Sustained Delivery of a Broadly Neutralizing Antibody in Nonhuman Primates Confers Long-Term Protection against Simian/Human Immunodeficiency Virus Infection

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ABSTRACT

Pathogen-specific neutralizing antibodies protect against many viral infections and can potentially prevent human immunodeficiency virus (HIV) transmission in humans. However, neutralizing antibodies have so far only been shown to protect nonhuman primates (NHP) against lentiviral infection when given shortly before challenge. Thus, the clinical utility and feasibility of passive antibody transfer to confer long-term protection against HIV-1 are still debated. Here, we investigate the potential of a broadly neutralizing HIV-1 antibody to provide long-term protection in a NHP model of HIV-1 infection. A human antibody was simianized to avoid immune rejection and used to sustain therapeutic levels for ~5 months. Two months after the final antibody administration, animals were completely protected against viral challenge. These findings demonstrate the feasibility and potential of long-term passive antibody for protection against HIV-1 in humans and provide a model to test antibody therapies for other diseases in NHP.

IMPORTANCE

Antibodies against HIV are potential drugs that may be able to prevent HIV infection in humans. However, the long-term protective capacity of antibodies against HIV has not been assessed. Here, we repetitively administered a macaque version of a human anti-HIV antibody to monkeys, after which the antibody persisted in the blood for >5 months. Moreover, the antibody could be sustained at protective levels for 108 days, conferring protection 52 days after the last dose in a monkey model of HIV infection. Thus, passive antibody transfer can provide durable protection against infection by viruses that cause AIDS in primates.

Neutralizing antibodies that confer protection against diverse viral strains are thought to be important for preventing human immunodeficiency virus type 1 (HIV-1) infection (1–6). In recent years, numerous HIV-1 broadly neutralizing monoclonal antibodies (bNAbs) have been isolated and serve as examples of the types of antibodies that a protective vaccine would aim to elicit (7–13). However, it has not yet been possible to elicit such antibodies by active immunization (14). Therefore, passive transfer of these bNAbs represents a potential prophylactic modality to limit the spread of HIV-1 infection (4). Previous studies have established the ability of neutralizing antibodies to protect nonhuman primates against simian/human immunodeficiency virus (SHIV) infection (15–21). In these experiments, relatively high doses of antibody were administered usually 24 to 48 h prior to intravenous or mucosal challenge and provided sterilizing protection (15–19, 22–24). While these studies demonstrated that antibodies were sufficient for protection, they raised questions about the practicality of passive transfer for clinical use if protection was dependent on high concentrations of antibody being infused immediately prior to virus exposure.

Due to the unpredictable nature of HIV-1 transmission, a clinically relevant immunoprophylactic treatment must provide prolonged protection against infection. The recent demonstration that the potent anti-HIV antibodies PGT121 and VRC01 can protect against SHIV challenge at relatively low concentrations of antibody suggests that modest levels of antibody may sustain pro-

tection in humans (22, 25, 26). Thus, the ability of passive transfer to confer sustained protection for more than a couple of days remains the fundamental question pertinent to the translation of passive neutralizing antibody transfer as a sustained prevention strategy in humans. To address this question in a nonhuman primate (NHP) model of HIV-1 infection, we simianized the human bNAb VRC01 (11, 27) to reduce cross-species immunogenicity. Using this simianized VRC01, we examined the durability of passive protection conferred by a bimonthly prophylactic treatment regimen in rhesus macaques. This approach of simianization and

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TABLE 1 Identification of macaque germ line genes homologous to VRC01 germ line genes

Human VRC01 gene	Macaque gene (GenBank accession no.) ^a	% identity
IGHV1-2*02	IGHV11 (AY161064)	92.6
IGHJ2*01	IGHJ2 (AY057983)	88.7
IGKV3-20 ^b	IGKV3d (AY963770)	90.6
IGKJ2*01	IGKJ2 ^c	94.6

^a The initial selection of IGKV3d was based on IGKV3-11 being inferred as the human VRC01 IGKV gene (93.3% identical).

^b The human VRC01 IGKV gene was later changed to IGKV3-20 after simian VRC01 had been produced.

