Published in final edited form as:

Hum Antibodies. 2012 ; 21(0): 65–73. doi:10.3233/HAB-130264.

Clonal analysis of human anti-V3 monoclonal antibodies selected by a V3 tetramer

Liuzhe Li^a, Xiao-Hong Wang^b, Sagarika Banerjee^a, Barbara Volsky^a, Constance Williams^a, M. Anthony Moody^c, Susan Zolla-Pazner^{a,b}, and Miroslaw K. Gorny^{a,*}

^aDepartment of Pathology, New York University School of Medicine, New York, NY, USA

^bVeterans Affairs New York Harbor Healthcare System, New York, NY, USA

^cDuke Human Vaccine Institute, Duke University Medical Center, Durham, NC, USA

Abstract

The production of human monoclonal antibodies (mAbs) has been improved recently using the single B cell and PCR technology. A number of new anti-HIV-1 mAbs directed to various epitopes were produced by selecting single B cells from HIV positive individuals using the HIV-1 envelope (Env) proteins, and we tested whether the peptide can select B cells specific to a particular Env epitope. Using the fluorescently-labeled peptide tetramer representative of the V3 loop of HIV-1 Env gp120 for staining the B cells derived from one HIV-1 infected donor, four clonal human mAbs were produced with specificity to the V3 region. The clonality of the four V3 mAbs was based on the usage of the same immunoglobulin genes and almost identical sequence of CDRs. The amino acid changes were present only in the framework and, possibly, they could be related to the differences observed in the relative affinity binding of these four mAbs to V3 antigen. One representative V3 mAb displayed very potent neutralizing activity to one of two viruses tested. This study shows the feasibility of utilizing a peptide tetramer to select epitope-specific B cells and produce mAbs.

Keywords

HIV-1; V3 region; immunoglobulin gene usage; human monoclonal antibodies; HIV neutralizing antibodies

1. Introduction

The production of antigen-specific human monoclonal antibodies (mAbs) improved recently when the new single B cell PCR technology was introduced into practice [1,2]. Particularly, the research on anti-HIV-1 envelope (Env) antibodies generated from the B cells of infected individuals benefited from the new technology and, in a few years, hundreds of such mAbs were produced [3–5].

One of the new approaches included the identification of B cells by high-throughput screening of activated memory B cell supernatants using a neutralization assay, which resulted in the production of broadly cross-neutralizing mAbs such as PG9 and PG16 [6]. Screening by the neutralization assay already proved very efficient in the generation of the

highly potent, though narrow, neutralizing mAb 2909 [7]. The use of fluorescently-labeled Env gp140 trimerized constructs to isolate Env-reactive B cells was proved to be a powerful tool for isolating antigen-specific B cells [3,8,9]. We have used green fluorescein protein-labeled virus-like particles (VLPs) to select single B cells and produce Env-specific mAbs [10]. These methods are directed at a wide range of epitopes which could necessitate the screening of many mAbs to identify those directed against particular epitopes.

The alternative method is to use an antigen, for example, peptides or mimotopes of linear or conformation epitopes, respectively, and select the epitopespecific B cells for production of mAbs with desired specificity. This approach was described by Newman, et al. who prepared B cell tetramers using a biotinylated peptide, known to inhibit a pathogenic ds-DNA antibody, which bound to four-valent streptavidine [11]. Using this reagent, the group did not produce mAbs, but was able to demonstrate a population of B cells induced by immunization, characterize them by flow cytometry, and demonstrate enrichment of these cells using an ELISPOT assay. Based on this approach one anti-gp41 HIV-1 human mAb was produced from single B cells using for selection a tetramer representing the sequence of the envelope gp41 distal membrane proximal external region (MPER) [12].

Our work, described in this paper, builds upon this technique and we tested the fluorescently-labeled tetramer of the HIV-1 gp120 V3 loop for selection of antigen-specific B cells. The utility of the V3 tetramer in identifying and sorting V3-specific B cells from the blood of HIV-1 infected patient and production of anti-V3 mAbs is demonstrated.

2. Materials and methods

2.1. Antibodies, cell lines and antigens

Monoclonal anti-V3 Abs, F39F [13], 447-52D [14], 2424 [15] and anti-gp41 Ab 167-7 [16] were produced from B cells of HIV-1 infected individuals. Human anti-parvovirus B19 mAb 1418 [17] was used as a negative control. All cell lines producing mAbs were grown in RPMI 1640 medium supplemented in 15% fetal bovine serum and mAbs were purified using Protein A or G columns.

