HIV

Antibodies targeting the fusion peptide on the HIV envelope provide protection to rhesus macaques against mucosal SHIV challenge

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The fusion peptide (FP) on the HIV-1 envelope (Env) trimer can be targeted by broadly neutralizing antibodies (bNAbs). Here, we evaluated the ability of a human FP-directed bNAb, VRC34.01, along with two vaccine-elicited anti-FP rhesus macaque mAbs, DFPH-a.15 and DF1W-a.01, to protect against simian-HIV (SHIV)$_{BG505}$ challenge. VRC34.01 neutralized SHIV$_{BG505}$ with a 50% inhibitory concentration (IC_{50}) of 0.58 μg/ml, whereas DF1W-a.01 and DFPH-a.15 were 4- or 30-fold less potent, respectively. VRC34.01 was infused into four rhesus macaques at a dose of 10 mg/kg and four rhesus macaques at a dose of 2.5 mg/kg. The animals were intrarectally challenged 5 days later with SHIV$_{BG505}$. In comparison with all 12 control animals that became infected, all four animals infused with VRC34.01 (10 mg/kg) and three out of four animals infused with VRC34.01 (2.5 mg/kg) remained uninfected. Because of the lower potency of DF1W-a.01 and DFPH-a.15 against SHIV$_{BG505}$, we infused both Abs at a higher dose of 100 mg/kg into four rhesus macaques each, followed by SHIV$_{BG505}$ challenge 5 days later. Three of four animals that received DF1W-a.01 were protected against infection, whereas all animals that received DFPH-a.15 were protected. Overall, the protective serum neutralization titers observed in these animals were similar to what has been observed for other bNAbs in similar SHIV infection models and in human clinical trials. In conclusion, FP-directed mAbs can thus provide dose-dependent in vivo protection against mucosal SHIV challenges, supporting the development of prophylactic vaccines targeting the HIV-1 Env FP.

INTRODUCTION

The fusion peptide (FP) on the HIV-1 envelope (Env) trimer is a conserved site of vulnerability that can be targeted by broadly neutralizing antibodies (bNAbs) (*1*–*7*). VRC34.01, PGT151, and ACS202 are FP-directed bNAbs isolated from individuals with chronic HIV-1 infection that have a neutralization breadth of up to 60% in a cross-clade panel of 208 HIV-1 strains (*3*, *4*, *7*). These FP-directed bNAbs provide templates for vaccine design to elicit such FP-directed antibodies in various animal models (*8*–*14*). From these vaccine studies, antibodies targeting FP have been isolated from mice (*12*), guinea pigs (*11*), and nonhuman primates (*10*). These vaccine-elicited antibodies have been shown to have neutralization breadth up to 59% in a 208–cross-clade virus panel, suggesting the potential for generating protective FP-directed responses (*10*).

However, the in vivo protective efficacy of FP-directed antibodies isolated from individuals with HIV-1, or from animals after vaccination, needs to be demonstrated to provide proof of concept for targeting FP in HIV-1 vaccine strategies. Simian-HIV (SHIV) challenge of naïve rhesus macaques is a widely used animal model for assessing the protective efficacy of bNAbs and HIV-1 vaccines (*15*–*17*). Recently, the antibody-mediated protection trial provided proof of concept for HIV-1 monoclonal antibody (mAb)–mediated protection in humans and further validated the nonhuman primate model as a useful model to predict the potential protective efficacy of bNAbs (*18*). Here, we assess the protective efficacy of the human FP bNAb, VRC34.01, along with two FP vaccine–elicited rhesus macaque mAbs, DFPH-a.15 and DF1W-a.01, to protect naïve rhesus macaques against mucosal challenge with SHIV$_{BG505}$.

RESULTS

Anti-FP antibodies potently neutralize SHIV$_{BG505}$ in vitro

We previously showed that the FP-directed mAb VRC34.01, isolated from an individual living with HIV-1, displays reasonably broad and potent neutralization of HIV-1 strains (*3*). In addition, FP-directed antibodies isolated from rhesus macaques immunized with FP immunogens also displayed neutralization of diverse HIV-1 strains (*10*). To evaluate the ability of such FP-directed antibodies to protect against viral challenge in vivo, we used the well-established animal model of SHIV challenge of naïve rhesus macaques (*15*). For the FP-directed antibodies, we used VRC34.01 a potent human anti-FP mAb and two vaccine-elicited macaque antibodies, DF1W-a.01 and DFPH-a.15. These were potent neutralizing antibodies that were isolated from two immunized rhesus macaques, DF1W and DFPH. DF1W and DFPH were primed with FP coupled to keyhole limpet hemocyanin and boosted with Env trimer. DF1W-a.01 neutralized 25% and DFPH-a.15 neutralized 40% of a cross-clade panel of 208 HIV-1 strains (*10*). We selected SHIV$_{BG505}$ as the challenge virus because it produces robust infection of rhesus macaques and was neutralized by these FP-directed antibodies. The human mAb VRC34.01 displayed more potent neutralization of

