Original Article

Higher Genetic Diversity and Viral Evolution in Key Regions of the Envelope Gene Are Associated with Broader Neutralizing Antibody Responses: a Report of Two Chronic HIV Infected Cases

Shuxu Shen¹,²,⁴†, Wen Tian¹,²,³†, Yangtao Ji¹,²,³, Yang Gao¹,²,³, Min Zhang¹,²,³, Xiaoxu Han¹,²,³, and Hong Shang¹,²,³,*

¹NHC Key Laboratory of AIDS Immunology (China Medical University), Department of Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang; ²Key Laboratory of AIDS Immunology of Liaoning Province, The First Affiliated Hospital of China Medical University, Shenyang; ³Key Laboratory of AIDS Immunology, Chinese Academy of Medical Sciences, Shenyang; and ⁴Yingkou Central Hospital, Yingkou, China

SUMMARY: Dozens of broadly neutralizing antibodies (bnAbs) have been identified from chronically infected HIV-1 patients, but it is still unclear what determines the acquisition of broad neutralizing activities. Two chronic HIV-1 infected cases with similar autologous neutralizing activities were followed up for two years to study the viral evolution of the envelope gene and the neutralizing activity against autologous and heterologous viruses. The neutralization activities against homologous viruses gradually increased in both patients. HA172 eventually developed a cross-clade neutralizing antibodies response, with a neutralization breadth of 88.9% (8/9) against tier 2 heterologous HIV-1. However, HA084 could only neutralize 44.4% (4/9) of the same virus panel. Higher genetic diversity of the env gene at baseline (0.027 vs. 0.002, p < 0.001), stronger immune pressure on V3 (3.08 vs. 0.99, p < 0.001) or V4 loops (2.63 vs. 0.62, p = 0.002), increasing length of V1V2 and V4 loops, and evolution on V1V2 and CD4-binding sites (CD4bs) were identified in HA172. The findings demonstrated that higher viral genetic diversity, viral evolution on V1V2 and CD4bs might contribute to the development of bnAbs. The findings indicate the possibility of inducing better neutralizing antibodies in immunodeficient patients and may help develop an immune therapy strategy based on bnAbs.

INTRODUCTION

Induction of broadly neutralizing antibodies (bnAbs) are one of the most important goals of human immunodeficiency virus (HIV) vaccine research. However, eliciting bnAbs that target HIV-1 in human by active immunization remains a big challenge. Although dozens of broad and potent monoclonal neutralizing antibodies have been isolated from HIV-1 infected individuals in recent years(1-3), we still failed to induce antibodies with similar activities that can neutralize individuals in recent years(1-3), we still failed to induce antibodies with similar activities that can neutralize individuals in recent years(1-3), we still failed to induce antibodies with similar activities. Two chronic HIV-1 infected cases with similar autologous neutralizing activities were followed up for two years to study the viral evolution of the envelope gene and the neutralizing activity against autologous and heterologous viruses. The neutralization activities against homologous viruses gradually increased in both patients. HA172 eventually developed a cross-clade neutralizing antibodies response, with a neutralization breadth of 88.9% (8/9) against tier 2 heterologous HIV-1. However, HA084 could only neutralize 44.4% (4/9) of the same virus panel. Higher genetic diversity of the env gene at baseline (0.027 vs. 0.002, p < 0.001), stronger immune pressure on V3 (3.08 vs. 0.99, p < 0.001) or V4 loops (2.63 vs. 0.62, p = 0.002), increasing length of V1V2 and V4 loops, and evolution on V1V2 and CD4-binding sites (CD4bs) were identified in HA172. The findings demonstrated that higher viral genetic diversity, viral evolution on V1V2 and CD4bs might contribute to the development of bnAbs. The findings indicate the possibility of inducing better neutralizing antibodies in immunodeficient patients and may help develop an immune therapy strategy based on bnAbs.