Recombinant gp120_{MN} and gp41_{MN} were purchased from Immunodiagnostics, Inc. (Woburn, MA). The V3_{JRCSF}-cholera toxin B (V3_B-CTB) fusion protein was produced as described [18] and kindly provided by Dr. Xiangpeng Kong (Dept. of Biochemistry, NYU School of Medicine, New York, NY). Seven 20- and 23-mer V3 peptides that span the tip of the V3 loop representing the sequence of WJ52, MN, JRCSF, BZ167, CM237, SF162 and RF were synthesized commercially; according to the manufacturers' information the peptides were analyzed by HPLC.

The two 33-mer peptides, one with a clade B V3 consensus03 (V3_{con03}), sequence (TRPNNNTRKSIHIGPGRAFYTTEIIGDIRQAH) and a second peptide with a scrambled V3 sequence (ITIDNIGHHNRTFRAANTRPISGQRGEYPGKT) were synthesized (PrimmBiotech, Inc., Cambridge, MA). The two peptides were biotinylated at the N-terminus using a short linker (GGG) and purified by reverse phase HPLC; the peptides were assessed for purity by HPLC (> 95% pure). The V3 consensus03 sequence is identical to the August 2004 clade B consensus sequence available from the Los Alamos National Laboratory HIV Sequence Database (<http://www.hiv.lanl.gov/content/>). The V3 sequence corresponds to gp120₃₀₆₋₃₃₈ and numbered relative to HIV-1_{HXB2CG}.

2.2. Production of B cell tetramers

The tetramer consists of four biotinylated peptides which are bound to one molecule of fluorochrome-labeled streptavidine, due to its valency of four. B cell tetramers were

produced as described [12,19]. Briefly, both biotinylated peptides, V3_{con03} and its scrambled version, were brought to 200 μ M in phosphate-buffered saline (PBS), mixed 1:1 with allophycocyanin (APC)-labeled streptavidin at 6.1 μ M, and incubated (4°C overnight). Final tetramer concentrations were determined using UV-Vis absorbance measurements on a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE).

2.3. Human PBMC flow cytometry analysis and single cell sorting

The subject donating a blood sample was recruited under IRB-approved protocols at the NYU School of Medicine. The subject was classified as a Long Term Non-Progressor (LTNP); during 18 years of follow-up his CD4⁺ T cell counts changed minimally (774 to 561 cells/ μ L); however, at the time of his recruitment and peripheral blood draw, his viral load had increased to ~10,000 RNA copies/mL and he was scheduled to start anti-retroviral treatment.

PBMCs were isolated from heparinized blood by gradient centrifugation and B cells were enriched using anti-CD19 magnetic beads (Miltenyi Biotec, Auburn, CA). The B cells were stained on ice for 30 min with 1 μ g/10⁶ cells of APC-labeled V3_{con03} tetramer, washed and stained with anti-human IgG (Fc)-Phycoerythrin (PE) and anti-CD27-FITC. During single cell sorting, the first gate is set to select for viable cells using forward and side scatter. The second gate was set to select for those viable cells that bind to APC-labeled V3-tetramer and anti-CD27-FITC. The V3 tetramer⁺ and CD27⁺ memory B cells were next gated for anti-IgG-PE⁺ cells to select for IgG producing memory B cells that bind to the V3 tetramer. Single cell sorting was performed using the FACSARIA in the NYU Langone Medical Center Cytometry Core at Bellevue Hospital, New York, NY. The single IgG memory B cells that bind specifically to the V3 tetramer were sorted into 96-well PCR plates (Eppendorf) containing ice-cold PBS, DTT, and RNAsin (Promega). Plates were spun and then frozen on dry ice and stored at -80°C.

2.4. Isolation of immunoglobulin genes from sorted single cells and immunoglobulin production

The V_H and V_L genes were amplified by RT-PCR as we recently described [10] according to the method by Tiller et al. [1]. Briefly, total RNA from single cells was reverse transcribed in the original 96-well sorting plate (Qiagen, Valencia, CA) using a random hexamer primer (Invitrogen, Carlsbad, CA), dNTP mix (Invitrogen, Carlsbad, CA), DTT (Invitrogen, Carlsbad, CA), Igepal CA-630 (Sigma, St. Louis, MO), RNAsin (Promega Corporation, Madison, WI), Prime RNase Inhibitor (Eppendorf, Hauppauge, NY) and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) as described [1]. Variable gene transcripts, IgH, Ig κ and Ig λ , were amplified by nested PCR using the primers of Tiller et al. [1] and sequenced with the respective reverse primer [1]. Sequence data were analyzed using Pregap4, BioEdit software and the International Im-MunoGene Tics (IMGT) information system. A phylogenetic tree was created using IMGT/Phylogene.

