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Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission

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The neutralizing activity of anti–HIV-1 antibodies is typically measured in assays where cell–free virions enter reporter cell lines. However, HIV–1 cell to cell transmission is a major mechanism of viral spread, and the effect of the recently described broadly neutralizing antibodies (bNAbs) on this mode of transmission remains unknown. Here we identify a subset of bNAbs that inhibit both cell–free and cell–mediated infection in primary CD4+ lymphocytes. These antibodies target either the CD4–binding site (NIH45–46 and 3BNC60) or the glycan/V3 loop (10–1074 and PGT121) on HIV–1 gp120 and act at low concentrations by inhibiting multiple steps of viral cell to cell transmission. These antibodies accumulate at virological synapses and impair the clustering and fusion of infected and target cells and the transfer of viral material to uninfected T cells. In addition, they block viral cell to cell transmission to plasmacytoid DCs and thereby interfere with type–I IFN production. Thus, only a subset of bNAbs can efficiently prevent HIV–1 cell to cell transmission, and this property should be considered an important characteristic defining antibody potency for therapeutic or prophylactic antiviral strategies.

Abbreviations used: bNAb, broadly neutralizing antibody; IC50, 50% inhibitory concentration; MPER, membrane–proximal external region; NVP, nevirapine; pDC, plasmacytoid DC; T/F, transmitted/founder.

HIV–1–infected individuals produce high titers of antibodies against the virus, but only a small fraction of the patients develop a broadly neutralizing serologic activity, generally after 2–4 yr of infection (Sather et al., 2009; Simsek et al., 2009; Stamatas et al., 2009; Walker et al., 2011; McCoy and Weiss, 2013). The serologic anti–HIV–1 activity in some of these individuals can be accounted for by a combination of antibodies targeting different sites on the HIV–1 envelope spike (Scheid et al., 2009; Bonsignori et al., 2012; Klein et al., 2012a; Georgiev et al., 2013) and in others, by a predominant highly expanded clone (Scheid et al., 2011; Walker et al., 2011; Burton et al., 2012; McCoy and Weiss, 2013). Although the presence of broad neutralizing activity does not correlate with a better clinical outcome, passive transfer of broadly neutralizing antibodies (bNAbs) can protect against infection in macaques or in mouse models (Hessell et al., 2009; Pietzsch et al., 2012; McCoy and Weiss, 2013). In addition, bNAbs can suppress viremia in humanized mice (Klein et al., 2012b). Moreover, antibodies against the HIV–1 envelope spike appear to be the unique correlate of protection in the RV144 HIV–1 vaccine trial (Haynes et al., 2012). Therefore, it has been proposed that vaccines that would elicit such antibodies may be protective against the infection in humans.

The recent development of efficient methods for cloning of human anti–HIV–1 antibodies from single cells (Scheid et al., 2009) led to the discovery of dozens of new bNAbs and new targets for neutralization (Burton et al., 2012; McCoy and Weiss, 2013). The new antibodies target at least six different sites of vulnerability on the HIV–1 spike. These include the CD4–binding site (VRC01, NIH45–46, 3BNC60/117, and CH103), the glycan–dependent V1/V2 loops (PG16 and PGT145) and V3 loop (PGT121, PGT135, and 2F5).

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In addition, this form of dissemination appears to be less susceptible to inhibition by antiretroviral drugs than cell-free virus transmission (Chen et al., 2007; Sigal et al., 2011; Abela et al., 2012).

Cell to cell spread of HIV-1 is in large part mediated through virological synapses, where viral particles accumulate at the interface between infected cells and targets (Sattentau, 2011; Dale et al., 2013). Synapse formation involves HIV-1 Env-CD4 co-receptor interactions and requires cytoskeletal rearrangements and adhesion molecules (Sattentau, 2011; Dale et al., 2013). Here, we examined the antiviral activity of a panel of 15 newly identified bNAbs targeting all known sites of vulnerability in conventional neutralization and cell to cell transmission assays. We show that only a subset of the bNAbs that target the CD4-binding site or the glycan/V3 loop efficiently neutralize cell to cell viral transfer in co-cultures of infected T cells with primary lymphocytes. We further characterized the antiviral mechanisms used by the effective antibodies and report that they affect multiple steps of viral cell to cell transfer.
We initially assessed the efficacy of the selected bNAbs to inhibit HIV-1 cell to cell transmission in culture using flow cytometry (Sourisseau et al., 2007). Primary CD4+ T cells infected with either NLAD8 or NL4.3 HIV-1 strains were incubated with bNAbs before co-culture with autologous target cells labeled with FarRed. Infection of target cells was measured by Gag expression. In the absence of bNAbs, 15–50% of the recipient cells were Gag+ after 48 h (representative experiments are shown in Fig. 1 a). Under these conditions, the contribution of cell-free virus to infection was negligible because Gag expression by the recipient cells was abrogated by separation of donors and targets in a transwell chamber or when cultures were gently shaken to avoid prolonged contacts (Sourisseau et al., 2007). Moreover, Gag expression in target cells was caused by de novo synthesis because it was significantly reduced in the presence of reverse transcription inhibitor (nevirapine [NVP]; Fig. 1 a).

