilizing protection if the antibodies are present in high enough titers in the bloodstream as well as in the mucosal tissue at the time of primary infection. After the establishment of infection, a vaccine-induced, cellular immune response would be beneficial to clear infected cells from the body before the virus can spread across the cellular compartments[83]. The successful studies about passive transfer of monoclonal NAb to protect monkeys from infection or to reduce viremia with a delayed disease progression, underline the importance of the humoral immune system in preventing HIV infection and they demonstrate that NAb can indeed be protective, provided their presence in high titers before infection. Much effort has been undertaken to identify protective immunogens. Thirty seven vaccine trials using recombinant protein units or peptides to induce an immune response (antibody as well as CTL) are listed on the IAVI report page; some of the trials are still ongoing (http://www.iavireport.org/trialsdb/). Among the trials, most of the proteins used for immunization consist of the HIV-1 Envelope derived from different clades (gp120, gp140 or gp160), but also combinations of various HIV-1 proteins, like p24 or Tat are being used. Peptide vaccines representing the V3 loop or CTL-epitopes for Nef, Gag and Env have also been included. Because the NAb response is mainly directed against the viral Envelope, gp120 was first thought to be an easy-to-make and promising vaccine. But the versatility of the virus and mutability of the envelope foiled these plans. Intensive structural studies on the envelope proteins and their trimeric structure on the viral surface revealed the virus' versatile defense systems (see above) and indicated that a subunit envelope vaccine cannot induce potent enough NAb. Consequently, a monomeric gp120 vaccine candidate, tested in phase III, was not efficient in protecting against HIV-1 infection[84-87]. The immune evasion of important envelope domains by shielding them with sugar moieties was bypassed in a study with monkeys infected with a mutated SIV, in which the glycosylation sites around the V1/V2 loops were removed to disclose the underlying CD4bs. Indeed, the NAb generated initially controlled the infection before the virus mutated and escaped[88]. But this example shows that vaccines presenting the immune system with the Achilles heel of the virus are capable of inducing broadly protective antibodies that are able to contain the infection, at least for a while. One critical issue for the outcome of infection seems to be the exact timing of the immune response. An already established immune protection before exposure to the virus gives the host a competitive advantage[89]. Another difficulty encountered in many trials is the correct presentation of the immunogens in order to induce NAb, even in cases where the epitopes of NAb are known. This is exemplified, for instance, by the NAb 2F5 directed against gp41 and interfering with membrane fusion during the late stage of infection (Fig. 3). The epitope of this antibody is very well known[90,91]; nevertheless, numerous trials failed to induce NAb with that epitope presented in different contexts to the immune system of vaccinated animals. A recent study, where the membrane-proximal regions including the 2F5 epitope were expressed in the context of the corresponding protein p15 from porcine endogenous retrovirus (PERV) describes the induction of HIV-1 NAb upon immunization with these hybrid proteins[92,93]. Also, the presentation of epitopes on phage may be suited to present those peptides in an appropriate manner. The immunization of macaques with a phage-displayed vaccine, comprising several peptides representing domains of the HIV-1 envelope and originally isolated with serum from HIV-1 infected individuals, induced a NAb response and protected from infection with SHIV89.6P[94,95]. However, this aggressive SHIV virus variant uses CXCR4 as a coreceptor, which is unlikely to be the primary infection route over mucosal tissues. Nevertheless, the benefit of small peptide vaccines mimicking several antibody targets on the viral envelope demonstrates the phage-display technology as a powerful tool in identifying new vaccine candidates.

References

1. Moog C, Fleury H, Pellegrin I, Kirn A, Aubertin A. Autologous and heterologous neutralizing antibody responses following initial seroconversion in HIV-1-infected individuals. J Virol 1997;71:3734-41.
2. Pilgrim A, Pantaleo G, Cohen O, et al. Neutralizing antibody responses to HIV-1 in primary infection and long-term nonprogressive infection. J Infect Dis 1997;176:924-32.
3. Bures R, Morris L, Williamson C, et al. Regional clustering of shared neutralization determinants on primary isolates of clade C HIV-1 from South Africa. J Virol 2002;76:2233-44.
4. Aasa-Chapman M, Hayman A, Newton P, et al. Development of the antibody response in acute HIV-1 infection. AIDS 2004;18:371-81.
5. Mascola J, D'Souza P, Gilbert P, et al. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate HIV-1 vaccines. J Virol 2005;79:10103-7.