Previous studies suggested that the determinants of bnAbs are rather complex, and that both viral and host factors are potentially involved(8, 9). Viral factors that include higher viral load, long-term antigenic stimulation, more complex genetic diversity, longer variable loops and the number of Potential N-linked glycosylation sites (PNGS) might contribute to the development of bnAbs(10-15). Host factors, including lower CD4+ cell counts and higher T follicular helper cell counts have been reported to benefit the development of bnAbs(16-18). However, the aforementioned factors are not always consistent and in some cases are controversial(19, 20).

Several recent studies focusing on viral-host “co-evolution” partially uncovered the developmental process of bnAbs(8, 21, 22). These studies were longitudinal analyses since the acute infection stage. It typically takes several years for bnAbs to develop and mature. In chronic HIV infection, HIV-1 has evolved to a complex quasispecies through several years of in vivo evolution, and some nAb responses have developed(21,22). In these conditions, what drives nAbs in the direction of a broader neutralizing activity is still unclear. With this goal in mind, two cases with similar baseline autologous neutralizing activities but distinct heterologous neutralizing activities 2 years later were selected in this study. A 2-year longitudinal study was carried out to determine the evolution of both HIV-1 and the heterologous neutralizing activities to elucidate the possible broader neutralizing activities associated...
with the viral factors. The findings indicate that higher genetic diversity of viral quasispecies and viral evolution on V1V2 and CD4bs might contribute to the development of bnAbs.

**MATERIALS AND METHODS**

**Study subjects:** HA172 and HA084 were chronic HIV-1 infected cases from a HIV infection natural history cohort in China. Both patients were male and both had been infected through unhygienic blood collection. Blood samples were collected annually in 2005 (T0), 2006 (T1), and 2007 (T2). Plasma was separated and stored at −80°C for viral evolution and neutralization assays. CD4+ T cell counts and HIV-1 viral load were determined from clinical records. Informed consent for blood donation and for the purpose of this study were obtained from both subjects. This study was approved by the Medical Research Ethics Committee of No.1 Hospital of China Medical University.

**Cloning of full-length envelope genes and sequence analysis:** Viral RNA extracted from the plasma of both patients was reverse transcribed into cDNA using the Superscript III Reverse Transcriptase kit (Invitrogen). Full-length envelope genes were amplified by nest PCR directly from cDNA as previously described(6). PCR products were purified using the Gel Extraction Kit (QIAGEN, Hilden, Germany) and cloned into pcDNA3.1 (Invitrogen, CA, USA) using the T4 DNA ligase kit (Invitrogen). Sequence fragments were assembled and edited using the Sequencher program (version 5.4) and aligned using BioEdit (version 5.0). Phylogenetic analyses were performed using the neighbor-joining method. The topology of the tree was tested by bootstrap analysis with 1000 replicates. The synonymous nonsynonymous analysis program (SNAP) was used to compare the average ratio of normalized nonsynonymous substitutions to synonymous substitutions (dN/dS) and was available at the HIV database website (https://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html).

**Neutralization assays:** HIV-1 neutralization testing was performed using a luciferase-based assay as previously described(6). The env-expression plasmid was co-transfected with the backbone construct pNL43R-E-luciferase into 293T cells using the transfection reagent Fugene 6 (Roche) to generate env-pseudotyped virus stocks. The lowest plasma dilution used in the assay was 1:20. Inhibitory dose 50 titer (ID50) was defined as the reciprocal of the plasma dilution that cause a 50% reduction in relative luciferase activity compared with controls after a single round of infection.

**Statistical analysis:** Statistical analysis and basic graphical delineation were carried out using GraphPad Prism 6 (GraphPad Software Inc.) based on a Mann–Whitney test or Wilcoxon test where appropriate. A p-value < 0.05 was considered significant.

**RESULTS**

**Clinical characteristics of the two cases:** HA172 and HA084 were diagnosed with HIV-1 subtype B’ (a variant of subtype B) infection in 1995 and 2000 respectively. During 2 years of observation, the CD4+ T cell counts of HA172 were much higher than HA084 (583 ± 38 cells/μl vs. 178 ± 10 cells/μl, p = 0.109), and the viral load of HA172 was lower than that of HA084 (3.61 ± 0.14 log_{10} copies/ml vs. 4.31 ± 0.30 log_{10} copies/ml, p = 0.109) (Fig. 1).

