

Supplemental Methods, Tables and Figures

(Deficient Synthesis of Class-Switched, HIV Neutralizing Antibodies to the CD4 Binding Site and Correction by Electrophilic gp120 Immunogen, Stephanie A. Planque et al)

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Supplemental Methods

Antigens and immunogens

Proteins were from these sources: ovalbumin and bovine serum albumin (BSA) – Sigma (St. Louis, MO); soluble CD4 (4 domain) – Protein Science (Meriden, CT); recombinant gp120 (MN) – Immunodiagnostics (Woburn, MA) or Protein Science (MN and IIIB strains); synthetic gp120 peptide 421-433 (MN strain) – NIH AIDS repository. E-gp120 contained 24.8-31.8 mol electrophilic phosphonate diester groups/mol gp120 (64.7-86.4% of available Lys side chains were derivitized) [1]. E-gp120 was lyophilized and stored at -80°C until use. E-C^{LIN}a and E-C^{LIN}b contained gp120 residues 416-433 and phosphonate groups at Lys421 and Lys432 (no change in the net peptide charge; **Table S1**) [2,3]. A biotin group was placed at the E-C^{LIN}a N-terminus and BSA was conjugated at the E-C^{LIN}b C-terminal Cys (6.1 mol peptide/mol BSA). The chemical identity of all peptides was verified by electrospray ionization mass spectrometry. Electrophilic E-hapten **1** was prepared as in ref [4].

Human subjects

Peripheral blood was collected from (a) Subjects with infected with HIV (The San Francisco Men's Health Study [5]) without AIDS (donor codes 2089-2091, 2093 and 2097; $393-1164 \times 10^3/\text{ml}$; 5 years post-seroconversion) or with AIDS (donor codes 1930-1933; CD4+T cells counts $56-188 \times 10^3/\text{ml}$; 0.5-5 years post-seroconversion); and (b) control non-infected subjects (Gulf Coast Blood bank; 13 donors, age 28-48 years). HIV seroconversion was determined by detection of antibodies to HIV-1 by ELISA and confirmed by Western blotting. The infected subjects had not received anti-HIV therapy (the blood samples were collected prior to availability of effective HIV drugs).

Immunization

BALB/c mice (4-7 weeks old; Jackson Laboratories, Bar Harbor, ME) were immunized periodically with gp120 or E-gp120 in 10 mM sodium phosphate pH 7.4, 137 mM NaCl, 2.7 mM KCl (PBS) mixed with adjuvant by the intraperitoneal (0.25-0.30 mL) or intranasal (0.02 mL) routes indicated in **Table S2**. Identical immunization protocols were applied for the two test immunogens in 3 studies (indicated by the suffix "a" for gp120 and "b" for E-gp120 in studies 1, 2 and 3). Studies 4 and 5 provided supporting data. Ribi and cholera toxin B adjuvants were from Sigma, and IL12, from R&D Systems (Minneapolis, MN). Serum was prepared from blood obtained from the retroorbital plexus.

Antibodies

IgG and IgM antibodies were purified from sera or hybridoma tissue culture fluids on columns of immobilized Protein G and anti-IgM antibody (directed to human or mouse IgM), respectively [4, 6]. The preparation and purification of recombinant 150 kDa IgG1 JL427 and 900 kDa IgM JL427 containing identical V-domains was described [7]. IgG 3A5 for the humanized mouse study was prepared from hybridoma cells grown in fermentation culture and purified on immobilized Protein A by BioXcell (West Lebanon, NH). Reference IgG1 VRC01 was from the AIDS Reagent Program, Division of AIDS, NIAID, NIH (deposited by Dr. John Mascola) [8]. The following isotype-matched antibodies were tested: for IgG YZ23, IgG2a/ κ CRL 1689 [4]; for IgG 3A5, IgG2b/ κ clones MPC11 and MG2b-57 (BioXcell); for IgG JL427, IgG1/ λ I5029 (Sigma); and for IgG VRC01, IgG1/ κ A50183H (BioDesign Intl, Sarco, ME). Monoclonal IgG 4B6 to phosphatidyl serine was from Abcam (Cambridge, MA). Functional tests were conducted following dialysis of the antibodies against PBS. The purified antibodies were electrophoretically homogeneous [9]. Their protein content was measured using a bicinchoninic acid kit (Pierce, Rockford, IL). The IgG and IgM content of unfractionated sera was measured using ELISA wells coated with anti-mouse κ/λ capture antibody (200 ng/well; Sigma) and the bound IgG or IgM were detected with peroxidase-conjugated antibodies to mouse IgG or IgM (1:1000; Sigma) [7]. The antibody concentrations were interpolated from standard curves constructed with authentic mouse IgG and IgM (Bethyl, Montgomery, TX).

