

RESEARCH ARTICLE

# Potent antibody-dependent cellular cytotoxicity of a V2-specific antibody is not sufficient for protection of macaques against SIV challenge

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## Abstract

Fc-mediated antibody effector functions, such as antibody-dependent cellular cytotoxicity (ADCC), can contribute to the containment HIV-1 replication but whether such activities are sufficient for protection is unclear. We previously identified an antibody to the variable 2 (V2) apex of the HIV-1 Env trimer (PGT145) that potently directs the lysis of SIV-infected cells by NK cells but poorly neutralizes SIV infectivity. To determine if ADCC is sufficient for protection, separate groups of six rhesus macaques were treated with PGT145 or a control antibody (DEN3) by intravenous infusion followed five days later by intrarectal challenge with SIV<sub>mac</sub>239. Despite high concentrations of PGT145 and potent ADCC activity in plasma on the day of challenge, all animals became infected and viral loads did not differ between the PGT145- and DEN3-treated animals. To determine if PGT145 can protect against a neutralization-sensitive virus, two additional groups of six macaques were treated with PGT145 and DEN3 and challenged with an SIV<sub>mac</sub>239 variant with a single amino acid change in Env (K180S) that increases PGT145 binding and renders the virus susceptible to neutralization by this antibody. Although there was no difference in virus acquisition, peak and chronic phase viral loads were significantly lower and time to peak viremia was significantly delayed

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in the PGT145-treated animals compared to the DEN3-treated control animals. Env changes were also selected in the PGT145-treated animals that confer resistance to both neutralization and ADCC. These results show that ADCC is not sufficient for protection by this V2-specific antibody. However, protection may be achieved by increasing the affinity of antibody binding to Env above the threshold required for neutralization.

## Author summary

Antibodies that bind to the human immunodeficiency virus (HIV-1) envelope glycoprotein (Env) on virions can neutralize viral infectivity. Antibodies may also bind to Env on the surface of virus-infected cells and recruit immune cells to eliminate the productively infected cells through a process known as antibody dependent cellular cytotoxicity (ADCC). In rare instances, certain antibodies are capable of mediating ADCC despite negligible neutralizing activity. Such antibodies are thought to have contributed to the modest protection observed in the RV144 HIV-1 vaccine trial and in some nonhuman primate studies. One antibody, PGT145, was found to cross-react with simian immunodeficiency virus (SIV) and to mediate potent ADCC against SIV-infected cells despite weak neutralization of viral infectivity. We therefore tested if the potent ADCC activity of PGT145 could protect rhesus macaques against mucosal challenge with pathogenic SIV. PGT145 did not protect against wild-type SIV<sub>mac239</sub>, but did protect against an SIV<sub>mac239</sub> variant with a single amino acid substitution in Env (K180S) that increases antibody binding to Env and makes the virus susceptible to neutralization. Thus, while ADCC may contribute to protection against immunodeficiency viruses through the elimination of productively infected cells, the higher affinity of Env binding necessary for potent neutralization is a critical determinant of antibody-mediated protection.

## Introduction

The RV144 trial remains the only clinical vaccine trial to show a modest reduction in the rate of HIV-1 infection [1]. Antibodies capable of binding to the variable 1 and 2 (V1V2) loops of HIV-1 gp120 were identified as a correlate of protection [2] and sequence analysis of breakthrough infections later revealed V2 signatures consistent with a sieving effect of vaccine-induced immune responses [3]. While these antibodies did not neutralize circulating HIV-1 isolates, a non-significant trend towards a lower risk of HIV-1 acquisition was observed among vaccinees with higher antibody-dependent cellular cytotoxicity (ADCC) [2]. It is widely cited that ADCC did correlate with a lower risk of HIV-1 acquisition after excluding vaccinees with high IgA titers. However, it is less well appreciated that so did all of the other primary variables, including tier 1 HIV-1 neutralization, IgG binding avidity and Env-specific CD4+ T cell responses [2]. Subsequent studies revealed the induction of Env-specific antibodies with enhanced Fc-mediated effector functions, but using methods that do not reflect physiologically relevant conformations of Env on virus-infected cells [4,5]. While the results of the RV144 trial continue to be debated [6], particularly in light of the failure of the similarly designed HVTN 702 trial [7], these studies have led to the hypothesis that Fc-mediated antibody effector functions may afford some protection against HIV-1 in the absence of detectable neutralization.

Attempts to model the types of immune responses that may have contributed to protection in the RV144 trial have yielded mixed results. While a few prime-boost vaccine studies in

macaques using different vectors, adjuvants and challenge viruses observed correlations of V2-specific antibodies with protection [8–13], others found no such association [14–18]. Passive administration of a V2-specific antibody with limited neutralization potency reduced viral loads in macaques following mucosal challenge with the tier 1 SHIV BaL.P4 [19]. Partial protection against mucosal SIV<sub>mac</sub>251 challenge was also achieved following the infusion of non-neutralizing IgG purified from vaccinated macaques with polyfunctional antibody signatures [20]. Furthermore, adeno-associated virus delivery of an antibody that directs ADCC against SIV-infected cells but lacks detectable neutralizing activity protected a single animal from mucosal SIV challenge [21]. However, the majority of passive transfer experiments with non-neutralizing antibodies (nnAbs) have failed to protect against SIV or SHIV challenge [22–24]. Thus, definitive evidence for protection by nnAbs has been elusive.

We and others have shown that ADCC correlates with neutralization and that nnAbs generally do not mediate ADCC against HIV- or SIV-infected cells [25–30]. This implies that most antibodies that are capable of binding to functional Env trimers on virions to block viral infectivity are also capable of binding to Env on the surface of virus-infected cells to mediate ADCC. Nevertheless, ADCC in the absence of detectable neutralization and neutralization in the absence of ADCC have been observed indicating that these antiviral functions may be uncoupled for certain antibodies [25,30].