**Moderate and increasing autologous neutralizing activities:** Although the two subjects displayed different CD4+ T cell counts and viral loads at the T0 baseline of the observation period, both displayed neutralization activities against the homologous virus. To better understand the evolution of homologous neutralization activities, plasmas from each individual at T1 and T2 were used to neutralize the representative homologous virus with the highest titer at T0. The homologous neutralization activities of plasma from HA084 increased gradually throughout the study period, with an ID50 of 1:457 at T0, 1:781 at T1 and 1:1371 at T2. For HA172, ID50 also increased from 1:112 to 1:181. The ID50 of T2 for HA172 was not available because of the limited sample volume (Fig. 2A).

**Distinct heterologous neutralizing activities against pseudovirus panel:** To compare the heterologous activities of nAbs for HA172 and HA084, 9 tier 2 pseudoviruses of subtype B’, B’C, and CRF01_AE derived from HIV-1 strains epidemic in China were used(6). Plasma from HA172 at T2 neutralized 89% (8/9) of the panel with a geometric mean ID50 titer (GMT) of 1:80. However, the plasma from HA084 at T2 could only neutralize 44% (4/9) of the panel with a GMT of 1:48 (Fig. 2B).

Since both HA172 and HA084 were infected with

![Fig. 1. The CD4+ T cell counts and viral loads of HA172 and HA084 in the observation period. T0, T1 and T2 represent baseline, 1 year and 2 year post baseline.](image)
subtype B’ virus, their plasma preferentially neutralized subtype B’ pseudoviruses also, with a neutralization breadth of 100\% (3/3) and 67\% (2/3) respectively. The GMT for clade B’ pseudoviruses in HA172 was significantly higher than that in HA084 (1:131 ± 66 vs. 1:37 ± 51, \( p = 0.007 \)). For non-clade B’ pseudoviruses, HA172 neutralized 83\% (5/6) of the viruses with a lower GMT of 1:63. HA084 neutralized 33\% (2/6) of the viruses with a GMT of 1:54 (Fig. 2B).

Viral quasispecies and evolution analyses: To explore the viral determinants of the distinct heterologous nAb responses, the quasispecies constituents and evolutions of HIV-1 env gene at different time-points was compared between HA172 and HA084. Firstly, the genetic diversity among viral quasispecies from HA172 at baseline were higher than that from HA084 (0.028 ± 0.007 vs. 0.002 ± 0.001, \( p < 0.001 \)). In contrast, the genetic diversity among the viral quasispecies were similar between HA172 and HA084 at both T1 and T2 (0.005 ± 0.002 vs. 0.002 ± 0.001, \( p = 0.172 \) and 0.008 ± 0.001 vs. 0.006 ± 0.002, \( p = 0.309 \), respectively). Secondly, the relationship among viral quasispecies from each case was further supported by the results of phylogenetic tree analysis. The branches of the viral quasispecies in HA172 were longer and more dispersed than those in HA084 at baseline, and became concentrated in both HA172 and HA084 at T1 and T2 (Fig. 3).

Viral amino acid and immune selection pressure analyses: To further explore the relationship between viral evolution and immune selection, the dN/dS of the env gene was used to evaluate the immune pressure on the envelope protein from the nAbs. The dN/dS of the five high hypervariable regions in the envelope gene, including V1/V2, V3, V4 and V5, were calculated. The dN/dS of V3 and V4 from HA172 were significantly higher than those from HA084 (3.08 ± 0.00 vs. 0.990 ± 0.00, \( p < 0.001 \); 2.63 ± 0.00 vs. 0.62 ± 0.00, \( p = 0.002 \), respectively). This difference continued to T2 for HA172 and HA084 (2.12 ± 0.51 vs. 0.58 ± 0.00, \( p = 0.003 \)), demonstrating a strong positive selective pressure in HA172, but a negative selective pressure in HA084. At T2, the dN/dS in V4 remained significantly distinct between HA172 and HA084. In contrast, dN/dS of V1/V2 was lower in HA172 than in HA084 (2.12 ± 0.51 vs. 0.58 ± 0.00, \( p = 0.003 \)), demonstrating a strong positive selective pressure in HA172, but a negative selective pressure in HA084. At T2, the dN/dS in V4 remained significantly distinct between HA172 and HA084. In contrast, dN/dS of V1/V2 was lower in HA172 than in HA084 (0.50 ± 0.03 vs. 1.59 ± 0.24, \( p = 0.01 \)). (Table 1).