Antigen binding and HIV neutralization

CS ratios were computed as the IgG/IgM binding ratios for individual antigen probes (gp120 or E-C^{LIN}b) as (A490/ μ g IgG)/(A490/ μ g IgM). ELISA was done using recombinant, immobilized E-C^{LIN}b (200 ng/well), gp120 (40 ng/well) or E-gp120 (40 ng/well) in duplicate [3,4]. Purified gp120 from subtype B strain MN (Immunodiagnostics) was used to estimate binding by human antibodies (3-80 μ g/mL IgM or IgG purified by chromatography on anti-IgM or Protein G columns). The same gp120 or E-gp120 immunogen applied to induce antibody synthesis was used as the antigen probe to determine binding by murine unfractionated sera collected in individual immunization studies (sera diluted 1:100 or 1:1000; corresponding, respectively, to 0.4-1.3 μ g IgM/ml and 1.5-3.7 μ g IgG/mL in the individual studies). The binding data were verified using purified IgM and IgG. The ELISA buffer contained excess BSA (1 mg/ml), minimizing nonspecific binding. The data were corrected for non-specific binding to wells coated with BSA instead of the test antigen (<0.05 A490). Polyclonal antibody specificity was evaluated from inhibition of binding to immobilized E-C^{LIN}b by soluble competitor polypeptides (human IgM, 80 μ g/mL; IgM or IgG from mice immunized with gp120 or E-gp120, respectively, 2.5 μ g/mL or 1 μ g/mL) [3]. Residual antibody binding was computed as: 100X(A490 in the presence of competitor/A490 in diluent). Unless otherwise indicated, the binding was tested in buffer containing excess E-hapten 1 (100 μ M). Bound IgM or IgG were quantified using peroxidase-conjugated antibodies to human IgM, human IgG, mouse IgM, or mouse IgG. Epitope specificity of purified monoclonal IgGs YZ23, 3A5, JL427 and VRC01 was verified from E-C^{LIN}b, gp120 and E-gp120 binding assays. Retention of antigen binding activity by recombinant IgM and IgG JL427 (74-150 μ g/ml) was verified by ELISA using immobilized gp120. IgM JL427 hydrolyzes gp120 detectably [7]. Negligible depletion of immobilized gp120 is anticipated at the reported hydrolytic rate. Antibody neutralizing activity was measured in quadruplicate using phytohemagglutinin-activated peripheral blood mononuclear cells as host cells (PBMCs; pooled from 4-12 donors) [2]. and primary HIV strains (from subtypes A, B, C, D and AE) or replication competent molecular HIV strains free of viral quasispecies. The panel included strains dependent on the C-C chemokine coreceptor 5 (CCR5) responsible for most initial infections, strains dependent on the C-X-C chemokine coreceptor 4 (CXCR4) that usually develop after prolonged infection, and dual-tropic strains. Infection was monitored by measuring HIV p24 antigen (primary strains) [2] or the provirally-encoded Renilla luciferase enzyme (molecular strains) [10]. Strains with mutations in the C^{LIN} sequence (Trp427Ala mutant; triple Arg419Ala/Val430Ala/Arg432Ala mutant) were prepared from subtype B *env* gene Bal26 (accession number DQ318211) by site-directed mutagenesis using the Quickchange kit (Agilent Technologies, Inc., Santa Clara, CA; primer for introducing Trp427Ala mutation – catgcagaataaaacaaattataaacatggcgcaggaagtaggaagagcaatg; primer for initial Arg419Ala replacement – aaataacacaatcacactcccatgcgcaataaaacaaattataaacatgtggc, and primer for subsequent Val430Ala/Arg432Ala replacements – taaacatgtggcaggaagcaggagcagcaatgtatgccctcc; Ala codons are underlined) [10]. The replacement mutations were confirmed by DNA sequence analysis. The

endotoxin (lipopolysaccharide) content of antibodies determined by the Limulus amoebocyte lysate test using the Endosafe®-PTS instrument was below the concentrations that interfere in the HIV neutralization (<0.05 EU/ml final concentration in the assays) [2].

