UDC 578.74:571.27

Requirements for the Induction of Broadly Neutralizing Antibodies against HIV-1 by Vaccination

A. N. Vzorov* and L. V. Uryvaev

*Ivanovsky Institute of Virology, Gamaleya Federal Research Center of Epidemiology and Microbiology,
Moscow, 123098 Russia*

*e-mail: anvzorov@mail.ru

Received March 26, 2017; in final form, April 18, 2017

Abstract—A study of the induction of broadly neutralizing antibodies (bNAbs) in HIV-infected patients and vaccinated subjects revealed the main criteria for the formation of bNAbs (the duration of exposure to a viral antigen, the effect of the diversity of HIV variants, and the removal of barriers associated with the Env-dependent defense mechanisms of HIV-1). Modified trimers of the HIV-1 envelope protein (Env) exposed on virus-like particles (VLP) have unique properties: they (i) modulate the exposure of binding sites (bs) of the CD4 receptor and co-receptor; (ii) create steric restrictions for contact with bNAbs; (iii) increase the Env presentation density, thus enhancing the immune response; (iv) form stable trimers that do not induce off-target immune responses; and (v) allow additional modifications to their structure and construction of a platform with immunostimulating molecules. Immunization using a heterologous subtype-cross prime–boost regime with modified trimeric Env is capable of inducing somatic hypermutation levels necessary for the formation of bNAbs.

Keywords: HIV-1, broadly neutralizing antibodies, protective neutralizing antibodies, Env trimer, virus-like particles

DOI: 10.1134/S0026893317060176

INTRODUCTION

Induction of broadly neutralizing antibodies (bNAbs) is the main objective in developing vaccines against the human immunodeficiency virus (HIV), but the mechanisms of their generation are still poorly understood. There is still no convincing evidence that virus neutralization in vitro correlates with the protective function of bNAbs in HIV-infected individuals, although many attempts were made to demonstrate such a correlation [1–3] (Table 1). The problem is that HIV-1 causes latent infection after entering the cell and evades the immune response by mutating and generating different variants of the envelope protein (Env), which is the main HIV-1 antigen, while specific neutralizing antibodies against rapidly changing antigenic determinants of the virus are produced with a lag [4–7]. Experiments on HIV-1 transmission showed that passive delivery of neutralizing antibodies totally prevents infection in animals [8–11]. Natural defense mechanisms help to achieve the goal, ensuring low transmission of HIV through mucous membranes [12, 13]. The induction of bNAbs against HIV and the influenza virus via vaccination was theoretically proved and

became potentially achievable over recent years [14, 15]. This gives grounds to expect a higher efficiency in inducing bNAbs and blocking virus entry into cells on mucosal surface by anti-HIV-1 vaccines [16, 17].

BROADLY NEUTRALIZING ANTIBODIES AND THEIR FEATURES

Four main sites of vulnerability are commonly recognized in the Env glycoprotein of HIV-1. They are a CD4-binding site (CD4-bs), glycan-dependent epitopes in the V1–V2 region and close to the V3/C3 base, and linear epitopes in the proximal outer domain (MPER) of gp41. Env most likely has other, still unmapped epitopes recognized by certain bNAbs as well [18]. The production of bNAbs in HIV-infected subjects is a result of immune responses to individual subdominant epitopes; i.e., serum with broadly neutralizing activity mostly reflect single-specificity immune responses (bNAbs against V1–V2 or CD4-bs), although multiple bNAb specificity (against V1–V2, CD4-bs, and other sites) was observed in rare HIV-infected patients [18–22]. Serum specificity strongly depends on the HIV-1 subtype. For instance, bNAbs against CD4-bs prevail in patients infected with subtype B viruses, while V2-glycan-specific bNAbs are mostly produced in patients infected with non-B viruses [23].

As is known, bNAbs are not produced in early HIV-1 infection [20, 24–27], but appear with time,

Abbreviations: HIV, human immunodeficiency virus; prime–boost, primary (initial)–repetitive (immunization); bNAbs, broadly neutralizing antibodies; bs, binding site; Env, envelope protein; Nabs, neutralizing antibodies; non-NAbs, non-neutralizing antibodies; OD, outer domain of gp120; pnnAbs, protective non-neutralizing antibodies; VLP, virus-like particle.

Table 1. Production of bNAbs

Obstacles in HIV-infected individuals	Overcoming by vaccination
Env spikes exposed on the surface of HIV-1 particles exhibit structural features: they have variable loops, are highly glycosylated, and occur in a metastable form	Construction of highly immunogenic Env trimers
Prolonged exposure to antigen	Duration of infection is independent driver of bNAbs induction; the V1–V2 -immune response does not require high-level somatic hypermutation,
Diversity with antigen exposure affects the development of breadth	A genetic diversity potential is ensured by heterologous prime–boost vaccination
High level of somatic mutations	Heterologous prime–boost regimen elicit of affinity maturation of Abs for developing breadth
Autoreactivity	10E8 bNAbs does not display autoreactivity
Complex mechanisms of bNAbs induction	Designation of a lineage approach to initiate a specific bNAbs development pathway ; discovered a critical role of CD4 ⁺ T (Tfh) cells for development of bNAbs
bNAbs do not protect HIV-infected from developing AIDS	bNAbs block HIV-1 entry on mucosal surface and protect from HIV-1 acquisition

the circumstance being associated with events of virus–host coevolution [2, 28, 29]. As a result, autologous (strain-specific) neutralizing antibodies (NAbs) are the first to form and lead to generation of escape mutations and high diversity of HIV immune variants and, later on, bNAbs. High-affinity bNAbs are capable of neutralizing a broad range of extracellular and cell-associated HIV strains [18, 30–38]. These antibodies ensure efficient protection from experimental virus infection in nonhuman primates and mice carrying functional genes of the human immune system [18, 39–44]. During coevolution with HIV, bNAbs may acquire unusual characteristics, such as a long complementarity-determining region H3 (CDRH3). Structural changes allow the antibodies to overcome the features that Env trimers have to prevent the binding and neutralization with antibodies [45]. Broadly neutralizing antibodies result from extensive somatic hypermutation of immune cells [32, 33, 46, 47]. Only 10–30% of patients produce bNAbs [1, 48–51], and attempts to induce bNAbs via vaccinations are often unsuccessful.

Polyreactivity and autoreactivity to the proximal region of gp41 were observed for bNAbs [52–54], arising possibly because hydrophobic residues of the CDRH3 region have to interact with the host cell membrane [55, 56]. At the same time, autoreactivity was not observed in autoimmunity studies for antibodies that belong to the same category, 10E8, and neutralize and suppress the majority of HIV-1 strains [57]. It seems that certain B-cell maturation pathways proceed without clonal deletion of B-cell precursors (tolerance mecha-

nism), making it more likely that such antibodies are generated as a result of vaccination (Table 1).