We tested a panel of 15 bNAbs to investigate their efficacy in inhibiting HIV-1 cell to cell transmission in culture using flow cytometry. The median inhibitory concentrations (IC50) were calculated with at least four independent experiments. Bold indicates IC50 < 0.5 µg/ml; single underline indicates IC50 = 2–10 µg/ml; and double underline indicates IC50 = 10–15 µg/ml. X, no neutralization (<25% inhibition at 15 µg/ml).

### RESULTS AND DISCUSSION

We investigated the mode and kinetics of cell to cell transmission of two T/F HIV-1 strains (WITO or SUMA). Infected cells were preincubated for 1 h with different doses of bNAbs before co-culture with autologous target cells stained with FarRed dye. After 48 h, the fraction of productively infected (Gag+) target cells was measured by flow cytometry. The median inhibitory concentrations (IC50) were calculated with cells from three to four independent donors. Bold indicates IC50 < 0.5 µg/ml; single underline indicates IC50 = 0.5–2 µg/ml; and double underline indicates IC50 = 2–10 µg/ml.

The indicated antibodies were tested against cell-free and cell-associated HIV-1 infection as indicated in Fig. 1. Median inhibitory concentrations (IC50) were calculated from at least four independent experiments. The corresponding inhibition curves are displayed Fig. S1. Bold indicates IC50 < 0.5 µg/ml; italics indicate IC50 = 0.5–2 µg/ml; single underline indicates IC50 = 2–10 µg/ml; and double underline indicates IC50 = 10–15 µg/ml. X, no neutralization (<25% inhibition at 15 µg/ml). 

### Table 1.

**Effect of bNAbs on cell-free and cell to cell HIV-1 transmission**

| bNAbs | IC50 (µg/ml) | NL4.3 | NLAD8 |
|-------|---------|-------|-------|
| NIH45-46 | 0.06 | 1.2 | 0.2 | 2.5 |
| 3BNC60 | 0.05 | 0.9 | 0.1 | 2.3 |
| VIRG9 | 0.2 | 7.2a | 0.3 | 12.1a |
| 1NC9 | 0.2 | 4.5a | 0.7 | 12.3a |
| 12A12 | 0.4a | X | 2.6 | X |
| 8ANC195 | 4.0a | X | 5.7a | X |
| 10-1074 | X | X | 0.1 | 1.6 |
| PGT121 | X | X | 0.1 | 1.3 |
| 10-1074GM | X | X | 0.3 | 6.2 |
| 10-996 | X | X | 0.1 | 1.7 |
| 10-1369 | X | X | 0.4 | 10a |
| PGT121 | 0.7a | >15 | 0.05 | 0.5a |
| 3BC176 | 0.7a | X | >15 | X |
| 1E8 | 0.1 | 6.7a | 1.1 | >15 |
| 4E10 | 4.3a | X | >15 | >15 |

The indicated antibodies were tested against cell-free and cell-associated HIV-1 infection as indicated in Fig. 1. Median inhibitory concentrations (IC50) were calculated from at least four independent experiments. The corresponding inhibition curves are displayed Fig. S1. Bold indicates IC50 < 0.5 µg/ml; italics indicate IC50 = 0.5–2 µg/ml; single underline indicates IC50 = 2–10 µg/ml; and double underline indicates IC50 = 10–15 µg/ml. X, no neutralization (<25% inhibition at 15 µg/ml).

