Supporting Information

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SI Materials and Methods

Peptides and Recombinant Proteins. Peptide ELDKWA, P1 (aa 630–685) clade B HXB2 HIV-1 and $W_{666}A$ mutated P1 (1) were chemically synthesized (purity >95%) by Eurogentec. P1-containing liposomes were prepared as described previously (2). The trimeric recombinant gp41 construct (524-SGGRGGS-618–682, with residue numbering assigned according to the position in gp160 of CXCR4 tropic HIV-1_{HXB2}, clade B) (3, 4) was kindly provided by Mymetics and Protein X'pert.

Cloning of 2F5 Antibody V Regions, Antibody Production, and Purification. DNA of 2F5 antibody V regions, VJ (for the light chain) and VDJ (for the heavy chain), were synthesized (5) and codon-optimized for expression in eukaryotic cells (Pasteur-Mérieux Connaught). For the production of 2F5 IgG1, DNA was cloned into two vectors, VHExpress and VKExpress, which direct the synthesis of the human gamma-1 heavy chain and light chain, respectively (6). For the 2F5 IgA2, DNA of 2F5 V regions was cloned into the pcDNA3:VHCα2m(1) and pcDNA3:VLCκ, (7) kindly provided by B. Corthésy (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland).

Monomeric 2F5 IgA2 and IgG1 were expressed in CHO *dhfr*$^-$ cells after electroporation with both heavy chain- and light chain-encoding plasmids. Selection was started 30–48 h later in the presence of G418 for the heavy chain and mycophenolic acid for the light chain. Clones were tested for antibody production by sandwich ELISA, allowing detection of only complete, covalently linked heterodimers (see below).

The 2F5 IgA2 was purified using the "CaptureSelect" human IgA affinity matrix (BAC) containing a 13-kDa Llama antibody fragment recognizing human IgA, in accordance with the manufacturer's instructions. After elution from the affinity media achieved with triethylamine 20 mM, the 2F5 antibody was dialyzed against PBS and concentrated using Amicon Ultra-50 centrifugal filter units (Millipore). Antibody purity was evaluated by SDS/PAGE, followed by analysis of Coomassie blue staining of a band at MW 110 kDa for the two heavy chains and another band at 50 kDa for the two light chain, as expected for the IgA2m1, whose heavy and light chains are not covalently linked, in contrast to IgA1 or IgA2m2 (8). The 2F5 IgG1 was purified by Protein A chromatography (Thermo Scientific Pierce) as described by the manufacturer.

ELISA. The concentration of 2F5 antibodies was measured by sandwich ELISA using goat anti-human IgA (Caltag) or IgG (Jackson ImmunoResearch), and biotinylated mouse anti-human Ig kappa light chains (BD Pharmingen), as described previously (4). Specificity of antibodies was measured by coating microtiter plates (NUNC-Immuno Plate MaxiSorp Surface or Peptide Immobilizer; Exiqon) with recombinant gp41 (trimeric rgp41 at 0.25 μg/well), peptide P1 (0.1 μg/well), or peptide ELDKWA (0.05 μg/well) overnight at 4 °C in carbonate buffer (pH 9). Antibody binding was detected either with streptavidin-HRP–coupled mouse anti-human IgA- (Jackson ImmunoResearch) or IgG- (Rockland Immunochemicals) specific heavy chains, or with anti-human Ig kappa light chains (BD Pharmingen).

For competitive binding ELISA, 2F5 IgG1 at indicated concentrations was used to compete with a constant concentration of 2F5 IgA2 (0.02 nM) for the binding to P1, ELDKWA, or rgp41. Binding of competing Abs was detected with a streptavidin-HRP–coupled mouse anti-human IgA, as mentioned earlier.

The affinity of 2F5 IgG1 and 2F5 IgA2 for P1 inserted in liposomes was evaluated as described previously (2). Specific binding was detected with streptavidin-HRP–coupled mouse anti-human IgG or IgA as before, and reading the absorbance at 490 nm using a SpectraMax 340 PC plate reader (Molecular Devices).

K_d values were obtained using GraphPad Prism 5.0 by nonlinear regression of the data obtained by ELISA, after subtraction of nonspecific binding of 2F5 IgG1 and 2F5 IgA2 to liposomes devoid of P1. Such computing is based on an equal amount of peptide bound on the surface of each well of the ELISA plate.