Purified PCR products were digested with the respective restriction enzymes AgeI, SalI, BsiWI, and XhoI (NEB), ligated into expression vectors and transformed into Competent *E.coli* DH10B bacteria (Invitrogen). Ampicillin resistant colonies were selected and screened by PCR for inserts of the expected size (650 bp for Ig γ , 700 bp for Ig κ and 590 bp for Ig λ) and then sequenced to confirm the identity with the original PCR amplicons. Plasmid DNA was isolated from bacterial cultures grown in Terrific Broth (Invitrogen, Carlsbad, CA) containing ampicillin using QIAprep Spin columns (Qiagen, Valencia, CA). Co-transfection of Ig vectors into 293T cells was performed using FuGENE HD (Roche, Indianapolis, IN) with equal amounts of IgH and corresponding IgL chain expression vector DNA. The cells were washed and then cultured for 4 days in DMEM supplemented with

10% FBS. Culture supernatants were harvested and IgG Abs purified by Protein A chromatography using Hi-Trap IgG columns (GE Healthcare, Piscataway, NJ).

2.5. Analysis of expressed antibodies

IgG concentrations in culture supernatants and in purified mAbs were determined by quantitative ELISA as described [14]. Reactivity of mAbs to recombinant proteins and V3 peptides was determined by standard ELISA [14] and compared to control mAbs to confirm the specificity of the assays.

2.6. Neutralization assay

Neutralization of two HIV-1 Env-pseudotyped viruses (psVs), SF162 and JRFL, by human mAbs was performed in our laboratory using the TZM-bl cell assay according to the described method [20]. Briefly, two psVs were produced by 293 cells co-transfected with plasmids containing the genes coding for envelope (Env) proteins and with an env-deleted backbone plasmid pNL4-3.Luc.R⁻E⁻, supplied by the NIH AIDS Research and Reference Reagent Program. The psVs-containing supernatants were tested for infectivity of the target TZM-bl cells and titrated to determine the input of psV for the assay. Monoclonal Abs were tested with three 10-fold dilutions, 50.0, 5.0 and 0.5 μ g/ml. Each mAb dilution was pre-incubated with the psV for an hour at 37°C and the mAb/psV mixture was then added on pre-plated TZM-bl cells expressing CD4, CXCR4 and CCR5 and incubated at 37°C for 48 h. The cell lysates were used to determine psVs infectivity by measuring tat-induced beta-galactosidase expression by TZM-bl cells. The reduction of psV infectivity due to neutralization by mAbs was expressed as percent neutralization by comparing the relative light units of beta-galactosidase activity in the presence and absence of mAbs [20,21].

3. Results

3.1. Tetramer binding specificity

The V3_{con03} tetramer and the scrambled peptide were assayed for specificity by surface plasmon resonance for interaction with captured anti-V3 mAb F39F according to the described method [22]. The V3_{con03} tetramer bound only to V3 mAb F39F and no binding was seen for the scrambled epitope tetramer; the data were kindly provided by Dr. Munir Alam, Duke University Medical Center, Durham, NC.

3.2. Selection of B cells from an HIV-1 infected subject using single-color tetramer staining

Enriched B cells from a chronically HIV-1 infected subject were stained with APC-labeled V3_{con03} tetramer and cells were sorted using the gating strategy shown in Fig. 1, sequentially gating on viable cells as defined by forward and side scatter (Fig. 1A), then on CD27⁺ and tetramer⁺ cells (Fig. 1B), and finally on surface-IgG⁺ and tetramer⁺ memory B cells (Fig. 1C). Of 10,000 cells selected in the first gate, 36 were present in the final gate (0.36%).

Single B cells were sorted into 96-well plates and the expressed immunoglobulin genes were isolated by reverse transcription and PCR. Twenty cognate pairs of the heavy and light chain genes were selected and expressed by co-transfection in 293T cells. The antibodies were secreted into culture supernatants and purified using Protein A columns.

3.3. Specificity of mAbs

The specificities of the mAbs were tested by ELISA against gp120_{MN}, gp41_{MN}, V3_{JRCSF}-CTB fusion protein and bovine serum albumin (BSA). Four of these 20 expressed mAbs were reactive with gp120_{MN} and V3_{JRCSF}-CTB but not to gp41_{MN} and BSA, confirming

their specificity to the V3 region (Table 1). None of the remaining 16 mAbs were reactive with any of HIV Env expressing antigens and their specificities were not determined (Table 1).

