Enhanced HIV-1 immunotherapy by commonly arising antibodies that target virus escape variants

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Antibody–mediated immunotherapy is effective in humanized mice when combinations of broadly neutralizing antibodies (bNAbs) are used that target nonoverlapping sites on the human immunodeficiency virus type 1 (HIV-1) envelope. In contrast, single bNAbs can control simian–human immunodeficiency virus (SHIV) infection in immune-competent macaques, suggesting that the host immune response might also contribute to the control of viremia. Here, we investigate how the autologous antibody response in intact hosts can contribute to the success of immunotherapy. We find that frequently arising antibodies that normally fail to control HIV-1 infection can synergize with passively administered bNAbs by preventing the emergence of bNAb viral escape variants.

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To determine whether HIV-1 can escape from all three antibodies when they are administered sequentially, we treated HIV-1YU2–infected mice with bNAbs starting with PG16 alone, and added 3BNC117 after 14 d, and 10–1074 after 28 d (Fig. 1A). We found a transient reduction (0.18 log10 to 0.78 log10) of the viral load shortly after each antibody was administered, followed by rapid rebound to baseline viremia (day 42; +0.14 log10 compared with day 0; Fig. 1A). Thus, sequential antibody administration differs from co-administration of the same tri-mix in that sequential therapy fails to control viral replication.

Figure 1. Sequential treatment of HIV-1YU2–infected humanized mice with bNAbs selects for triple-escape mutants. (A) HIV-1YU2–infected mice were sequentially treated with PG16 (orange), PG16, and 3BNC117 (green) and finally with the tri-mix consisting of PG16, 3BNC117, and 10–1074 (blue) as indicated. Graph shows the log10 change in viral RNA copies in plasma plotted on the y-axis and time in days after starting treatment on the x-axis. The red line shows the mean of changes in viral load. Individual mice IDs are listed at the right. (B) gp120 envelope sequence analysis before and after 14, 28, and 42–49 d of treatment revealed the emergence of HIV-1YU2 escape variants at the respective target sites of the bNAbs (i.e., PNGS at position N160 for PG16; 280–282 and 458 for 3BNC117; PNGS at position N332 for 10–1074). Each dotted line represents an independent sequence and changes to gp120YU2 are shown in bold. Red letters and gray highlights indicate regions corresponding to known escape sites as identified in previous monotherapy experiments (Klein et al., 2012; Horwitz et al., 2013). Residues in HIV-1YU2 (top) were numbered according to HXBc2 (bottom). Presented data were obtained from five treated mice in a single experiment, and sequence information was retrieved and analyzed from at least three mice at each indicated time point.
Failure to suppress viremia with sequential tri-mix administration suggested that this form of therapy selects for viral variants that are resistant to all three antibodies (Fig. 1 A). Consistent with this idea, viral envelope sequence analysis at day 0, 14, 28, and 42–49 revealed sequential development of specific antibody-resistant HIV-1YU2 escape variants (Fig. 1 B). For example, 14 d after starting PG16 therapy, all gp120 sequences analyzed carried mutations at position N160 or T162 that remove the epitope targeted by PG16 (Fig. 1 B). Sequential addition of 3BNC117 and 10–1074 selected for viral escape variants (Fig. 1 B).

Figure 2. Infection of humanized mice with HIV-1YU2 triple mutant (HIV-1YU2TM2). (A) Viral loads in humanized mice infected with WT HIV-1YU2 (blue) and HIV-1YU2TM2 (green) at day 34 after infection. P-value was determined using a two-sided Mann-Whitney U test. **, P < 0.001. Plot shows data of 30 infected mice of 1 representative experiment of 2 performed. (B) Graph shows viral RNA copies/ml (y-axis) versus days after infection (x-axis) for HIV-1YU2TM2–infected humanized mice. Each black line represents a single mouse, and the red line represents the geometric mean. (C) env sequence analysis of individual mice that are shown in (B). Time of sequence analysis is indicated in days after infection. (D) Tri-mix (PG16, 3BNC117, 10–1074) therapy in mice infected with HIV-1YU2 or HIV-1YU2TM2 (right). Changes in viral load in ∆log_{10} (y-axis) plotted against days after infection (x-axis) compared with baseline (day 0). Each black line represents a single mouse and the red line illustrates the mean. Treatment response in mice infected with HIV-1YU2 or HIV-1YU2TM2 was analyzed in parallel in a single experiment and each group consisted of at least five individual mice (D).
variants that carry mutations in all three antibody target sites (Fig. 1 B; Klein et al., 2012; Horwitz et al., 2013). Thus, sequential triple bNAb therapy selects for HIV-1YU2 variants that are resistant to all three bNAbs.