hu-PBMC-NSG mouse model

NOD/*scid*-IL-2Rgc^{null} mice (NSG, stock #005557; Jackson Laboratories, Bar Harbor, ME) were bred under specific pathogen-free conditions in compliance with ethical guidelines for care of laboratory animals at the University of Nebraska Medical Center. Detailed procedures for mouse humanization and monitoring of HIV infection were described [11-13]. PBMCs from healthy humans (pool of 4 donors; 2×10^7 cells/mouse) were injected intraperitoneally. Graft efficiency was confirmed in all mice prior to HIV challenge on day 13 by flow cytometry using antibodies to human pan-CD45, CD3, CD4 and CD8 antigens. The human CD45+ cell/total lymphocyte number ratios in the blood of mice that had not been challenged with HIV (n=3) on days 21 and 28, respectively, were 4.2 ± 1.0 and $45.4 \pm 14.6\%$. The corresponding ratio in spleen of the same mice on day 28 at euthanasia was $16.0 \pm 1.5\%$. The mice received vehicle (PBS) or IgG 3A5 (2.5 mg in 0.35 ml PBS per injection) on day 13 and were challenged intraperitoneally on day 14 with HIV strain ADA (100 TCID₅₀) pretreated for 1 h at 37°C with vehicle or IgG 3A5, followed by intraperitoneal injections of vehicle or IgG 3A5 on days 13, 17, 20, 23 and 26 (2.5 mg each). Viral RNA was quantified in blood from the submandibular vein using strain ADA-specific gag primers by the reverse transcriptase-polymerase chain reaction (UCLA CFAR Virology Core Facility). HIV gag RNA in spleen homogenates was determined by real-time PCR and normalized for the tissue content of RNA for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). HIV p24+ cells and total human leucocytes in serial spleen sections were determined by staining, respectively, with antibodies to p24 or HLA-DR/DP/DQ counterstained with hematoxylin. Data are expressed as % of total number of human cells observed in spleen sections. CD4+ and CD8+ T cells were measured by flow cytometry in blood and splenocyte suspensions. IgG in blood on day 28 was measured by ELISA as before using reference mouse IgG as standard. The approximate blood half-life of the IgG was 3.3 days determined after a single antibody injection in a PBMC-grafted mouse (computed from the equation $[IgG] = [IgG]_{max} \cdot \exp(-kt)$ where $[IgG]_{max}$ is the interpolated maximum IgG concentration, k is the decay rate, and half-life is $\ln 2/k$).

Supplemental Tables

Supplemental Table S1: (A) Consensus C^{LIN} sequence, and (B) Probe and immunogen C^{LIN} sequences.

(A) But for positions 417, 429 and 432, the C^{LIN} residues are identical or contain conservative substitutions at >90% frequency across HIV strains from subtypes A, B, C, D, F, G, H, I, J, K and circulating recombinant forms (4211 strains; <http://www.hiv.lanl.gov/content/sequence/VIRALIGN/viralalign.html>) [4].

(B) The E-C^{LIN}b antigen probe sequence is identical to the consensus sequence except for replacement of Cys418 by Ser (which eliminates formation of S-S linked dimers). Immunogens with variant C^{LIN} sequences were tested in studies 1-5 listed in **Table S2**. Red and green C^{LIN} residues denote non-conservative and conservative deviations compared to the E-C^{LIN}b antigen probe, respectively (clustalW2 algorithm). Strain MN gp120 immunogens (and the corresponding E-gp120 immunogens) in studies 1-3 and 5 were from two different commercial sources and contained certain C^{LIN} sequence deviations, likely due to envelope diversification during virus passage in tissue culture (sequence deviation over the entire length of gp120, 2.6%). Study 4 immunogen was from strain IIIB.

A. C^{LIN} consensus sequence

Residue number	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433
Consensus sequence	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A
% conservation	99	76	98	97	98	98	98	98	98	90	98	98	98	46	95	98	68	98

B. Peptide probe and immunogen C^{LIN} sequences

Residue number	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433
Probe C ^{LIN} peptide	L	P	S	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A
Immunogen C ^{LIN} , Study 1,2,5	L	Q	C	K	V	K	Q	I	I	N	M	W	Q	K	V	G	K	A
Immunogen C ^{LIN} , Study 3	L	Q	C	K	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A
Immunogen C ^{LIN} , Study 4	L	P	C	R	I	K	Q	F	I	N	M	W	Q	E	V	G	K	A

Supplemental Table S2: Mouse immunization studies.

Identical immunization protocols were applied to compare the gp120 and E-gp120 immunogens (designated by the letters “a” and “b”, respectively) in each of studies 1-3. n, number of immunized mice. Immunogen dose, route, adjuvant and immunization schedule were varied as indicated. gp120 in studies 1, 2 and 5 was from Immunodiagnostics Inc, and in studies 3 and 4, from Protein Sciences Inc. MN and IIIB denote the virus strain from which the gp120 immunogen was isolated. The immunogen was administered mixed with the indicated adjuvant except the intravenous administration conducted in diluent alone in study 3 on day 42. Study 5 mice received additional IL12 intranasal administrations without immunogen on days 1, 2 and 3 (1 µg). CTB, Cholera Toxin subunit B. One hundred µL RIBI is composed of 25 µg monophosphoryl Lipid A, 25 µg synthetic trehalose dicorynomycolate in 2% squalene oil and 0.2% Tween 80.