3.4. Immunoglobulin gene usage, mutations and phylogenetic trees

The four anti-V3 mAbs used the same IGHV1-69*01 and IGKV1-5*03 gene segments according to analysis using the IMGT system (Table 2). The CDR3 of the heavy (CDR H3) and light chains (CDR L3) are 17 and 8 AA long, respectively, and have the same AA sequence which confirm their origin from one clone of B cells producing anti-V3 antibodies (Table 2).

Alignment of the nucleotide sequences for the variable fragment of the V3 mAbs with the corresponding germline sequences for IGHV1-69*01 and IGLV1-5*03 (without CDR H3) revealed the comparable percent of mutations in the range between 8.33% to 9.32% for both heavy and light chain genes (Table 2). For three V3 mAbs, 1a13, 1a61 and 1d11, the percent of mutations was slightly higher in the light chain compared to heavy chain genes (Table 2).

These four clonal V3 mAbs do not have identical nucleotide (GenBank accession numbers: HM222540-HM222547) and AA sequences (Fig. 2). The AA changes are observed both in the frameworks (FR 1-3) and in CDRs (CDR 1 and 2); there are 16-17 and 13-15 changes in AA for the heavy and light chains, respectively (Fig. 1). Some AA changes are in the same position for all four V3 mAbs (11 and 9 common AA changes in the heavy and light chains, respectively) and at several positions the changes are individual for each V3 mAb (10 and 12 positions have individual AA changes in the heavy and light chains, respectively) (Fig. 2).

Phylogenetic trees for heavy and light chain nucleotide sequences show the relationships among the nucleotide sequences of the four variable fragments (without CDR H3) of the anti-V3 mAbs compared to the corresponding germline sequence. For the heavy and light chain tree, mAb 1a13 appears in the closest distance to IGHV1-69*01 germline sequence but more distant to the remaining three mAbs (Fig. 3). Distances shown in each tree are relative and the equivalent distances between trees do not imply identical phylogenetic distance for the two chains. However, for two mAbs, 1d11 and 1a61, their heavy chain sequences are in different distances than light chain sequences to the corresponding germline sequences, in contrast to the other two clonal mAbs, 1a13 and 1a6, which have the same distance for both Ig chains (Fig. 3).

3.5. Binding activity of four clonal V3 mAbs

The binding studies revealed the substantial difference between four clonal V3 mAbs in their relative affinities. Titration of the V3 mAbs for binding to V3_{JRCSF} peptide allowed the measurement of the amount of mAbs ($\mu\text{g/ml}$) needed for 50% maximal binding. The experiment showed that three mAbs, 1a13, 1d11 and 1a61, had close relative affinities, 0.07, 0.09 and 0.18 $\mu\text{g/ml}$, respectively, and comparable to the value of the positive control and the well-characterized human V3 mAb 447-52D (Fig. 4). The fourth mAb, 1a6, did not reach the saturation level required for determination of maximal binding and its relative affinity was significantly lower compared to other tested V3 mAbs (Fig. 4).

Monoclonal antibody 1d11 was selected for further binding studies using seven V3 peptides representing clade B sequences. The reactivity of 1d11 appeared to be limited to only three V3 peptides, WJ52, JRCSF and MN, in contrast to control V3 mAb 447-52D which bound by ELISA to all tested V3 peptides (Table 3).

3.6. Neutralization

The same mAb 1d11 was tested in the TZM-bl cell neutralization assay against two psVs, the Tier 1 (sensitive to neutralization) SF162 and Tier 2 (resistant to neutralization) JRFL, and compared to control mAbs (Fig. 5). It neutralized only psV SF162 with high potency comparable to the activity of V3 mAb 447-52D while it did not neutralize (did not reach 50% neutralization) the psV JRFL. In contrast, psV JRFL was neutralized by 447-52D which reached over 50% neutralization at 50 μ g/ml while another V3 mAb 2424, particularly potent against this psV, reached 50% neutralization at 5.0 μ g/ml (Fig. 5). Negative control mAb 1418 did not neutralize any of the psVs. These experiments confirmed that the V3 mAb 1d11 displays limited cross-reactivity and cross-neutralization with high neutralizing potency against Tier 1 psV SF162.