To determine whether tri-mix resistant HIV-1YU2 retains infectivity we compared infection with WT HIV-1YU2 and a variant harboring the N160K, N332K, and G458D mutation (HIV-1YU2TM2). 34 d after infection, HIV-1YU2TM2-infected mice showed geometric mean viral loads of 4.2 log10 and 5.42 log10, respectively (P = 0.0003; Fig. 2 A). Viremia was long lasting in HIV-1YU2TM2-infected mice (Fig. 2 B) and in most cases the N160K, N332K, and G458D mutations were maintained even in the absence of antibody selection pressure (Fig. 2 C). Finally, HIV-1YU2TM2-infected mice were resistant to tri-mix therapy (Fig. 2 D). We concluded that HIV-1YU2 can escape from sequential tri-mix therapy in vivo without measurable loss of infectivity or impaired viral fitness in humanized mice.

To determine whether chronically SHIVAD8-infected NHPs or HIV-1–infected humans harbor antibodies that might neutralize bNAb-resistant variants, we assayed plasma/serum samples for neutralizing activity against escape variants in vitro. Plasma samples from chronically SHIVAD8–infected macaques (Shingai et al., 2012, 2013) were tested against WT SHIVAD8 and SHIVAD8 variants. The SHIVAD8 variants carried either an N332K mutation (SHIVAD8N332K) that rendered the virus resistant to the bNAb 10–1074 or the G458D mutation (SHIVAD8G458D) that strongly reduced (200-fold) sensitivity to the bNAb 3BNC117. In addition, we included an SHIVAD8 variant that harbored both mutations (N332K-G458D; SHIVAD8DM). Although plasma from 16 SHIV AD8–infected macaques showed varying levels of neutralizing activity against the WT virus SHIVAD8 (Fig. 3 A; ID50), a significant increase in neutralizing activity was detected for bNAb-resistant variants of HIV-1YU2 with mutations that naturally arose in vivo experiments (single mutations: N160K, N332K, and N280Y; HIV-1YU2 triple mutations: TM1–3; Fig. 4 B; Klein et al., 2012; Horwitz et al., 2013). Only one of the tier-1 neutralizing antibodies showed activity against the WT HIV-1YU2 at a very high concentration (IC50 = 93 µg/ml; Fig. 4 B). In contrast, three V2 loop–, four V3 loop–, two CD4bs–, and one CD4i–directed antibodies showed activity against the HIV-1YU2 mutant viruses with IC50 as low as 0.5 µg/ml (Fig. 4 B). The three V2

![Figure 3](https://example.com/fig3.png)

**Figure 3.** Increased neutralizing NHP serum activity against bNAb escape variants. (A) Table shows serum neutralization (ID50) of NHPs after established infection with SHIVAD8 (days post infection [PI] as indicated in NHP ID). Neutralizing activity was measured against SHIVAD8 wt (gray), N332K, and G458D single mutants (blue), as well as an SHIVAD8 double mutant (double; N332K and G458D, blue). NHP plasma was also tested against WT HIV-1YU2 (gray) and HIV-1YU2 triple mutants TM1–3 (N160K, N332K, and N280Y; HIV-1YU2 triple mutations: TM1–3; Fig. 3 A; ID50). Neutralizing activity was measured against the HIV-1YU2 mutant viruses with IC50 as low as 0.5 µg/ml (Fig. 4 B). The three V2