The mechanisms essential for bNAbs production in the human body are complex and are still poorly understood. However, CD4⁺ follicular T-helper cells (Tfh) are known to play a crucial role in primary B-cell responses within lymph-node germinal centers, leading to bNAbs development [58, 59]. Tfh cells are phenotypically characterized by expression of the CXCR5 surface receptor, and their developmental program is regulated by the Bcl-6 transcription factor [60, 61]. Tfh cells affect the class switching and maturation of immunoglobulins in B cells, acting via secretion of cytokines, such as interleukin 21 (IL-21) and (IL-4) [62, 63], and contact mechanisms [64, 65]. Data from experiments with model animals showed that antigen presentation by dendritic cells is necessary and sufficient for triggering the Tfh developmental program [66, 67]. Martin-Gayo et al. [68] found that the CXCR5⁺CXCR3⁺PD-1⁺ Tfh cell population serves as precursors of PD-1^{hi} cells, which sustain B-cell development by secreting cytokines. These cells can be selectively primed by dendritic cells from HIV-1 controller with neutralizing antibody responses and can induce bNAbs against HIV-1 in the absence of high-level HIV-1 viremia. Such studies will help to understand the mechanisms responsible for the induction of bNAbs against HIV-1 and to develop preventive vaccines on the basis of their results.

Fig. 1. The development rate of bNAbs against the HIV-1 Env trimer is epitope-specific. Precursor antibodies lack strain-specific neutralizing activity, while antibody lineages against CD4-bs (a, 1) and V1–V2 (b, 1) possess this activity. Somatic hypermutation is necessary for maturation of both strain-specific (a, 2) and CD4-bs lineage (a, 3) antibodies. (b) In contrast, bNAbs against V1–V2 form in a germline cell lineage that express bNAbs with pre-existing long CDRH3 regions. Subjects with antibodies against V1–V2 develop bNAbs quicker (within several months) (b, 2) than subjects with antibodies against CD4-bs (2–3 years) (a, 3). Black lines, somatic hypermutations; black bars, bNAbs; Abs, antibodies.

INDUCTION OF BROADLY NEUTRALIZING ANTIBODIES IS INDEPENDENT OF THE DURATION OF ANTIBODY–ANTIGEN INTERACTION

Individual specifics of the immune system and the course of infection in HIV-infected patients may limit or facilitate the development of specific bNAbs. Activity of anti-HIV bNAbs is known to increase in patients after several years of infection, and the increase is mostly associated with viremia and a low CD4⁺ cell count [1, 2, 69, 70]. Long persistence of a virus antigen in the body was thought to be necessary for stimulating affinity maturation of antibodies and bNAb induction [35, 71]. However, recent studies showed that bNAb induction no longer depends on the infection duration after 3 years of the infection process [23] (Table 1). The finding was supported by several case reports, including those of mother-to-child virus transmission [72]. In newborns, bNAb activity develops in early infection [72]. The observation indicates that an efficient induction of bNAbs via vaccination is principally possible.

When an anti-CD4-bs antibody lineage forms in infected patients, a B-cell recognizes, but is incapable to neutralize the infecting virus. Synthesis of bNAbs is achieved after long-term B-cell maturation to high affinity, and the resulting antibodies against CD4-bs neutralize the autologous virus and, later, heterologous strains (Fig. 1) [73]. A study of the mechanisms sustaining the production of anti-V1–V2 antibodies showed that an unmutated common ancestor (UCA) is capable of binding and neutralizing the infecting virus [74]. In addition, a special structure (a long CDRH3 region) of antibodies of the class forms as a result of recombination events in the cell prior to its contact with the antigen (Fig. 1). In contrast to the CD4-bs lineage, a broad immune response of V1–V2

antibodies is accompanied by a moderate somatic hypermutation level (i.e., a long-term contact with the antigen is unnecessary) [74]. The finding explains why a broad range of neutralizing antibodies may develop in one particular subject (with a certain bNAb specificity and a moderate somatic hypermutation level) quicker than in another subject (with another bNAb specificity and higher affinity) [74]. The long CDRH3 region possibly allows V1–V2 antibodies to interact with the sensitive epitope through the protective glycan layer of an Env trimer [75–78]. Prolonged maturation of bNAbs against CD4-bs is most likely explained by the fact that the region is poorly accessible for antibodies (the CD4–gp120 interface is laden with cavities) [79].

In spite of the above findings, it is possible to expect that construction of Env trimers with special modifications and a capability of modulating CD4-bs [80–83] will help to accelerate maturation of bNAbs against newly exposed functional CD4-bs regions upon immunization.

MODIFICATION OF THE ENV PROTEIN TO INDUCE BROADLY NEUTRALIZING ANTIBODIES

In HIV-1 infection, antibody production is additionally induced by nonfunctional Env proteins, such as noncleaved or defective trimers, dissociated gp120 monomers, intermediate 6-helix bundle (6-HB)-containing gp41 forms, and Env degradation products, which expose predominantly immunodominant epitopes for non-neutralizing antibodies (non-NAbs) [84, 85]. It is possible that non-NAbs hinder the induction of NAb against trimers. The induction and binding of NAb depend on the conformational flexibility of the Env trimer, which can change in quater-

Fig. 2. Stabilization of the HIV-1 Env trimer. (a) The Env conformation changes (is stabilized) upon binding with the CD4 receptor. (b) The GCN4 sequence, which forms a triple-helix knot and is incorporated in the CT domain, stabilizes the Env trimer. The N and C ends of the Env transmembrane gp41 protein are indicated.

nary structure from a closed (with epitopes not exposed on the surface) to an open (with epitopes exposed on the surface) conformation [86–89].