4. Discussion

In this paper we describe the production of four clonal human anti-V3 mAbs from single IgG⁺ memory B cells selected by a V3 tetramer carrying four linear sequences. The study shows the utility of this reagent to identify and sort V3 specific CD19⁺ B cells from an HIV-1 infected subject, and shows that transfected 293T cells, expressing the recovered IGHV and IGLV genes, produce antibodies that bind specifically to the V3 region of HIV-1 Env proteins.

Along with the four V3 mAbs, we also produced 16 mAbs which did not react with the HIV-1 Env proteins, gp120 and gp41, and their specificity was not determined but could be directed against any antigens known to humans. Thus, the efficiency of specific mAb production was low at 20%, which may depend on many factors, including the non-specific or polyspecific serum antibodies which are present at any stage of HIV-1 infection and which have tendency to increase during the course of infection as we previously reported [23]. The efficiency probably also depends on the particular donor of the B cells; this hypothesis is supported by our other studies, where we produced 10 of 34 (29%) Env-specific mAbs from the single B cells from one infected subject using the GFP-labeled JRFL-VLPs [10]. Other studies showed approximately 45% and 54% efficiency in two different patients using fluorochrome-labeled gp140 [3] or 57% efficiency using double-labeled gp120 for staining the B cells and production of Env-specific mAbs [5].

This technique using the B cell tetramer could be applied to any linear epitope recognized by an anti-pathogen response. However, it cannot be excluded that the nature of the bait may also play a role in the efficiency of the technique and the constrained antigen, in contrast to a linear peptide, can bind more specifically to the B cell receptor. It is probably inevitable that some non-specific mAbs from the single B cells of HIV-1 infected subjects will be produced unless the B cells are pre-adsorbed with an irrelevant antigen.

As the four anti-V3 mAbs are derived from one clone of B cells based on the usage of the same IGHV1-69*01 and IGLV1-5*03 genes and the identity of AA sequence of CDR H3 and L3 are the same, one would expect that they would also display the same binding activity to the V3 antigen. It appeared that one of four mAbs did not bind well to the V3 antigen (did not reach the saturation level) and its relative affinity was approximated by extrapolation. The percent of mutations in the variable fragment of the heavy and light chain genes (without CDR H3) was very similar (in the range of 8.33% to 9.32% for the nucleotide sequence). The percent of mutations in the heavy chain genes is comparable to the mean of 8.4% for the heavy chain genes of 48 other human V3 mAbs (Gorny et al., unpublished data) and indicate that these mAbs are affinity matured and this is supported by the fact that these mAbs originated from memory B cells.

The AA sequence of the CDRs H1, H2, L1 and L2 domains, which are involved in the interaction with the epitope, for all four V3 mAbs was very similar. There were some differences, however, in the position of single AA changes in the FR 1–3 of the heavy and light chains. As the FRs are not involved in the antigen binding activity they may influence the shape of the CDR domains and indirectly determine, probably minimally, the antibody affinity.

In summary, we have designed a V3 tetramer for exploring the possibility of staining the single B cells from HIV-1 infected individuals to produce V3-specific mAbs. Although the efficiency was relatively low, which was probably related to the particular donor of the B cells, four clonal anti-V3 mAbs were generated from the V3 tetramer stained and sorted B cells.

Acknowledgments

This study was supported in part by NIH grants AI091543, AI096977 (MKG), AI067854 (B.F. Haynes), HL59725 (SZP) and the Department of Veterans Affairs (SZP). The authors thank Drs. Barton F. Haynes and Munir Alam of the Duke Human Vaccine Institute, Duke University Medical Center, Durham, NC for providing the B cell tetramers and for studies using the surface plasmon resonance, respectively, and Dr. Xiangpeng Kong of the Department of Biochemistry, NYU School of Medicine, New York, NY, for providing the reagent V3 β -CTB.