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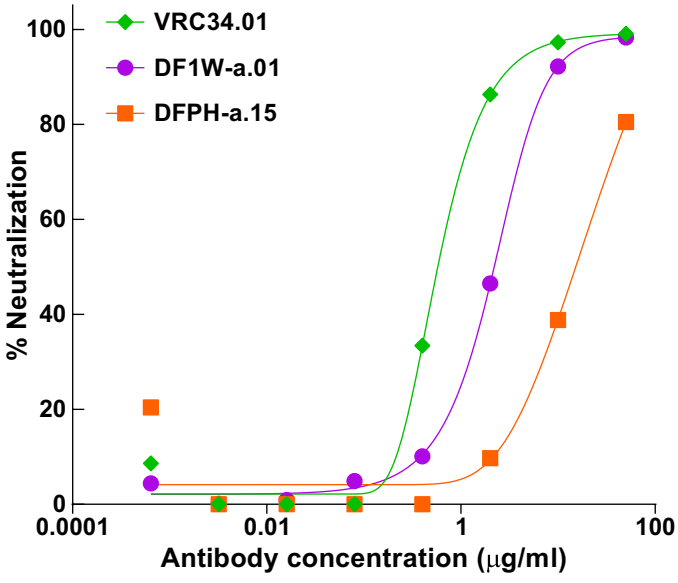
$SHIV_{BG505}$ than the vaccine-elicited rhesus macaque mAbs DF1W-a.01 and DFPH-a.15 (Fig. 1). DFPH-a.15 was the least potent in neutralization of $SHIV_{BG505}$, with a 50% inhibitory concentration (IC_{50}) of 15.1 μg/ml and an IC_{80} of 48.9 μg/ml, and, unlike VRC34.01 and DF1W-a.01, it was not able to achieve a maximum plateau for $SHIV_{BG505}$ neutralization, even at the highest concentration tested.

VRC34.01 infusion confers dose-dependent protection against $SHIV_{BG505}$ in rhesus macaques

We first evaluated the protective efficacy of VRC34.01 against the $SHIV_{BG505}$ challenge in rhesus macaques. We administered a single intravenous infusion of VRC34.01, either 2.5 or 10 mg/kg, to two groups of four naïve rhesus macaques each and measured plasma concentrations of VRC34.01 after infusion. We observed a dose-dependent pharmacokinetic profile (Fig. 2, A and B) with calculated half-lives ranging from 18 to 31 days (mean half-life of 27 days) (Table 1). Mean peak plasma concentrations were 130 and 30 μg/ml at the earliest time point tested after infusion with the 10 and 2.5 mg/kg doses, respectively. These animals were then challenged intrarectally with approximately 5 to 10 animal infectious doses (AIDs) of $SHIV_{BG505}$ 5 days after antibody infusion. On the day of the challenge, the average plasma concentrations for VRC34.01 in these animals were 118 and 33 μg/ml for the 10 and 2.5 mg/kg doses, respectively. A control group of four naïve rhesus macaques that did not receive any antibody infusion were challenged intrarectally at the same time as the VRC34.01-infused animals with the same dose of the identical $SHIV_{BG505}$ challenge stock. All four of the control animals became productively infected and viremic by week 1 after the $SHIV_{BG505}$ challenge (fig. S1). In contrast, none of the animals administered the 10 mg/kg dose of VRC34.01 became infected and only one out of the four animals in the 2.5 mg/kg dose group became infected after the $SHIV_{BG505}$ challenge (Fig. 2, C and D). Viremia in this animal was delayed to 21 days after the SHIV challenge. Overall, VRC34.01 provided significant protection against mucosal SHIV challenge when compared with the control animals ($P < 0.0001$, log-rank test).



	IC_{50} (μg/ml)	IC_{80} (μg/ml)	IC_{90} (μg/ml)	Neut max (%)
VRC34.01	0.58	1.43	2.62	99.1
DF1W-a.01	2.2	5.17	8.41	98.3
DFPH-a.15	15.1	48.9	>50	80.5

Fig. 1. FP-targeting antibodies can neutralize $SHIV_{BG505}$ in vitro. The neutralizing activity of VRC34.01, DF1W-a.01, and DFPH-a.15 against replication-competent $SHIV_{BG505}$ was tested in a neutralization assay using a luciferase reporter-based Tzm-bl cell line. The table lists the IC_{50}, IC_{80}, IC_{90}, and neutralization maximum (Neut max) values for the neutralization of $SHIV_{BG505}$ by the three mAbs.

Protection against the $SHIV_{BG505}$ challenge in rhesus macaques was conferred by vaccine-elicited rhesus macaque anti-FP antibodies