6. Louder M, Sambor A, Chertova E, et al. HIV-1 envelope pseudotyped viral vectors and infectious molecular clones expressing the same envelope glycoprotein have a similar neutralization phenotype, but culture in peripheral blood mononuclear cells is associated with decreased neutralization sensitivity. Virology 2005;339:226-38.
7. Zhu P, Chertova E, Bess J, et al. Electron tomography analysis of envelope glycoprotein trimers on HIV and SIV virions. Proc Natl Acad Sci USA 2003;100:15812-7.

8. Kwong P, Wyatt R, Majeed S, et al. Structures of HIV-1 gp120 envelope glycoproteins from laboratory-adapted and primary isolates. Structure Fold Des 2000;8:1329-39.
9. Sattentau Q, Moore J. HIV-1 neutralization is determined by epitope exposure on the gp120 oligomer. J Exp Med 1995;182:185-96.
10. Zolla-Pazner S. Identifying epitopes of HIV-1 that induce protective antibodies. Nat Rev Immunol 2004;4:199-210.
11. Pantophlet R, Burton D. GP120: Target for neutralizing HIV-1 antibodies. Annu Rev Immunol 2006;24:739-69.
12. Wu L, Paxton W, Kassam N, et al. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1 in vitro. J Exp Med 1997;185:1681-91.
13. Khurana S, Kennedy M, King L, Golding H. Identification of a linear peptide recognized by monoclonal antibody 2D7 capable of generating CCR5-specific antibodies with HIV-neutralizing activity. J Virol 2005;79:6791-6800.
14. Königs C, Rowley M, Thompson P, et al. Monoclonal antibody screening of a phage-displayed random peptide library reveals mimotopes of chemokine receptor CCR5: implications for the tertiary structure of the receptor and for an N-terminal binding site for HIV-1 gp120. Eur J Immunol 2000;30:1162-71.
15. Burton D. Antibodies, viruses and vaccines. Nat Rev Immunol 2002;2:706-13.
16. Parren P, Burton D. The antiviral activity of antibodies *in vitro* and *in vivo*. Adv Immunol 2001;77:195-262.
17. Parren P, Mondor I, Naniche D, et al. Neutralization of HIV-1 by antibody to gp120 is determined primarily by occupancy of sites on the virion irrespective of epitope specificity. J Virol 1998;72:3512-9.
18. Schonning K, Lund O, Lund O, Hansen J. Stoichiometry of monoclonal antibody neutralization of T-cell line-adapted HIV-1. J Virol 1999;73:8364-70.
19. Yang X, Kurteva S, Lee S, Sodroski J. Stoichiometry of antibody neutralization of HIV-1. J Virol 2005;79:3500-8.
20. Layne S, Merges M, Dembo M, et al. Factors underlying spontaneous inactivation and susceptibility to neutralization of HIV. Virology 1992;189:695-714.
21. Bourinbaiar A. The ratio of defective HIV-1 particles to replication-competent infectious virions. Acta Virol 1994;38:59-61.
22. Chertova E, Bess J, Crise B, et al. Envelope glycoprotein incorporation, not shedding of surface envelope glycoprotein (gp120/SU), is the primary determinant of SU content of purified HIV-1 and SIV. J Virol 2002;76:5315-25.
23. Binley J, Ditzel H, Barbas C, et al. Human antibody responses to HIV type 1 glycoprotein 41 cloned in phage display libraries suggest three major epitopes are recognized and give evidence for conserved antibody motifs in antigen binding. AIDS Res Hum Retroviruses 1996;12:911-24.
24. Schneider J, Kaaden O, Copeland T, Oroszlan S, Hunsmann G. Shedding and interspecies type sero-reactivity of the envelope glycopolypeptide gp120 of HIV. J Gen Virol 1986;67:2533-8.
25. Poignard P, Fouts T, Naniche D, Moore J, Sattentau Q. Neutralizing antibodies to HIV-1 gp120 induce envelope glycoprotein subunit dissociation. J Exp Med 1996;183:473-84.
26. Moore J, McKeating J, Weiss R, Sattentau Q. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. Science 1990;250:1139-42.
27. Hart T, Klinkner A, Ventre J, Bugelski P. Morphometric analysis of envelope glycoprotein gp120 distribution on HIV-1 virions. J Histochem Cytochem 1993;41:265-71.
28. Ho D, Sarngadharan M, Hirsch MS, et al. HIV neutralizing antibodies recognize several conserved domains on the envelope glycoproteins. J Virol 1987;61:2024-8.
29. Herrera C, Spenlehauer C, Fung M, Burton D, Beddows S, Moore J. Non-neutralizing antibodies to the CD4-binding site on the gp120 subunit of HIV-1 do not interfere with the activity of a neutralizing antibody against the same site. J Virol 2003;77:1084-91.