References

1. Tiller T, Meffre E, Yurasov S, Tsuiji M, Nussenzweig MC, et al. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J Immunol Methods*. 2008; 329:112–124. [PubMed: 17996249]
2. Liao HX, Levesque MC, Nagel A, Dixon A, Zhang R, et al. High-throughput isolation of immunoglobulin genes from single human B cells and expression as monoclonal antibodies. *J Virol Methods*. 2009; 158:171–179. [PubMed: 19428587]
3. Scheid JF, Mouquet H, Feldhahn N, Seaman MS, Velinzon K, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature*. 2009; 458:636–640. [PubMed: 19287373]
4. Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, et al. Sequence and Structural Convergence of Broad and Potent HIV Antibodies That Mimic CD4 Binding. *Science*. 2011; 333:1633–1637. [PubMed: 21764753]
5. Moody MA, Yates NL, Amos JD, Drinker MS, Eudailey JA, et al. HIV-1 gp120 Vaccine Induces Affinity Maturation in both New and Persistent Antibody Clonal Lineages. *J Virol*. 2012; 86:7496–7507. [PubMed: 22553329]
6. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, et al. Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target. *Science*. 2009; 326:285–289. [PubMed: 19729618]
7. Gorny MK, Stamatatos L, Volsky B, Revesz K, Williams C, et al. Identification of a new quaternary neutralizing epitope on human immunodeficiency virus type 1 virus particles. *J Virol*. 2005; 79:5232–5237. [PubMed: 15795308]
8. Pietzsch J, Scheid JF, Mouquet H, Seaman MS, Broder CC, et al. Anti-gp41 antibodies cloned from HIV-infected patients with broadly neutralizing serologic activity. *J Virol*. 2010; 84:5032–5042. [PubMed: 20219932]
9. Scheid JF, Mouquet H, Feldhahn N, Walker BD, Pereyra F, et al. A method for identification of HIV gp140 binding memory B cells in human blood. *J Immunol Methods*. 2009; 343:65–67. [PubMed: 19100741]
10. Li L, Wang X-H, Banerjee S, Volsky B, Williams C, et al. Different pattern of immunoglobulin gene usage by HIV-1 compared to non-HIV-1 antibodies derived from the same infected subject. *e. PLoS One*. 2012; 7:e39534. [PubMed: 22761815]
11. Newman J, Rice JS, Wang C, Harris SL, Diamond B. Identification of an antigen-specific B cell population. *J Immunol Methods*. 2003; 272:177–187. [PubMed: 12505722]

12. Morris L, Chen X, Alam M, Tomaras G, Zhang R, et al. Isolation of a human anti-HIV gp41 membrane proximal region neutralizing antibody by antigen-specific single B cell sorting. *PLoS One*. 2011; 6:e23532. [PubMed: 21980336]
13. Gao F, Weaver EA, Lu Z, Li Y, Liao HX, et al. Antigenicity and immunogenicity of a synthetic human immunodeficiency virus type 1 group m consensus envelope glycoprotein. *J Virol*. 2005; 79:1154–1163. [PubMed: 15613343]
14. Gorny MK, VanCott TC, Hioe C, Israel ZR, Michael NL, et al. Human monoclonal antibodies to the V3 loop of HIV-1 with intra- and interclade cross-reactivity. *J Immunol*. 1997; 159:5114–5122. [PubMed: 9366441]
15. Gorny MK, Revesz K, Williams C, Volsky B, Louder MK, et al. The V3 Loop Is Accessible on the Surface of Most Human Immunodeficiency Virus Type 1 Primary Isolates and Serves as a Neutralization Epitope. *J Virol*. 2004; 78:2394–2404. [PubMed: 14963135]
16. Xu J-Y, Gorny MK, Palker T, Karwowska S, Zolla-Pazner S. Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodeficiency virus type 1, using ten human monoclonal antibodies. *J Virol*. 1991; 65:4832–4838. [PubMed: 1714520]
17. Gigler A, Dorsch S, Hemauer A, Williams C, Kim S, et al. Generation of neutralizing human monoclonal antibodies against parvovirus B19 proteins. *J Virol*. 1999; 73:1974–1979. [PubMed: 9971777]
18. Totrov M, Jiang X, Kong XP, Cohen S, Krachmarov C, et al. Structure-guided design and immunological characterization of immunogens presenting the HIV-1 gp120 V3 loop on a CTB scaffold. *Virology*. 2010; 405:513–523. [PubMed: 20663531]
19. Verkoczy L, Moody MA, Holl TM, Bouton-Verville H, Searce RM, et al. Functional, non-clonal IgMa-restricted B cell receptor interactions with the HIV-1 envelope gp41 membrane proximal external region. *PLoS One*. 2009; 4:e7215. [PubMed: 19806186]
20. Seaman MS, Janes H, Hawkins N, Grandpre LE, Devoy C, et al. Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessment of neutralizing antibodies. *J Virol*. 2010; 84:1439–1452. [PubMed: 19939925]
21. Hioe CE, Wrin T, Seaman MS, Yu X, Wood B, et al. Anti-V3 monoclonal antibodies display broad neutralizing activities against multiple HIV-1 subtypes. *PLoS ONE*. 2010; 5:e10254. [PubMed: 20421997]
22. Alam SM, Searce RM, Parks RJ, Plonk K, Plonk SG, et al. Human immunodeficiency virus type 1 gp41 antibodies that mask membrane proximal region epitopes: Antibody binding kinetics, induction, and potential for regulation in acute infection. *J Virol*. 2008; 82:115–125. [PubMed: 17942537]
23. Gorny MK, Gianakakos V, Sharpe S, Zolla-Pazner S. Generation of human monoclonal antibodies to HIV. *Proc Natl Acad Sci*. 1989; 86:1624–1628. [PubMed: 2922401]

Fig. 1.