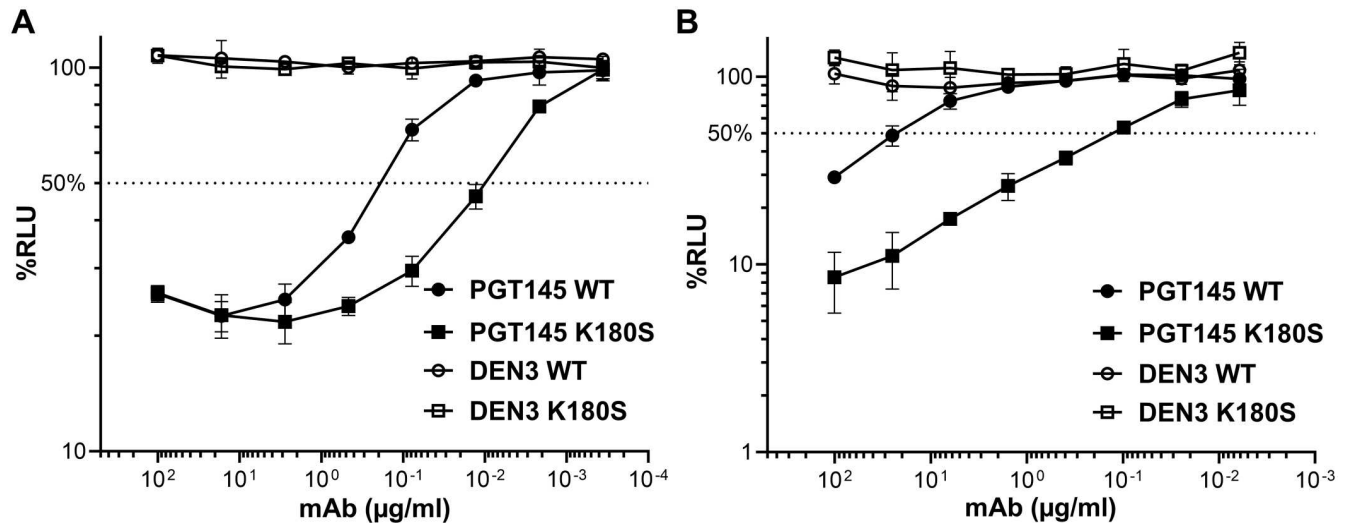
One such antibody showing an uncoupling of neutralization and ADCC for SIV<sub>mac</sub>239 is PGT145. This antibody binds to a proteoglycan epitope at the V2 apex of HIV-1 Env trimers and potently neutralizes genetically diverse HIV-1 isolates [31]. We previously showed that PGT145 also binds to Env on the surface of SIV-infected cells and directs efficient NK cell killing of SIV-infected cells but only very weakly neutralizes SIV infectivity [32]. We proposed that the affinity of PGT145 for SIV Env is sufficient to allow enough binding to virus-infected cells to promote ADCC but not enough for potent neutralization. Indeed, a single amino acid substitution in the V2 core epitope of SIV Env increases PGT145 binding and confers notable sensitivity to neutralization [32]. In the present study, we took advantage of these properties of PGT145 to investigate the extent to which ADCC is sufficient for protection and the effect of a minimal change in Env that makes the virus sensitive to neutralization.

## Results

Neutralization of HIV-1 by PGT145 is dependent on antibody binding to a quaternary proteoglycan epitope at the V2 apex that includes an N-linked glycan at position 160 (N160) and a polybasic region at the convergence of all three gp120 protomers [31]. PGT145 is therefore specific for Env trimers and does not bind to monomeric gp120 [33]. Using an assay designed to measure ADCC against virus-infected cells expressing natural conformations of Env [34], we found that PGT145 efficiently directs ADCC against SIV<sub>mac</sub>239-infected cells (Fig 1A), but only weakly neutralizes replication-competent SIV<sub>mac</sub>239 at high concentrations ( $\geq 25$   $\mu\text{g/ml}$ ) (Fig 1B) [32]. The cross-reactivity of PGT145 with SIV is a consequence of the binding of this antibody to a conserved epitope at the V2 apex of SIV Env trimers that includes an N-linked glycan at position 171 (N171) and basic residues in the V2 core epitope [32]. Further analysis of Env substitutions in this region identified a single amino acid substitution (K180S) that enhances PGT145 binding to Env, increases the susceptibility of SIV-infected cells to ADCC (Fig 1A), and is sufficient to confer sensitivity to neutralization (Fig 1B) [32].

### PGT145 does not protect rhesus macaques against wild-type SIV<sub>mac</sub>239

To determine if ADCC is sufficient for protection, separate groups of six rhesus macaques of Indian ancestry were treated with PGT145 or a control antibody (DEN3). PGT145 and DEN3



**Fig 1. Sensitivity of SIV<sub>mac239</sub> and SIV<sub>mac239</sub> K180S to PGT145-mediated ADCC and neutralization.** (A) CEM.NKR<sup>-CCR5-sLTR</sup>-Luc cells were infected with wild-type SIV<sub>mac239</sub> (WT) or SIV<sub>mac239</sub> K180S (K180S) and incubated with an NK cell line (KHYG-1 cells) expressing rhesus macaque CD16 at a 10:1 effector to target (E:T) ratio for eight hours in the presence of serial dilutions of mAbs PGT145 and DEN3. ADCC was calculated as the percent relative light units (% RLU) of luciferase activity remaining in SIV-infected cells incubated with antibody relative to SIV-infected cells without antibody after correcting for the background luciferase activity present in uninfected cells. (B) Wild-type SIV<sub>mac239</sub> (WT) and SIV<sub>mac239</sub> K180S (K180S) were incubated with serial dilutions of PGT145 and DEN3 for one hour before addition to TZM-bl cells. Neutralization was measured as the dose-dependent reduction in luciferase activity (% RLU) for viruses incubated with antibody relative to viruses without antibody after subtracting the background luciferase activity present in uninfected cells. ADCC and neutralization values reflect the mean and standard deviation (error bars) for triplicate wells at each antibody concentration. The dotted lines indicate half-maximal responses.

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were administered intravenously at doses of 30 mg/kg. After a five-day interval to allow the antibodies time to become distributed throughout the tissues [35], the animals were challenged intrarectally with an animal-titred stock of SIV<sub>mac239</sub> at a dose sufficient to infect all the control animals (6,000 TCID<sub>50</sub>). On the day of challenge, the average concentration of PGT145 in plasma for the animals that received this antibody was  $307 \pm 58.5$  µg/ml (Table 1). Plasma from each of the PGT145-treated animals collected on the day of challenge also mediated ADCC against SIV<sub>mac239</sub>-infected cells with a mean 50% ADCC titer of  $239 \pm 50$  (Fig 2A and Table 1). Consistent with only weak neutralization of wild-type SIV at high concentrations of PGT145, neutralizing antibodies to SIV<sub>mac239</sub> were not detectable in serum from any of the animals (Fig 2B). However, as expected these samples did neutralize SIV<sub>mac239</sub> K180S (Table 1). Following intrarectal challenge, all of the animals became infected and viral loads did not differ significantly during acute or chronic (8–20 weeks post-challenge) infection between PGT145- and DEN3-treated animals ( $p = 0.4$ , SE = 0.20, linear regression and  $p = 0.4$ , SE = 0.46, linear mixed models, respectively) (Fig 2C). Sequence analysis of the virus population in plasma at week 3 post-infection revealed two Env substitutions (V67M and R751G) that emerged in both PGT145- and DEN3-treated animals, as well as a few low frequency substitutions that were present in only one or two animals (S1 Fig). Hence, there was also no evidence of selective pressure for resistance to PGT145. Thus, the potent ADCC activity of PGT145 against SIV-infected cells as measured in a standard *in vitro* assay does not predict protection against SIV<sub>mac239</sub> and suggests that this antiviral function alone is not sufficient for protection against viral challenge.