There are many ways to construct immunogens for inducing bNAbs, which include gp120 monomer-lineages, native gp140, gp120-cor, and outer domain (OD) proteins, epitope-specific structures (scaffolds), epitope-based peptides, etc. [90]. We focused on developing and using the immunogens that closely imitate the natural Env complex occurring on the envelope of HIV-1 particles [82, 83] and have an open structure for the recognition of mature B-cell receptors. In contrast, a lineage B-cell vaccine is specifically targeted to the precursors of desirable antibodies and is based on the use of several sequential Env trimers as immunogens [34]. A trimer-based approach is preferred for engaging the bNAb precursors, in particular, because the trimers have structural features that create steric limitations for epitopes as compared with monomeric gp120-based constructs, which lack such steric limitations. For instance, a construct based on gp120 OD monomers induces only non-bNAbs in VRC01 knock-in mice, which express the restored heavy chain of germline VRC01 lineage antibodies against CD4-bs [91]. Specific stabilizing mutations are introduced to stabilize the trimers of soluble gp140 and to ensure natural gp120–gp41 cleavage; e.g., a disulfide bridge can be used to covalently link two subunits. This helps to maintain the gp41 ectodomain in a transitional conformation occurring prior to fusion to the cell [92, 93] and prevents trimer aggregation [94, 95]. At the same time, various approaches to trimer construction often yield nonfunctional Env forms, which are capable of inducing off-target non-NAbs [96]. An alternative approach is stabilizing the Env trimers via an indirect

effect on the gp120 surface unit (SU) [83] (Fig. 2). Modification of the cytoplasmic (CT) domain was used for the purpose [82, 83, 97, 98].

Fusion of the virus and cell membranes proceeds through a series of transitional conformations and is possible to simulate by outward signaling through the CT domain [99]. These changes strongly affect the functions of receptor-binding segments of gp120 and increase the exposure of the coreceptor-binding site in the modified Env protein. Like the above recombinant trimers used to activate the bNAb precursors, these trimers provide optimal conditions for epitope-recognizing antibodies. The epitopes are sterically limited by structural specifics that are characteristic of the native stabilized trimers and protect them from non-specific binding with antibodies. Such conditions may play a critical role, especially in the case of epitopes associated with CD4-bs [80, 81]. Products of Env degradation are incorporated in the virus envelope in infection and in the case of recombinant trimers [96]. When Env trimers are stabilized through the CT domain, functional trimers are mostly incorporated in the membrane [83]. Additional modification consisting in a substitution of the highly conserved transmembrane spanning (TMS) domain of HIV Env with the TMS domain of a mouse mammary tumor virus (MMTV) protein substantially increases the incorporation of trimers in virus-like particles (VLPs) and their exposure on the VLP surface [82]. Immunogen design is one of the central problems in constructing a vaccine. A proper choice of the Env protein is also of important for immunogen construction. The ZM53 HIV-1 primary isolate is a transmitted founder (T/F) strain [100]. The strain is distinguished by a more open Env conformation because its gp120 has shorter V1–V4 loops as com-

Table 2. Characteristics of the Env trimer with a modified CT domain on the basis of a VLP platform

- Stabilized Env trimers, but not the degradation products or other forms of Env, are incorporated in VLPs, which do not induce off-target immune responses
- Env presentation density is increased (owing to modification of the TMS domain), thus increasing the efficiency of the immune response
- The bNAb approach angle is optimized as a result of Env trimer stabilization
- The modified CT domain affects the functional regions of the Env surface subunit and modulates exposition of the binding sites for CD4 and coreceptor
- The modified CT domain may affect the presence of steric or structural hindrances to epitope recognition and bNAb induction
- Immunostimulating molecules are possible to include to improve the efficacy
- HIV VLPs with systemic modifications in Env glycosylation are possible to construct (changes in glycosylation affect the demasking of Env antigenic sites)

pared with other strains. Biological characteristics were studied for the Env protein of HIV-1 subtype D strain 92UG046, which was isolated from a HIV-infected patient. The parental 92UG046 Env with the full-size CT domain interacted with CD4 molecules and infected CD4⁺ HeLa cells. Its quasi form with a shortened CT domain interacted with CD8 molecules and infected CD8⁺ HeLa cells. Thus, the 92UG046 HIV-1 Env has a potential to adopt various modifications in both outer and CT domains and can be used as an immunogen [81]. It is known additionally that the Env specificity (belonging to a particular subtype) affects the specificity of induced bNAbs [23], and modifications introduced in Env of a certain subtype are therefore aimed at exposing subtype-specific epitopes.

VLP-BASED IMMUNOGENIC PLATFORMS WITH THE Env TRIMER DESIGNED TO INDUCE BROADLY NEUTRALIZING ANTIBODIES

Vaccines based on VLPs have several advantages over vaccines based on soluble Env proteins. First, VLP-based immunogens contain membrane-anchored Env with the cytoplasmic CT tail; i.e., Env occurs in a natural conformation wherein exposed epitopes correspond to the epitopes present in a virion (in contrast to soluble monomeric Env antigens). Second, Env trimers contained in particles are far more efficient as immunogens than their soluble forms. Third, immunogenicity is possible to increase by incorporating additional membrane-associated immunostimulating molecules in the VLP envelope and introducing combined modifications (modification of the CT domain to affect exposition of the functional Env regions + systemic modification of glycans or modification of the CT domain + deletion of the V1–V3 loops) (Table 2).

New composite forms and means to deliver them are currently developed to improve the efficiency of immunogens. Immunostimulating molecules are

expedient to use on the VLP platform for inducing bNAbs. B-cell activation by Env present in the form of stabilized trimers on VLPs increased in the presence of immunostimulators. For instance, the membrane-associated glycosylphosphatidylinositol (GPI)-anchored granulocyte macrophage colony-stimulating factor (GM-CSF), which is a ligand of Toll-like receptor 5 (TLR5), enhanced the induction of bNAbs against a Env trimer [101, 102].

The role that immunostimulating molecules play in enhancing the immune response upon vaccination was demonstrated with the examples of the simian immunodeficiency virus (SIV) and influenza virus. Immunization of mice with synthetic nanoparticles containing SIV or influenza virus antigens plus ligands involved in TLR4 and TLR7 signaling led to synergistic increase in antigen-specific NAb level as compared with immunization with nanoparticles containing the same antigens and only one TLR ligand [103]. Persistence of germinal centers and plasma cell reactions in lymph nodes for more than 1.5 years was reported as one of the effects [103, 104].

The above and other data [101, 105] demonstrate that the incorporation of immune response-stimulating molecules in particles or VLPs is promising for the development of anti-HIV vaccines [83].

INDUCTION OF BROADLY NEUTRALIZING ANTIBODIES WITH LINEAGE B-CELL VACCINES

The main problem to solve when constructing a lineage B-cell vaccine is to overcome the obstacles to bNAb induction in HIV-infected patients. Hence, construction of immunogens targeting bNAb precursors underlies the design of B-cell vaccines against HIV [34]. Native HIV-1 Env trimers can induce autologous NAbs, and, therefore, trimeric Env variants and special immunization protocols can be used to simulate the bNAb formation. The approach is known as the natural clonal vaccine and is based on using the *env*

gene sequences from HIV-infected subjects to induce bNAbs [18, 34, 71, 73, 74, 106]. An alternative, but not mutually exclusive, means to obtain a lineage vaccine is to design the Env immunogens so that the antibody response targets a particular epitope [91, 107–115].