^c The macaque IGKJ germ line gene was determined by a BLAST search of the Baylor University macaque genome database using human IGKJ2*01 as the query.

repetitive passive transfer in NHPs provides a preclinical model not only to assess efficacy for HIV-1 prevention but also for diverse antibody therapies.

MATERIALS AND METHODS

Study design. Simian immunodeficiency virus (SIV)-negative Indian-origin rhesus macaques (*Macaca mulatta*) weighing between 4 and 5 kg were used for nonhuman primate studies. Macaques allocated to control and experimental groups were age and weight matched. For passive-transfer experiments, Indian rhesus macaques were anesthetized and administered each antibody by slow intravenous injection. On the day of challenge, macaques were inoculated intrarectally with 1 ml of SHIV-BaLP4 (obtained from Norman Letvin) at 12,800 tissue culture infective doses (TCID₅₀)/ml, when the titer was determined in TZM-bl cells (28). Macaques challenged with SHIV were subjected to routine clinical evaluation. Macaque plasma was isolated from whole blood to measure VRC01 concentrations by a quantitative resurfaced HIV core derivative 3 (RSC3) enzyme-linked immunosorbent assay (ELISA), as stated below. Pharmacokinetic profiles were determined by using WinNonLin software (Pharsight). The plasma viral load was quantified by lysing virions and isolating RNA using a QIAasympyphony virus/pathogen midikit (Qiagen). RNA was reverse transcribed with Superscript III (Invitrogen) and analyzed by real-time PCR in a StepOne PCR machine (Applied Biosystems) using a SIV gag-specific TaqMan primer set. Veterinary procedures were performed as approved by the Vaccine Research Center Animal Care and Use Committee. All animals were housed and cared for in accordance with local, state, federal, and institute policies in an American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited facility.

Antibodies. Human and simian VRC01 IgGs were produced in 293F cells by transient cotransfection of heavy and light chain plasmids. The antibodies were purified by protein A resin and dialyzed into phosphate-buffered saline (PBS). A recombinant macaque IgG against desmipramine was used as a negative control for *in vitro* and *in vivo* experiments (NIH Nonhuman Primate Reagent Resource). The endotoxin concentration in the antibody preparations used for *in vivo* administration was measured by a *Limulus* amebocyte lysate (LAL) assay (Lonza). If the endotoxin concentration was >1 endotoxin unit (EU) per mg of antibody, it was removed by using an EndoTrap column (Hyglos).

Simianization of VRC01 IgG. The macaque germ line genes with the highest sequence identity to the human VRC01 germ line genes were identified (Table 1). To create the variable regions of simian VRC01, all three mature complementarity-determining regions (CDRs) of the human VRC01 heavy and light chains were grafted onto the inferred macaque germ line genes. To reduce species differences in the variable region, only framework residues that were implicated in supporting the CDR loop structure (29) or predicted to be important for VRC01 binding based on analysis of a VRC01-gp120 complex structure (27) were transplanted onto the macaque germ line genes. Therefore, only seven somatic muta-

tions in the framework regions (FWRs) from human VRC01 were grafted onto the macaque light or heavy germ line genes (Fig. 1). The variable region was attached to the macaque gamma constant region (GenBank accession number AF045537), excluding any introns. The light chain variable region was attached to the macaque kappa constant region (GenBank accession number FJ795843; GenBank accession number AF050635) similarly. The DNA sequence was synthesized (GeneArt) and cloned into expression vectors. The full-length immunoglobulin amino acid sequence was >90% identical to the macaque reference sequence.

RSC3 ELISA. ELISAs were performed by coating Nunc Maxisorp plates (Thermo Fisher) with 2 µg/ml of RSC3 (11) overnight at 4°C. The ELISA plates were blocked with a solution containing 5% skim milk and 2% bovine serum albumin (BSA) in Tris-buffered saline–0.05% Tween (TBS-T). For quantitation of simian VRC01 levels in plasma, a standard curve was derived by serially diluting purified simian VRC01 in blocking buffer and adding it to the ELISA plate. Heat-inactivated macaque plasma was incubated in the plate for 1 h and washed away, and simian VRC01 was detected with a horseradish peroxidase (HRP)-linked anti-macaque Fc gamma antibody (Southern Biotech). The concentration of simian VRC01 in the macaque plasma was calculated based on the standard curve using Softmax Pro V5.0 software (Molecular Devices). Quantification of human VRC01 in plasma was performed similarly except that an HRP-linked anti-human Fc gamma antibody (Southern Biotech) was used.