### Table 2.

**Antiviral activity of bNAbs on cell to cell transmission of two T/F HIV-1 strains**

| bNAbs | WITO | SUMA |
|-------|-------|-------|
| NIH45-46 | 3.1 | 13.9a |
| 3BNC60 | 3.4 | 3.3 |
| VIRG9 | 14.3a | X |
| 8ANC195 | X | 3.7a |
| 10-1074 | 1.8 | 1.9 |
| PG16 | 0.05 | 0.3 |
| 1E8 | 9.4a | >15 |
| 3BC176 | X | X |

Primary HIV-1 CD4+ T cells were infected with two T/F HIV-1 strains (WITO or SUMA). Infected cells were preincubated for 1 h with different doses of bNAbs before co-culture with autologous target cells stained with FarRed dye. After 48 h, the fraction of productively infected (Gag+) target cells was measured by flow cytometry. The median inhibitory concentrations (IC50) were calculated with cells from three to four independent donors. Bold indicates IC50 < 0.5 µg/ml; single underline indicates IC50 = 0.5–2 µg/ml; and double underline indicates IC50 = 2–10 µg/ml.

The indicated antibodies were tested against cell-free and cell-associated HIV-1 infection as indicated in Fig. 1. Median inhibitory concentrations (IC50) were calculated with at least four independent experiments. The corresponding inhibition curves are displayed Fig. S1. Bold indicates IC50 < 0.5 µg/ml; italics indicate IC50 = 0.5–2 µg/ml; single underline indicates IC50 = 2–10 µg/ml; and double underline indicates IC50 = 10–15 µg/ml. X, no neutralization (<25% inhibition at 15 µg/ml). 

The first step in cell to cell spread is the formation of conjugates between infected cells and targets, a process which is dependent on Env (Massanella et al., 2009). To examine one inactive (8ANC195) bNAbs are shown in Fig. 1 b. The most active antibodies in the cell to cell transmission assay were NIH45-46 and 3BNC60, which target the CD4bs, and conally related anti-glycan/V3 antibodies, 10-1074, 10-996, and PGT121 (Table 1 and Fig. S1). IC50s < 0.9–2.5 µg/ml. Time of addition experiments showed that NIH45-46 and 3BNC60 remained strongly active when added up to 6 h after the beginning of the co-culture (not depicted). However, the antibody concentrations required to inhibit cell to cell transmission were 10–20-fold higher than cell-free transmission (Table 1 and Fig. S1). As previously reported (Abela et al., 2012), VRC01, a less active clonal relative of NIH45-46, was only partially effective in blocking cell to cell transmission, with 65 and 85% inhibition of NLAD8 and NL4.3, respectively, at concentrations of 15 µg/ml. NIH45-46 and 3BNC60 also inhibited viral spread when CD4+ T cells were co-cultured with MOLT cells chronically infected with BaL (not depicted). In contrast, most of the other antibodies tested were relatively ineffective, including the two anti-MPER antibodies and PG16. 1E8 was only partially active against NL43, with 75% inhibition at a concentration of 15 µg/ml, and PG16 showed only 60% inhibition at this concentration.

Some of the bNAbs were also tested for their ability to block cell to cell transmission of two transmitted/founder (T/F) HIV-1 viruses (Salazar-Gonzalez et al., 2009; Ochesenbauer et al., 2012). The IC50s of the antibodies against these viruses were generally similar to those observed with NLAD8 (Table 2). The more active bNAbs against T/F viruses were PG16, 10-1074, and 3BNC60 (Table 2).
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Figure 2. Effect of bNAbs on formation of conjugates of infected and target cells. (a) CFSE-stained MOLT cells chronically infected with HIV-1 NL4.3 or BaL strains were preincubated for 1 h with 15 µg/ml NI45-46 bNAb and then co-cultured with FarRed-stained primary CD4+ T cells. After 2 h, conjugates of donors and targets (CFSE-FarRed+) were quantified by flow cytometry. (left) Flow plots from one representative experiment are shown. (right) Frequency of conjugates with noninfected (NI) and NL4.3- or BaL-infected MOLT cells in the absence of bNAb. Each dot represents an experiment with primary target T cells from independent donors. The bars represent SD. *, P < 0.05. (b) Conjugate formation in the presence of the indicated bNAbs (15 µg/ml) as determined in panel a. Data are shown from six independent experiments. The percentage of cell-forming conjugates is shown. *, P < 0.05 (Wilcoxon matched pairs test). (c) HeLa cells stably expressing HIV-1 Env (NL4.3) and Tat were preincubated for 1 h with the indicated bNAbs (15 µg/ml) before overnight co-culture with HeLa P4C5 cells, which carry an HIV-1 LTR–β-gal reporter cassette. Upon syncytia formation, Tat will transactivate the HIV-1 LTR. Levels of syncytia were quantified by measuring β-gal activity. Data represent mean ± SD of triplicate samples from six independent experiments. *, P < 0.05.