The lineage approach includes designing the immunogens that trigger a specific bNAb development pathway. A first step of the bNAb response involves naive B cells, which express a germline bNAb precursor without any mutation. However, recombinant Env glycoproteins of a standard design (with a wild-type Env conformation) are usually weak in recognizing the precursors [109, 110, 116, 117]. Various constructs based on the gp120 monomer, such as eOD or core-gp120, were designed to activate a proper (desired) B-cell germline bNAb precursor. The V1–V2 variable loops or glycosylation sites are usually deleted from these modified proteins in order to create optimal conditions for bNAbs to target the CD4-bs region [109, 110, 116, 117]. The lineage approach takes advantage of the structural and other features of HIV Env trimers (e.g., SOSIP, where SOS stands for double Cys mutations and IP is a substitution of Ile with Pro) to engage multiple germline bNAbs. Immunological studies were performed to examine the use of the triers to initiate a bNAb lineage with a subsequent boost with one or more mature SOSIP trimers (targeting a germinal center → an antibody lineage within a patient → within a subtype → cross-subtype) [118]. Promising results were obtained in studying the immunogenicity of BG505 SOSIP.664 and other trimers in knock-in mouse models expressing the heavy chain of human 3BNC60-class Ig (3BNC60-bNAbs) targeting the HIV-1 CD3-bs [112]. It is important to note that certain mature SOSIP.664 trimers bind with different human bNAb precursor antibodies in vitro [111, 119]. The efficacy of anti-HIV vaccines of the type is currently tested with mouse models.

Knock-in mouse models are useful for testing how Env-based immunogens activate specific germline precursors of human bNAbs, although this in vivo system fails to report how a mixed germline repertoire, where epitope immunodominance and subdominance come into play, affect the immunogenicity of an antigen [120]. Thus, lineage immunogens are capable of inducing bNAbs in special knock-in mice, although the response is expected to be associated with the induction of additional immunodominant non-NAbs in the presence of the total human germline repertoire.

ACHIEVEMENT OF A BROADLY PROTECTIVE ANTIBODY RESPONSE WITH A PRIME–BOOST STRATEGY

Genetic diversity (mutants and quasispecies) of HIV-1 in infected patients is one of the main factors that affect the breadth of the bNAb spectrum. The effect of HIV diversity remained significant and strong even after a correction for time as a parameter used to

evaluate the extent of virus diversity and neutralizing activity of antibodies. The finding indicates that the effect of diversity is independent of the duration of infection and the time point at which genotypic resistance testing (GRT) is carried out [23]. The effect of virus diversity was demonstrated in the cases of bNAb evolution after superinfection [106, 121] and is supported by the genetic diversity potential in vaccination using a cross-subtype prime–boost regimen [82]. A heterologous prime–boost immunization regimen [122–125] is capable of facilitating affinity maturation of antibodies and the breadth of the immune response (Table 1) [126]. There are data that the heterologous cross-subtype prime–boost regimen of immunization with proper Env proteins is necessary for achieving a broader response and seems to induce the somatic mutation levels that are essential for the production of bNAbs targeting diverse HIV variants [82].

Six efficacy studies have been performed to date with anti-HIV vaccines, which utilized potential HIV antigens (Env, Gag, etc.) and were based on four different concepts [125]. However, an acceptable efficacy was observed only for one vaccine, RV144 [127], whose testing was completed in 2009 and involved more than 16 thousand adult subjects. The study evaluated a combination of two vaccines, a prime vaccine, which was injected first, and a boost vaccine, which was injected two weeks later. The prime vaccine was ALVAC-HIV (vCP1521), which was based on a canarypox virus carrying *env* (subtype E/B), *gag*, and *pro* (subtype B). The vaccine is designed to stimulate the cell immune response. The vaccine does not prevent initial infection, but vaccine-induced cytotoxic T lymphocytes (CTLs, or CD8 cells) decrease HIV replication and thereby reduce the virus load and decelerate or stop disease progression to AIDS. The investigational VAX003 vaccine (with the AIDSVAX B/EB Env gp120 antigen) was used as a boost component. The vaccine is designed to induce the antibody response against HIV-1 gp120. The vaccine was found to be ineffective in earlier clinical trials [128], the lack of efficacy being possibly due to genetic hypervariability of HIV-1 gp120; i.e., any antibody response to native Env will be inefficient. The clinical study showed a 31.2% efficacy in preventing HIV infection for the RV144 vaccine, which was actually a combination of two vaccines, ALVAC-HIV (vCP1521) and AIDSVAX B/EB gp120, and was used in a heterologous prime–boost regimen. On the one hand, this was an unprecedented achievement and certain success in HIV vaccinology. On the other hand, the efficacy was not high enough to recommend RV144 as an anti-HIV vaccine for broad use. The protective response was associated with the production of protective nonneutralizing antibodies (pnnAbs) to the V1–V2 region of gp120 [129].

The GOVX-B11 preventive anti-HIV-1 vaccine is based on VLPs. A heterologous prime–boost immunization regimen was used again with this vaccine, including a recombinant DNA to stimulate the

immune response and a recombinant modified vaccinia Ankara (MVA) virus to enhance the response. Both of the two vaccine components produced VLPs with a membrane-anchored Env trimer [130]. The protective efficacy of a vaccine based on the SIVmac239 strain and analogous to the GOVX-B11 anti-HIV vaccine was evaluated in indian origin rhesus macaques. [131]. A strong protective effect against mucosal infection with the heterologous pathogenic SIVsmE660 strain was observed in macaques. It is important that protective activity of the vaccine was not affected by the presence or absence of restrictive *TRIM5 α* alleles or the use of GM-CSF as an adjuvant. A key role in preventing infection was attributed to pnnAbs rather than to T cells [131]. Immunization was found to stimulate an immune response with a production of V1–V2-targeting protective antibodies [129].

Immunization with a modified Env trimer of the ZM53 founder strain induced specific bNAbs and high-avidity broadly acting antibodies in Guinea pigs [82, 132]. It remains unclear whether the protective high-avidity antibodies induced by the vaccine are precursors of bNAbs.

New data on bNAbs generation were obtained in a study of virus–bNAbs coevolution in a HIV-infected patient [73]. Synthesis of bNAbs occurs when multiple diverse virus variants are present simultaneously. B cells of a germinal center must have a broad activity and high avidity for interactions with various virus variants on the surfaces of follicular dendritic cells. As a result, B cells with high avidity and a broad activity are predominantly selected from B cells with a narrow activity (even those having higher affinity) [126]. The finding makes it possible to conclude that broadly acting high-avidity antibodies induced in a heterologous vaccination regimen may have a common ancestor with bNAbs.