Isolation of cells using single-color tetramer sorting. B cells were enriched using CD19 magnetic beads prior to staining with APC-labeled V3_{con03} tetramer, anti-CD27-FITC, and anti-IgG-PE. Sequential gating was used to identify the tetramer-positive memory B cells. Forward and side scatter identified viable cells (A), the next gating was on viable B cells that binds to APC-labeled V3-tetramer and anti-CD27-FITC (B) and finally, those memory B cells that bind to V3-tetramer were gated for surface-IgG⁺ population (C).

(A)	CDR s				CDR s		
	H1	H2	H3		L1	L2	L3
L22582	GGTFSSYA	IIPIFGTA	AR	X72813	QSISSW	KAS	QQYNSYS
1a6	-----D-V	F-----SP	VI		-D-	---	---D-HW
1a61	-----D-V	F-----SP	VI		-T--D-	---	---D-HW
1d11	-----D-V	F-----SP	VI		-----D-	---	---D-HW
1a13	-----D--	F-----SP	VI		-----D-	---	---D-HW
(B)							
	FR1				FR2		FR3
L22582	QVQLVQSGAEVKKPGSSVKVSCKAS				ISWVRQAPGQGLEWMGG		NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC
1a6H	E-----D-V-----R--				VT-----A-		-----V-----R---YD---W-
1a61H	E-----D-V-----R--				VT-----A-		-----V-----R---YD---W-
1d11H	E-----D-V-----R--				V-----A-		-----V-----V---YD---L-W-
1a13H	E-----L-----R--				-----A-		-----H-----V-A-----D---L-W-
X72813	DIQMTQSPSTLSASVGDRVTITCRAS				LAWYQQKPGKAPKLLIY		SLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYC
1a6K	-----VS-----I-----				-----N---M---		I-A-----A-----I--G-----
1a61K	-----S-----I-----				-----H---N---M---		I-A-----A-----E-----G-----
1d11K	-----VS-----I-----				-----N---M---		I-A-----A-----I--G-----
1a13K	-----S-----I-----				-----R---N---M---		L-AT-A-A-----G-----R

Fig. 2.

Amino acid sequence analysis of the variable fragment of human anti-V3 mAbs which corresponds to the germline sequence. (A) Sequence alignment of the heavy and light chain CDRs. The germline sequence of the VH segment (IGHV1-69*01) is shown on the upper line; the position of the frameworks (FR) and CDRs are shown above the germline sequence. (B). Sequence alignment of three frameworks of the heavy and light chain. The kappa chain sequences of the four V3 mAbs are shown aligned to the germline sequence (IGKV1-5*03). The nucleotide sequences of the heavy and kappa chains have been deposited in GenBank under accession numbers: HM222540- HM222547. The alignment was performed using the IMGT system; hyphens (-) show nucleotide identity, the gaps were removed to simplify comparison. The CDR3s include only these AA which have corresponding germline sequence. AA mutation in the sequence of mAb 1a6 which is different than in other three mAbs is highlighted grey while position of AA which was not mutated compared to other mAbs is labeled dark grey.

Fig. 3.

Phylogenetic trees for heavy and light chain sequences. Relationships among the nucleotide sequences of four variable fragments (without CDR H3) of the anti-V3 mAbs compared to corresponding heavy chain gene IGHV1-69*01 (left) and kappa light chain gene IGKV1-5*03 (right) of germline sequences are shown. The phylogenetic trees found by NJ from the four sequences and rooted using the midpoint procedure was analyzed by IMGT/PhyloGene program (www.imgt.org). Additional horizontal boxes indicate the heavy and kappa light chain sequences of two V3 mAbs with comparable distances to germline sequences in contrast to other two mAbs with different distances.

Fig. 4.