30. Poignard P, Moulard M, Golez E, et al. Heterogeneity of envelope molecules expressed on primary HIV-1 particles as probed by the binding of neutralizing and non-neutralizing antibodies. J Virol 2003;77:353-65.
31. Ahmad A, Menezes J. Antibody-dependent cellular cytotoxicity in HIV infections. FASEB J 1996;10:258-66.
32. Ahmad R, Sindhu S, Toma E, et al. Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti-HIV-1 antibodies and prognostic predictors of HIV infection. J Clin Immunol 2001;21:227-33.
33. Verrier F, Moog C, Barre-Sinoussi F, Van der Ryst E, Spenlehauer C, Girard M. Macaque immunization with virions purified from a primary isolate of HIV-1 induced enhancement antibodies. Bull Acad Natl Med 2000;184:67-84.
34. Beirnaert E, Nyambi P, Willems B, et al. Identification and characterization of sera from HIV-infected individuals with broad cross-neutralizing activity against group M (env clade A-H) and group O primary HIV-1 isolates. J Med Virol 2000;62:14-24.
35. Kostrikis L, Cao Y, Ngai H, Moore J, Ho D. Quantitative analysis of serum neutralization of HIV-1 from subtypes A, B, C, D, E, F, and I: lack of direct correlation between neutralization serotypes and genetic subtypes and evidence for prevalent serum-dependent infectivity enhancement. J Virol 1996;70:445-8.
36. Spenlehauer C, Kirn A, Aubertin A, Moog C. Antibody-mediated neutralization of primary HIV-1 isolates: investigation of the mechanism of inhibition. J Virol 2001;75:2235-45.
37. Poignard P, Klasse P, Sattentau Q. Antibody neutralization of HIV-1. Immunol Today 1996;17:239-46.
38. Wyatt R, Kwong P, Desjardins E, et al. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 1998;393:705-11.
39. Rizzuto C, Sodroski J. Fine definition of a conserved CCR5-binding region on the HIV-1 glycoprotein 120. AIDS Res Hum Retroviruses 2000;16:741-9.
40. Johnson W, Desrosiers R. Viral persistence: HIV's strategies of immune system evasion. Annu Rev Med 2002;53:499-518.
41. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 1998;280:1884-8.
42. Broder C, Earl P, Long D, Abedon S, Moss B, Doms R. Antigenic implications of HIV-1 envelope quaternary structure: oligomer-specific and -sensitive monoclonal antibodies. Proc Natl Acad Sci USA 1994;91:11699-703.
43. Burton D. A vaccine for HIV type 1: the antibody perspective. Proc Natl Acad Sci USA 1997;94:10018-23.
44. Nyambi P, Mbah H, Burda S, et al. Conserved and exposed epitopes on intact, native, primary HIV-1 virions of group M. J Virol 2000;74:7096-107.
45. Thali M, Moore J, Furman C, et al. Characterization of conserved HIV-1 gp120 neutralization epitopes exposed upon gp120-CD4 binding. J Virol 1993;67:3978-88.
46. Labrijn A, Poignard P, Raja A, et al. Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary HIV-1. J Virol 2003;77:10557-65.
47. McCann C, Song R, Ruprecht R. Antibodies: can they protect against HIV infection? Curr Drug Targets Infect Disord 2005;5:95-111.
48. Trkola A, Kuster H, Rusert P, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med 2005;11:615-22.
49. Ruppach H, Nara P, Raudonat I, Elanjikal Z, Rubsamen-Waigmann H, Dietrich U. HIV-positive sera obtained shortly after seroconversion neutralize autologous HIV type 1 isolates on primary macrophages but not on lymphocytes. J Virol 2000;74:5403-11.
50. Richman D, Wrin T, Little S, Petropoulos C. Rapid evolution of the neutralizing antibody response to HIV-1 infection. Proc Natl Acad Sci USA 2003;100:4144-9.
51. Beirnaert E, De Zutter S, Janssens W, van der Groen G. Potent broad cross-neutralizing sera inhibit attachment of primary HIV-1 isolates (groups M and O) to peripheral blood mononuclear cells. Virology 2001;281:305-14.
52. Broliden K, Hinkula J, Devito C, et al. Functional HIV-1 specific IgA antibodies in HIV-1 exposed, persistently IgG seronegative female sex workers. Immunol Lett 2001;79:29-36.