CONCLUSIONS

The inefficiency of bNAbs generation in HIV-infected patients is possible to overcome via vaccination. Designing the immunogen with due regard to the factors that stimulate or suppress bNAbs induction makes it possible to obtain functional stabilized trimers that have an open Env form, are efficiently incorporated in VLPs, and induce a HIV-specific immune response. Adding immunostimulating agents, such as membrane-associated TLR5 ligands or GM-CSF, to the vaccine will increase the efficiency of antigen presentation or enhance the antibody response. Modification of the V1–V2 and V3 regions and the glycan component of Env will improve the exposition of conserved functional regions of Env, thus accelerating bNAbs development. A heterologous prime–boost immunization regimen, which stimulates an accumulation of somatic hypermutations, will induce bNAbs capable of affecting the total diversity of HIV-1 variants on mucous membranes, which are the HIV portal of entry. More-

over, such trimers induce high-affinity antibodies or pnnAbs to ensure an additional protective effect of the resulting vaccine against HIV-1 [82, 83, 133].

As is known, such pnnAbs act by “tagging” virus or infected cells for elimination by the innate immunity system [133]. Antibodies induced by Env trimers play their protective role by facilitating virus aggregation, inhibiting the virus via complement, and inducing antiviral cytokines.

REFERENCES

1. Sather D.N., Armann J., Ching L.K., et al. 2009. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. *J. Virol.* **83**, 757–769.
2. Gray E.S., Madiga M.C., Hermanus T., et al. 2011. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. *J. Virol.* **85**, 4828–4840.
3. McMichael A., Hanke T. 2002. The quest for an AIDS vaccine: Is the CD8+ T-cell approach feasible? *Nat. Rev. Immunol.* **2**, 283–291.
4. Swiggard W.J., Baytop C., Yu J.J., et al. 2005. Human immunodeficiency virus type 1 can establish latent infection in resting CD4+ T cells in the absence of activating stimuli. *J. Virol.* **79**, 14179–14188.
5. Seu L., Kutsch O. 2015. The host cell side of latent HIV-1 infection. *Oncotarget.* **6**, 19920–19921.
6. Sanders R.W., Moore J.P. 2017. Native-like Env trimers as a platform for HIV-1 vaccine design. *Immunol. Rev.* **275**, 161–182.
7. Mouquet H. 2014. Antibody B cell responses in HIV-1 infection. *Trends Immunol.* **35**, 549–561.
8. Shibata R., Igarashi T., Haigwood N., et al. 1999. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat. Med.* **5**, 204–210.
9. Mascola J.R., Montefiori D.C. 2010. The role of antibodies in HIV vaccines. *Annu. Rev. Immunol.* **28**, 413–444.
10. Burke B., Barnett S.W. 2007. Broadening our view of protective antibody responses against HIV. *Curr. HIV Res.* **5**, 625–641.
11. Hessel A.J., Poignard P., Hunter M., et al. 2009. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat. Med.* **15**, 951–954.
12. Pope M., Haase A.T. 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nat. Med.* **9**, 847–852.
13. Joseph S.B., Swanstrom R., Kashuba A.D., et al. 2015. Bottlenecks in HIV-1 transmission: Insights from the study of founder viruses. *Nat. Rev. Microbiol.* **13**, 414–425.
14. Burton D.R., Ahmed R., Barouch D.H., et al. 2012. A blueprint for HIV vaccine discovery. *Cell Host Microbe.* **12**, 396–407.

15. Krammer F., Palese P. 2014. Universal influenza virus vaccines: Need for clinical trials. *Nat. Immunol.* **15**, 3–5.
16. Burton D.R., Poignard P., Stanfield R.L., et al. 2012. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. *Science*. **337**, 183–186.
17. Burton D.R., Hessel A.J., Keele B.F., et al. 2011. Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 11181–11186.
18. Klein F., Mouquet H., Dosenovic P., et al. 2013. Antibodies in HIV-1 vaccine development and therapy. *Science*. **341**, 1199–1204.
19. Mikell I., Stamatatos L. 2012. Evolution of cross-neutralizing antibody specificities to the CD4-BS and the carbohydrate cloak of the HIV Env in an HIV-1-infected subject. *PLoS ONE*. **7**, e49610.
20. Tomaras G.D., Binley J.M., Gray E.S., et al. 2011. Polyclonal B cell responses to conserved neutralization epitopes in a subset of HIV-1-infected individuals. *J. Virol.* **85**, 11502–11519.
21. Wibmer C.K., Bhiman J.N., Gray E.S., et al. 2013. Viral escape from HIV-1 neutralizing antibodies drives increased plasma neutralization breadth through sequential recognition of multiple epitopes and immunotypes. *PLoS Pathog.* **9**, e1003738.
22. Bonsignori M., Montefiori D.C., Wu X., et al. 2012. Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: Implications for vaccine design. *J. Virol.* **86**, 4688–4692.
23. Rusert P., Kouyos R.D., Kadelka C., et al. 2016. Determinants of HIV-1 broadly neutralizing antibody induction. *Nat. Med.* **22**, 1260–1267.
24. Wei X., Decker J.M., Wang S., et al. 2003. Antibody neutralization and escape by HIV-1. *Nature* **422**, 307–312.
25. Richman D.D., Wrin T., Little S.J., et al. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 4144–4149.
26. Gray E.S., Moore P.L., Choge I.A., et al. 2007. Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. *J. Virol.* **81**, 6187–6196.
27. Mikell I., Sather D.N., Kalams S.A., et al. 2011. Characteristics of the earliest cross-neutralizing antibody response to HIV-1. *PLoS Pathog.* **7**, e1001251.
28. Doria-Rose N.A., Klein R.M., Manion M.M., et al. 2009. Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. *J. Virol.* **83**, 188–199.
29. van Gils M.J., Euler Z., Schweighardt B., et al. 2009. Prevalence of cross-reactive HIV-1-neutralizing activity in HIV-1-infected patients with rapid or slow disease progression. *AIDS*. **23**, 2405–2414.
30. Walker L.M., Phogat S.K., Chan-Hui P.Y., et al. 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science*. **326**, 285–289.
31. Walker L.M., Huber M., Doores K.J., et al. 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature*. **477**, 466–470.
32. Wu X., Yang Z.Y., Li Y., et al. 2010. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science*. **329**, 856–861.
33. Scheid J.F., Mouquet H., Ueberheide B., et al. 2011. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science*. **333**, 1633–1637.
34. Haynes B.F., Kelsoe G., Harrison S.C., et al. 2012. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat. Biotechnol.* **30**, 423–433.
35. Kwong P.D., Mascola J.R. 2012. Human antibodies that neutralize HIV-1: Identification, structures, and B cell ontogenies. *Immunity*. **37**, 412–425.
36. Kwong P.D., Mascola J.R., Nabel G.J. 2013. Broadly neutralizing antibodies and the search for an HIV-1 vaccine: The end of the beginning. *Nat. Rev. Immunol.* **13**, 693–701.
37. Mascola J.R., Haynes B.F. 2013. HIV-1 neutralizing antibodies: Understanding nature's pathways. *Immunol. Rev.* **254**, 225–244.
38. Gruell H., Klein F. 2014. Opening fronts in HIV vaccine development: Tracking the development of broadly neutralizing antibodies. *Nat. Med.* **20**, 478–479.
39. Klein K., Veazey R.S., Warrier R., et al. 2013. Neutralizing IgG at the portal of infection mediates protection against vaginal simian/human immunodeficiency virus challenge. *J. Virol.* **87**, 11604–11616.
40. Veselinovic M., Neff C.P., Mulder L.R., et al. 2012. Topical gel formulation of broadly neutralizing anti-HIV-1 monoclonal antibody VRC01 confers protection against HIV-1 vaginal challenge in a humanized mouse model. *Virology*. **432**, 505–510.
41. Barouch D.H., Whitney J.B., Moldt B., et al. 2013. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature*. **503**, 224–228.
42. McCoy L.E., Weiss R.A. 2013. Neutralizing antibodies to HIV-1 induced by immunization. *J. Exp. Med.* **210**, 209–223.
43. Shingai M., Nishimura Y., Klein F., et al. 2013. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature*. **503**, 277–280.
44. West A.P., Jr., Scharf L., Scheid J.F., et al. 2014. Structural insights on the role of antibodies in HIV-1 vaccine and therapy. *Cell*. **156**, 633–648.
45. van Gils M.J., Sanders R.W. 2013. Broadly neutralizing antibodies against HIV-1: Templates for a vaccine. *Virology*. **435**, 46–56.
46. Wu X., Zhou T., Zhu J., et al. 2011. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science*. **333**, 1593–1602.