ELISA reactivity of human anti-V3 mAbs with V3_{JRCSF} peptide. The four clonal V3 mAbs listed in Table 2, positive control V3 mAb 447 and negative control mAb 1418 (anti-parvovirus B19) were tested against V3 peptide coated onto ELISA plate at concentration 1.0 $\mu\text{g/ml}$. The concentration ($\mu\text{g/ml}$) of mAb needed for 50% maximal binding that reflects relative affinity was determined when the binding reached the saturation point. *This value was determined by extrapolation; mAb 1418 (>) did not react with V3 peptide.

Fig. 5. Neutralization of clade B pseudotyped HIV-1 viruses by mAb 1d11 using the TZM-bl cell assay. Human mAbs 447-52D, 2424 (anti-V3) and 1418 (anti-parvovirus B19), were used as a positive and negative control, respectively.

Human recombinant antibodies produced from single IgG⁺ memory B cells selected using V3_B tetramer

Table 1

#	mAb	Site	V3 _{JRCSF} -CTB O.D. ¹	gp120 _{MN} O.D. ¹	gp41 _{MN} O.D. ¹	BSA O.D. ¹
1	1a6	V3	2.6	3.1	0.1	0.1
2	1a13	V3	3.3	2.6	0.1	0.1
3	1d11	V3	3.8	3.2	0.1	0.1
4	1a61	V3	2.5	3.5	0.1	0.1
5	1a4	nd	0.1	0.1	0.1	0.1
6	1a18	nd	0.1	0.1	0.1	0.1
7	1a32	nd	0.1	0.1	0.1	0.1
8	1a34	nd	0.1	0.1	0.1	0.1
9	1a48	nd	0.1	0.1	0.1	0.1
10	1a52	nd	0.1	0.1	0.1	0.1
11	1a57	nd	0.1	0.1	0.1	0.1
12	1a65	nd	0.1	0.1	0.1	0.1
13	1a69	nd	0.1	0.1	0.1	0.1
14	1a74	nd	0.1	0.1	0.1	0.1
15	1a81	nd	0.1	0.1	0.1	0.1
16	1a94	nd	0.1	0.1	0.1	0.1
17	1b12	nd	0.1	0.1	0.1	0.1
18	1d34	nd	0.1	0.1	0.1	0.1
19	1d42	nd	0.1	0.1	0.1	0.1
20	1d50	nd	0.1	0.1	0.1	0.1
C (+)	447-52D	V3	3.9	3.5	0.1	0.1
C (+)	167-7	gp41	0.1	0.1	3.3	0.2
C (-)	1418	B19	0.1	0.1	0.1	0.1

¹ A standard ELISA was used to determine the binding activity of mAbs to antigens. Antigens were coated onto ELISA plates at a concentration of 1.0 μg/ml and mAbs were used at a concentration of 10.0 μg/ml. The numbers are O.D. values; bold numbers indicate specific reactivity based on the value above cutoff which was defined as the mean binding of mAb 1418 (specific to parvovirus B19) + 3 standard deviations described above. C(+) – positive control; C(-) – negative control; nd – not determined.

Table 2
Clonal anti-V3 mAbs produced from single IgG+ memory B cells stained with V3_B tetramers

mAb	Site	IGHV	Mut ¹	CDR H3	IGLV	Mut ¹	CDR L3
1a6	V3	1-69	9.03%	VIMYYSGDDDDYPPDFQH	K1-5	8.96%	QQYDSHWT
1a13	V3	1-69	8.33%	VIMYYSGDDDDYPPDFQH	K1-5	9.32%	QQYDSHWT
1a61	V3	1-69	9.03%	VIMYYSGDDDDYPPDFQH	K1-5	9.32%	QQYDSHWT
1d11	V3	1-69	8.68%	VIMYYSGDDDDYPPDFQH	K1-5	8.96%	QQYDSHWT

¹Percent of mutation in VH and VL genes indicates the difference in nucleotide sequence between the tested mAbs and corresponding germline.

Table 3

Binding of purified mAb 1d11 to V3 peptides^J

mAb	Site	WJ52	JRC5F	MN	BZ167	CM237	SF162	RF
1d11	V3	3.3	3.6	1.6	0.2	0.2	0.2	0.3
447	V3	3.7	3.8	3.6	3.9	3.2	3.9	1.6
1418	B19	0.1	0.1	0.1	0.1	0.1	0.1	0.1

^J Binding of mAbs to V3 peptides was determined by ELISA; all V3 peptides were coated onto ELISA plates at 1.0 μ g/ml while mAbs were tested at 10.0 μ g/ml.