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To determine whether antibodies that neutralize bNAb escape variants can also be elicited by vaccination, we analyzed plasma from NHPs immunized with YU2 gp140-F trimers (Sundling et al., 2010). Although plasma samples from the vaccinated animals showed little or no neutralizing activity against HIV-1 YU2 (WT; Fig. 4 C), there was a significant increase in neutralizing activity against HIV-1 YU2 escape variants. Loop-directed antibodies were active against HIV-1 YU2 N332K, HIV-1 YU2 TM1, and HIV-1 YU2 TM2. The four V3 loop-directed antibodies showed activity against HIV-1 YU2 N280Y, HIV-1 YU2 N160K, HIV-1 YU2 N332K, as well as all three HIV-1 YU2 TMs. Finally, at least one of each of the antibodies targeting CD4bs and CD4i was active against the HIV-1 YU2 TMs in vitro (Fig. 4 B).
in neutralizing activity against the HIV-1YU2 triple mutants (HIV-1YU2TM1, HIV-1YU2TM2; Fig. 4 C). To determine whether macaque antibodies recognizing the V3 loop might account for this activity, we tested 6 different anti-V3 mAbs isolated from these immunized macaques against WT HIV-1YU2, HIV-1YU2N280Y, HIV-1YU2N160K, HIV-1YU2N332K, and two HIV-1YU2TM (Fig. 4 D). Of the 6 antibodies we tested, 2 showed high levels of activity against HIV-1YU2 (WT; IC50 of 33.8 and 49.6 µg/ml), whereas the other four did not reach an IC50 when measured up to a concentration of 50 µg/ml. In contrast, 5 out of these 6 V3 loop-directed antibodies showed activity against the HIV-1YU2 single mutants. Moreover, neutralization activity was strongly increased against both HIV-1YU2TM as reflected by IC50 in the range of 1 µg/ml (Fig. 4 D). Thus, although tier-1 neutralizing human and macaque anti–HIV-1 antibodies do not neutralize WT HIV-1YU2, they are active against bNAb escape variants in TZM-bl assays in vitro.

Of the tested human tier-1 neutralizing antibodies, 10–188 and 1–79 demonstrated the best neutralizing activity against the triple mutated HIV-1YU2 (Fig. 4 B). Both antibodies recognize the crown of the V3 loop. Notably, 10–188 and 1–79 bind to different regions of the V3 loop: 1–79 targets the hydrophobic V3 crown at aa 307, 309, and 317 (CRADLE-type; Burke et al., 2009; Almond et al., 2010; Jiang et al., 2010; and unpublished data) and 10–188 targets the V3 β-turn at aa 312–314 (LADLE-type; Burke et al., 2009). To determine whether similar antibodies are present in NHPs chronically infected with SHIVAD8, we measured reactivity of plasma samples against selected peptides by ELISA (Fig. 5; Totrov et al., 2010). All tested NHP sera showed activity against both peptides, demonstrating the presence of CRADLE- and LADLE-type antibodies in SHIVAD8-infected NHPs (Fig. 5).

To examine the possibility that HIV-1–directed tier-1 neutralizing antibodies can actively suppress viremia in vivo, we infected humanized mice with HIV-1YU2 (WT) or with HIV-1YU2TM2. Infected mice were treated with either of the tier-1 neutralizing human V3 loop-directed antibodies 10–188 or 1–79. Although only a small effect on the viral load was detected in HIV-1YU2–infected mice (Fig. 6 A, left), a reduction in viremia was observed in mice infected with the mutant virus HIV-1YU2TM2 (Fig. 6 A, middle and right). We conclude that the tier-1 neutralizing anti-V3 loop antibodies 10–188 and 1–79 do not alter the viral load in HIV-1YU2–infected mice, but can suppress HIV-1YU2TM2 infection in vivo.

To determine whether 10–188 and 1–79 exerted selective pressure on HIV-1YU2 and/or HIV-1YU2TM2, we cloned and sequenced cDNA encoding gp120 from HIV-1YU2 and HIV-1YU2TM2–infected mice treated with these antibodies. Although 10–188 showed little activity against HIV-1YU2 in vitro (IC50 93 µg/ml) and had no measurable effect on the viral load in HIV-1YU2–infected mice, 2 out of the 4 mice showed mutations in the β-turn in the crown of the V3 loop, which is the epitope targeted by 10–188 (Zolla-Pazner and Cardozo, 2010; Mouquet et al., 2011; Fig. 6 B, top). In addition, all gp120 sequences analyzed from HIV-1YU2TM2–infected mice (2 animals) carried mutations in the same region (Fig. 6 B, bottom). In the case of HIV-1YU2–infected mice treated with 1–79, we also detected mutations in the V3 crown in some but not all of the sequences in the 3 animals we analyzed, indicating incomplete selection (Fig. 6 C). In contrast, all sequences obtained from HIV-1YU2TM2–infected mice treated with 1–79 harbored the same K305R mutation (Fig. 6 C).

HIV-1YU2TM2 is an engineered virus that carries bNAb escape mutations that naturally occurred in mice treated with single antibodies (Klein et al., 2012; Horwitz et al., 2013). However, it is not a naturally arising strain that might also contain compensatory mutations as part of a heterogeneous swarm.