### PGT145 partially protects rhesus macaques against SIV<sub>mac239</sub> K180S

We previously demonstrated that the relatively low binding affinity of PGT145 for SIV Env is sufficient to trigger ADCC by cross-linking multiple Env trimers on the surface of

**Table 1. PGT145 concentration, ADCC and neutralizing antibody titers on the day of challenge.**

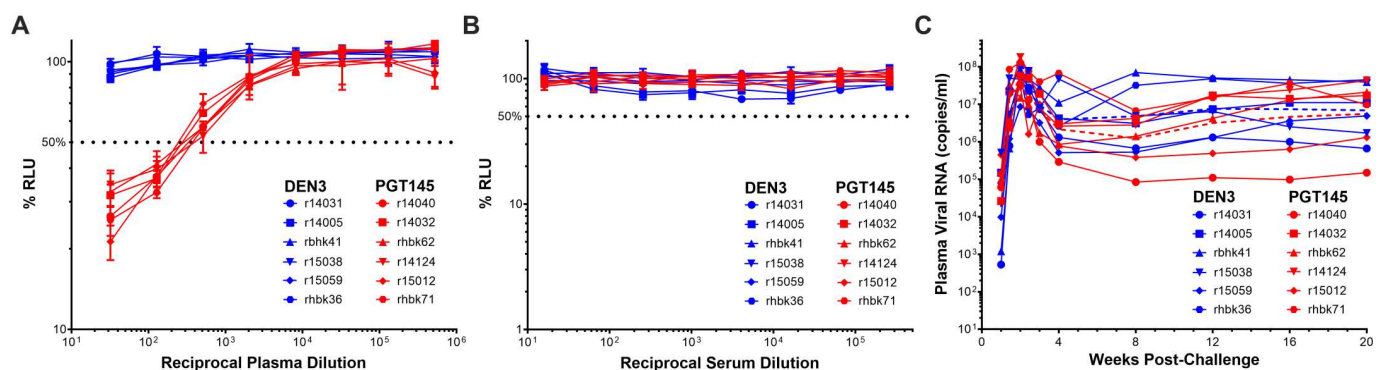
Study	Animal	PGT145 ( $\mu\text{g/ml}$ )	50% ADCC titer <sup>a</sup>	50% Neut. titer <sup>b</sup>
Wt 239 challenge	r14040	367	245	695
	r14032	340	201	483
	rhbk62	319	217	461
	r14124	293	313	850
	r15012	214	181	927
	rhbk71	-	279	466
	Mean $\pm$ SD	307 $\pm$ 58.5	239 $\pm$ 50.0	647 $\pm$ 208
K180S challenge	rhbj64	330	434	454
	rh2761	280	398	725
	rhbj89	280	888	1251
	r09070	395	452	685
	r14054	252	1406	1205
	r15003	243	1399	1571
	Mean $\pm$ SD	296 $\pm$ 57.1	830 $\pm$ 479	982 $\pm$ 425

<sup>a</sup>ADCC responses were measured against SIV<sub>mac239</sub>-infected cells for the animals challenged with wild-type SIV<sub>mac239</sub> and against SIV<sub>mac239</sub> K180S-infected cells for the animals challenged with SIV<sub>mac239</sub> K180S.

<sup>b</sup>Neutralizing antibody titers were measured against SIV<sub>mac239</sub> K180S for the animals challenged with both wild-type SIV<sub>mac239</sub> and SIV<sub>mac239</sub> K180S.

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SIV<sub>mac239</sub>-infected cells to Fc $\gamma$  receptors on NK cells, but is not high enough to block viral infectivity [32]. However, a single amino acid substitution in the V2 core epitope (K180S) increases PGT145 binding to Env and ADCC by approximately 100-fold [32]. This substitution also renders SIV<sub>mac239</sub> K180S susceptible to neutralization by PGT145 (Fig 1B). To determine if the combined antiviral effects of neutralization and ADCC translate into better protection, PGT145 and DEN3 were administered intravenously to two additional groups of six rhesus macaques at doses of 30 mg/kg and protection was assessed five days later by



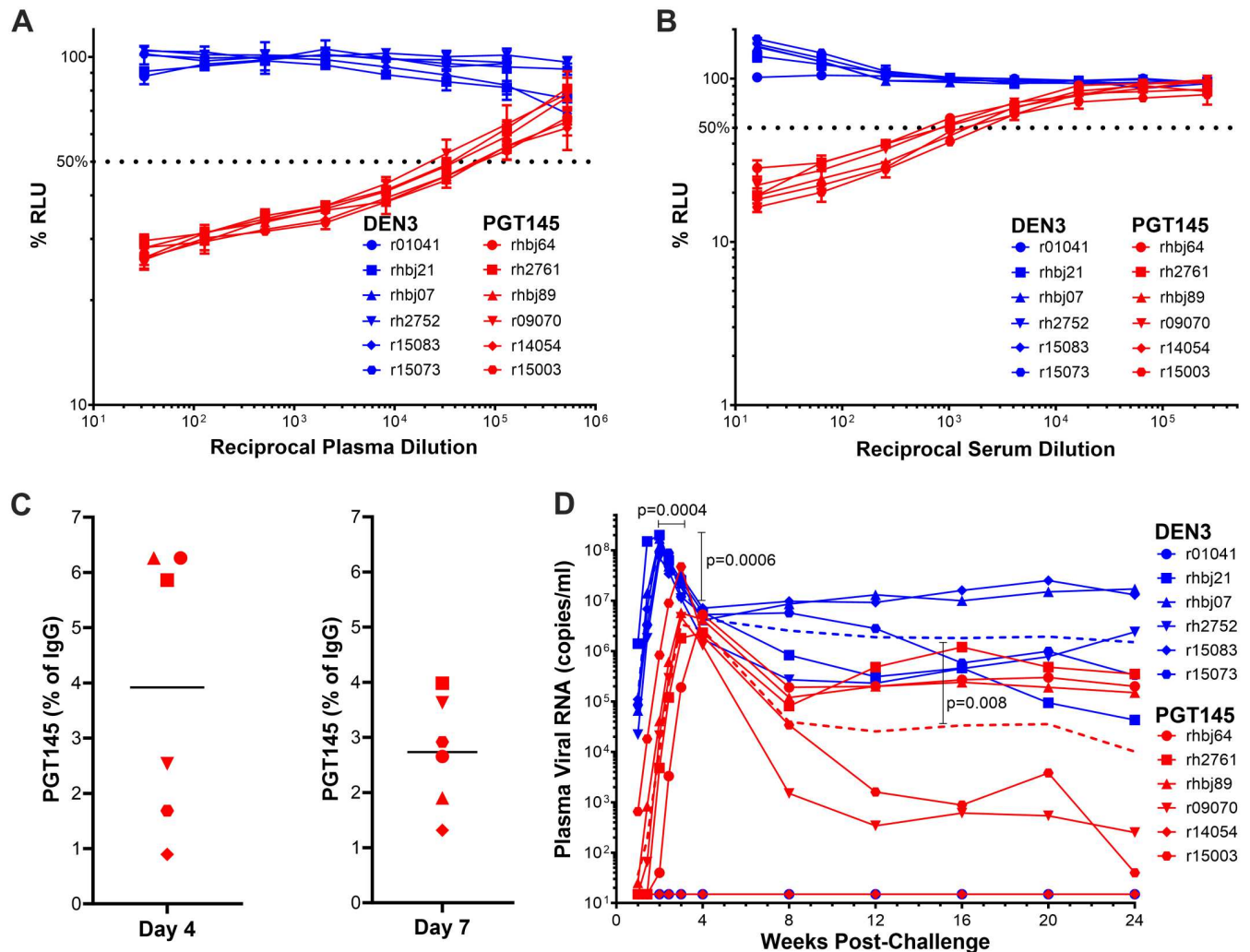
**Fig 2. PGT145 does not protect against wild-type SIV<sub>mac239</sub> challenge.** (A) ADCC responses in plasma at the time of challenge. CEM<sub>10</sub>-<sub>NKR</sub>-CCR5-sLTR-Luc cells infected with SIV<sub>mac239</sub> were incubated with a NK cell line expressing rhesus macaque CD16 at a 10:1 effector/target ratio in the presence of serial dilutions of plasma collected on the day of challenge from animals treated with either PGT145 or DEN3. (B) Neutralization activity in plasma at the time of challenge. ADCC was calculated from the dose-dependent loss of luciferase activity (% RLU) after an 8 hour incubation. SIV<sub>mac239</sub> was incubated in the presence of serial dilutions of plasma for 1 hour before addition to TZM-bl cells. Luciferase induction was measured after 48 hours and neutralization was calculated from the dose-dependent inhibition of luciferase (% RLU). (C) Post-challenge viral loads. Viral RNA loads in plasma were measured using a qRT-PCR assay with a detection threshold of 15 copies/ml. Geometric mean viral loads for the DEN3 and PGT145 groups are shown as dashed lines. Peak and chronic phase (8–20 weeks post-challenge) viral loads were not significantly different in the animals treated with PGT145 and DEN ( $p = 0.4$ ,  $SE = 0.20$ , linear regression and  $p = 0.4$ ,  $SE = 0.46$ , linear mixed models, respectively).