SHIV and HIV-1 *in vitro* neutralization assay. *In vitro* neutralization by human or simian VRC01 IgG was assessed by using single-cycle pseudovirus infection of TZM-bl cells, as previously described (30). Similarly, heat-inactivated plasma from macaques infused with simian VRC01 IgG or preimmune plasma was analyzed by the TZM-bl neutralization assay at various dilutions. The neutralization titer was calculated by JMP (SAS Institute) as the concentration or dilution at which 50% of virus replication was inhibited (IC₅₀ or ID₅₀, respectively).

Simian VRC01 immunohistochemistry. Pinch biopsy specimens were taken from the rectum and jejunum of rhesus macaques 7 days before, 2 days after, and 14 days after simian VRC01 passive immunization. The biopsy specimens were fixed for 3 h at room temperature in SafeFix II (Fisher Scientific), washed three times with 80% ethanol, and stored overnight in 80% ethanol. The fixed tissues were paraffin embedded until they were stained for simian VRC01. For analysis of simian VRC01 IgG, the tissue was deparaffinized and incubated at 98°C in EDTA Decloaker (Biocare Medical) for 10 min to allow epitope retrieval. The tissue sections were then blocked overnight with Sniper (Biocare Medical) at 4°C. Twenty micromolar Avi-tagged RSC3 was biotinylated by using the BirA kit (Avidity) and purified by gel filtration. The tissue sections were stained with biotinylated RSC3 for 2 h at room temperature. Alkaline phosphatase-conjugated antibiotin was used as the detection antibody in combination with Warp Red (Biocare Medical).

Quantification of simian VRC01 in rectal tissue. Rectal pinch biopsy specimens from rhesus macaques were frozen in liquid nitrogen and stored at –80°C. On the day of quantification, one rectal biopsy specimen was homogenized for 30 s in 100 µl of PBS containing EDTA-free Complete protease inhibitors (Roche). The homogenizer was rinsed with an additional 100 µl and combined with 100 µl of the homogenate. The homogenate was cleared by centrifugation for 20 min at 4°C, the supernatant was filtered, and simian VRC01 IgG was quantified by an RSC3 ELISA, as stated above. The simian VRC01 concentration was normalized to the total protein concentration of the supernatant, determined by the QuickStart Bradford assay (Bio-Rad).

Anti-human and anti-simian VRC01 ELISA. ELISA plates were coated with 2 µg/ml of Fab or IgG overnight at 4°C. ELISA plates were blocked with TBS-T containing 5% milk and 2% BSA. Heat-inactivated macaque plasma was diluted in blocking buffer and added to the ELISA plate in duplicate. Fab molecules for ELISAs were generated by papain digestion using the Pierce Fab preparation kit (Thermo Scientific). Macaque IgG reactivity with antigen on the plate was detected with an HRP-linked anti-macaque IgG Fc secondary antibody (Southern Biotech). The