53. Brown L, Souberbielle B, Marriott J, et al. The conserved carboxy terminal region of HIV-1 gp120 is recognized by seronegative HIV-exposed people. AIDS 1999;13:2515-21.
54. Buchacz K, Parekh B, Padian N, et al. HIV-specific IgG in cervicovaginal secretions of exposed HIV-uninfected female sexual part-

ners of HIV-infected men. AIDS Res Hum Retroviruses 2001; 17:1689-93.
55. Cavacini L, Kuhrt D, Duval M, Mayer K, Posner M. Binding and neutralization activity of human IgG1 and IgG3 from serum of HIV-infected individuals. AIDS Res Hum Retroviruses 2003;19:785-92.
56. Montefiori D, Pantaleo G, Fink L, Neutralizing and infection-enhancing antibody responses to HIV-1 in long-term nonprogressors. J Infect Dis 1996;173:60-7.
57. Pinter A, Honnen W, He Y, Gorny M, Zolla-Pazner S, Kayman S. The V1/V2 domain of gp120 is a global regulator of the sensitivity of primary HIV-1 isolates to neutralization by antibodies commonly induced upon infection. J Virol 2004;78:5205-15.
58. Vincent N, Malvoisin E, Pozzetto B, Genin C. Detection of IgA inhibiting the interaction between gp120 and soluble CD4 receptor in serum and saliva of HIV-1-infected patients. AIDS 2004;18:37-43.
59. Devito C, Hinkula J, Kaul R, et al. Mucosal and plasma IgA from HIV-exposed seronegative individuals neutralize a primary HIV1 isolate. AIDS 2000;14:1917-20.
60. Devito C, Hinkula J, Kaul R, et al. Cross-clade HIV-1-specific neutralizing IgA in mucosal and systemic compartments of HIV-1-exposed, persistently seronegative subjects. J Acquir Immune Defic Syndr 2002;30:413-20.
61. Scarlatti G, Albert J, Rossi P, et al. Mother-to-child transmission of HIV-1: correlation with neutralizing antibodies against primary isolates. J Infect Dis 1993;168:207-10.
62. Devash Y, Calvelli T, Wood D, Reagan K, Rubinstein A. Vertical transmission of HIV is correlated with the absence of high-affinity/avidity maternal antibodies to the gp120 principal neutralizing domain. Proc Natl Acad Sci USA 1990;87:3445-9.
63. Rossi P, Moschese V, Broliden P, et al. Presence of maternal antibodies to HIV-1 envelope glycoprotein gp120 epitopes correlates with the uninfected status of children born to seropositive mothers. Proc Natl Acad Sci USA 1989,86:8055-8.
64. Xu W, Smith-Franklin B, Li P, et al. Potent neutralization of primary HIV clade C isolates with a synergistic combination of human monoclonal antibodies raised against clade B. J Hum Virol 2001;4:55-61.
65. Kitabwalla M, Ferrantelli F, Wang T, et al. Primary African HIV clade A and D isolates: effective cross-clade neutralization with a quadruple combination of human monoclonal antibodies raised against clade B. AIDS Res Hum Retroviruses 2003;19:125-31.
66. Zwick M, Wang M, Poignard P, et al. Neutralization synergy of HIV-1 primary isolates by cocktails of broadly neutralizing antibodies. J Virol 2001;75:12198-208.
67. Ferrantelli F, Hofmann-Lehmann R, Rasmussen R, et al. Post-exposure prophylaxis with human monoclonal antibodies prevented SHIV89.6P infection or disease in neonatal macaques. AIDS 2003;17:301-9.
68. Mascola J, Lewis M, Stiegler G, Protection of Macaques against pathogenic SHIV 89.6PD by passive transfer of neutralizing antibodies. J Virol 1999;73:4009-18.
69. Baba T, Liska V, Hofmann-Lehmann R, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal SHIV infection. Nat Med 2000;6:200-6.
70. Parren P, Marx P, Hessell A, et al. Antibody protects macaques against vaginal challenge with a pathogenic R5 SHIV at serum levels giving complete neutralization in vitro. J Virol 2001;75:8340-7.
71. Mascola J, Stiegler G, VanCott T, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000;6:207-10.
72. Hofmann-Lehmann R, Vlasak J, Rasmussen R, et al. Postnatal passive immunization of neonatal macaques with a triple combination of human monoclonal antibodies against oral SHIV challenge. J Virol 2001;75:7470-80.
73. Ferrantelli F, Ruprecht R. Neutralizing antibodies against HIV – back in the major leagues? Curr Opin Immunol 2002;14:495-502.