We next evaluated the protection provided by DFPH-a.15 and DF1W-a.01 using the same $SHIV_{BG505}$ challenge virus stock. Because the neutralization potencies for these antibodies were 4- to 30-fold lower than VRC34.01 against $SHIV_{BG505}$ (Fig. 1), we decided to use a higher dose of 100 mg/kg for infusion into four animals for each antibody. After infusion, the plasma concentrations of DFPH-a.15 (the less potent of the two rhesus mAbs) were two- to threefold higher compared with DF1W-a.01 (Fig. 3, A and B, and Table 1). DFPH-a.15 also displayed a significantly slower clearance rate ($P = 0.03$, Mann-Whitney test) compared with DF1W-a.01 (Table 1). As in the previous challenge study with VRC34.01, we intrarectally challenged these animals 5 days after antibody infusion with 5 to 10 AIDs of $SHIV_{BG505}$. Control groups of four naïve rhesus macaques that did not receive any antibody infusion were challenged intrarectally at the same time as either DF1W-a.01 or DFPH-a.15–infused animals with the same dose of the identical $SHIV_{BG505}$ challenge stock. All eight of these control animals became productively infected and viremic by week 1 after the $SHIV_{BG505}$ challenge (fig. S1). On the day of the challenge, the mean concentration for DFPH-a.15 and DF1W-a.01 was 1281 and 284 μg/ml, respectively, with a significant difference in these concentrations observed between the two antibodies ($P = 0.03$, Mann-Whitney test). All four animals that received DFPH-a.15 were protected against infection (Fig. 3C). For DF1W-a.01, one out of the four animals became infected after $SHIV_{BG505}$ challenge (Fig. 3D). The onset of viremia in this animal was delayed to 21 days after SHIV challenge, similarly to one animal in the 2.5 mg/kg dose group of VRC34.01.

Because the DFPH-a.15–infused animals exhibited the expected declining concentrations of plasma antibody after SHIV challenge, we rechallenged these animals with $SHIV_{BG505}$ intrarectally at day 35 to identify an approximate antibody threshold of protection (Fig. 3C). On the day of second $SHIV_{BG505}$ challenge, the range of plasma concentrations of DFPH-a.15 was 180 to 484 μg/ml (mean, 334 μg/ml). Two out of these four animals became infected, suggesting that these concentrations were close to the threshold for protecting against infection. In both infected animals, the onset of viremia was delayed to 14 days after SHIV rechallenge. Overall, both antibodies provided significant protection against mucosal SHIV challenge compared with controls ($P < 0.0001$, log-rank test; fig. S1), demonstrating the protective efficacy of FP-directed rhesus antibodies against mucosal transmission.

We also estimated plasma neutralization ID_{50} and ID_{80} titers needed for protection for these antibodies, as previously reported for other anti–HIV-1 antibodies (*15*). This was done using the plasma concentration of the mAb on the day of challenge and the known in vitro IC_{50} or IC_{80} against $SHIV_{BG505.}$ Similar to our previously published findings with the same challenge $SHIV_{BG505}$ (*15*), we found that plasma ID_{50} titers of 111 (range: 21 to 215) were associated with full protection compared with that of ID_{50} titers of 155 (range: 36 to 664) that were associated with near complete (95%) protection for CD4 binding site bNAbs, although the current dataset was too small to generate regression curves to more accurately quantify the plasma neutralization titer associated with protection (Table 1).

Breakthrough infections in animals receiving anti-FP antibodies were established by neutralization-sensitive wild-type $SHIV_{BG505}$ virus

Incomplete protection by passively administered bNAbs could result from infection by neutralization-resistant variants present in the challenge stock or by neutralization-sensitive viruses if antibody titers were subprotective. Previously, we analyzed the homogeneity of the $SHIV_{BG505}$ (SHIV.BG505.332 N.375Y) challenge stock and found it to be >99.5% (*19*). The frequency of known FP bNAb resistance mutations was further determined to be <1%, making the likelihood of transmission of neutralization-resistant viral variants low. However, to obtain additional evidence to distinguish between breakthrough infections resulting from neutralization-sensitive versus neutralization-resistant viruses, we sequenced plasma viral RNA (vRNA) from animals soon after virus transmission. For the three infected rhesus macaques that received DF1W-a.01 (34558) or DFPH-a.15 (DGAC and A14V136), plasma vRNA from peak viremia time points 21 to 28 days after SHIV challenge was subjected to single-genome sequencing of full-length gp160 genes (Fig. 4A). In each animal, the consensus plasma vRNA sequence was identical to the molecular clone of SHIV.BG505.332 N.375Y and to the viral consensus in the challenge stock that was derived from this cloned virus (*19*). In animal 34558, 2 of 37 (5.4%) sequences contained an I515M substitution in the amino terminus of the FP, which represents a known FP bNAb resistance mutation (*10*). We tested the effect of a mutation at this residue on viral neutralization sensitivity to the mAb DF1W-a.01 and found that it conferred >261-fold resistance (IC_{50} > 50 μg/ml) (Fig. 4B). In animal DGAC, 7 of 19 (36.8%) sequences contained an S241N substitution, which again represents a known resistance-conferring mutation for some FP bNAbs (*10*). We tested this mutation compared with wild-type S241 in the BG505 Env background and found that it conferred threefold resistance to DFPH-a.15 (Fig. 4B). In animal A14V136, mutations were exceedingly rare, and none occurred in the FP or in motifs known to confer resistance to FP bNAbs. Thus, in animals receiving DFPH-a.15 or DF1W-a.01 antibodies, breakthrough infections likely resulted from transmission of a neutralization-sensitive wild-type virus with IC_{50}, IC_{80}, and IC_{90} values shown in Fig. 1, with a minority of plasma vRNA sequences containing resistance mutations resulting from positive selection by declining concentrations of