47. Zhou T., Zhu J., Wu X., et al. 2013. Multidonor analysis reveals structural elements, genetic determinants, and maturation pathway for HIV-1 neutralization by VRC01-class antibodies. *Immunity*. **39**, 245–258.
48. Moog C., Fleury H.J., Pellegrin I., et al. 1997. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. *J. Virol.* **71**, 3734–3741.
49. Burren R., Salmon-Ceron D., Richert S., et al. 2001. Immunoglobulin G (IgG) and IgA, but also nonantibody factors, account for in vitro neutralization of human immunodeficiency virus (HIV) type 1 primary isolates by serum and plasma of HIV-infected patients. *J. Virol.* **75**, 5421–5424.
50. Binley J.M., Lybarger E.A., Crooks E.T., et al. 2008. Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C. *J. Virol.* **82**, 11651–11668.
51. Simek M.D., Rida W., Priddy F.H., et al. 2009. Human immunodeficiency virus type 1 elite neutralizers: Individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J. Virol.* **83**, 7337–7348.
52. Haynes B.F., Fleming J., St Clair E.W., et al. 2005. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. *Science*. **308**, 1906–1908.
53. Alam S.M., McAdams M., Boren D., et al. 2007. The role of antibody polyspecificity and lipid reactivity in binding of broadly neutralizing anti-HIV-1 envelope human monoclonal antibodies 2F5 and 4E10 to glycoprotein 41 membrane proximal envelope epitopes. *J. Immunol.* **178**, 4424–4435.
54. Matyas G.R., Wiczorek L., Beck Z., et al. 2009. Neutralizing antibodies induced by liposomal HIV-1 glycoprotein 41 peptide simultaneously bind to both the 2F5 or 4E10 epitope and lipid epitopes. *AIDS*. **23**, 2069–2077.
55. Sun Z.Y., Oh K.J., Kim M., et al. 2008. HIV-1 broadly neutralizing antibody extracts its epitope from a kinked gp41 ectodomain region on the viral membrane. *Immunity*. **28**, 52–63.
56. Ofek G., McKee K., Yang Y., et al. 2010. Relationship between antibody 2F5 neutralization of HIV-1 and hydrophobicity of its heavy chain third complementarity-determining region. *J. Virol.* **84**, 2955–2962.
57. Huang J., Ofek G., Laub L., et al. 2012. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature*. **491**, 406–412.
58. Crotty S. 2014. T follicular helper cell differentiation, function, and roles in disease. *Immunity*. **41**, 529–542.
59. Craft J.E. 2012. Follicular helper T cells in immunity and systemic autoimmunity. *Nat. Rev. Rheumatol.* **8**, 337–347.
60. Nurieva R.I., Chung Y., Martinez G.J., et al. 2009. Bcl6 mediates the development of T follicular helper cells. *Science*. **325**, 1001–1005.
61. Crotty S. 2011. Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* **29**, 621–663.
62. Avery D.T., Bryant V.L., Ma C.S., et al. 2008. IL-21-induced isotype switching to IgG and IgA by human naive B cells is differentially regulated by IL-4. *J. Immunol.* **181**, 1767–1779.
63. Bentebibel S.E., Schmitt N., Banchemareau J., et al. 2011. Human tonsil B-cell lymphoma 6 (BCL6)-expressing CD4⁺ T-cell subset specialized for B-cell help outside germinal centers. *Proc. Natl. Acad. Sci. U. S. A.* **108**, E488–497.
64. Vinuesa C.G., Linterman M.A., Goodnow C.C., et al. 2010. T cells and follicular dendritic cells in germinal center B-cell formation and selection. *Immunol. Rev.* **237**, 72–89.
65. Liu D., Xu H., Shih C., et al. 2015. T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. *Nature*. **517**, 214–218.
66. Goenka R., Barnett L.G., Silver J.S., et al. 2011. Cutting edge: Dendritic cell-restricted antigen presentation initiates the follicular helper T cell program but cannot complete ultimate effector differentiation. *J. Immunol.* **187**, 1091–1095.
67. Yao C., Zurawski S.M., Jarrett E.S., et al. 2015. Skin dendritic cells induce follicular helper T cells and protective humoral immune responses. *J. Allergy Clin. Immunol.* **136**, 1387–1397 e7.
68. Martin-Gayo E., Cronin J., Hickman T., et al. 2017. Circulating CXCR5⁺CXCR3⁺PD-1^{lo} Tfh-like cells in HIV-1 controllers with neutralizing antibody breadth. *JCI Insight*. **2**, e89574.
69. Moore P.L., Williamson C., Morris L. 2015. Virological features associated with the development of broadly neutralizing antibodies to HIV-1. *Trends Microbiol.* **23**, 204–211.
70. Landais E., Huang X., Havenar-Daughton C., et al. 2016. Broadly neutralizing antibody responses in a large longitudinal Sub-Saharan HIV primary infection cohort. *PLoS Pathog.* **12**, e1005369.
71. Klein F., Diskin R., Scheid J.F., et al. 2013. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. *Cell*. **153**, 126–138.
72. Goo L., Chohan V., Nduati R., et al. 2014. Early development of broadly neutralizing antibodies in HIV-1-infected infants. *Nat. Med.* **20**, 655–658.
73. Liao H.X., Lynch R., Zhou T., et al. 2013. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature*. **496**, 469–476.
74. Doria-Rose N.A., Schramm C.A., Gorman J., et al. 2014. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature*. **509**, 55–62.
75. Pejchal R., Walker L.M., Stanfield R.L., et al. 2010. Structure and function of broadly reactive antibody PG16 reveal an H3 subdomain that mediates potent neutralization of HIV-1. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 11483–11488.
76. McLellan J.S., Pancera M., Carrico C., et al. 2011. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. *Nature*. **480**, 336–343.
77. Pancera M., Shahzad-Ul-Hussan S., Doria-Rose N.A., et al. 2013. Structural basis for diverse N-glycan recog-