**Figure 5.** Presence of V3-specific antibodies in plasma of SHIVΔ68–infected NHPs. Values reflect OD405 measurements (values >0.3 were considered positive). PI, post infection (days). mAbs were measured at 10 µg/ml. Plasma samples were measured at a titer of 1:100. ID50 titers of plasma are highlighted in red (>2.5), orange (1–2.5), and yellow (0.3–1).
Figure 6. Suppression of HIV-1\textsubscript{YU2\textsuperscript{TM2}} but not HIV-1\textsubscript{YU2} viremia by V3-loop antibodies 10–188 and 1–79. (A, left and middle) Graphs show changes in viral load in response to 10–188 or 1–79 treatment in $\Delta$log$_{10}$ (y-axis) plotted against days after infection (x-axis) compared with baseline (day 0) for mice infected with HIV-1\textsubscript{YU2} or HIV-1\textsubscript{YU2\textsuperscript{TM2}}. Each black line represents a single mouse, and the red line illustrates the mean. Blue colored symbols indicate a viral load measurement below 200 copies/ml. (A, right) Statistical analysis to determine group differences between HIV-1\textsubscript{YU2} (green) and HIV-1\textsubscript{YU2\textsuperscript{TM2}} (orange) using repeated measures ANOVA; 10–188, $P = 0.003$; 1–79, $P = 0.032$. (B and, C) gp120 sequences obtained from HIV-1\textsubscript{YU2}– or HIV-1\textsubscript{YU2\textsuperscript{TM2}}–infected mice treated with 10–188 (B) or 1–79 (C) between days 14 and 28. Residues in HIV-1\textsubscript{YU2} (top) were numbered according to HXBc2 (bottom). Treatment response of 10–188 and 1–79 in mice infected with HIV-1\textsubscript{YU2} or HIV-1\textsubscript{YU2\textsuperscript{TM2}} was analyzed in a single experiment in which each group consisted of at least four individual mice and sequence data were obtained from at least two mice per group.
et al., 2012). In contrast to monotherapy with any of the three antibodies alone (Figs. 6 A and 7 A), both combinations produced a prolonged and sustained drop in viremia (Fig. 7, B–D). Moreover, the combination of 10–1074 and 1–79 continued to suppress viremia below the limit of detection, with no viral escape during 6 wk of therapy (Fig. 7 C). Escape from 10–1074 monotherapy was associated with N332K/S in 6 out of 6 mice analyzed (Fig. 7 E). When rebound occurred with the combination of 10–1074 and 10–188, it was associated with selection of mutations in both the 10–1074 and 10–188 target sites at position 332 and the β-turn of the V3 crown (position 312–315), respectively (Fig. 7 F).

We conclude that tier-1 neutralizing antibodies with little demonstrable activity against primary isolates in vitro can make significant contributions to control HIV-1 infection when combined with potent bNAbs in vivo.

Most individuals infected with HIV-1 develop antibodies that neutralize autologous but not primary heterologous viral strains (Doria-Rose et al., 2009; Simek et al., 2009). As a result of the rapid rate of viral evolution in the host, the effect of these antibodies on effectively controlling viremia is limited (Schmitz et al., 2003; Miller et al., 2007; Gauvin et al., 2009a,b; Huang et al., 2010). Nevertheless, these antibodies put selective pressure on HIV-1 as indicated by the emergence of antibody-resistant escape variants (Wei et al., 2003). Escape from tier-1 neutralizing antibodies frequently involves changes that indirectly conceal the epitope (Ly and Stamatatos, 2000; Wei et al., 2003; Pinter et al., 2004; Blish et al., 2008; Bunnik et al., 2008;
Bosch et al., 2010; O’Rourke et al., 2010). For example, the glycan at position N332 can shield the V3 loop from tier-1 neutralizing anti-V3 loop antibodies, but does not alter the antibody target sequence directly (Wei et al., 2003; McCaffrey et al., 2004). Similarly, glycans at position N276 or N301 can shield the CD4bs from tier-1 neutralizing anti-CD4bs antibodies (Koch et al., 2003; McGuire et al., 2013). These changes are often favored over direct changes in the target site that might interfere with viral fitness. For example, a glycan can be added to shield the V3 loop without reducing infectivity. This shielding mechanism appears to be preferred over escape mutations in the highly conserved crown of the V3-loop that could interfere with co-receptor binding and alter viral fitness (Zolla-Pazner and Cardozo, 2010). However, the same glycans that shield V3 also make important contributions to the epitopes of some of the most potent bNAbs, such as 10–1074 or PGT121 (Walker et al., 2011; Mouquet et al., 2012). These antibodies have profound effects on viremia in NHPs but less so in immune compromised humanized mice when used as a single reagent (Klein et al., 2012; Barouch et al., 2013; Shingai et al., 2013, Fig. 7 A). Our data suggest that one of the reasons that escape from 10–1074 or PGT121 immunotherapy in macaques is difficult might be because the autologous antibodies, which are present in macaques but not in humanized mice, prevent escape by the N332 mutation. Interestingly, several of the 10–1074-escape variants in SHIV AD8-infected NHPs carried a mutation that removes the potential N-linked glycosylation site (PNGS) at position 332 but also generated a new PNGS at position 334 (Shingai et al., 2013). Therefore, the glycan was shifted from N332 to N334, allowing escape from 10–1074 as well as retaining a glycan at the V3-stem that is likely to protect the V3-loop from autologous antibodies.