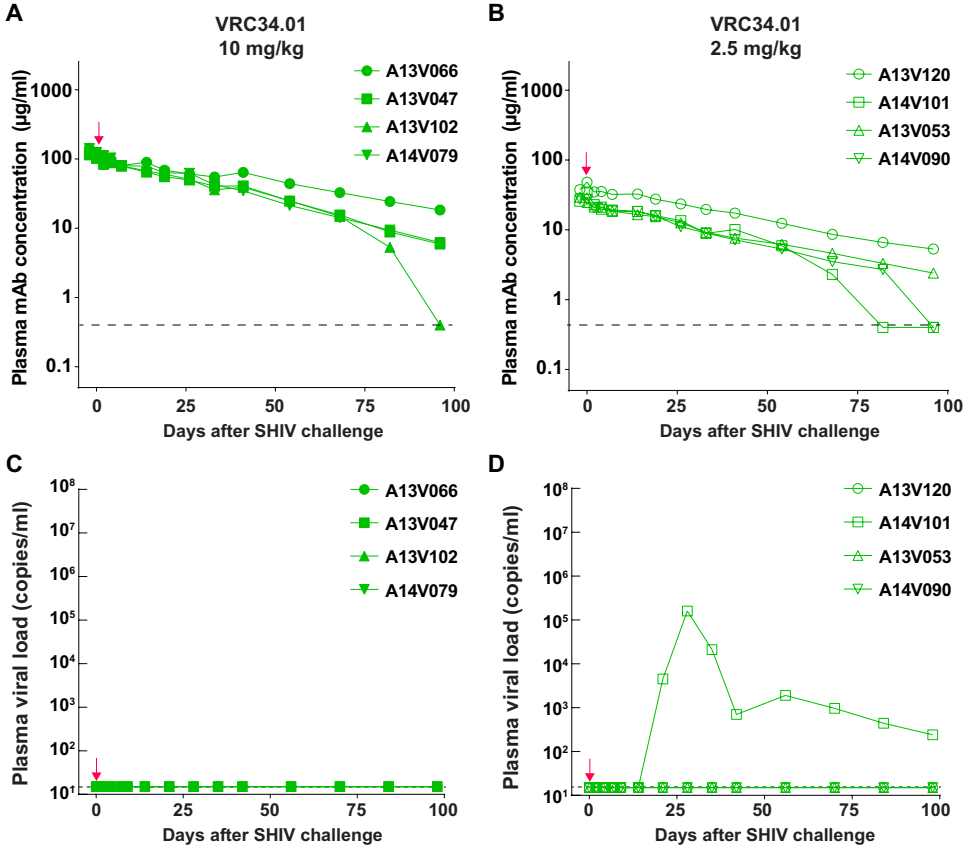


Fig. 2. Plasma mAb concentrations and plasma viral loads in rhesus macaques administered VRC34.01 followed by a single mucosal challenge with $SHIV_{BG505}$. (**A** and **B**) The plasma concentrations of VRC34.01 IgG1 were measured using an FP-based enzyme-linked immunosorbent assay (ELISA) after intravenous administration of either a 10 (A) or a 2.5 (B) mg/kg dose of the antibody. (**C** and **D**) Plasma viral loads were measured in rhesus macaques administered either a 10 (C) or a 2.5 (D) mg/kg dose of VRC34.01 and rectally challenged 5 days later with a single high dose of $SHIV_{BG505}$. The red arrows indicate the time of the mucosal SHIV challenge. The dashed horizontal lines indicate the limit of detection for each assay.

Table 1. Passive immunization studies performed with VRC34.01, DFPH-a.15, and DF1W-a.01.

Antibody	Animal identification	Dose (mg/kg)	mAb half-life (day)	Clearance (ml/day per kg)	Day of challenge plasma mAb concentration (μg/ml)	Infection status	Estimated ID_{50}	Estimated ID_{80}
VRC34.01	A13V066	10	37.9	1.5	124.5	Uninfected	215	87
	A14V079	10	22.9	2.2	123.9	Uninfected	214	87
	A13V102	10	24.2	2.4	123.7	Uninfected	213	87
	A13V047	10	22.2	2.4	101.8	Uninfected	176	71
	A13V120	2.5	32.9	1.2	48.0	Uninfected	83	34
	A14V090	2.5	26.8	2.6	27.2	Uninfected	47	19
	A13V053	2.5	30.5	2.3	24.7	Uninfected	43	17
	A14V101	2.5	17.5	2.6	33.8	Infected	58	24
DFPH-a.15	DGEV	100	34.5	1.6	1035.7	Uninfected	69	21
	A14V019	100	27.1	2.3	1130.5	Uninfected	75	23
	DGAC	100	18.1	2.0	1452.1	Uninfected	96	30
	A14V136	100	13.2	2.8	1504.7	Uninfected	100	31
	DGEV	Rechallenge at day 35	34.5	1.6	484.2	Uninfected	32	10
	A14V019	Rechallenge at day 35	27.1	2.3	313.8	Uninfected	21	6
	DGAC	Rechallenge at day 35	18.1	2.0	358.1	Infected	24	7
	A14V136	Rechallenge at day 35	13.2	2.8	179.8	Infected	12	4
DF1W-a.01	34557	100	15.6	11.6	295.9	Uninfected	135	57
	16C024	100	18.0	12.2	294.6	Uninfected	134	57
	15C333	100	21.3	12.4	266.2	Uninfected	121	51
	34558	100	8.8	16.0	279.6	Infected	127	54