Study	N	Immunogen (strain, µg/administration)	Route (number of administrations)	Adjuvant (amount/administration)	Immunogen administration schedule, day
1a	10	gp120 (MN, 5)	Intranasal (4X)	CTB (10 µg); IL-12 (1 µg)	0, 14, 28, 99
1b	10	E-gp120 (MN, 5)	Intranasal (4X)	CTB (10 µg); IL-12 (1 µg)	0, 14, 28, 99
2a	10	gp120 (MN, 5)	Intraperitoneal (4X)	RIBI (100 µL)	0, 14, 28, 99
2b	10	E-gp120 (MN, 5)	Intraperitoneal (4X)	RIBI (100 µL)	0, 14, 28, 99
3a	4	gp120 (MN, 11.5)	Intraperitoneal (3X); intravenous (1X)	RIBI (100 µL)	0, 14, 28, 42
3b	4	E-gp120 (MN, 11.5)	Intraperitoneal (3X); intravenous (1X)	RIBI (100 µL)	0, 14, 28, 42
4	3	gp120 (IIIB, 10)	Intraperitoneal (2X)	RIBI (100 µL)	0, 14
5	5	E-gp120 (MN, 5)	Intranasal (4X)	CTB (10 µg); IL-12 (1 µg)	0, 14, 28, 42

Supplemental Table S3: Cross-subtype HIV neutralizing activity of IgG YZ23 and IgG 3A5. IC50 and IC80 values were interpolated from IgG dose-response curves (IgG YZ23: 0.002-20 µg/mL, except the strain Du422 assay, which included an additional 80 µg/mL data point; IgG 3A5: 0.002-37.5 µg/mL). r^2 corresponds to correlation coefficient for curve fitting. Primary HIV strains containing a mixture of quaspecies and molecular strains (subtype B Bal, Bal26, 9010 and WEAU strains expressing a single envelope sequence) were analyzed. The strain employed to challenge humanized mice (ADA) is included. A smaller panel of strains neutralized by IgG YZ23 was reported previously [4]. Irrelevant isotype-matched IgGs did not neutralize any strain tested (IgG MPC11, IgG MG2b-57 and IgG CRL1689; subtype B ADA and SF162 strains, subtype C 97ZA009 and 98BR004 strains).

A. IgG YZ23

HIV subtype, strain, coreceptor	IC ₅₀ , µg/mL	IC ₈₀ , µg/mL	r^2
A, 96USSN54, R5	3.14	14.75	0.89
A, 92RW008, R5	0.23	4.29	0.63
B, US1, R5	2.00	>20	0.80
B, SF162, R5	0.33	2.63	0.91
B, 92BR020, R5	7.79	24.0	0.83
B, PAVO, R5	3.10	10.5	0.73
B, Bal, R5	0.66	0.77	0.82
B, Bal26, R5	0.44	1.68	0.80
B, 9010, R5	0.34	4.52	0.75
B, WEAU, R5X4	3.00	>20	0.82
C, 97ZA009, R5	1.56	4.88	0.91
C, 98BR004, R5	1.40	3.21	0.99
C, Du172, R5	2.21	20.5	0.96
C, Du422, R5	3.51	55.7	0.89
C, 98TZ017, R5	1.13	3.37	0.94
AE, 90CM235, R5	0.82	3.58	0.94
Geometric mean [95%C.L.], µg/mL	1.28 [0.74-2.20]	6.80 [3.68-12.6]	
Median, µg/mL	1.48	4.70	
Range, µg/mL	0.23-7.79	0.77-55.7	

B. IgG 3A5

HIV subtype, strain, coreceptor	IC ₅₀ , µg/mL	IC ₈₀ , µg/mL	r^2
B, 92BZ167, R5X4	0.84	2.17	0.80
B, ADA, R5	0.78	2.97	0.89
B, Bal26, R5	1.20	18.5	0.73
C, 97ZA009, R5	1.04	4.07	0.94
C, 98TZ017, R5	0.10	1.10	0.66
D, 92UG046, X4	0.49	0.57	0.67
Geometric mean [95%C.L.], µg/mL	0.59 [0.22-1.53]	2.59 [0.73-9.10]	
Median, µg/mL	0.74	4.89	
Range, µg/mL	0.10-1.20	0.57-18.50	

Supplemental Table S4: C^{LIN} sequence of strains neutralized by IgGs YZ23, 3A5 and JL427. Deviations from the consensus sequence are in color (color coding as in Table S1B). The C^{LIN} sequence for strain 97USSN54 listed in Tables S3 and S6 is not available. # denotes an unidentified residue.