Virus capture was adapted a procedure described previously (9). To capture 2F5Abs, ELISA plates (NUNC-Immuno Plate MaxiSorp Surface; Exiqon) were coated overnight with goat anti-human IgG (H+L) or goat anti-human serum IgA alpha chain-specific (Jackson ImmunoResearch). After blocking, 2F5 Abs IgG1 and IgA2 (1 μg/mL) were incubated for 1 h at room temperature. The respective amount of 2F5 isotype actually bound to the plate, evaluated using the same mouse anti-human Ig kappa light chain antibody, showed that coating with IgG1 2F5 was 90% of that for 2F5 IgA2. This was taken into account for comparative evaluation of the amount of HIV-1 p24 bound to each isotype. Normal serum IgG or purified IgA2 was used as a negative control. HIV-1 JR-CSF (clade B, R5 tropic) at 300 ng or 30 ng p24 was then added for 1 h at 37 °C. p24 released from the captured virus by incubation with Nonidet P-40 diluted in RPMI 1640 containing 10% FBS was measured using a commercial ELISA (Innotest HIV-1 Antigen mAb; Innogentetics) according to the manufacturer's instructions.

Surface Plasmon Resonance. All experiments were performed in duplicate, as described previously (10), with a Biacore 3000 instrument at 20 °C in HBS-EP running buffer [10 mM Hepes (pH 7.4), 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20]. Immobilization of ligand, the P1 peptide, to CM5 chips (GE Healthcare) followed the standard procedures recommended by the manufacturer. The final immobilization levels were between 300 and 500 resonance units (RU) to avoid rebinding events, as described previously (10). Initial comparative binding experiments with 2F5 IgG1, 2F5 IgA2, and nonspecific isotypes as analytes were performed. No specific signal was observed with nonspecific isotypes (recorded value of 0 RU), indicating that the observed binding of 2F5 IgG1 and IgA2 to P1 was specific. For kinetic measurements, sensorgrams were obtained by passing various concentrations of the analyte, 2F5 IgG1, or IgA2 at various concentrations between 0 and 3 nM over the ligand surface at a flow rate of 5 μL/min, with a 5-min association phase and a 10-min dissociation phase. The sensor surface was regenerated between each experiment with a single injection of 35 mM NaOH and 1.3 M NaCl at a flow rate of 10 μL/min. Identical injections over blank surfaces run in parallel (and giving a value of 0 RU) were subtracted from the data for kinetic analysis. Binding kinetics were evaluated by linearization using BiaEvaluation "fit kinetics Langmuir binding type" (Biacore). Relative Pearson's χ^2 test values assessing goodness of fit were always <0.3, indicating that the models used for fitting adequately describe recorded data.

Target Cells. ***CD4$^+$ T cells.*** $CD4^+$ T cells were purified from peripheral blood samples obtained from 20 healthy donors using a human $CD4^+$ T cell enrichment kit (StemCell Technologies)

and stimulated for 2 d with 5 μg/mL of phytohemagglutinin (Sigma-Aldrich).

CEM-CCR5$^+$ cells. CEM-CCR5$^+$ cells (AIDS Research and Reference Program, National Institutes of Health) were maintained in RPMI 1640 containing 10% (vol/vol) FBS on a precise passage regimen (1:10 split, twice a week). To prepare cells for neutralization assays, cells were split 1:3 on the day of passage and used the next day.

Virus Stock Preparation. A stock of HIV-1JR-CSF (clade B, R5 tropic) was prepared by transfecting 293T cells with a plasmid containing the DNA sequence of JR-CSF (National Institutes of Health) (4). The HIV-1 primary isolate 93BR029 (clade B, R5, obtained through the National Institutes of Health AIDS Reagent Program), was amplified on peripheral blood mononuclear cells (PBMCs), as described previously (11).

HIV-1 Neutralization Assays. ***HIV-1 transcytosis.*** Inhibition of HIV-1 transcytosis across monolayers of the HEC-1 endometrial epithelial cell line induced on contact with PBMCs infected with clade B, R5 HIV, either JR-CSF or 93BR029 (both clade B, R5), and with appropriated controls, was performed as described previously (12–14).

Inhibition of HIV-1 transfer from Langerhans cells to T cells. The inhibition of HIV-1 transfer from Langerhans cells (LCs) to T cells was evaluated using monocyte-derived LCs obtained from PBMCs as described previously (15), along with autologous CD4$^+$ T cells. In brief, LCs were incubated with HIV-1 JR-CSF for 2 h at 37 °C, washed extensively to remove the free virus, and distributed in 96-well plates at 100,000 cells/well. 2F5 IgG1 and IgA2 antibodies of the desired concentration were then added to the corresponding wells. Finally, either medium alone or resting CD4$^+$ T cells in medium (100,000 cells/well) was added. The LC–T cell cocultures were incubated at 37 °C for 7 d. Virus transfer was evaluated by measuring p24-Ag by ELISA (INNOTEST HIV-1 Antigen mAb; Innogenetics) according to the manufacturer's instructions. Results are expressed as percentage of transfer in the absence of Abs.