bNAb. In animal A14V101, which received VRC34.01, the earliest plasma viral sequences analyzed were from 5 weeks after the SHIV challenge. Most of these sequences (27 of 30, or 90%) contained V172I + G514E mutations. G514E is in the amino terminus of the FP and was shown previously to confer resistance to neutralization by VRC34.01 (*20*). We confirmed this resistance (27-fold) in the BG505 Env background (Fig. 4B). The V172I mutation, which was present in all sequences, likely represented an incidental variant in the challenge stock. Two of the 30 sequences contained a G516E mutation (but not a G514E mutation), and one sequence contained a V513G mutation (also without a G514E mutation). Mutations at residues 513 and 516 were shown previously to confer virus escape from VRC34.01 (*10*, *20*). The finding of three resistance mutations at residues 513, 514, and 516 in different nonoverlapping sets of sequences can be explained most plausibly by postulating that a neutralization-sensitive wild-type SHIV.BG505.332 N.375Y virus lacking these mutations was responsible for breakthrough infection when circulating bNAb titers were subprotective and persistent but declining titers of VRC34.01 selected for neutralization escape in the evolving viral quasi-species at the three affected residues. Thus, we conclude that macaques 34558, DGAC, A14V136, and A14V101 were each infected by neutralization-sensitive wild-type SHIV.BG505.332 N.375Y viruses.

DISCUSSION

The identification and characterization of conserved neutralization epitopes on the HIV-1 Env trimer have led to antibody-based design approaches for HIV-1 vaccines (*1*, *21*, *22*). Targeting the FP on the HIV-1 envelope has been increasingly used as an HIV-1 vaccine strategy in animal models with successful elicitation of FP-directed antibodies that can neutralize diverse strains of HIV-1 (*8*, *9*, *11*–*14*). However, the protective efficacy of FP-directed antibodies against viral infection had not been previously demonstrated. Here, we show that both an FP-directed human bNAb from an individual living with HIV-1 and mAbs from vaccinated macaques can provide complete protection against SHIV infection. These results support the ongoing efforts to elicit FP-directed antibodies by prophylactic HIV-1 vaccination.

The protective titers for FP-directed antibodies observed in this study were similar to those that have been reported for antibodies targeting other conserved sites on the HIV-1 envelope glycoprotein in similar nonhuman primate models using mucosal SHIV challenges (*15*). The calculated plasma neutralization titers from this and prior passive infusion studies are also in line with the titers observed to be protective in the recently reported human trials of VRC01 in preventing HIV-1 transmission in high-risk individuals, although these titers tended to be slightly higher in humans (*18*, *23*). In the current study, the human FP-directed bNAb VRC34.01

displayed dose-dependent protection against mucosal SHIV challenge, with breakthrough infection observed only at plasma ID_{50} titers below 100. Between the two rhesus FP-directed mAbs, the more potent DF1W-a.01 was not more effective in providing protection compared to the other mAb, DFPH-a.15. This may be a function of the better in vivo pharmacokinetic profile for DFPH-a.15 compared with DF1W-a.01, because we observed higher plasma concentrations and slower clearance rate for DFPH-a.15 compared with DF1W-a.01. It is probable that concentrations of DF1W-a.01 were lower than DFPH-a.15 at the rectal mucosa where SHIV challenge occurred in this study. Therefore, faster removal of DF1W-a.01 from circulation compared with DFPH-a.15 could have limited the amount of antibody reaching the rectal mucosa. Overall, we saw protection provided by three different FP-directed antibodies against the SHIV challenge, validating the FP as a viable epitope to target for prophylactic HIV-1 vaccines.

A key feature of this study was the genetic and phenotypic characterization of breakthrough viruses, which allowed us to better understand the mechanisms of breakthrough infections. In four of four animals, breakthrough infections resulted from the transmission of wild-type, neutralization-sensitive viruses. This indicates that local and systemic concentrations of antibodies in these animals were insufficient, given their respective potencies, to prevent infection of susceptible cells. Conversely, in other animals where antibody concentrations were sufficiently high, the results indicate that FP-targeting bNAbs conferred sterilizing immunity and did not simply delay or retard infection. Together, the findings support a growing consensus that an effective HIV-1 vaccine will likely need to elicit antibodies targeting multiple bNAb epitopes so as to prevent infection by partially resistant viruses or the rapid acquisition of resistance when circulating antibody concentrations are suboptimal.