- nitition by HIV-1-neutralizing V1-V2-directed antibody PG16. *Nat. Struct. Mol. Biol.* **20**, 804–813.
78. Julien J.P., Lee J.H., Cupo A., et al. 2013. Asymmetric recognition of the HIV-1 trimer by broadly neutralizing antibody PG9. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 4351–4356.
  79. Kwong P.D., Wyatt R., Robinson J., et al. 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature*. **393**, 648–659.
  80. Vzorov A.N., Compans R.W. 2016. Cytoplasmic domain effects on exposure of co-receptor-binding sites of HIV-1 Env. *Arch. Virol.* **161**, 3011–3018.
  81. Vzorov A.N., Yang C., Compans R.W. 2015. An amphipathic sequence in the cytoplasmic tail of HIV-1 Env alters cell tropism and modulates viral receptor specificity. *Acta Virol.* **59**, 209–220.
  82. Vzorov A.N., Wang L., Chen J., et al. 2016. Effects of modification of the HIV-1 Env cytoplasmic tail on immunogenicity of VLP vaccines. *Virology*. **489**, 141–150.
  83. Vzorov A.N., Compans R.W. 2016. VLP vaccines and effects of HIV-1 Env protein modifications on their antigenic properties. *Mol. Biol. (Moscow)*. **50**, 353–361.
  84. Moore P.L., Crooks E.T., Porter L., et al. 2006. Nature of nonfunctional envelope proteins on the surface of human immunodeficiency virus type 1. *J. Virol.* **80**, 2515–2528.
  85. Parren P.W., Burton D.R., Sattentau Q.J. 1997. HIV-1 antibody: Debris or virion? *Nat. Med.* **3**, 366–367.
  86. Munro J.B., Gorman J., Ma X., et al. 2014. Conformational dynamics of single HIV-1 envelope trimers on the surface of native virions. *Science*. **346**, 759–763.
  87. Guttman M., Cupo A., Julien J.P., et al. 2015. Antibody potency relates to the ability to recognize the closed, pre-fusion form of HIV Env. *Nat. Commun.* **6**, 6144.
  88. Pugach P., Ozorowski G., Cupo A., et al. 2015. A native-like SOSIP.664 trimer based on an HIV-1 subtype B env gene. *J. Virol.* **89**, 3380–3395.
  89. Harris A.K., Bartesaghi A., Milne J.L., et al. 2013. HIV-1 envelope glycoprotein trimers display open quaternary conformation when bound to the gp41 membrane-proximal external-region-directed broadly neutralizing antibody Z13e1. *J. Virol.* **87**, 7191–7196.
  90. Sliepen K., Sanders R.W. 2016. HIV-1 envelope glycoprotein immunogens to induce broadly neutralizing antibodies. *Expert. Rev. Vaccines*. **15**, 349–365.
  91. Jardine J.G., Ota T., Sok D., et al. 2015. HIV-1 vaccines: Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. *Science*. **349**, 156–161.
  92. Binley J.M., Sanders R.W., Clas B., et al. 2000. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. *J. Virol.* **74**, 627–643.
  93. Sanders R.W., Vesanen M., Schuelke N., et al. 2002. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J. Virol.* **76**, 8875–8889.
  94. Khayat R., Lee J.H., Julien J.P., et al. 2013. Structural characterization of cleaved, soluble HIV-1 envelope glycoprotein trimers. *J. Virol.* **87**, 9865–9872.
  95. Klasse P.J., Depetris R.S., Pejchal R., et al. 2013. Influences on trimerization and aggregation of soluble, cleaved HIV-1 SOSIP envelope glycoprotein. *J. Virol.* **87**, 9873–9885.
  96. Crooks E.T., Tong T., Osawa K., et al. 2011. Enzyme digests eliminate nonfunctional Env from HIV-1 particle surfaces, leaving native Env trimers intact and viral infectivity unaffected. *J. Virol.* **85**, 5825–5839.
  97. Vzorov A.N., Gernert K.M., Compans R.W. 2005. Multiple domains of the SIV Env protein determine virus replication efficiency and neutralization sensitivity. *Virology*. **332**, 89–101.
  98. Vzorov A.N., Compans R.W. 2011. Effects of stabilization of the gp41 cytoplasmic domain on fusion activity and infectivity of SIVmac239. *AIDS Res. Hum. Retroviruses*. **27**, 1213–1222.
  99. Waning D.L., Russell C.J., Jardetzky T.S., et al. 2004. Activation of a paramyxovirus fusion protein is modulated by inside-out signaling from the cytoplasmic tail. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 9217–9222.
  100. Derdeyn C.A., Decker J.M., Bibollet-Ruche F., et al. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. *Science*. **303**, 2019–2022.
  101. Skountzou I., Quan F.S., Gangadhara S., et al. 2007. Incorporation of glycosylphosphatidylinositol-anchored granulocyte-macrophage colony-stimulating factor or CD40 ligand enhances immunogenicity of chimeric simian immunodeficiency virus-like particles. *J. Virol.* **81**, 1083–1094.
  102. Vassilieva E.V., Wang B.Z., Vzorov A.N., et al. 2011. Enhanced mucosal immune responses to HIV virus-like particles containing a membrane-anchored adjuvant. *MBio*. **2**, e00328-10.
  103. Kasturi S.P., Skountzou I., Albrecht R.A., et al. 2011. Programming the magnitude and persistence of antibody responses with innate immunity. *Nature*. **470**, 543–547.
  104. Kasturi S.P., Kozlowski P.A., Nakaya H.I., et al. 2017. Adjuvanting a simian immunodeficiency virus vaccine with Toll-like receptor ligands encapsulated in nanoparticles induces persistent antibody responses and enhanced protection in TRIM5- α restrictive macaques. *J. Virol.* **91** (4), e01844-16. doi 10.1128/JVI.01844-16
  105. Feng H., Zhang H., Deng J., et al. 2015. Incorporation of a GPI-anchored engineered cytokine as a molecular adjuvant enhances the immunogenicity of HIV VLPs. *Sci. Rep.* **5**, 11856.
  106. Bhiman J.N., Anthony C., Doria-Rose N.A., et al. 2015. Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. *Nat. Med.* **21**, 1332–1336.
  107. Xiao X., Chen W., Feng Y., et al. 2009. Maturation pathways of cross-reactive HIV-1 neutralizing antibodies. *Viruses*. **1**, 802–817.
  108. Xiao X., Chen W., Feng Y., et al. 2009. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: Implications for evasion of immune responses and