Single-cycle neutralization assay. The neutralization activity of 2F5 IgG1 and IgA2 was evaluated on purified CD4$^+$ T cells and on CEM-CCR5$^+$ cells infected with HIV-1 JR-CSF by flow cytometric detection of intracellular p24-Ag, as described previously (4). Neutralization was defined in terms of percentage of cells infected in the absence of antibody. The neutralizing titer was defined as the respective inhibitory concentration (IC) of the antibody (interpolated between successive dilutions performed in triplicate) that neutralized infection by 50% (IC_{50}), 90% (IC_{90}), and 99% (IC_{99}).

Neutralization assays in TZM-bl cells with envelope-pseudotyped viruses. Neutralization was also evaluated using a highly sensitive test based on viruses pseudotyped with an HIV-1 envelope, here the HIV-1 R5 tropic QH0692.42 envelope. Neutralization directly correlates to the reduction in luciferase reporter gene expression after a single round of virus infection in JC53-BL cells (also termed TZM-bl cells), as described previously (13). The antibody IC_{50} and IC_{90} for luciferase reporter gene production were determined by regression analysis.

Epitope Mapping. Epitope mapping of both isotypes was performed as described previously (16), using linear 12-mer peptide libraries displayed on the protein pIII of M13 phages (New England Biolabs) as recommended by the manufacturer. In brief, IgA- or IgG-coated beads were incubated with 2F5 IgA2 or IgG1 (2 μg for the first round, 1 μg for the second round, and 0.5 μg for the third round) in PBS/BSA 0.1% (wt/wt)/Tween-20, 0.05% (vol/vol) on a rotating wheel for 2 h at room temperature. Epitope screening was initiated by incubating each bead of 2F5 IgA2 or IgG1 with 10 μL of the original 12-mer (1×10^{11}) phage-displayed peptide library containing different phages, on a rotating wheel overnight at 4 °C. The beads were washed, and bound phages were eluted by pH shift with 0.2 M glycine-HCl (pH 2.2) and then immediately neutralized with 1 M Tris-HCl (pH 9.1; Sigma-Aldrich). Eluted phages were subjected to negative selection using beads coated with normal human IgA or IgG (Jackson ImmunoResearch). Phages remaining from the negative selection were amplified in *Escherichia coli* ER2738 (New England Biolabs), precipitated overnight at 4 °C with 20% (wt/vol) PEG-8000/2.5 M NaCl (Sigma-Aldrich), and used for second and third rounds of selection similar to the first round, but with increased buffer stringency [Tween-20 (vol/vol) concentration raised to 0.1% (vol/vol) for the second round and to 0.5% for the third round]. After the third positive selection, the phages were tittered, and single clones were picked and tested by phage ELISA for specific binding on each 2F5 IgA2 or IgG1. Positive clones were amplified, and each specific peptide insert was sequenced.

Mimotope Sequence Analyses and Mapping on gp41. For each set of 2F5 IgA2 and IgG1 mimotopes, epitope mapping on HIV-1 gp41 was performed using the Pepitope software (http://pepitope.tau.ac.il) (17) and the recently published structure of a trimeric gp41 (18) (PDB code: 2X7R). The Pepitope software is a Web-based tool that aims to predict discontinuous or 3D eptitopes based on a set of peptides that were affinity-selected against monoclonal antibody of interest (19, 20). The pepitope program takes into account the number of times each sequence peptide was isolated during the experiment, and provides 0 to n clusters that reveal locations of key residues. We considered all predicted clusters and we computed amino acids predicted frequencies (amino acids predicted occurrences normalized by the highest occurrence) for each chain of the gp41 structure. These frequencies are plotted all along the resolved monomer structure sequence, and mapped onto the protein structure to visualize the epitope in the surface of gp41.

Given a pair of aligned residues, this algorithm searches the remaining sequences in the examined set to find any residue aligning to both residues, called a "triplet." The weight for a pair of aligned residues is determined by the sum of all triplets involving that residue pair. More consistent pairs will get higher weights and will more likely be aligned. Weight is translated into a color scheme, the CORE index that uses the consistency among pairwise alignments to estimate reliability; red bits are very reliable, whereas green and blue bits are unreliable.

Statistics. P values were calculated using the Student t test.

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