One limitation of the current study is the use of a SHIV strain having the FP sequence that is well recognized by VRC34.01, DFPH-a.15, and DF1W-a.01. $SHIV_{BG505}$ is derived from a clade A HIV-1 Env, and there is some conservation of the FP sequence across clades (*3*). However, there is some variation in the FP sequence among various strains of HIV-1 (*9*), and it would be informative to assess the protection provided by these antibodies against viruses with different FP sequences. In vitro neutralization data indicate some cross-reactivity of these mAbs against viruses with alternative FP sequences. Nonetheless, a cocktail of two or three mAbs or polyclonal vaccine serum targeting multiple FP sequences may potentially be required to confer broad protection against the majority of the prevalent HIV-1 strains with diverse FP sequences. In addition, the titers needed for protection, although in line with other passive transfer studies, may be difficult to achieve through vaccination. Overall, this study represents proof of concept that FP-directed antibodies can provide protection against SHIV infection. Because FP-directed protective antibodies can be elicited in some animals by vaccination, this work supports efforts to design and develop prophylactic HIV-1 vaccines that can elicit broad and potent FP-directed responses.

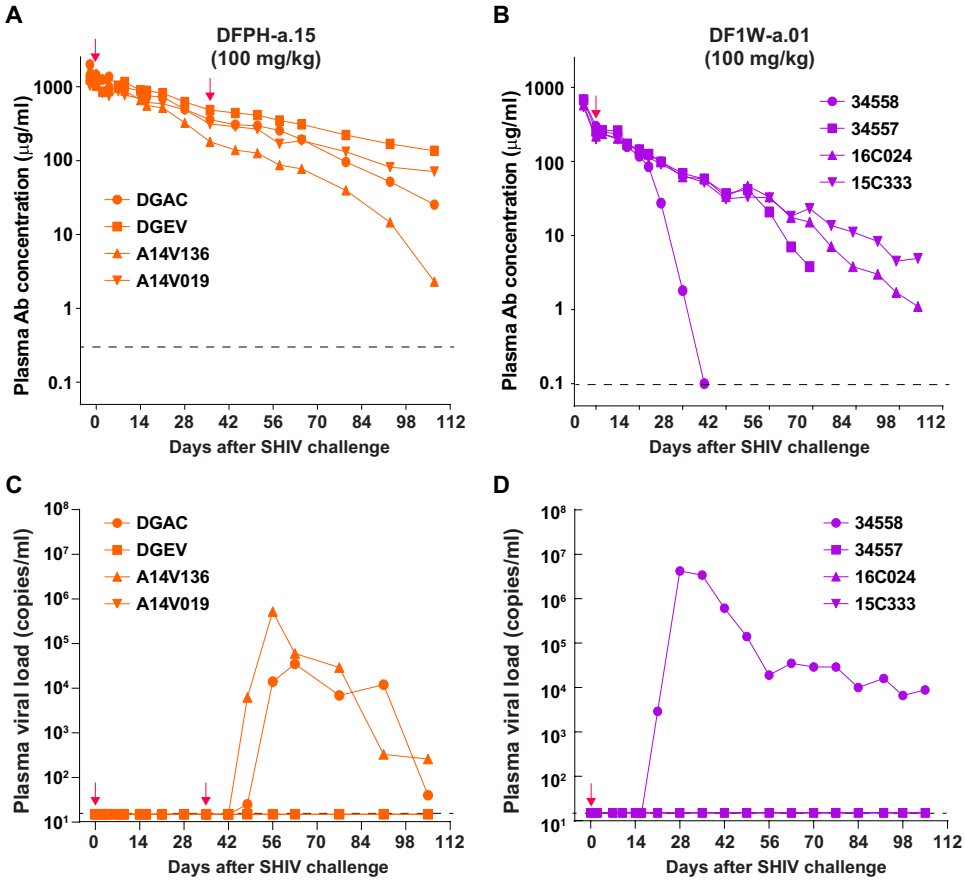


Fig. 3. Plasma mAb concentrations and plasma viral loads in rhesus macaques administered DFPH-a.15 or DF1W-a.01 followed by mucosal challenges with $SHIV_{BG505}$. (**A** and **B**) The plasma concentration of DFPH-a.15 (A) or DF1W-a.01 (B) IgG1 was measured using an FP-based ELISA after intravenous administration of 100 mg/kg dose of the antibody. (**C**) Plasma viral loads were measured in rhesus macaques that were administered a 100 mg/kg dose of DFPH-a.15 and rectally challenged either 5 or 35 days later with a single high dose of $SHIV_{BG505}$. (**D**) Plasma viral loads were measured in rhesus macaques that were administered a 100 mg/kg dose of DF1W-a.01 and rectally challenged 5 days later with a single high dose of $SHIV_{BG505}$. The red arrows indicate the time of mucosal SHIV challenges. The dashed horizontal lines indicate the limit of detection for each assay.

MATERIALS AND METHODS

Study design

To test the in vivo protective efficacy of anti-FP antibodies, we designed passive transfer experiments in rhesus macaques that were administered either human or rhesus anti-FP antibodies followed by a mucosal SHIV challenge. For the VRC34.01 protection experiment, the animal study was designed to have two groups of four animals each, receiving two different doses of VRC34.01 such that at least at one dose we will be able to get complete protection from infection. There was also one control group of four animals that did not receive any antibody before the $SHIV_{BG505}$ challenge. Therefore, having all animals protected in one of the groups gave enough statistical power to observe a significant difference between the control group and the antibody group. For the protection studies with DFPH-a.15 and DF1W-a.01, there was a single group of four animals receiving each antibody at one dose along with a control group of four animals that did not receive any antibody before the $SHIV_{BG505}$ challenge. The dose of antibody was selected such that all the animals would be protected after antibody infusion based on previous passive transfer studies. Therefore, having all animals protected in the antibody group compared with the control group gave enough statistical power to observe a significant difference between the control group and the antibody groups. In addition, all the animals were randomized into the antibody and control groups based on their weight to ensure even weight distribution. There was no blinding done during the administration of the antibody into the animals.