Our experiments indicate that HIV-1 infection is in part more readily controlled during immunotherapy in immunocompetent hosts because escape from bNAbs can create holes in the glycan shield that render the virus susceptible to otherwise ineffective autologous antibodies that are present in nearly all infected individuals. Thus, although antibodies with tier-1 neutralizing activity, such as V3 loop-directed monoclonals (Hoe et al., 2010), generally display weak and sporadic neutralizing activity against most tier-2 viruses, they can effectively synergize with bNAbs in anti–HIV-1 immunotherapy.

**MATERIALS AND METHODS**

**Human samples.** Serum samples from HIV-1–infected individuals were collected under informed consent and in accordance with the Institutional Review Board (IRB) protocol number 09–281, University of Cologne, Cologne, Germany. All samples were heat-inactivated for 1 h at 56°C and the IgG fraction was purified with Protein G–Sepharose 4 Fast Flow (GE Healthcare). Sterile filtration and buffer exchange to PBS was performed for all IgGs before testing for neutralizing activity.

**Nonhuman primate samples.** Plasma samples were obtained from nonhuman primates (NHPs; *Macaca mulatta*) 90–198 wk after infection with SHIVAD8 (Shingai et al., 2012, 2013) or from uninfected NHPs (*Macaca mulatta*) before and after immunization with soluble YU2 gp140-F trimers (Yang et al., 2002), as previously described (Sundling et al., 2010). SHIVAD8–infected macaques were housed in a biosafety level 2 National Institute of Allergy and Infectious Disease (NIAID) facility and cared for in accordance with standards of the American Association for Accreditation of Laboratory Animal Care (AAALAC) in AAALAC-accredited facilities. All animal procedures were performed according to NIAID animal protocol LMM32, approved by the Institutional Animal Care and Use Committees of NIAID/NIH. The animals used for immunizations were housed at the AAALAC-accredited Astrid Fagraeus Laboratory animal facility in Stockholm in compliance with the guidelines of the Swedish Board of Agriculture. Isolation of mAbs from NHPs was performed essentially as previously described (Sundling et al., 2012a) but by sorting for total Env–specific memory B cells and using optimized primers for amplification of NHP VDJ sequences (Sundling et al., 2012b). For antibody expression, equal amounts of heavy- and light-chain plasmid DNAs were transfected into FreeStyle 293F cells and the secreted IgGs were purified by protein A–Sepharose columns (GE Healthcare). The specificities of these V3 loop–directed antibodies were determined by ELISA binding analyses using a set of different YU2 Env probes.

**HIV-1 infection.** HIV-1 YU2 and HIV-1 YU2TM2 were produced by transiently transfecting HEK 293T/17 cells using a construct consisting of HIV-1 NL4/3 backbone carrying the HIV-1 YU2 envelope (Zhang et al., 2002). HIV-1 YU2TM2 harbors the mutations N160K, N332K, and G458D that were introduced by site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis kit (Agilent Technologies). The concentration of the virus was determined by measuring p24 using the Alliance HIV-1 p24 Antigen ELISA kit (PerkinElmer). Humanized mice were infected by intraperitoneal injection of HIV-1 YU2 or HIV-1 YU2TM2. All experiments were performed under approval of the Institutional Review Board and the Institutional Animal Care and Use Committee of The Rockefeller University.