Except for the point mutations, HIV-1 usually escapes the immune selection pressure of nAbs through insertion, deletion, or glycosylation of the envelope protein. We further compared the length polymorphisms and the number of PNGS in the variable region of the
Determinants Associated with BnAbs

The length of V1V2 and V4 significantly increased for HA172. The length of V1V2 increased by 12.00 ± 1.10 amino acids at T0 to 65.00 ± 0.00 amino acids at T1 (p = 0.001). Similarly, the length of V4 increased by 24.00 ± 0.00 amino acids at T1 to 29.00 ± 0.00 amino acids at T2 (p = 0.003). In contrast to HA172, the length of the variable region decreased significantly only in V4 in HA084, with the number of amino acids dropping from 32.00 ± 0.00 at T0 to 8.62 ± 13.45 at T1 (p < 0.001) (Table 1). In addition, the number of PNGS in V1V2 increased by 2.86 ± 0.38 PNGS per sequence from T0 to T1 in HA172, but decreased by 3.77 ± 1.92 PNGS in V4 per sequence from T0 to T1 in HA084.

**Evolution of sites targeted by known bnAbs:** It is believed that the neutralization activities of some bnAbs against viruses depend on amino acids or the PNGS at some key sites of the envelop proteins(21, 23-27). In HA172 and HA084, the polymorphisms and evolution on amino acids sites targeted by V1V2-glycan and CD4bs bnAbs were different. Half of the viral quasispecies from HA172 carried the wild-type arginine residues on site 166 at T0, but mutated to asparagine residues at T1 and T2, implying positive selection pressure. In contrast, the arginine residues were present at site 166 in HA084 at all time points. Similarly, on site 234, which was the key target site for CD4bs binding targeted by bnAbs VRC01, continuous mutations occurred from wild-type asparagine residue at T0 to lysine (K) at T1 then to Serine (S) at T2 in HA172. In contrast, the wild-type asparagine residue was always fixed in all quasispecies in HA084. However, HA084 presented another viral evolution pattern in sites 160, 162 and 167. The PNGS in site 160 was absent in all the viral quasispecies at T0 in HA084, but emerged at later time-points. Additionally, the epitope-switch from one mutant-type at T0 to another mutant-type at later time-points was observed in site 162 and 167 in HA084. As

---

**Table 1. The virological characters of HA172 and HA084 at 3 follow-up time-points**

| Clones   | No. of clones | Genetic Diversity | dN/dS | No. of residues | No. of PNGS |
|----------|---------------|-------------------|-------|----------------|-------------|
|          |               |                   |       | V1V2 | V3 | V4 | V5 | V1V2 | V3 | V4 | V5 | V1V2 | V3 | V4 | V5 | gp41-ectodomain |
| HA172-T0 | 6             | 0.028             | ——    | 58   | 33 | 24.67 | 9.5 | 5     | 1     | 2.5 | 1.17 | 4 |
| HA172-T1 | 7             | 0.005             | 1.51  | 3.08 | 2.63 | 0.55 | 65 | 33 | 24 | 10 | 7.86 | 1 | 4 | 2 | 4 |
| HA172-T2 | 3             | 0.008             | 0.50  | NA   | 2.12 | 0.31 | 60 | 33 | 29 | 10 | 6 | 1 | 5 | 2 | 4 |
| HA084-T0 | 9             | 0.002             | ——    | 58 | 33 | 32 | 9 | 5 | 1 | 5 | 1 | 4 |
| HA084-T1 | 13            | 1.62              | 0.99  | 0.62 | NA | 57 | 33 | 8.62 | 9 | 5.92 | 1 | 1.23 | 1 | 4 |
| HA084-T2 | 7             | 1.59              | NA    | 0.58 | 0.72 | 60 | 32 | 29 | 10.29 | 6 | 1 | 6 | 1 | 4 |

All data in this table are displayed with mean values. dN/dS, the ratio of substitution at nonsynonymous and synonymous sites; NA, not applicable.