SHIV neutralization assays

Neutralization of the replication-competent $SHIV_{BG505}$ challenge stock was evaluated in vitro using Tzm-bl target cells (RRID:CVCL_B478) and a luciferase reporter assay as described previously (*24*–*26*). $SHIV_{BG505}$ (SHIV.BG505.332 N.375Y) was

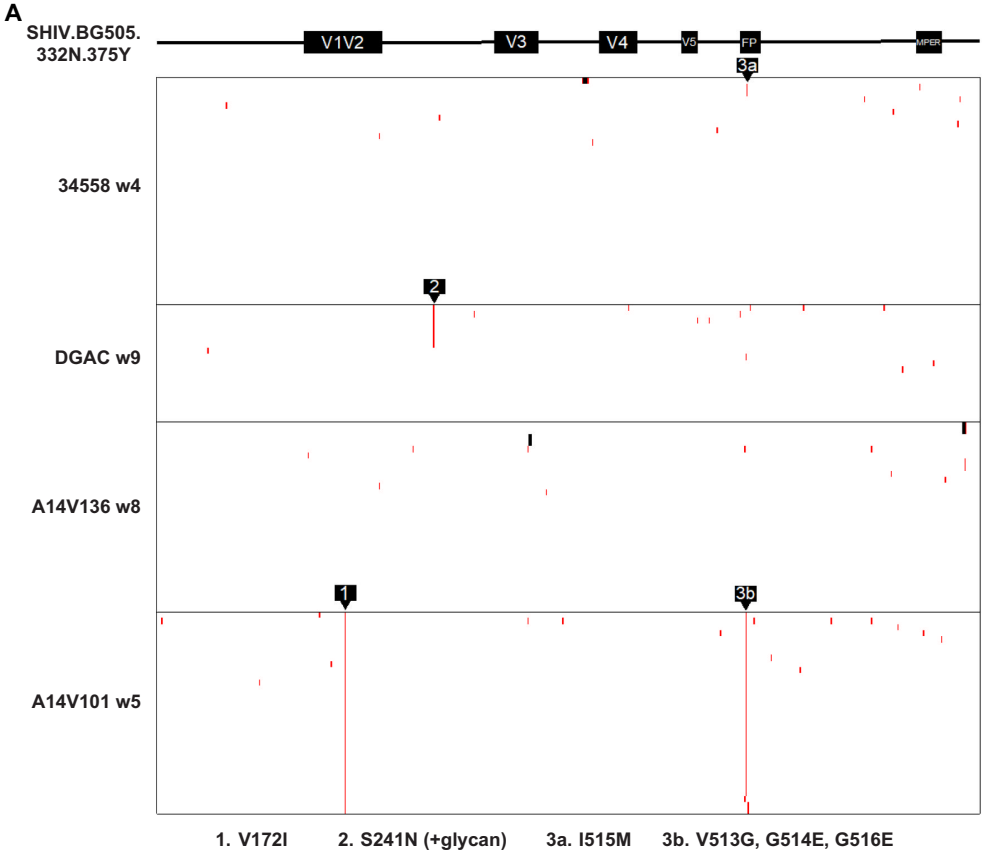


Fig. 4. Genotypic and phenotypic analysis of breakthrough infections in anti-FP bNAb-infused, SHIV-challenged macaques. (**A**) Pixel alignment (http://hiv.lanl.gov/content/sequence/pixel/pixel.html) of the Env gp160 single-genome sequences (rows) compared with the SHIV.BG505.332 N.375Y inoculum Env ectodomain sequence (shown with annotation at top). Amino acid substitutions (red) and deletions (black) are depicted, with positively selected mutations highlighted in the main text and denoted as 1, 2, 3a, and 3b. All sequences shown were deposited in GenBank (accession numbers OR343944 to OR344060). (**B**) IC_{50} values (μg/ml) and IC_{50} fold changes of FP bNAb mAbs against BG505.T332N wild-type (WT) and mutant pseudoviruses are shown in the tables. Neutralization against murine leukemia virus (MLV) is used as a negative control.