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intrarectal challenge with SIV<sub>mac239</sub> K180S. The challenge dose for this study was increased five-fold to 30,000 TCID<sub>50</sub>, as this was determined to be the minimum dose required to infect three of three naïve macaques by intrarectal inoculation in a prior animal titration experiment.

On the day of challenge, the average PGT145 concentration in plasma among the animals that received this antibody was 296 ± 57.1 µg/ml (Table 1). Potent ADCC and neutralizing antibody responses to SIV<sub>mac239</sub> K180S were detectable with mean 50% titers of 830 ± 479 and 982 ± 425, respectively (3A, Fig 3B and Table 1). PGT145 was also detectable in rectal transudate at 0.90–6.3% and 1.3–4.0% (median 4.2% and 2.8%) of total IgG confirming that the



**Fig 3. PGT145 affords partial protection against SIV<sub>mac239</sub> K180S challenge.** (A) ADCC responses in plasma at the time of challenge. CEM<sub>NKR</sub>-CCR5-sLTR-Luc cells infected with SIV<sub>mac239</sub> K180S were incubated with a NK cell line expressing rhesus macaque CD16 at a 10:1 E:T ratio in the presence of serial dilutions of plasma collected from animals treated with either PGT145 or DEN3. ADCC was calculated from the dose-dependent loss of luciferase activity (% RLU) after an 8 hour incubation. (B) Neutralization activity in serum at the time of challenge. SIV<sub>mac239</sub> K180S was incubated in the presence of serial dilutions of serum for 1 hour before addition to TZM-bl cells. Luciferase induction was measured after 48 hours and neutralization was calculated from the dose-dependent inhibition of luciferase (% RLU). (C) PGT145 concentrations as a percentage of total IgG in rectal transudate. PGT145 and total IgG concentrations were measured by ELISA in mucosal secretions eluted from rectal swabs collected on days 4 and 7 post-challenge. (D) Post-challenge viral loads. Viral RNA loads in plasma were measured using a qRT-PCR assay with a detection threshold of 15 copies/ml. Geometric mean viral loads for the DEN3 and PGT145 groups (excluding uninfected animals) are shown as dashed lines. Peak viral loads were delayed 1.4 weeks ( $p = 0.0004$ ,  $SE = 0.24$ , linear regression) and were 1.28 log lower ( $p = 0.0006$ ,  $SE = 0.24$ , linear regression) in the PGT145-treated animals. Chronic phase (8–24 weeks post-challenge) viral loads were also lower in the PGT145-treated animals ( $p = 0.008$ ,  $SE = 0.7$ , linear mixed models).

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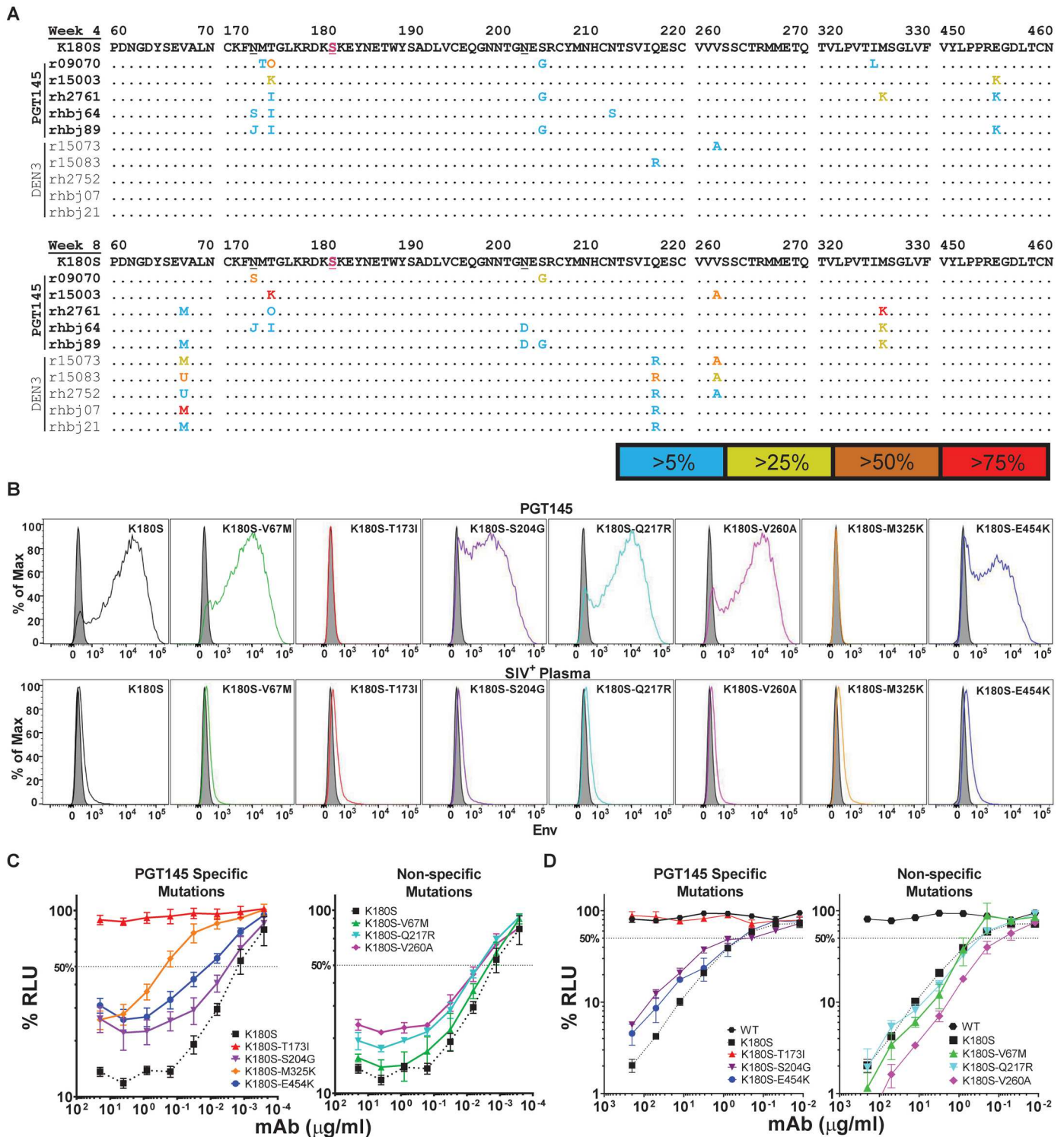
antibody was present at the site of challenge (Fig 3C). Following SIV<sub>mac239</sub> K180S challenge, five of the six animals in each group became infected (Fig 3D). PGT145 therefore did not prevent virus acquisition, even at a challenge dose that was not quite high enough to infect all the control animals. However, significant differences in the timing and peak of viremia were observed. Compared to the DEN3-treated control group, peak viral RNA loads in plasma during acute infection were delayed by 1.4 weeks ( $p = 0.0004$ ,  $SE = 0.24$ , linear regression) and were 1.28 log lower ( $p = 0.0006$ ,  $SE = 0.24$ , linear regression) in the PGT145-treated animals (Fig 3D). During chronic infection (8–24 weeks post-challenge) viral loads were also significantly lower in the PGT145-treated animals ( $p = 0.008$ ,  $SE = 0.7$ , linear mixed models) (Fig 3D). Therefore, even though PGT145 did not prevent SIV<sub>mac239</sub> K180S transmission, these viral load differences reveal a significant impact of the antibody on virus replication.