HIV subtype, strain, coreceptor	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	IgG tested
A, 92RW008, R5	L	Q	C	R	I	K	Q	I	I	N	M	W	Q	R	A	G	Q	A	YZ23
B, US1, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A	YZ23, JL427
B, SF162, R5	L	P	C	R	I	K	Q	I	I	N	R	W	Q	E	V	G	K	A	YZ23, JL427
B, 92BR020, R5	L	P	C	R	I	K	Q	I	V	N	M	W	Q	E	V	G	K	A	YZ23, JL427
B, PAVO, R5	L	P	C	R	I	K	Q	I	V	N	M	W	Q	E	V	G	K	A	YZ23
B, 92BR021, R5	L	P	#	R	I	K	#	I	I	#	M	W	Q	E	V	G	K	A	JL427
B, BX08, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A	JL427
B, NP1538, R5	L	P	C	R	I	R	Q	I	V	N	M	W	Q	E	V	G	K	A	JL427
B, 92BZ167, R5X4	L	P	C	R	I	K	Q	F	V	N	M	W	Q	E	V	G	K	A	3A5, JL427
B, TYBE, X4	L	Q	C	R	I	K	Q	I	V	N	M	W	Q	R	V	G	R	A	JL427
B, ADA, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A	3A5
B, Bal, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	K	V	G	R	A	YZ23
B, Bal26, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	R	A	YZ23, 3A5
B, 9010, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A	YZ23
B, WEAU, R5X4	L	P	C	R	I	K	Q	I	I	N	R	W	Q	E	V	G	K	A	YZ23
C, 97ZA009, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	R	A	YZ23, 3A5, JL427
C, 98BR004, R5	I	Q	C	R	I	K	Q	I	I	N	M	W	Q	E	V	R	R	A	YZ23
C, 98Du172, R5	L	Q	C	R	I	K	Q	I	I	R	M	W	Q	G	V	G	Q	A	YZ23
C, 98Du422, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	R	A	YZ23
C, 98TZ017, R5	L	Q	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	R	A	YZ23, 3A5
D, 92UG046, X4	L	P	C	R	I	K	Q	I	I	N	M	W	Q	G	V	G	K	A	3A5
D, NKU3006, R5	L	P	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A	JL427
D, 94UG114, R5	L	Q	C	R	I	K	Q	I	I	N	M	W	Q	E	V	G	K	A	JL427
AE, 90CM235, R5	L	P	C	K	I	K	Q	I	I	N	M	W	Q	G	A	G	Q	A	YZ23, JL427

Supplemental Table S5: Sequence diversity of gp120 V-domains expressed by HIV strains sensitive to neutralization by IgGs YZ23 and 3A5. % sequence identity was computed relative to the corresponding V1-V5 domains of the E-gp120 immunogen from strain MN employed to induce IgG YZ23 and IgG 3A5. The immunodominant gp120 epitopes are located in the gp120 V-domains. The gp120 sequence for test strain 97USSN54 listed in Tables S3 and S6 is not available.

A. IgG YZ23

HIV subtype, strain, coreceptor	V-domain identity, %				
	V1	V2	V3	V4	V5
A, 92RW008, R5	17	44	63	41	46
B, US1, R5	42	69	80	52	77
B, SF162, R5	38	73	74	46	69
B, 92BR020, R5	31	58	77	45	69
B, PAVO, R5	41	48	74	46	54
B, Bal, R5	41	68	77	46	62
B, Bal26, R5	38	73	80	46	54
B, 9010, R5	42	52	77	46	62
B, WEAU, R5X4	35	65	66	63	69
C, 97ZA009, R5	21	53	63	40	54
C, 98BR004, R5	28	43	63	40	54
C, 98Du172, R5	20	48	60	44	54
C, 98Du422, R5	24	41	63	29	46
C, 98TZ017, R5	27	49	66	41	77
AE, 90CM235, R5	17	53	60	34	54
Average	31	56	70	44	60

B. IgG 3A5

HIV subtype, strain, coreceptor	V-domain identity, %				
	V1	V2	V3	V4	V5
B, 92BZ167, R5X4	34	64	66	40	62
B, ADA, R5	47	75	83	57	62
B, Bal26, R5	38	75	83	46	62
C, 97ZA009, R5	24	58	66	40	54
C, 98TZ017, R5	30	56	69	41	69
D, 92UG046, X4	20	49	57	37	54
Average	29	56	67	43	59

Supplemental Table S6: Cross-subtype HIV neutralization by C^{LIN}-directed IgG JL427 (A) and reference IgG VRC01 (B).

IC50 and IC80 values were interpolated from plots of IgG concentration (0.066-21 µg/mL) versus HIV neutralization. *, >50% neutralization observed at lowest IgG concentration tested. NA, r² value not available because of near-complete neutralization at lowest IgG concentration. The IC₅₀ geometric mean and 95% confidence limits (C.L.) for IgG JL427 neutralization of the 6 strains neutralized by IgG VRC01 in panel B were, respectively: 0.095 µg/ml and 0.022-0.401 µg/ml. Neutralization of HIV pseudovirions by IgG VRC01 using TZM-BI host cells was reported previously [8]. Irrelevant isotype-matched IgGs tested in parallel did not neutralize any of the strains tested (subtype A 97USSN54; subtype B US1, 92BR020, BX08, 92BZ167, Tybe; subtype C 97ZA009; subtype D 94UG114; subtype AE CM235).