B

IC_{50} (μg/ml)	DF1W-a.01	DFPH-a15	VRC34.01
MLV	>50	>50	>50
BG505.332N WT	0.1915	1.743	0.09365
S241N	0.4464	5.124	0.2208
A512W	>50	>50	>50
G521E	>50	>50	2.519
I515L	>50	1.134	0.1617

>50 μg/ml
1–10 μg/ml
0.1–1 μg/ml
0.01–0.1 μg/ml

IC_{50} fold change	DF1W-a.01	DFPH-a15	VRC34.01
BG505.332N WT	1.00	1.00	1.00
S241N	2.33	2.94	2.36
A512W	>261	>28.6	>534
G514E	>261	>28.6	26.90
I515L	>261	0.65	1.73

<2-fold difference
2- to 10-fold resistance
>10-fold resistance
Complete knockout

incubated with the FP-targeting bNAb mAbs for 30 min at 37°C before Tzm-bl cells were added. The protease inhibitor indinavir was added to a final concentration of 1 μM to limit infection of target cells to a single round of viral replication. Luciferase expression was quantified 48 hours after infection upon cell lysis and the addition of luciferin substrate (Promega). Additional neutralization assays were conducted with site-directed mutants of SHIV.BG505.332 N.375Y (S241N, A512W, G514E, and I515L) Env-expressing pseudoviruses.

Nonhuman primates

All animals (naïve male or female rhesus macaques, aged 2 to 4 years) were housed and cared for in accordance with Guide for Care and Use of Laboratory Animals Report number NIH 82-53 (Department of Health and Human Services, Bethesda, MD, USA, 1985) in a biosafety level 2 National Institute of Allergy and Infectious Diseases (NIAID) facility. All animal procedures and experiments were performed according to protocols (protocol number VRC-16-686) approved by the Institutional Animal Care and Use Committee of the NIAID. The animals were intrarectally challenged with $SHIV_{BG505}$. Briefly, animals were inoculated intrarectally with 1 ml of a 1:8 dilution of challenge stock (p27 concentration: 154.2 ng/ml). This corresponds to an AID of approximately 5 to 10 based on the reported AID_{50} titer, which was 1 ml of 1:120 dilution of the challenge stock (*19*). Plasma viremia was quantitated using a polymerase chain reaction–based method to quantify simian immunodeficiency virus *gag* RNA concentrations with a detection limit of 15 copies/ml as described previously (*27*).

Generation of monoclonal antibodies

All anti-FP mAbs were produced from transiently transfected Expi293 cells and purified using a protein A column. Briefly, VRC34.01 variable sequences were cloned into vectors expressing the human heavy (IgG1, immunoglobulin G 1) and kappa light chain constant regions. In addition, the LS (M428L/N434S) mutation was included in the heavy constant domain that has been shown to increase in vivo half-life (*28*). Similarly, DF1W-a.01 and DFPH-a.15 variable sequences were cloned into vectors expressing the rhesus macaque kappa light and heavy (IgG1) chain constant regions; these sequences also included the LS mutation. The heavy and light chain plasmids for each mAb were then used for transient transfection of Expi293 cells. Cells were then incubated for 4 to 5 days. The mAbs were purified from the culture supernatants using a protein A column.

ELISA to measure plasma concentration of antibody infusions

VRC34.01, DF1W-a.01, and DFPH-a.15 concentrations were measured using quantitative enzyme-linked immunosorbent assay (ELISA)–based methods in which microtiter plates coated overnight at 4°C with biotinylated FP probe (2 μg/ml) in phosphate-buffered saline (PBS) were used to capture the administered antibodies followed by detection using horseradish peroxidase (HRP)–conjugated anti-human IgG antibody (Jackson Immunoresearch, catalog no. 709-005-098, RRID: AB_2340482). After coating, microtiter plates were washed with PBS-T (PBS with 0.05% Tween 20) and blocked with tris-buffered saline containing 5% skim milk, 2% bovine serum albumin, and 0.1% Tween 20 (blocking buffer). Plasma from macaques was diluted in a blocking buffer. Diluted samples were incubated on the plates alongside standard curves of known antibody concentration for 1 hour at room temperature, followed by a PBS-T wash. The plates were then incubated with a 1:10,000 dilution of HRP-conjugated anti-human IgG antibody in a blocking buffer for 30 min at room temperature. The plates were washed with PBS-T, and then the SureBlue TMB (Kirkegaard & Perry Laboratories) substrate was added. The plates were allowed to develop for 15 min in the dark and then stopped with 1 N H_2SO_4 before reading the optical density at 450 nm using a SpectraMax plus 96 spectrophotometer (Molecular Devices). ELISAs were performed two independent times for each sample, and the data reported are an average from these two independent experiments.

SHIV envelope sequencing and analysis

Single-genome sequencing of SHIV 3′ half genomes was performed as previously described (*29*, *30*). Geneious Prime was used for alignments and sequence analysis, and sequences were visualized using the LANL Pixel tools (www.hiv.lanl.gov/content/sequence/pixel/pixel.html) (*31*). All sequences have been submitted to GenBank under the accession numbers OR343944 to OR344060.

Statistical analysis

All raw, individual data are presented in data file S1. For comparison of pharmacokinetic parameters like day of challenge plasma concentrations, half-lives, and clearance for DF1W-a.01 and DFPH-a.15, we performed a nonparametric unpaired two-tailed Mann-Whitney test in GraphPad Prism. For comparison of the proportion of protected animals between bNAb-treated and control groups, we used the log-rank (Mantel-Cox) test analysis in GraphPad Prism. All statistical tests were done with significance determined as $P < 0.05$.

Supplementary Materials

This PDF file includes:
Fig. S1

Other Supplementary Material for this manuscript includes the following:
Data file S1
MDAR Reproducibility Checklist

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