Nevertheless, the protection afforded by PGT145 was not as complete as might have been expected based on a previous meta-analysis of the relationship between neutralizing antibody titers and protection in previous passive immunization studies [36]. The mean 50% neutralization titer (ID50) in serum against SIV<sub>mac239</sub> K180S on the day of challenge was  $982 \pm 425$ , which exceeded the ID50 titer of 685 (95% CI 319, 1471) predicted to afford 95% protection against mucosal SHIV challenge [36]. The explanation for this difference is presently unclear but may reflect resistance of the challenge virus to complete neutralization by PGT145. To investigate this possibility, serial dilutions of the SIV<sub>mac239</sub> K180S challenge stock were incubated with 50  $\mu\text{g/ml}$  of PGT145 and K11 before the addition of TZM-bl cells. In contrast to K11, which binds to a glycan hole on the gp120 surface of the SIV Env trimer and completely neutralizes SIV<sub>mac239</sub> K180S at high multiplicities of infection [37], residual infectivity became detectable at a 10-fold virus dilution (1.4 ng/ml p27) in the presence of PGT145 (S2 Fig). These results are similar to the previously reported incomplete neutralization of HIV-1 by V2 apex bnAbs [38,39]. The limited protection against SIV<sub>mac239</sub> K180S challenge may therefore be due to a fraction of the challenge virus that is resistant to PGT145, possibly as a consequence of heterogenous Env glycosylation.

### PGT145 selects for escape mutations in Env

To better understand incomplete protection against SIV<sub>mac239</sub> K180S, the virus population in plasma was sequenced at weeks 4 and 8 post-challenge. These time points were selected to capture the emergence of antibody escape mutations when virus replication was occurring in the presence of passively administered PGT145 (S3 Fig). While the K180S change was retained as a fixed substitution in all the animals at both time points, several Env changes were observed in two or more of the PGT145-treated animals that were not observed in the DEN3-treated control animals. With a couple of exceptions, these Env substitutions were present at a low frequency that did not exceed 75% of the virus population (Fig 4A). The low frequency of these substitutions probably reflects the incomplete and transient nature of PGT145 selection, owing to a fraction of neutralization-resistant virus in the challenge stock (S2 Fig) coupled with declining concentrations of PGT145 in serum after passive transfer (S3 Fig).

At week 4 post-infection, the virus population of all five of the PGT145-treated animals acquired changes in residues 171–173 that coincide with the loss of an N-linked glycosylation site previously shown to be essential for PGT145 binding (Fig 4A) [32]. Two additional substitutions were also detected in three of these animals (S204G and E454K). The S204G substitution disrupts another N-linked glycosylation site (residues 202–204) that was not previously implicated in Env binding by PGT145. At week 8 post-infection, substitutions in residues 171–173 persisted in four of the PGT145-treated animals and increased in frequency in two of them (Fig 4A). Amino acid changes in residues 202–204 were also present in three animals



**Fig 4. PGT145 treatment selects for amino acid changes in Env that confer resistance to ADCC and to neutralization.** (A) Viral RNA was isolated from plasma at weeks 4 and 8 post-infection and sequenced. The predicted amino acid sequences in Env from PGT145-treated animals (top, bold) and from DEN3-treated animals (bottom, plain text) were aligned to SIV<sub>mac</sub>239 K180S Env. Regions of Env with substitutions in multiple animals are shown. The K180S substitution is indicated in magenta and residues N171 and N202 are underlined in the SIV<sub>mac</sub>239 K180S Env reference sequence. Positions of identity are indicated by periods and amino acid differences are identified by their single letter code. Amino acid ambiguities are indicated with non-standard letters as follows: U = M/L, J = S/K, O = A/I (week 4) and O = I/K (week 8). The frequencies of each substitution within the virus population are indicated by color. (B) Antibody binding to Env was assessed by staining CEM<sub>NKR</sub>.CCR5-sLTR-Luc cells infected with each of the indicated SIV<sub>mac</sub>239 K180S mutants with PGT145 (top row) and with SIV+ plasma



(bottom row) followed by AF647-conjugated goat anti-human IgG F(ab')<sub>2</sub> after gating on virus-infected (CD4<sub>low</sub>, p27<sup>+</sup>) cells. The shaded histograms represent non-specific staining with DEN3. (B & C) SIV<sub>mac239</sub> K180S mutants with substitutions in Env selected only in PGT145-treated animals (specific) or in both PGT145- and DEN3-treated animals (non-specific) were tested for susceptibility to ADCC (B) and neutralization (C) mediated by PGT145. The dotted lines indicate half-maximal ADCC or neutralization and the error bars indicate standard deviation of the mean for triplicate wells at each antibody concentration.