1) Genetic Diversity was calculated as the genetic distance among viral quasispecies at each time point within individual. The unit of diversity is “mutations/bp.”

---

![Fig. 3. Phylogenetic analyses of full length env gene for HA172 and HA084. The analyses were performed using the neighbor-joining method using MEGA version 6.0. All viral quasispecies derived from T0 (black circle), T1 (black triangle), T2 (black diamond) are shown. Sequence labels include time-points of sampling and sequence number. Clone with asterisk was selected as autologous virus in neutralization assays.](image-url)
Table 2. The evolution of epitopes commonly targeting by bnAbs among the clones of HA172 and HA084

| Clones   | V1V2-Glycan | V3-Glycan | CDabs | MPER |
|----------|-------------|-----------|-------|------|
|          | PG9/PG16    | VRC26     | 2G12  | PGT135 |
|          | 156 158 159 | 160 162 167 173 | 295 332 339 | 301 332 459 |
|          | N S F N S K Y | N S R G K V | N N N N | G G E N S G |
|          |              |           |        |       |
| HA172-T0 | - - + T - | + T - - S T | - + - - + | - - - - - |
| HA172-T1 | - - + T - | + T N T T | - + - - + | - - - - K |
| HA172-T2 | - - + T - | + T N T T | - + - - + | - - - - S |
| HA084-T0 | - - + T - | + T N T T | - + - - + | - - - - A |
| HA084-T1 | - - + T - | + T K T T | - + - - + | - - - - A |
| HA084-T2 | - - + T - | + T K T T | - + - - + | - - - - A |

Below the bold "N" , "+" indicates the presence of glycosylation site, and "-" indicates the absence of glycosylation site. Others, "-" indicates the amino-acid residue at this site was the same as the residue of the epitope. Letters indicate that the amino-acid residue at this site was mutated to the residue represented by the letter.

far as the targeting sites of other bnAbs, such as V3-Glycan targeted by 2G12 and PGT135, and MPER targeted by 10E8, both cases presented wild-type amino acid residues all the time (Table 2).

**DISCUSSION**

**High viral diversity benefits development of bnAbs:** Both HA172 and HA084 experienced chronic HIV-1 infection and suffered some degree of immune injury. The CD4 T cell count of HA084 decreased to <200 copies/ml. However, both cases exhibited moderate neutralization activities against a contemporaneous autologous virus at baseline, which increased gradually over time, suggesting that relatively normal humoral immune responses were present in both cases. Interestingly, only the plasma of HA172 eventually developed potent neutralizing activities against a wide range of heterologous viruses of the same and different clades. We focused on the viral factors that drove the development of better neutralizing antibody responses and found that viral quasispecies of HA172 had more than 10 times higher genetic diversity than HA084 at baseline. Higher viral diversity increases the development of a broad neutralization activity during acute or early HIV infection(12-14). Although higher genetic diversity was only observed at one of the three time-points, we speculate that viruses with higher genetic diversity might provide more stimulations to B cell and induce stronger nAbs responses later in the infection. On the other hand, broad and strong nAbs responses might act as a strong immune selection pressure and help select the predominant quasispecies with better fitness. In this case, the escaped virus with lower sensitivity to auto-nAbs responses may survive as dominant virus, since the genetic diversity subsequently decreases. The data revealed that the interplay between virus and nAbs responses in HA172 were completely different from the responses in HA084, whose genetic diversity remained steady at all the three time-points. Unlike previous studies that focused on viral diversity at early stages of infection, HA172 and HA084 were diagnosed 5 to 10 years before the baseline in this study. The infecting virus had experienced extensive evolution in vivo. So, the findings demonstrated that even in advanced stages of HIV infection, the higher genetic diversity of the viral quasispecies might benefit the development of bnAbs.