FIG 1 Structure-guided simianization of VRC01. (A) Amino acid sequence alignment of the macaque germ line (Mac) and simian and human VRC01 light chain (V_K) and heavy chain (V_H) variable regions. The percent identity and number of residues grafted onto the macaque germ line gene for each section of the variable region are denoted above the alignment. The amino acid residues of the complementarity-determining regions (CDRs) are shown in red. CDR3s are omitted from the macaque VRC01 light chain and heavy chain germ line sequences since CDR3s result from recombination, and there is uncertainty in their exact germ line sequences in this instance. Somatic mutations in the framework regions (FWRs) that were transplanted onto simian VRC01 are shown in violet. All remaining framework somatic mutations in human VRC01 are shown in blue. (B) Tertiary structural location of residues selected for simianization. The light-chain (LC) and heavy-chain (HC) FWR somatic mutations (blue and violet spheres) and CDRs (red) of human VRC01 are shown on the crystal structure in relation to HIV-1 gp120 (27). (C) Comparison of HIV-1 neutralization by simian and human VRC01 IgGs in the TZM-bl assay. Neutralization titers (IC₅₀) are shown in micrograms per milliliter of the indicated antibody and are color-coded as follows: red, <0.05 μg/ml; orange, 0.051 to 0.5 μg/ml; yellow, 0.51 to 5 μg/ml; green, 5.1 to 50 μg/ml; white, >50 μg/ml. NA indicates that the fold reduction could not be calculated due to a lack of neutralization by one or both of the antibodies.

ELISAs were developed with tetramethylbenzidine (TMB) substrate according to the manufacturer's instructions (KPL).

Anti-simian VRC01 SPR. For surface plasmon resonance (SPR) analysis, control macaque IgG (NIH NHP Reagent Resource) and simian VRC01 IgG (10 μg/ml in 10 mM acetate, pH 5.5) were immobilized (10 min at a 10-μl/min flow rate) on a Biacore CM5 sensor chip to an immobilization level of ~5,000 response units (RU). Plasma immunoglobulins were purified with an equal mixture of CaptureSelect anti-human kappa

and anti-human lambda resins (Life Technologies) and dialyzed into PBS. Purified IgG was injected for 1.5 min and allowed to dissociate for 2.5 min at a 40-μl/min flow rate. The flow cell surface was regenerated by two sequential injections of 2 M MgCl₂ for 0.5 min at an 80-μl/min flow rate. Injection of a buffer sample was performed after each purified IgG sample injection.

Statistical analyses. Statistical tests were performed with Prism version 6 (GraphPad).

FIG 2 Simian VRC01 possesses enhanced plasma pharmacokinetics and traffics to gastrointestinal mucosal sites in rhesus macaques. (A) Comparison of plasma pharmacokinetics of simian and human VRC01 IgGs following passive transfer of 20 mg/kg. Values are means $\pm$ standard errors of the means from at least quadruplicate independent measurements. The terminal half-life and clearance for human VRC01 were 4.2 days and 31.9 ml/day/kg, respectively. The terminal half-life and clearance for simian VRC01 were 7.9 days and 9.6 ml/day/kg, respectively. (B) Anti-VRC01 plasma IgG in macaques administered human or simian VRC01 IgG as described above for panel A. Binding to human and simian VRC01 Fabs is shown on the left and right, respectively. (C) Reverse immunohistochemical detection of simian VRC01 in cross sections of rectal and jejunal tissues from the macaques in panel A, performed as stated in Materials and Methods. Simian VRC01 was detected in the basal region of the mucosal and cryptal columnar epithelial cells at weeks 1 and 2 after passive immunization but not prior to passive transfer (week 0). Yellow arrows indicate epithelial cells. Representative images from staining performed on biopsy specimens from two different macaques are shown. Images are $\times 200$ the original magnification.

RESULTS

Structure-guided design of simianized VRC01 IgG. The assessment of long-term protection by human antibodies in NHPs is complicated by the xenogeneic immune response that limits their persistence *in vivo* (31). To overcome this limitation, we engineered a macaque version of VRC01 that would be less immunogenic and suitable for repetitive administration in monkeys. To simianize VRC01, we identified the homologous macaque germ line genes that were 89 to 95% identical to the human VRC01 germ line genes (32, 33) (Table 1). To create the variable regions of simian VRC01, we grafted all three mature CDRs and seven framework somatic mutations of the human VRC01 heavy and light chains onto the inferred macaque germ line genes (Fig. 1A and B).

To confirm that simian VRC01 behaves similarly to human VRC01, we compared the neutralization activities of both antibodies on a panel of 20 HIV-1 Env pseudoviruses and replication-competent SHIV-BaLP4. Simian VRC01 displayed a mean 2.8-fold-lower neutralization potency overall and neutralized one less virus in the panel than did the human antibody (Fig. 1C). Simian VRC01 neutralized several viruses almost identically to human VRC01, including the challenge stock SHIV-BaLP4, suggesting

that the simianized antibody maintains breadth and potency similar to those of human VRC01.