the mechanism of inhibition of cell to cell transmission by bNAbs, we initially assayed their effects on conjugate formation. Preincubation of NL4.3- or BaL-infected lymphocytes with NIH45–46, 3BNC60, or VRC01 decreased the formation of conjugates, and 10-1074 and PGT121 inhibited the formation of clusters with cells expressing HIV-BaL (Fig. 2, a and b). In contrast, the other bNAbs did not show measurable inhibitory effects (Fig. 2, a and b). Of note, 10E8 increased formation of conjugates with NL4.3 and was ineffective against BaL (Fig. 2, a and b). It is possible that...
10E8, which blocks Env-mediated fusion without affecting Env–CD4 interaction, may stabilize cell conjugates in the absence of cell fusion.

Contact between Env+ cells and target cells can lead to formation of syncytia. Therefore, we assessed the effect of bNAbs on syncytia formation using a quantitative assay, in which HeLa cells expressing Env (from NL4.3) and Tat are mixed with HeLa cells expressing CD4 and HIV-1 LTR–β-gal reporter cassette (P4C5 cells; Schwartz et al., 1994). β-gal expression is induced upon fusion and transfer of Tat between the two cell types. NIH45-46, 3BNC60, and VRC01 blocked fusion as did the anti-gp41 MPER 10E8 (Fig. 2 c). In contrast, bNAbs that failed to inhibit cell to cell transmission of NL4.3 had little or no effect on fusion (Fig. 2 c).

After formation of cell conjugates, viral particles are transferred from infected cells to targets. To measure viral transfer, we examined Gag expression in target cells by flow cytometry. 4 h after initiation of the co-culture, 4–6% of target cells were Gag+ (Fig. 3 a). NIH45–46, 3BNC60, VR.C01, and 10-1074 decreased transfer of NL4.3 and/or NLAD8, whereas the other antibodies were inactive (Fig. 3 a). Immunofluorescence and confocal imaging further demonstrated that preincubation of donor cells with NIH45-46 or 3BNC60 led to accumulation of the bNAb at the virological synapse and to colocalization with Gag (Fig. 3 b). Moreover, an examination of target cells that captured viral particles despite the presence of bNAbs demonstrated that these viruses were generally, if not always, coated with bNAbs (Fig. 3 c).
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findings, it was reported to be poorly active (Chen et al., 2007; Massanella et al., 2009; Martin et al., 2010; Abela et al., 2012). Here, we show that many of the 15 bNAbs tested, including some of the most active antibodies to six different sites of vulnerability on the envelope, were ineffective in HIV-1 cell to cell transmission. However, five bNAbs, isolated from four different patients (NIH45-46, 3BNC60, PGT121, 10-1074, and PG16) blocked the intercellular spread of the tested viruses at IC50s of 0.05–2.5 µg/ml. NIH45-46 and 3BNC60 target the CD4-binding site, whereas 10-1074 and PGT121 bind to a complex epitope composed of glycans and the V3 loop epitope on HIV-1 gp120 (Scheid et al., 2009; Walker et al., 2011; Wu et al., 2011; Kwong and Mascola, 2012; Mouquet et al., 2012; Liao et al., 2013). Of note, 10-1074 and PGT121 efficiently inhibited cell-free and cell to cell transmission of all R5 viruses tested but were inactive against the X4 strain NL4.3. Further work will help determining whether V3-directed bNAbs inhibit other X4 isolates.

The bNAbs inhibiting cell to cell viral spread were those acting at a low concentration against free virus (IC50 < 0.2 µg/ml; Scheid et al., 2009; Walker et al., 2011). This suggests that very high levels of potency in cell-free virion assays are...
required for antibodies to block viral cell–cell spread. But potency alone may not be sufficient, as indicated by 10E8, which fails to reach 80% neutralizing activity in cell to cell transmission despite potencies that are nearly comparable with the effective antibodies in cell-free assays. It is noteworthy that the concentrations required to inhibit cell to cell transmission were at least 10–20-fold higher than for free virus. Similarly, the serum concentrations of bNAbs required to inhibit infection in mouse or monkey models of HIV-1 infection are also 1–2 logs higher than in cell-free assays (Balazs et al., 2012; Klein et al., 2012a; Moldt et al., 2012; Pietzsch et al., 2012). Cell to cell transmission may therefore provide a more reliable method for predicting the potency of bNAbs in vivo.