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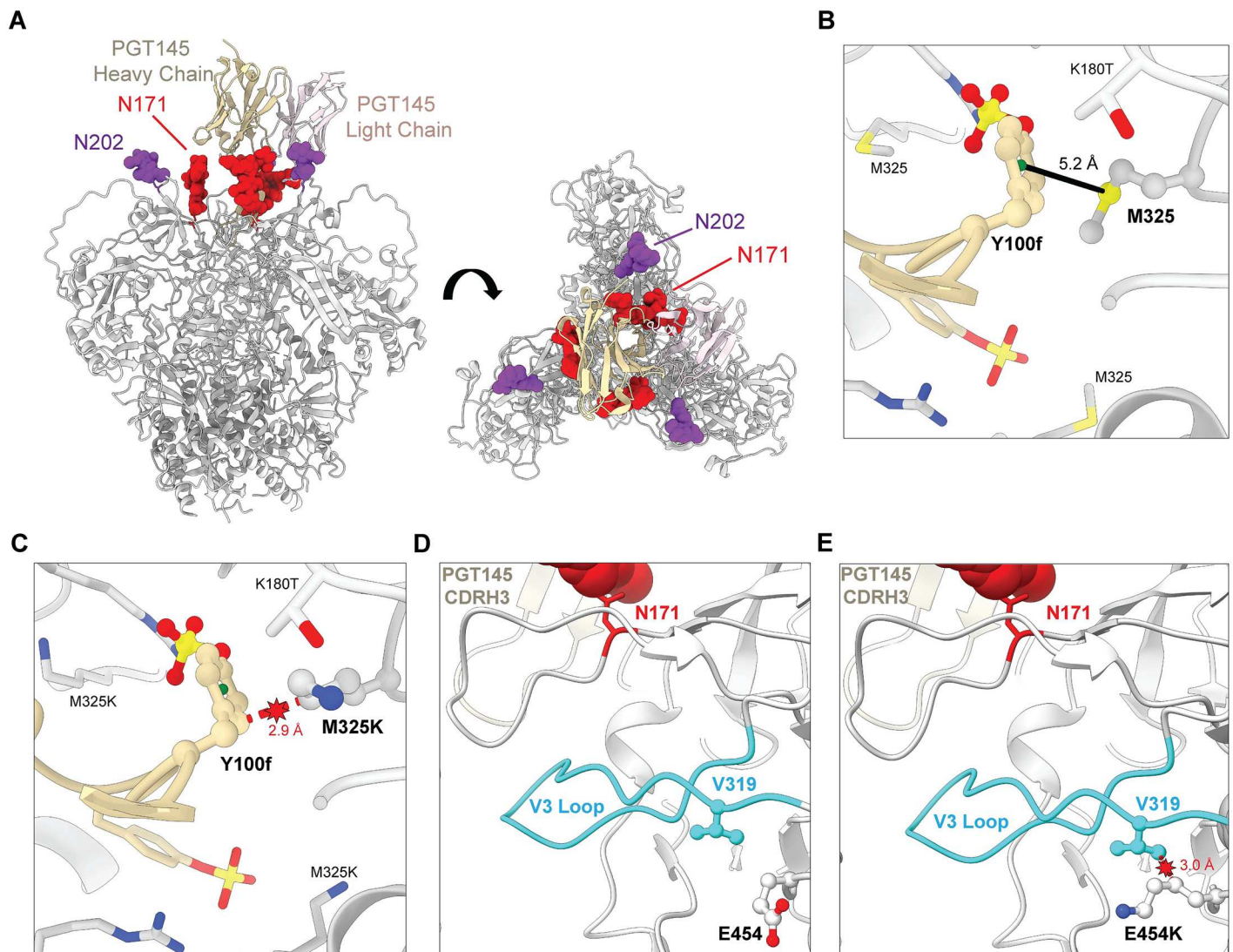
(Fig 4A). Although the E454K substitution was not detected at week 8, the virus acquired another substitution (M325K) in three of the PGT145-treated animals (Fig 4A). Additional Env changes were also observed that were independent of PGT145 selection. Two of these substitutions, V67M and R751G, occurred in both PGT145- and DEN3-treated animals following infection with either wild-type SIV<sub>mac239</sub> or SIV<sub>mac239</sub> K180S (Figs 4A and S1). Two others, Q217R and V260A, emerged in the DEN3-treated animals after infection with SIV<sub>mac239</sub> K180S and may be related to the adaptation of this variant for more efficient replication in macaques (Fig 4A).

Env substitutions selected in SIV-infected macaques were introduced into SIV<sub>mac239</sub> K180S to assess their impact on the sensitivity of virus-infected cells to PGT145 binding and ADCC. These included four changes that emerged in the PGT145-treated animals (T173I, S204G, M325K and E454K) and three in the DEN3-treated control animals (V67M, Q217R and V260A). None of these substitutions impaired Env expression on the surface of infected cells as indicated by similar levels of Env staining using plasma pooled from SIV-infected animals of other studies (Fig 4B). The V67M, Q217R and V260A substitutions that arose in the control animals also did not reduce PGT145 staining (Fig 4B). In contrast, each of the substitutions selected in the PGT145-treated animals partially or completely impaired PGT145 binding to virus-infected cells. PGT145 staining was diminished by S204G and E454K and nearly eliminated by T173I and M325K (Fig 4B). These differences in Env binding corresponded to differences in the susceptibility of virus-infected cells to ADCC. Whereas the Env changes acquired in the control animals had little effect on sensitivity to PGT145, the substitutions selected in the PGT145-treated animals conferred partial or complete resistance to ADCC (Fig 4C). In accordance with their effects on Env binding, sensitivity to ADCC was impaired to a greater extent by T173I and M325K than by S204G or E454K (Fig 4C).

Similar effects were observed on the sensitivity of each of the Env variants to neutralization. The Env substitutions that arose in the DEN3-treated control animals did not alter (V67M & Q217R) or slightly increased (V260A) the sensitivity of SIV<sub>mac239</sub> K180S to neutralization by PGT145 (Fig 4D). In contrast, the substitutions selected in the PGT145-treated animals conferred partial (S204G and E454K) or complete (T173I) resistance to neutralization (Fig 4D). The T173I substitution is therefore sufficient for complete resistance to both neutralization and ADCC, which is consistent with the requirement for glycosylation of N171 for PGT145 binding [32].

### Structural analysis of PGT145 escape mutations

Two cryo-electron microscopy structures of the SIV<sub>mac239</sub> Env trimer were recently solved [37,40], one of which was determined for PGT145 in complex with an Env variant (SIV<sub>mac239</sub> Env:K180T) with a lysine-to-threonine (K180T) substitution that stabilizes the binding of this antibody to SIV Env similar to our K180S substitution [32,40]. This structure reveals extensive asymmetric contacts between PGT145 and the N171 glycans on each of the three gp120 protomers, primarily involving the complementarity determining region 3 and 1 of the heavy and light chains (CDRH3 and CDRL1) (Fig 5A). This is consistent with the complete resistance of the T173I variant of SIV<sub>mac239</sub> K180S to ADCC and neutralization as a result of the loss of these N-linked glycans (Fig 4). PGT145 is also near another glycan attached to residue N202



**Fig 5. Structural analysis of Env substitutions selected in SIV<sub>mac239</sub> K180S.** The amino acid changes that emerged in PGT145-treated animals infected with SIV<sub>mac239</sub> K180S were mapped onto a cryo-EM structure of PGT145 in complex with the SIV<sub>mac239</sub>:K180T Env trimer (PDB:8DVD) [40]. (A) Orthogonal views parallel (left) and perpendicular (right) to the viral membrane are shown for the variable heavy (tan) and light (pink) chains of PGT145 bound to the V2 apex of the SIV<sub>mac239</sub>:K180T Env trimer (gray). The N-linked glycans on residues N171 (red) and N202 (purple) are indicated. (B & C) An interaction between M325 of SIV Env and the aromatic ring of Y100f in the PGT145 VH chain contributes to PGT145-Env binding (B) and M325K is predicted to clash with Y100f of the PGT145 VH chain and K180T of SIV Env (C). (D & E) E454 is not part of the PGT145 binding interface (D). However, the E454K substitution may interfere allosterically with PGT145 binding by clashing with V319 and altering the V3 loop (light blue) (E). Three-dimensional models were generated using ChimeraX (ver 1.5) [52].