Systemic and mucosal pharmacokinetics of simian VRC01.

To assess the potential for simian VRC01 to sustain therapeutic levels *in vivo*, we characterized its pharmacokinetics and immunogenicity in rhesus macaques after a single administration. Two macaques each were infused with 20 mg/kg of body weight of simian VRC01 IgG or human VRC01 IgG for comparison. Simian VRC01 exhibited a high peak concentration, a 2-fold increase in the antibody half-life, and a 3-fold decrease in the antibody clearance rate in plasma compared to human VRC01 (Fig. 2A). In contrast to human VRC01, no antibodies against simian VRC01 were detected (Fig. 2B). Thus, simianization reduced the immunogenicity of the VRC01 antibody in macaques. These results indicated that this simianized version of VRC01 would be amenable for a prolonged prophylactic course of antibody administrations that otherwise would have been precluded by the immunogenicity of the therapeutic antibody.

Since mucosal transmission is a major route of infection by HIV-1, we next determined the localization of simian VRC01 at mucosal sites after passive transfer in macaques. Similar to previous studies performed with human VRC01 (34), immunohistochemistry

FIG 3 Simian VRC01 is maintained in the plasma and mucosal tissue by repetitive passive transfer. (A) Plasma concentrations of simian VRC01 or simian VRC01-LS IgG in macaques during four 5-mg/kg passive immunizations. Arrows on the x axis indicate time points for antibody transfers and SHIV-BaLP4 challenge. Each curve represents an individual macaque, and values are means $\pm$ standard errors of the means from four to six independent measurements. The plasma terminal half-lives were 7.33 ± 0.12 and 11.80 ± 0.16 days for wild-type and LS mutant simian VRC01 IgGs, respectively. The minimum concentrations were 7.84 ± 0.30 and 19.98 ± 0.43 $\mu\text{g/ml}$ for wild-type and LS mutant simian VRC01 IgGs, respectively. Values represent the means $\pm$ standard errors of the means for the four animals in each group, excluding time points where anti-simian VRC01 responses were detected. (B) Concentrations of simian VRC01 and simian VRC01-LS IgGs in rectal tissue homogenates during the first three 5-mg/kg infusions. Values are means $\pm$ standard errors of the means from triplicate independent measurements.

using a labeled HIV-1 envelope molecule showed abundant cytoplasmic staining of simian VRC01 IgG in the basal region of the mucosal cryptal columnar epithelial cells of the rectum up to 2 weeks after infusion (Fig. 2C). Early after transmission, HIV-1 replicates in the gut-associated T cell compartment (35); therefore, we also examined the localization of the prophylactic antibody to the jejunum. Simian VRC01 was found to be present in this tissue in a localization pattern similar to that in the rectum (Fig. 2C). Localization to the initial sites of transmission and replication for 2 weeks suggested that prophylactic antibody treatments could provide a barrier to infection for prolonged durations of time.

Long-term protection by simian VRC01 immunoprophylaxis. We hypothesized that long-term protection would be influenced by antibody persistence at the above-mentioned mucosal sites of viral exposure and replication as well as in circulation. The persistence of antibodies of the gamma isotype *in vivo* is regulated by their interaction with the neonatal Fc receptor (FcRn), which can be augmented by mutations in the heavy chain constant region (36–38). We have previously found that these mutations increase mucosal levels of human VRC01 and provide enhanced short-term protection against SHIV challenge in macaques (34). We therefore asked whether a repeat-dose immunoprophylaxis regimen of simian VRC01, either alone or in combination with an