74. Cao Y, Qin L, Zhang L, Safrit J, Ho D. Virologic and immunologic characterization of long-term survivors of HIV-1 infection. N Engl J Med 1995;332:201-8.
75. Carotenuto P, Looij D, Keldermans L, de Wolf F, Goudsmit J. Neutralizing antibodies are positively associated with CD4+ T-cell counts and T-cell function in long-term AIDS-free infection. AIDS 1998;12:1591-600.
76. Zhang Y, Fracasso C, Fiore J, et al. Augmented serum neutralizing activity against primary HIV-1 isolates in two groups of HIV-1-infected long-term nonprogressors. J Infect Dis 1997;176:1180-7.
77. Magierowska M, Theodorou I, Debre P, et al. Combined genotypes of CCR5, CCR2, SDF1, and HLA genes can predict the long-term nonprogressor status in HIV-1-infected individuals. Blood 1999; 93:936-41.
78. Clegg A, Ashton L, Biti R, et al. CCR5 promoter polymorphisms, CCR5 59029A and CCR5 59353C, are under represented in HIV-1-infected long-term non-progressors. The Australian Long-Term Non-Progressor Study Group. AIDS 2000;14:103-8.
79. Saksena N, Wang B, Dyer W. Biological and molecular mechanisms in progression and non-progression of HIV disease. AIDS Rev 2001;3:133-44.
80. Gea-Banacloche J, Migueles S, Martino L, et al. Maintenance of large numbers of virus-specific CD8+ T-cells in HIV-infected progressors and long-term nonprogressors. J Immunol 2000; 165:1082-92.
81. Harrer T, Harrer E, Kalams S, et al. Cytotoxic T lymphocytes in asymptomatic long-term nonprogressing HIV-1 infection. Breadth and specificity of the response and relation to *in vivo* viral quasispecies in a person with prolonged infection and low viral load. J Immunol 1996;156:2616-23.
82. Schreiber A, Humbert M, Benz A, Dietrich U. 3D-Epitope-Explorer (3DEX): Localization of conformational epitopes within three-dimensional structures of proteins. J Comput Chem 2005;26:879-87.
83. McMichael A, Hanke T. HIV vaccines 1983-2003. Nat Med 2003;9:874-80.
84. Graham B, Mascola J. Lessons from failure–preparing for future HIV-1 vaccine efficacy trials. J Infect Dis 2005;191:647-9.
85. Gilbert P, Peterson M, Follmann D, et al. Correlation between immunologic responses to a recombinant gp120 vaccine and incidence of HIV-1 infection in a phase III HIV-1 preventive vaccine trial. J Infect Dis 2005;191:666-77.
86. Flynn N, Forthal D, Harro C, Judson F, Mayer K, Para M. Placebo-controlled phase III trial of a recombinant gp120 vaccine to prevent HIV-1 infection. J Infect Dis 2005;191:654-65.
87. Gilbert P, Ackers M, Berman P, et al. HIV-1 virologic and immunologic progression and initiation of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant gp120 vaccine. J Infect Dis 2005;192:974-83.
88. Reitter J, Means R, Desrosiers R. A role for carbohydrates in immune evasion in AIDS. Nat Med 1998;4:679-84.
89. Graham B. Clinical trials of HIV vaccines. Annu Rev Med 2002;53:207-21.
90. Barbato G, Bianchi E, Ingallinella P, et al. Structural analysis of the epitope of the anti-HIV antibody 2F5 sheds light into its mechanism of neutralization and HIV fusion. J Mol Biol 2003;330:1101-15.
91. Ofek G, Tang M, Sambor A, et al. Structure and mechanistic analysis of the anti-HIV-1 antibody 2F5 in complex with its gp41 epitope. J Virol 2004;78:10724-37.
92. Langhammer S, Hubner J, Kurth R, Denner J. Antibodies neutralizing feline leukemia virus (FeLV) in cats immunized with the transmembrane envelope protein p15E. Immunology 2006;117:229-37.
93. Fiebig U, Langhammer S, Kurth R, Denner J. Induction of HIV-neutralizing antibodies of the 2F5/4E10 type. International Meeting of The Institute of Human Virology. Baltimore, USA, 29 August -2 September 2005.
94. Scala G, Chen X, Liu W, et al. Selection of HIV-specific immunogenic epitopes by screening random peptide libraries with HIV-1-positive sera. J Immunol 1999;162:6155-61.
95. Chen X, Scala G, Quinto I, et al. Protection of rhesus macaques against disease progression from pathogenic SHIV-89.6PD by vaccination with phage-displayed HIV-1 epitopes. Nat Med 2001; 7:1225-31.