- design of vaccine immunogens. *Biochem. Biophys. Res. Commun.* **390**, 404–409.
109. McGuire A.T., Hoot S., Dreyer A.M., et al. 2013. Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. *J. Exp. Med.* **210**, 655–663.
110. Jardine J., Julien J.P., Menis S., et al. 2013. Rational HIV immunogen design to target specific germline B cell receptors. *Science*. **340**, 711–716.
111. Sliepen K., Medina-Ramirez M., Yasmeen A., et al. 2015. Binding of inferred germline precursors of broadly neutralizing HIV-1 antibodies to native-like envelope trimers. *Virology*. **486**, 116–120.
112. Dosenovic P., von Boehmer L., Escolano A., et al. 2015. Immunization for HIV-1 broadly neutralizing antibodies in human Ig knockin mice. *Cell*. **161**, 1505–1515.
113. Scharf L., West A.P., Sievers S.A., et al. 2016. Structural basis for germline antibody recognition of HIV-1 immunogens. *eLife*. **5**, e13783
114. McGuire A.T., Gray M.D., Dosenovic P., et al. 2016. Specifically modified Env immunogens activate B-cell precursors of broadly neutralizing HIV-1 antibodies in transgenic mice. *Nat. Commun.* **7**, 10618.
115. Medina-Ramirez M., Sanders R.W., Klasse P.J. 2014. Targeting B-cell germ lines and focusing affinity maturation: The next hurdles in HIV-1-vaccine development? *Expert Rev. Vaccines*. **13**, 449–452.
116. Hoot S., McGuire A.T., Cohen K.W., et al. 2013. Recombinant HIV envelope proteins fail to engage germline versions of anti-CD4bs bNAbs. *PLoS Pathog.* **9**, e1003106.
117. McGuire A.T., Glenn J.A., Lippy A., et al. 2014. Diverse recombinant HIV-1 Envs fail to activate B cells expressing the germline B cell receptors of the broadly neutralizing anti-HIV-1 antibodies PG9 and 447-52D. *J. Virol.* **88**, 2645–2657.
118. de Taeye S.W., Moore J.P., Sanders R.W. 2016. HIV-1 Envelope trimer design and immunization strategies to induce broadly neutralizing antibodies. *Trends Immunol.* **37**, 221–232.
119. Andrabi R., Voss J.E., Liang C.H., et al. 2015. Identification of common features in prototype broadly neutralizing antibodies to HIV envelope V2 apex to facilitate vaccine design. *Immunity*. **43**, 959–973.
120. McGuire A.T., Dreyer A.M., Carbonetti S., et al. 2014. HIV antibodies. Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies. *Science*. **346**, 1380–1383.
121. Cortez V., Wang B., Dingens A., et al. 2015. The broad neutralizing antibody responses after HIV-1 superinfection are not dominated by antibodies directed to epitopes common in single infection. *PLoS Pathog.* **11**, e1004973.
122. Lu S. 2009. Heterologous prime-boost vaccination. *Curr. Opin. Immunol.* **21**, 346–351.
123. Bolhassani A., Kardani K., Vahabpour R., et al. 2015. Prime/boost immunization with HIV-1 MPER-V3 fusion construct enhances humoral and cellular immune responses. *Immunol. Lett.* **168**, 366–373.
124. Brown S.A., Surman S.L., Sealy R., et al. 2010. Heterologous prime-boost HIV-1 vaccination regimens in pre-clinical and clinical trials. *Viruses*. **2**, 435–467.
125. Iyer S.S., Gangadhara S., Victor B., et al. 2016. Virus-like particles displaying trimeric simian immunodeficiency virus (SIV) envelope gp160 enhance the breadth of DNA/modified vaccinia virus Ankara SIV vaccine-induced antibody responses in rhesus macaques. *J. Virol.* **90**, 8842–8854.
126. Victora G.D., Mesin L. 2014. Clonal and cellular dynamics in germinal centers. *Curr. Opin. Immunol.* **28**, 90–96.
127. Rerks-Ngarm S., Pitisuttithum P., Nitayaphan S., et al. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N. Engl. J. Med.* **361**, 2209–2220.
128. Pitisuttithum P., Gilbert P., Gurwith M., et al. 2006. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J. Infect. Dis.* **194**, 1661–1671.
129. Haynes B.F., Gilbert P.B., McElrath M.J., et al. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N. Engl. J. Med.* **366**, 1275–1286.
130. Smith J.M., Amara R.R., McClure H.M., et al. 2004. Multiprotein HIV type 1 clade B DNA/MVA vaccine: Construction, safety, and immunogenicity in Macaques. *AIDS Res. Hum. Retroviruses*. **20**, 654–665.
131. Chamcha V., Kannanganat S., Gangadhara S., et al. 2016. Strong, but age-dependent, protection elicited by a deoxyribonucleic acid/modified vaccinia Ankara simian immunodeficiency virus vaccine. *Open Forum Infect. Dis.* **3**, ofw034.
132. Derdeyn C.A., Moore P.L., Morris L. 2014. Development of broadly neutralizing antibodies from autologous neutralizing antibody responses in HIV infection. *Curr. Opin. HIV AIDS*. **9**, 210–216.
133. Robinson H.L. 2013. Non-neutralizing antibodies in prevention of HIV infection. *Expert Opin. Biol. Ther.* **13**, 197–207.

Translated by T. Tkacheva