**Greater immune pressure drives viral evolution and might help the exposure of neutralizing epitopes:** HA172 was characterized by a higher genetic diversity of viral quasispecies at baseline, which quickly evolved to highly homologous viral quasispecies at T1 and T2, suggesting a strong immune pressure on the envelope protein. This suggestion was further supported by dN/ dS results of the variable regions in the env gene. The dN/dS of the V3 or V4 on viral quasispecies at T1 and V4 on viral quasispecies at T2 in HA172 were all much higher than 1, implying that the above regions experienced obvious immune pressures.

Recent studies on virus-antibody co-evolution have demonstrated that virus escape under contemporaneous immune pressure might change the structure or the physicochemical properties of the envelope protein and therefore create or expose new bnAbs epitopes, which could and contribute to the maturation of nAbs(10, 21, 22). The stronger immune pressure experienced by HA172 might have driven the infecting virus to develop or expose key epitopes that might be helpful for the development of bnAbs.

**Ever-changing epitopes promote the development of bnAbs:** Another remarkable difference between HA172 and HA084 was the mutation of amino acid residues targeted by bnAbs. Sites 166 and 234 of the envelop protein were targeted by V1V2-glycan and CD4bs dependent bnAbs respectively. In HA172, the amino acids at site 166 and site 234 of the envelope protein mutated from wild type to mutant type, implying an ongoing immune selection. The amino acid residue at site 166 has been reported as the key contact site for VRC26 neutralization, and R166A mutation significantly decreased the viral sensitivity to VRC26(9, 25). The phenotype of R166N mutation in HA172 is still unclear. However, we can speculate that the mutation from an alkaline amino acid R to a neutral amino acid N might impact the binding of V1V2-glycan dependent antibodies to HIV-1 and mediate immune escape. Similar to site 166, N234K in HA172, a mutation from an alkaline amino acid R to a neutral amino acid N might also be driven by CD4bs dependent bnAbs and mediate immune escape. However, the mutant type at sites 160, 162 and 167 in HA084 at T0 suggested immune escape, and reverse or new mutations detected at subsequent time-points were less likely to be associated with
the selection of bnAbs. The collective results partly explain the considerable differences in the heterologous neutralizing responses between individuals HA172 and HA084.

This study has several limitations. The findings were from two B’ HIV-1 chronically infected cases. And some factors other than viral factors, such as the length of infection time and host genetic factors, might also have influenced the development of nAbs. Second, the heterologous neutralizing activity at baseline was not tested because of sample limitations. Thus, we cannot rule out the possibility of a baseline difference. Furthermore, we selected only one representative T0 virus, rather than more genetically-diverged viruses, as the autologous virus to be neutralized by the plasma collected at different time-points from these two individuals. Third, we did not evaluate if non-nAbs played a role in the viral evolution. The present findings must be validated with more studies.

In summary, in two chronic HIV infected cases, high viral diversity, and viral evolution on V1V2 and CD4bs might have aided the development of broader nAbs. The study findings support the potential of inducing better bnAbs in chronic HIV infected patients and may help to develop immune therapy strategy based on bnAbs.

Acknowledgments This work was supported in part by mega-projects of national science research for the 13th Five-Year Plan (2017ZX10011030), the Innovation Team Development Program of the Ministry of Education (IRT_16R70), and the Natural Science Foundation (81371787).

Conflict of interest None to declare.