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(Fig 5A). The N202 glycans are located on the periphery of the antibody binding site at a distance of 6–10 Å from the PGT145 light chain and may have a greater role in the coordination of antibody binding rather than forming direct protein contacts. This could account for the modest effect of the loss of these glycans on the sensitivity of the S204G variant to ADCC and neutralization (Fig 4).

The methionine at position 325 of SIV Env also participates directly in PGT145 binding. M325 is located in the V3 loop within the core of the Env trimer where it contacts a sulfated tyrosine residue (Y100f) of the PGT145 CDRH3 (Fig 5B) [40]. M325 and Y100f form a characteristic methionine-aromatic interaction that is predicted to contribute an additional 1–1.5

kcal/mol of energy to protein stability beyond a purely hydrophobic interaction [41]. Modeling of the M325K substitution in the SIV Env trimer suggests that a lysine at this position would disrupt the interaction with PGT145 by clashing with Y100f (Fig 5C), which would account for the decreased sensitivity of the M325K variant to PGT145 binding and ADCC.

The glutamic acid at position 454 of SIV Env is distal to the PGT145 interface, so the E454K substitution is unlikely to alter direct contacts with PGT145 (Fig 5D). However, a non-conservative change to a lysine residue at this position with a bulkier side chain is predicted to clash with V319 on the V3 loop, which may have allosteric effects on PGT145 binding (Fig 5E). Accordingly, such allosteric effects may account for the diminished binding and ADCC activity of PGT145 against cells infected with the E454K variant of SIV<sub>mac239</sub> K180S.

## Discussion

Although neutralizing antibodies have repeatedly demonstrated protection against SHIVs in nonhuman primates [36], definitive evidence of protection by non-neutralizing antibodies has been elusive. To determine the extent to which neutralizing and non-neutralizing activities are required for protection, we took advantage of the unique cross-reactivity of the HIV-1 bnAb PGT145 with the SIV envelope glycoprotein. PGT145 binds to Env on the surface of SIV-infected cells and directs efficient NK cell killing of virus-infected cells, but poorly neutralizes SIV infectivity. Despite mediating potent ADCC both *in vitro* and *ex vivo*, this antibody failed to prevent mucosal transmission or reduce virus replication in macaques after intrarectal challenge with wild-type SIV<sub>mac239</sub>. However, PGT145 was able to significantly delay and reduce virus replication in animals challenged with a variant of SIV<sub>mac239</sub> that contains a single amino acid substitution that increases its binding to Env and renders the virus susceptible to neutralization [32].

The inability of PGT145 to protect against wild-type SIV<sub>mac239</sub> challenge despite potent ADCC against this virus raises questions about the extent to which ADCC alone is sufficient to prevent immunodeficiency virus transmission. The ADCC assay used here is the same assay that we used to measure ADCC against HIV-infected cells in the immune correlates analysis of the RV144 trial [2]. The ADCC titers in our animals are much higher than ADCC titers in RV144 vaccine recipients, which were often undetectable and never exceeded 35% killing at the highest serum concentration tested [2]. Thus, the absence of protection against wild-type SIV<sub>mac239</sub> cannot simply be explained by suboptimal ADCC responses. There are of course important differences that limit the extent to which the results of this study may be compared with clinical vaccine trials. The passive administration of a single monoclonal antibody does not represent the breadth of polyclonal antibodies elicited in response to vaccination. Our animals were also challenged with a sufficiently high dose of SIV<sub>mac239</sub> to establish infection in enough control animals that the outcome of protection would be interpretable after a single intrarectal inoculation. It is conceivable that this challenge dose may have overwhelmed partial protection that might have been observed under more physiological conditions, such as repeated low-dose mucosal challenge. However, the absence of any detectable effect on post-challenge viral loads after SIV<sub>mac239</sub> transmission suggests otherwise.

PGT145 did however confer partial protection against SIV<sub>mac239</sub> K180S, as indicated by a delay in peak viremia as well as significant reductions in viral loads during acute and chronic infection. Since the K180S substitution increases PGT145 binding to Env by approximately 100-fold and confers sensitivity to neutralization [32], these results are consistent with studies showing that neutralization is a key determinant of antibody-mediated protection [36,42,43]. However, since five of the six animals in the experimental and control groups became infected,

the protection afforded by PGT145 was not as complete as might have been expected based on a meta-analysis of the relationship between neutralizing antibody titers and protection in previous passive immunization studies [36]. On the day of challenge, the mean ID50 titer in serum against SIV<sub>mac239</sub> K180S ( $982 \pm 425$ ) exceeded the ID50 titer of bnAbs predicted to achieve 95% protection against virus acquisition by mucosal SHIV challenge (685, 95% CI 319, 1471) [36]. The explanation for the lack of an effect on virus acquisition is not certain but may reflect a fraction of the SIV<sub>mac239</sub> K180S challenge stock that is resistant to complete neutralization by PGT145 as a consequence of heterogeneous Env glycosylation. Indeed, this possibility is supported by previous reports of incomplete neutralization of HIV-1 by V2 apex bnAbs [38,39].

The rapid emergence of amino acid changes in Env that further confer resistance to PGT145 helps to explain breakthrough replication of SIV<sub>mac239</sub> K180S in the presence of high *in vivo* antibody concentrations. Substitutions that disrupt a conserved N-linked glycosylation site at position 171 were observed in each of the PGT145-treated animals. We previously demonstrated that glycosylation of residue N171 is essential for PGT145 binding to the SIV Env trimer [32] and that this site corresponds to an N-linked glycan at position 160 of HIV-1 Env (N160) that is part of the V2 epitope for this antibody [31]. Extensive contacts between PGT145 and the glycans attached to N171 on each of the gp120 protomers were confirmed in a recent cryo-EM structure of PGT145 bound to the SIV<sub>mac239</sub> K180T Env trimer [40]. Accordingly, a threonine-to-isoleucine substitution at residue 173 (T173I), which arose in four of the five animals by week 4 post-infection, completely abrogated the sensitivity of SIV<sub>mac239</sub> K180S to neutralization and ADCC. A serine-to-glycine substitution at position 204 (S204G) that is predicted to eliminate another N-linked glycan attached to residue N202 also impaired the susceptibility of SIV<sub>mac239</sub> K180S to ADCC and neutralization. This glycan is located at the periphery of the PGT145 binding site and does not appear to make direct contact with the antibody, which may account for the more modest effect of the loss of this glycan on PGT145 binding, ADCC, and neutralization compared to T173I [40].