Our experiments show that the most active bNAbs interfere with cell to cell transmission by efficiently impairing formation of clusters between infected cells and targets, the appearance of syncytia, and transfer of viral material to recipient cells and by accumulating at the virological synapse. Moreover, they also decorate any free viral particles that may still have been captured by target cells. Additional experimentation will be required to determine whether the various bNAbs display differing abilities to accumulate at sites of cell–cell contacts and to impair the formation of virological synapses. It will also be of interest to further examine how these bNAbs interfere with viral cell to cell transmission between myeloid DCs and lymphocytes.

HIV-1 cell to cell transmission is likely playing a predominant role in infected individuals (Murooka et al., 2012). Our results confirm that this mode of viral spread may represent a means to escape the selection pressure exerted by most of the bNAbs (Ganesh et al., 2004; Abela et al., 2012). Our observations may also help explain why some bNAbs like JBC176 are ineffective in vivo (Klein et al., 2012b), whereas others like 10–1074 and a derivative of NIH45–46 (45–46W) suppressed viral loads below detection (Klein et al., 2012b). Consistent with this idea, 10E8, which does not efficiently inhibit HIV-1 cell to cell transmission, failed to suppress viremia in vivo and failed to select antibody-resistant HIV-1 variants (Fig. S2). We speculate that bNAbs that effectively interfere with cell to cell transmission in vitro will also display efficient and long-lasting therapeutic or prophylactic properties in vivo.

**Viral cell to cell transmission assay.** Donor cells were infected with the indicated HIV-1 strains and used a few days later, when ~10–75% of the cells were Gag+. Target cells were labeled with FarRed or 2.5 µM CFSE (Molecular Probes). Donors were preincubated with the indicated doses of bNAbs. Donor and target cells were then mixed at a 1:2 ratio in 96-well plates at a final concentration of 1.5 × 10^6/ml in 200 µl. After 48 h, cells were stained for intracellular Gag (KC57 mAb; Beckman Coulter) and analyzed by flow cytometry. When stated, 12.5 nM NVP was added 1 h before co-culture.

**Cell-free infection of P4C5 cells.** The neutralization activity of bNAbs was evaluated on P4C5 cells (HeLa CD4+CCR5+ cells carrying an HIV-1 LTR–β-gal reporter cassette). 1 d before infection, 7 × 10^3 cells were plated in 96-well plates. Cells were infected in triplicate with 1 or 5 ng Gag p24.Viruses were incubated with the indicated bNAbs for 1 h before infection. After 36 h, cells were lysed in PBS, 0.1% NP-40, and 5 mM MgCl2 and incubated with the β-gal substrate CPRG (Roche), before measurement of 570-nm OD.

**Calculation of IC50.** Dose–response inhibition curves were drawn by fitting data from three to six independent experiments to sigmoid dose–response curves (variable slope) using Prism software (GraphPad Software). Percentage of inhibition was defined as (percent signal in nontreated target cells − percent signal in bNAb-treated cells)/percent signal in nontreated target cells) × 100. The IC50 was calculated with Prism.

**Analysis of conjugate formation between infected and target cells.** Chronically infected MOLT cells (2 × 10^5/well) were stained with CFSE and preincubated in 96-well plates with 15 µg/ml bNAbs for 1 h at 37°C, before adding 2 × 10^5 FarRed-labeled CD4 T cells for 2 h (Massanella et al., 2009). Cellular contacts were measured by flow cytometry. CFSE+FarRed+ cells were considered as cellular conjugates. The percentage of conjugates was calculated as follows: (conjugates/total conjugates + MOLT cells + CD4 T cells) × 100.

**Analysis of HIV-1 capture by target cells.** Primary donor cells were infected with HIV-1 and used a few days later, when 10–75% of the cells were Gag+. The indicated target cells were labeled with FarRed or 2.5 µM CFSE (Molecular Probes). Donors were incubated with bNAbs for 1 h. Donor and target cells were then mixed at a 1:2 ratio in 96-well plates at a final concentration of 1.5 × 10^5/ml in 200 µl. After 4 h, target cells were stained for Gag and analyzed by flow cytometry.