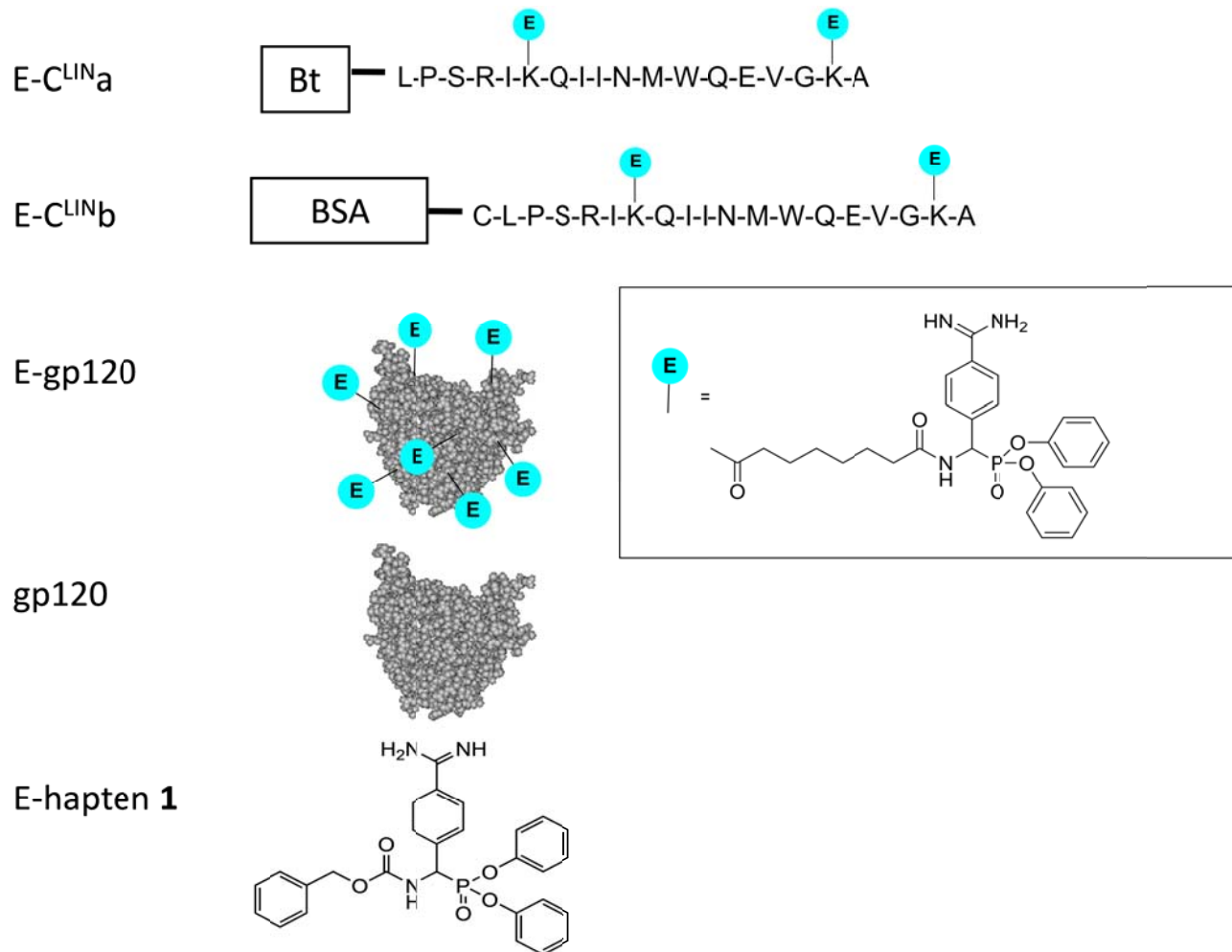
A. IgG JL427

HIV subtype, strain, coreceptor	IC ₅₀ , µg/mL	IC ₈₀ , µg/mL	r ²
A, 97USSN54, R5	0.062	7.443	0.67
B, US1, R5	< 0.066*	0.090	0.96
B, SF162, R5	0.026	0.401	0.65
B, 92BR020, R5	0.066	0.180	0.83
B, 92BR021, R5	0.015	0.076	0.90
B, BX08, R5	< 0.066*	0.181	0.87
B, NP1538, R5	<0.066*	<0.066*	NA*
B, 92BZ167, R5X4	1.460	6.000	0.44
B, Tybe, X4	0.227	18.09	0.81
C, 97ZA009, R5	0.030	0.112	0.91
D, NKU3006, R5	<0.066*	<0.066*	NA*
D, 94UG114, R5	0.045	0.334	0.90
AE, 90CM235, R5	0.092	0.863	0.64
Geometric mean [95% C.L.], µg/mL	0.071 [0.035-0.141]	0.429 [0.126-1.460]	
Median, µg/mL	0.066	0.181	
Range, µg/mL	0.015-1.460	0.066-29.60	