enhanced FcRn interaction, could maintain the antibody in the plasma and at mucosal sites to confer long-term protection. We constructed a variant of simian VRC01 with an increased affinity for FcRn. This variant contained a methionine-to-leucine substitution and an asparagine-to-serine substitution at amino acid positions 428 and 434, respectively, and was termed simian VRC01-LS. Consistent with data from previous reports, the LS mutation had no effect on simian VRC01 binding to HIV-1 Env (34). Rhesus macaques received four administrations of 5 mg/kg of wild-type simian VRC01 or the simian VRC01-LS variant over an 8-week period. Detectable levels of simian VRC01 were maintained in the plasma during the repeat-dosing regimen (Fig. 3A). Simian VRC01-LS exhibited an increased plasma half-life, resulting in a trough concentration ~ 2.5 -fold higher than that of wild-type simian VRC01 (Fig. 3A). To determine whether protective concentrations were maintained between infusions of simian VRC01, a separate group of naive rhesus macaques was administered an equivalent dose of wild-type simian VRC01 in parallel and challenged with SHIV-BaLP4 at day 14 instead of receiving another dose of wild-type simian VRC01 (Fig. 4A). On the day of challenge, plasma concentrations of simian VRC01 ranged from 6.4 to 3.5 $\mu\text{g/ml}$, which afforded plasma neutralization ID_{50} titers against the challenge stock ranging from 1:69 to 1:101 (Fig. 4B).

FIG 4 Protection from SHIV infection 2 weeks after wild-type simian VRC01 infusion. (A) Plasma concentrations of simian VRC01 after a 5-mg/kg passive transfer in rhesus macaques. The arrow on the x axis indicates the challenge time point. Values are means $\pm$ standard errors of the means from quadruplicate independent measurements. (B) Plasma neutralization titers (ID_{50}) on the day of challenge, shown as reciprocal plasma dilutions and color-coded as follows: red, $>1,000$; orange, 601 to 1,000; yellow, 301 to 600; green, 20 to 300; white, <20 . MLV, murine leukemia virus. (C) Plasma viral loads of rhesus macaques that received a single administration of nonimmune macaque IgG (control) or simian VRC01 IgG and were challenged with SHIV-BaLP4 2 weeks after antibody infusion. The numbers of infected macaques were lower in the simian VRC01-infused group than in the control IgG-infused group ($n = 4$ per group; $P = 0.0143$ by one-tailed Fisher's exact test).

All four simian VRC01-treated animals were protected against infection, whereas four macaques given control macaque IgG were infected ($P = 0.0143$ by one-tailed Fisher's exact test) (Fig. 4C). Therefore, protective concentrations of simian VRC01 were maintained between antibody infusions.

The ability of the antibody to traffic to and persist in the mucosal tissue was assessed by quantifying the simian VRC01 IgG present in rectal biopsy specimens over a period of 54 days. Detectable concentrations of both antibodies were maintained over the 54-day period, which encompassed three antibody administrations (Fig. 3B). The enhanced FcRn binding mutant was maintained at a higher concentration over the 54 days ($P = 0.0191$ by two-way analysis of variance [ANOVA]) (Fig. 3B). Moreover, on the final day of follow-up (day 54), simian VRC01-LS was present in rectal tissue at concentrations ~ 3 -fold higher than those of the wild-type antibody (Fig. 3B). This observation extends our previous findings obtained with human VRC01 to show that the LS mutation has an enduring effect on the mucosal persistence of passively transferred antibodies (26, 34).

One of the four macaques in each treatment group (simian VRC01 and simian VRC01-LS) showed a rapid decline in plasma antibody levels (Fig. 3A) that was associated with an antibody response against the human residues transplanted to make simian VRC01 (Fig. 5). Thus, even with a simianized antibody, xeno-

genic responses can occur in some cases after multiple exposures to the antibody. Importantly, while human antibody immunogenicity is a pitfall of nonhuman primate models, it is not predictive of immunogenicity in humans (39). These two animals were therefore omitted from the subsequent SHIV challenge portion of the study.