**Production and administration of mAbs.** For mouse treatment experiments we used the following mAbs: PG16 (V1/V2; Walker et al., 2009), 10–1074 (V3-stem; Mouquet et al., 2012), 3BNC117 (CD4bs; Scheid et al., 2011), 10–188 (V3-crown; Mouquet et al., 2011), and 1–79 (V3-crown; Scheid et al., 2009). With the exception of 3BNC117, all antibodies were produced by transiently transfected HEK 293-6E cells with equal amounts of immunoglobulin heavy and light chain expression vectors. After 7 d, the supernatant was harvested and antibodies were concentrated by ammonium sulfate precipitation. IgG was purified with Protein G–Sepharose 4 Fast Flow. 3BNC117 was produced in CHO cells by Celladex Therapeutics, Inc. All antibodies were filtered (Ultrafree-CL Centrifugal Filters, 0.22 μm; Millipore) and administered s.c. to humanized mice. For treatment of HIV-1–infected mice, 1 mg (each) of 3BNC117, PG16, and 10–1074 was injected as loading dose followed by 0.5 mg of each antibody/mouse twice a week. 10–188 and 1–79 were injected at 4 mg/mouse for the loading dose followed by 2 mg/mouse twice a week.

**HIV-1 plasma viral load.** Plasma viral load in HIV-1–infected mice was determined as previously described (Klein et al., 2012).

**HIV-1 envelope glycoprotein sequence analysis.** Total RNA from 100 μl EDTA-plasma was extracted using the MiniElute Virus Spin kit (QIAGEN)
Electrophoretic Mobility Shift Assay

Amplification of HIV-1 gp120 sequences was performed by a double-nested PCR, using the Expand High Fidelity PCR System (Roche). Primers for first round PCR were FW_5 and Rev_5 and for second round PCR FW_5 and Rev_5. Primers were gel purified and cloned into the pCR-4-TOPO vector (Invitrogen) and transfected into MAX Efficiency Stbl2 Competent Cells (Life Technologies). Individual colonies were sequenced with 5 different primers and assembled according to HXk2 (http://www.hiv.lanl.gov/content/sequence/LOCATE/locate.html).

Peptide ELISA. ELISA plates (SpectraWell; Roche) were coated at 37°C for 1.5 h with 1 µg/ml biotinylated peptides (Clade B CRADLE peptide, Cyclic Biotin-ACQAFYASSPRKSIHIGACA-OH; Clade B LADLE peptide, Cyclic Biotin-RCRIHIGPGRAFYACG-OH; Clade B TM1 peptide, Cyclic Biotin-RCRIHIGPGRAFYACG-OH; Clade B TM2 peptide, Cyclic Biotin-RCRIHIGPGRAFYACG-OH). Plates were washed 6 times with PBS containing 0.05% Tween-20, pH 7.4, and incubated for 1.5 h (37°C) with 100 µl/well and each sample was run in duplicate.

Neutralization. Neutralizing activities of NHP plasma samples, purified IgGs, and mAbs were determined in a TZM.bl neutralization assay (Li et al., 2005; Seaman et al., 2010). Mutations in HIV-1 and SHIV envelope glycoprotein sequences were produced using the QuikChange Site-Directed Mutagenesis kit (Agilent Technologies) according to the manufacturer’s instructions and used for pseudovirus production. SHIV and HIV-1 pseudovirus mutants included the single mutations SHIV_160 (N332K), SHIV_160 (G458D), HIV-1YU2 (N160K), HIV-1YU2 (N332K), and HIV-1YU2 (N280K), as well as the double and triple mutations SHIV_160 (N160K, N332K), HIV-1YU2 (N160K, N332K, N280K), HIV-1YU2 (N160K, N332K, N280Y), HIV-1YU2 (N160K, N332K, G458D), and HIV-1YU2 (N162I, N332K, N279K).

Statistics. Statistical significance between IgG neutralizing activity against HIV-1YU2m and HIV-1YU2TM (Fig. 4 A), and differences in viral load between HIV-1YU2m and HIV-1YU2TM–infected mice (Fig. 2 A) was determined by performing a two-sided Mann–Whitney U test in which P < 0.05 was considered significant. Statistical analysis for comparing neutralizing activity of NHP sera against SHIV and HIV-1 variants was performed by Friedman test followed by Dunn multiple comparison. Significant differences in log10 changes of viral load between mice treated with 10–1074 + 1–79, as well as HIV-1YU2m and HIV-1YU2TM–infected mice treated with 10–188 or 1–79 were determined by using repeated measures ANOVA with a Bonferroni post-hoc test considering P < 0.05 significant. Sample size of humanized mice in antibody treatment experiments was estimated based on previous results testing single antibodies in HIV-1–infected humanized mice (Hovrutz et al., 2013; Klein et al., 2012). All analyses were performed using GraphPad Prism version 5.0b for Mac OS X, GraphPad Software.

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