B. IgG VRC01

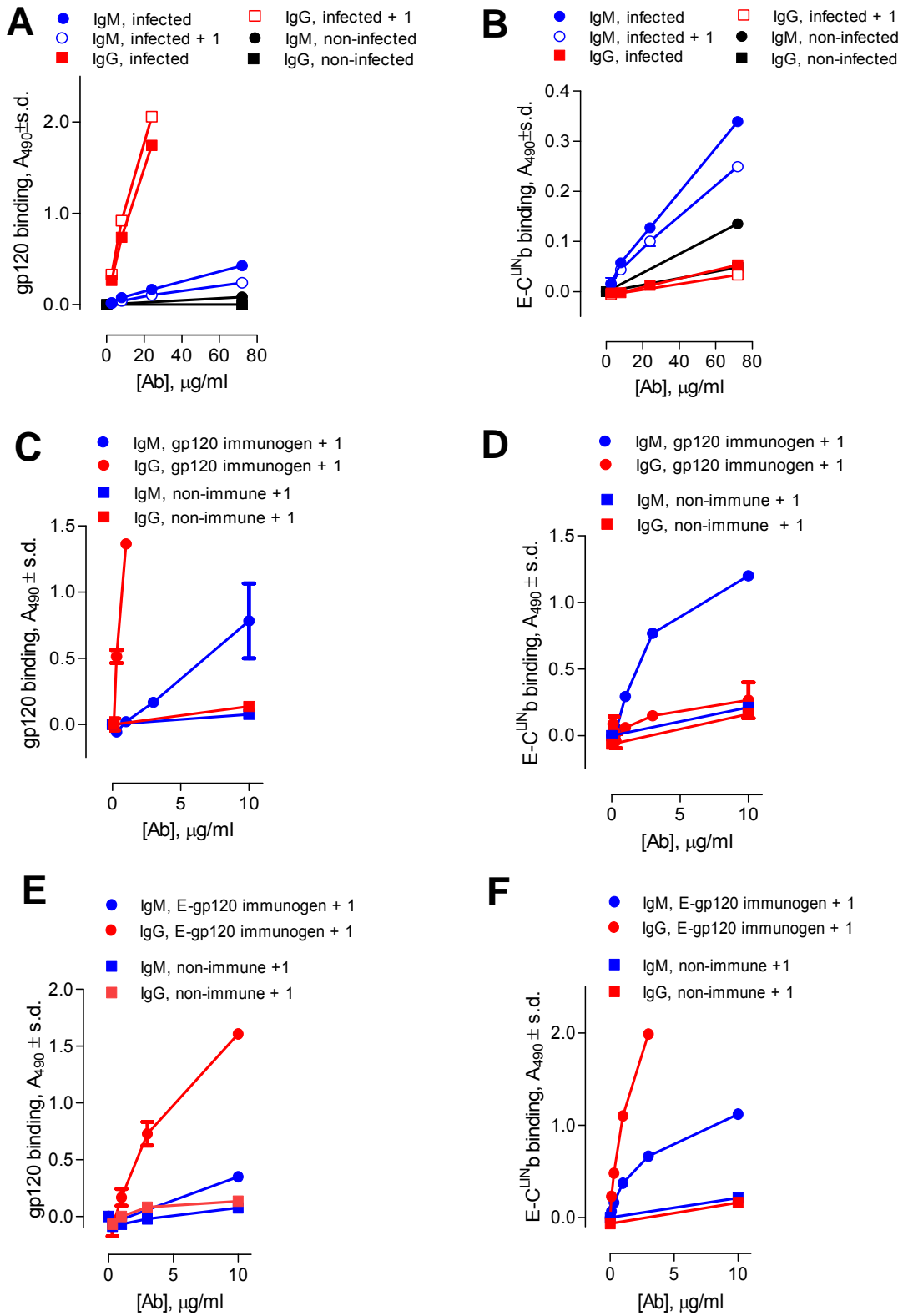
HIV subtype, strain, coreceptor	IC ₅₀ , µg/mL	IC ₈₀ , µg/mL	r ²
A, 97USSN54, R5	0.534	2.007	0.70
B, 92BR020, R5	0.081	0.291	0.84
B, Tybe, X4	0.359	6.154	0.66
C, 97ZA009, R5	0.045	0.264	0.95
D, 94UG114, R5	0.166	1.201	0.83
AE, 90CM235, R5	1.113	16.286	0.66
Geometric mean [95% C.L.], µg/mL	0.225 [0.063-0.796]	1.627 [0.290-9.119]	
Median, µg/mL	0.263	1.604	
Range, µg/mL	0.045-1.113	0.264-16.286	

Supplemental Figures



Supplemental Fig. S1: Antigen and immunogen structures.