The six simian VRC01-infused macaques (3 wild-type-infused and 3 LS variant-treated macaques) were challenged with SHIV-BaLP4 nearly 2 months (52 days) after the last antibody administration. On the day of challenge, the plasma neutralization titers ranged from 1:62 to 1:104 for the macaques that received wild-type simian VRC01 (Fig. 6A). In agreement with the simian VRC01 plasma concentrations, the neutralization titer was higher for the three macaques that received the LS mutant than for those that received wild-type simian VRC01 (Fig. 6A). All four macaques that received control IgG became infected (Fig. 6B). Two of the three macaques that received wild-type simian VRC01 IgG were protected (Fig. 6B). All three of the simian VRC01-LS-infused macaques possessed detectable simian VRC01-LS at the time of challenge and were protected against infection (Fig. 6B). Therefore, simian VRC01-LS conferred complete, long-term protection against SHIV infection, providing a proof of concept that an antibody treatment regimen can durably protect against mucosal challenge.

FIG 5 Characterization of anti-simian VRC01 humoral immune responses elicited by repetitive passive transfer. (A and B) Macaque pretransfer (open symbols and dashed lines) and postimmune (solid symbols and lines) plasma IgG ELISA binding to simian VRC01 Fab (A) or a negative-control macaque Fab (B). (C and D) Surface plasmon resonance binding of purified total immunoglobulin to simian VRC01 (C) and simian VRC01-LS (D) IgGs. (E and F) Direct ELISA binding to human VRC01 IgG (E) or normal human IgG (F) by plasma IgG from macaques repetitively administered simian VRC01 IgG.

DISCUSSION

The isolation of numerous bNAbs against HIV-1 has revitalized interest in passive antibody transfer as a potential prophylactic measure. However, no study has addressed the ability of antibody transfer to provide sustained protection from mucosal lentiviral

infection. Using a structure-based design, we constructed a simianized human bNAb with minimally mutated simian FWRs that could potentially neutralize HIV-1, maintain levels in plasma, and circulate to mucosal sites. We demonstrated that simian VRC01 could protect macaques from mucosal SHIV challenge almost 2

FIG 6 Long-term protection from SHIV infection after repetitive passive transfer of wild-type and LS mutant simian VRC01. (A) Plasma neutralization titers (ID₅₀) on the day of challenge, shown as reciprocal plasma dilutions. The color-code is described in the legend of Fig. 4. (B) Plasma viral loads for each macaque administered macaque IgG, wild-type simian VRC01, or simian VRC01-LS and challenged with SHIV-BaLP4 52 days (week 15.5) after the final antibody infusion. The numbers of infected macaques were lower in the simian VRC01-infused group (animals receiving either wild-type simian VRC01 or simian VRC01-LS) than in the control IgG-infused group ($n = 6$ for simian VRC01 and $n = 4$ for control IgG; $P = 0.0238$ by one-tailed Fisher's exact test).

months after the last antibody administration. This finding provides a significant extension in protection by passively transferred antibody far beyond the typical 6- to 48-h intervals that have been shown previously. The duration of protection supports the notion that antibody immunoprophylaxis represents a feasible long-term prevention strategy.

The long-term protection observed here is likely a function of the potent neutralization of SHIV-BaLP4 by our simianized VRC01 antibody, the long systemic persistence of this simianized antibody, and its ability to localize to the mucosal compartment after passive immunization. Mucosal localization and persistence of antibodies are potentially important aspects of protection afforded by passive immunization (40). Mucosal concentrations of antibodies are augmented by enhanced FcRn interactions (34), which we found to improve systemic and mucosal antibody levels. A similar effect could potentially occur in humans, since the M428L and N434S mutations used here increase the half-life of human antibodies in human FcRn transgenic mice (38).

Our findings provide support for the concept that neutralizing antibody immunoprophylaxis could prevent HIV-1 infection in humans over a sustained period of time, perhaps months. With this increased duration of protection, antibody immunoprophylaxis could require less frequent administrations than microbicides (41) or oral antivirals (42), improving the chance of treatment adherence and reducing the cost of this approach. More specifically, our study suggests that an HIV prevention strategy containing a loading phase of frequent antibody infusions (every 2 weeks) followed by a maintenance phase of infusions (every 2 months) could translate to six antibody infusions providing complete protection for a year. The creation of increasingly potent neutralizing antibodies by structure-based optimization and rational design (26, 43), in combination with the results presented here, suggests that such a goal for long-term antibody prophylaxis against HIV-1 infection in humans may be attainable.

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