REFERENCES

1. Derdeyn CA, Moore PL, Morris L. Development of broadly neutralizing antibodies from autologous neutralizing antibody responses in HIV infection. Curr Opin HIV AIDS. 2014;9:210-6.
2. West AP Jr., Scharf L, Scheid JF, et al. Structural insights on the role of antibodies in HIV-1 vaccine and therapy. Cell. 2014;156:633-48.
3. Klein F, Mouquet H, Dosenovic P, et al. Antibodies in HIV-1 vaccine development and therapy. Science. 2013;341(6151):1199-204.
4. Hemelaar J, Gouws E, Ghys PD, et al. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. AIDS. 2006;20:W13-23.
5. Taylor BS, Sobieszczyn ME, McCutchan FE, et al. The challenge of HIV-1 subtype diversity.
6. Shang H, Han X, Shi X, et al. Genetic and neutralization sensitivity of diverse HIV-1 env clones from chronically infected patients in China. J Biol Chem. 2011;286:14531-41.
7. Karlsson Hedestam GB, Fouchier RA, Phogat S, et al. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. Nature Rev Microbiol. 2008;6:143-55.
8. Liao HX, Lynch R, Zhou T, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 2013;496:469-76.
9. Doria-Rose NA, Schramm CA, Gorman J, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies.
10. Moore PL, Williamson C, Morris L. Virological features associated with the development of broadly neutralizing antibodies to HIV-1. Trends Microbiol. 2015;23:204-11.
11. van Gils MJ, Sanders RW. Broadly neutralizing antibodies against HIV-1: templates for vaccine. Virology. 2016;485:45-56.
12. Sather DN, Armann J, Ching LK, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol. 2009;83:757-69.
13. Piantadosi A, Panteleeff D, Blish CA, et al. Breadth of neutralizing antibody response to human immunodeficiency virus type 1 is affected by factors early in infection but does not influence disease progression. J Virol. 2009;83:10269-74.
14. Gray ES, Madiga MC, Hermanus T, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD8+ T cell decline and high viral load during acute infection. J Virol. 2011;85:4828-40.
15. Hraber P, Korber BT, Lapedes AS, et al. Impact of clade, geography, and age of the epidemic on HIV-1 neutralization by antibodies. J Virol. 2014;88:12623-43.
16. Llocre M, Havenaar-Daughton C, Landais E, et al. Human circulating PD-1+ CXCR3–CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. Immunity. 2013;39:758-69.
17. Cohen K, Alfheid M, Alter G, et al. Early preservation of CXCR5+ PD-1+ helper T cells and B cell activation predict the breadth of neutralizing antibody responses in chronic HIV-1 infection. J Virol. 2014;88:13310-21.
18. Simek MD, Rida W, Priddy FH, et al. 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. 2009;83:7337-48.
19. Powell RL, Kinge T, Nyambi PN. Infection by discordant strains of HIV-1 markedly enhances the neutralizing antibody response against heterologous virus. J Virol. 2010;84:9415-26.
20. Gao Y, Tian W, Han X, et al. Immunological and virological characteristics of human immunodeficiency virus type 1 superinfection: implications in vaccine design. Front Med. 2017;11:480-9.
21. Moore PL, Gray ES, Wibmer CK, et al. Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. Nature Med. 2012;18:1688-92.
22. Wibmer CK, Bluman JN, Gray ES, et al. Viral escape from HIV-1 neutralizing antibodies drives increased plasma neutralization breadth through sequential recognition of multiple epitopes and immunotypes. PLoS Pathog. 2013;9:e1003738.
23. Zhou T, Georgiev I, Wu X, et al. Structural basis for broad and potent neutralizing HIV-1 by antibody VRC01. Science. 2010;329:811-7.
24. Julien JP, Sok D, Khayat R, et al. Broadly neutralizing antibody PGT121 allosterically modulates CD4 binding via recognition of the HIV-1 gp120 V3 base and multiple surrounding glycans. PLoS Pathog. 2013;9:e1003342.
25. Moore PL, Gray ES, Sheward D, et al. Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. J Virol. 2011;85:3128-41.
26. Chen D, He X, Ye J, et al. Genetic and phenotypic analysis of CRF01_AE HIV-1 env clones from patients residing in Beijing, China. AIDS Res Hum Retroviruses. 2016;32:1113-24.
27. Boonchawalit S, Harada S, Shirai N, et al. Impact of the maraviroc-resistant mutation M434I in the C4 region of HIV-1 gp120 on sensitivity to antibody-mediated neutralization. Jpn J Infect Dis. 2016;69:236-43.

Nature. 2014;509:55-62.
10. Moore PL, Williamson C, Morris L. Virological features associated with the development of broadly neutralizing antibodies to HIV-1. Trends Microbiol. 2015;23:204-11.