E denotes the electrophilic phosphonate inserted at Lys side chains. E-C^{LIN}a contains gp120 residues 416-433 and an N-terminal biotin (Bt). E-C^{LIN}b contains BSA conjugated at the C-terminal Cys. gp120 and E-gp120 were tested as immunogens. E-gp120 contains electrophilic phosphonates at Lys side chains. E-hapten **1** is the electrophilic phosphonate devoid of a peptide epitope.



Supplemental Fig. S2: Example data showing concentration-dependent gp120 and E-C^{LIN}b binding by antibodies from an infected human (A,B), mice immunized with gp120 (C,D) and mice immunized with E-gp120 (E,F).

(A) and (B), respectively, binding by IgM or IgG from an HIV-infected subject in the presence or absence of E-hapten 1 (100 μ M; shown as “+ 1”). Low-level C^{LIN} recognition by antibodies from non-infected humans was described previously [3].

(C) and (D), respectively, binding by IgM or IgG in the presence of E-hapten **1** (100 μ M) from a Study 2a mouse immunized with gp120.

(E) and (F), respectively, binding by IgM or IgG in the presence of E-hapten **1** (100 μ M) from a Study 2b mouse immunized with E-gp120.

SUPPLEMENTAL REFERENCES

1. Paul S, Planque S, Zhou YX, Taguchi H, Bhatia G, Karle S, *et al.* **Specific HIV gp120-cleaving antibodies induced by covalently reactive analog of gp120.** *J Biol Chem* 2003,278:20429-20435.
2. Planque S, Salas M, Mitsuda Y, Siencyk M, Escobar MA, Mooney JP, *et al.* **Neutralization of genetically diverse HIV-1 strains by IgA antibodies to the gp120-CD4-binding site from long-term survivors of HIV infection.** *AIDS* 2010,24:875-884.
3. Planque SA, Mitsuda Y, Nishiyama Y, Karle S, Boivin S, Salas M, *et al.* **Antibodies to a superantigenic glycoprotein 120 epitope as the basis for developing an HIV vaccine.** *J Immunol* 2012,189:5367-5381.
4. Nishiyama Y, Planque S, Mitsuda Y, Nitti G, Taguchi H, Jin L, *et al.* **Toward effective HIV vaccination: induction of binary epitope reactive antibodies with broad HIV neutralizing activity.** *J Biol Chem* 2009,284:30627-30642.
5. Lang W, Perkins H, Anderson RE, Royce R, Jewell N, Winkelstein W, Jr. **Patterns of T lymphocyte changes with human immunodeficiency virus infection: from seroconversion to the development of AIDS.** *J Acquir Immune Defic Syndr* 1989,2:63-69.
6. Paul S, Karle S, Planque S, Taguchi H, Salas M, Nishiyama Y, *et al.* **Naturally occurring proteolytic antibodies: selective immunoglobulin M-catalyzed hydrolysis of HIV gp120.** *J Biol Chem* 2004,279:39611-39619.
7. Sapparapu G, Planque S, Mitsuda Y, McLean G, Nishiyama Y, Paul S. **Constant domain-regulated antibody catalysis.** *J Biol Chem* 2012,287:36096-36104.
8. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, *et al.* **Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1.** *Science* 2010,329:856-861.
9. Planque S, Bangale Y, Song XT, Karle S, Taguchi H, Poindexter B, *et al.* **Ontogeny of proteolytic immunity: IgM serine proteases.** *J Biol Chem* 2004,279:14024-14032.
10. Edmonds TG, Ding H, Yuan X, Wei Q, Smith KS, Conway JA, *et al.* **Replication competent molecular clones of HIV-1 expressing Renilla luciferase facilitate the analysis of antibody inhibition in PBMC.** *Virology* 2010,408:1-13.
11. Gorantla S, Sneller H, Walters L, Sharp JG, Pirruccello SJ, West JT, *et al.* **Human immunodeficiency virus type 1 pathobiology studied in humanized BALB/c-Rag2-*l*-gammac-*l*- mice.** *J Virol* 2007,81:2700-2712.
12. Gorantla S, Makarov E, Finke-Dwyer J, Castanedo A, Holguin A, Gebhart CL, *et al.* **Links between progressive HIV-1 infection of humanized mice and viral neuropathogenesis.** *Am J Pathol* 2010,177:2938-2949.
13. Kitchen SG, Levin BR, Bristol G, Rezek V, Kim S, Aguilera-Sandoval C, *et al.* **In vivo suppression of HIV by antigen specific T cells derived from engineered hematopoietic stem cells.** *PLoS Pathog* 2012,8:e1002649.