**Syncytia assay.** HeLa 243 cells, which stably express the HIV-1 NL4.3 Env and Tat proteins (Schwartz et al., 1994), seeded at 8 × 10^3 cells in 96-well plates, were preincubated for 1 h with 15 µg/ml bNAbs, before addition of 8 × 10^3 P4C5 cells for 8 h. Upon cell fusion, Tat transactivates the HIV-1 LTR and drives expression of β-gal, at levels which correlate with the amount of syncytia (Schwartz et al., 1994).

**Immunofluorescence analysis.** HIV-infected primary donor CD4+ T cells were treated with the indicated bNAbs for 1 h before addition of autologous targets. After 1 h of co-culture, cells were fixed and double Gag/bNAb stainings were performed with a mouse mAb anti-p24 and a secondary anti-human antibody to visualize the bNAb. Confocal microscopy analysis was performed on an LSM700 (Carl Zeiss) using a 40 or 63 objective. Z-series of optical sections were performed at 0.2–0.5-µm increments.

**Sensing of HIV-infected cells by hematopoietic cells.** The indicated infected donor cells were incubated for 1 h with the bNAbs and mixed with target cells (PBMCs or Gen2.2) at a ratio of 1:2 in 96-well plates at a final concentration of 2–3 × 10^5/ml in 100 µl for PBMCs, or 10^6/ml in 200 µl for Gen2.2, as described previously (Lepelley et al., 2011). After a co-culture of 18 h (PBMCs) or 48 h (Gen2.2), type I IFN levels and expression of the protein MxA were measured as described previously (Lepelley et al., 2011; Puigdomènech et al., 2013).
Infection of humanized mice. HIV-1 infection of humanized mice, bNAb treatments, and analysis of viral loads and sequences were performed as described previously (Klein et al., 2012b). Animal experiments were performed with authorization from the Institutional Review Board and the Institutional Animal Care and Use Committee at the Rockefeller University.

Statistical analyses. Wilcoxon–matched paired Student’s t tests (and Mann–Whitney unpaired Student’s t test in Fig. 3 b) were performed using Prism software.

Online supplemental material. Fig. S1 shows dose–response analysis of the antiviral effect of bNAb on cell-free and cell-associated infections. Fig. S2 shows that 10E8 treatment does not impose selective pressure on HIV-1 infection in humanized mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20131244/DC1.

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Figure S1. Dose–response analysis of the antiviral effect of bNAb on cell-free and cell-associated infections. Cell-free infections were performed on HeLa-derived P4C5 target cells and quantified by measuring β-gal after 36 h. Virions were preincubated with bNAb before infection. For cell-associated infections, primary HIV-1–infected CD4+ T cells were preincubated for 1 h with bNAb before co-culture with autologous target cells stained with FarRed. After 48 h, the fraction of productively infected (Gag+) target cells was measured by flow cytometry. The bNAb were used at the indicated doses, and the percentage of inhibition was calculated. Data are mean ± SD from two to eight independent experiments. Lines represent fitted results.
**Figure S2.** 10E8 treatment does not impose selective pressure on HIV-1 infection in humanized mice. (a) Viral loads before and during 10E8 antibody therapy for acute (left) and chronically infected hu-mice. Each line represents a single mouse. Gray shading indicates 10E8 therapy, 0.5 mg twice per week s.c. Red arrows indicate infection with HIV-1 YU2. Symbols indicate viral load measurements. 10E8 therapy did not have any apparent effect on viral load in acutely or chronically infected animals. In contrast, some bNAbs like 10-1074 and a derivative of NIH45-46 [45–46G54W] significantly reduced viral loads after 1 wk of therapy in this animal model (Klein et al., 2012b). (b) gp41 sequences cloned from mice during (top) and before 10E8 therapy. Dots indicate nonsynonymous (red) and synonymous (green) mutations relative to wild-type HIV-1 YU2. The MPER region (residues 665–683) is highlighted in orange. Scale bar gives HXBc2-aligned gp160 residue numbers. One mutation was found in the MPER region (K665E), but the same mutation was found in a chronically infected mouse before 10E8 therapy. No apparent resistance mutations were observed in any 10E8-treated mouse. In contrast, resistance mutations were always observed for mice treated with the bNAbs 10–1074 or 45–46G54W (Klein et al., 2012b).

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