Additional Env changes selected in the PGT145-treated animals may also have contributed to viral breakthrough. A methionine-to-lysine substitution at position 325 (M325K) emerged in three animals by eight weeks post-challenge that disrupts a methionine-aromatic interaction between M325 and a sulfated tyrosine residue (Y100f) in the HCDR3 region of PGT145 [40]. Consistent with the replacement of this stabilizing interaction with a predicted clash between these two residues, the M325K substitution reduced the sensitivity of virus-infected cells to PGT145 binding and ADCC by more than two orders of magnitude. The glutamic acid-to-lysine substitution at position 454 (E454K), which was present in three of the animals at week four but had disappeared by week eight post-challenge, is unlikely to affect PGT145 binding directly since it is located distal to the antibody binding site [40]. However, this substitution may have allosteric effects on Env as a result of conformational changes to the V3 loop that could account for diminished PGT145 binding and ADCC responses to virus-infected cells.

Overall, this study shows that in the case of a V2-specific antibody with potent ADCC against immunodeficiency virus-infected cells, that ADCC in the absence of detectable neutralization is not sufficient for protection. However, partial protection may be achieved by increasing the affinity of antibody binding to Env above the threshold required for neutralization of viral infectivity. Therefore, while ADCC and potentially other Fc-mediated effector functions may contribute to protection through the elimination of productively infected cells, the higher affinity of Env binding necessary for potent neutralization is a critical determinant of antibody-mediated protection.



## Materials and methods

### Ethics statement

Rhesus macaques (*Macaca mulatta*) were housed at the Wisconsin National Primate Research Center (WNPRC) in accordance with the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the University of Wisconsin Research Animal Resources Center and Compliance unit (UWRARC). Animal experiments were approved by the University of Wisconsin College of Letters and Sciences and the Vice Chancellor for Research and Graduate Education Centers Institutional Animal Care and Use Committee (protocol number G005141) and performed in compliance with the principles described in the *Guide for the Care and Use of Laboratory Animals* [44]. Fresh water was always available, commercial monkey chow was provided twice a day and fresh produce was supplied daily. To minimize any pain and distress related to experimental procedures, Ketamine HCL was used to sedate animals prior to blood collection and animals were monitored twice a day by animal care and veterinary staff. The animals were socially housed in pairs or groups of compatible animals whenever possible.

### Animals

Twenty-four rhesus macaques of Indian ancestry were used for these studies. MHC class I genotyping was performed using genomic DNA isolated from PBMCs by sequencing a 150 bp region of exon 2 (Illumina MiSeq system). Sequences were analyzed by comparison to an in-house database as previously described [45]. Animals positive for MHC class I alleles associated with spontaneous control of SIV infection (*Mamu-B\*008* and *-B\*017*) were excluded and experimental and control groups were structured with an equal distribution of male and female animals. The sex, age, weight and MHC class I genotype of the animals are shown in [Table 2](#).

### Antibody infusion

Purified, low-endotoxin stocks of PGT145 and DEN3 were produced by Catalent Pharma Solutions (Madison, WI) and The Scripps Research Institute (La Jolla, CA), respectively. These antibodies were diluted in sterile phosphate buffered saline (PBS) and gradually administered to rhesus macaques at doses of 30 mg/kg by intravenous infusion through a catheter placed aseptically in the saphenous vein of anesthetized animals over a period of approximately 20–30 minutes to prevent anaphylaxis.

### Antibody concentrations in plasma and rectal transudate

Rectal mucosal secretions were collected and processed with a modified wick method using Weck-Cel spears (Beaver Visitec, # 0008680) as described previously [46]. Total IgG content was determined by ELISA using purified mAbs PGT121 or PGT145 as a standard. Plates were coated with 2 µg/ml anti-human F(ab')<sub>2</sub>-specific antibody (Jackson ImmunoResearch Laboratories #109-005-097) and blocked using PBS containing 3% BSA. Sera and rectal samples were diluted 1:200 or 1:50 in 1% BSA, respectively, and serially diluted in PBS/1% BSA. Antibodies were allowed to bind for 1 hour at room temperature before plates were washed 3x with PBS/0.05% Tween 20. Bound antibodies were then detected using 1:2000 diluted alkaline phosphatase-labelled anti-human IgG Fcγ (Jackson ImmunoResearch Laboratories #109-055-098) and 4-Nitrophenyl phosphate disodium salt hexahydrate substrate (Sigma #S0942). Optical density was measured at 405 nm and IgG concentrations were calculated based on the PGT121 or PGT145 standards.

Table 2. Rhesus macaques.

Study	Animal	Antibody	Sex	Age <sup>a</sup>	Weight (kg) <sup>a</sup>	MHC Class I Haplotypes			
Wt 239 challenge	r14040	PGT145	F	3.51	5.68	A002.01	A008.01	B047.01	B055.01
	r14032	PGT145	F	3.56	6.69	A028.01	A055.01	B012.03	B043.01
	rhbk62	PGT145	M	3.31	4.24	A008.01	A028.01	B071.01	B069.01
	r14124	PGT145	M	2.98	4.69	A055.01	A224.01	B028.01	B043.01
	r15012	PGT145	M	2.62	4.02	A001.01	A008.01	B001.01	B015.01
	rhbk71	PGT145	M	3.27	4.16	A004.01	A023.01	B012.03	B012.03
	r14031	DEN3	F	3.56	6.24	A004.01	A008.01	B055.01	B045.01
	r14005	DEN3	F	3.85	3.62	A001.01	A008.01	B015.01	B055.01
	rhbk41	DEN3	M	3.48	4.54	A025.01	A224.01	B045.01	B045.01
	r15038	DEN3	M	2.30	5.22	A004.01	A023.01	B015.01	B015.01
	r15059	DEN3	M	2.15	3.64	A004.01	A028.01	B001.01	B069.01
	rhbk36	DEN3	M	3.51	4.76	A011.01	A023.01	B015.02	B012.03
K180S challenge	rhbj64	PGT145	M	4.53	9.3	A004.01	A008.01	B012.03	B001.01
	rh2761	PGT145	F	10.86	8.9	A004.01	A007.01	B055.01	B001.01
	rhbj89	PGT145	M	4.10	8.55	A004.01	A023.01	B012.03	B012.03
	r09070	PGT145	F	8.58	6.97	A007.01	A023.01	B047.01	B055.01
	r14054	PGT145	F	3.59	6.58	A004.01	A028.01	B001.01	B012.03
	r15003	PGT145	M	3.10	6.38	A055.01	A019.01	B043.01	B047.01
	r01041	DEN3	F	16.65	8.21	A008.01	A023.01	B001.01	B047.01
	rhbj21	DEN3	M	4.86	8.2	A004.01	A023.01	B012.03	B012.03
	rhbj07	DEN3	M	4.94	9.15	A004.01	A023.01	B012.03	B024.01
	rh2752	DEN3	F	11.75	7.22	A004.01	A041.01	B055.01	B043.03
	r15083	DEN3	M	2.21	5.94	A002.01	A007.01	B012.01	B001.01
	r15073	DEN3	F	2.25	5.68	A004.01	A008.01	B012.01	B043.01

<sup>a</sup>Ages and weights are at the time of SIV challenge.

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For the detection of specific IgG, plates were coated with 2 µg/ml anti-His (Invitrogen #MA1-21315) over night and blocked for 1 hour as described above. Soluble HIV-1 BG505 SOSIP trimers containing a hexahistidine tag were then allowed to bind at 3 µg/ml for 1 hour before plates were washed 3 times with PBS/0.05% Tween 20. Serially diluted samples were then incubated for 1 hour. After washing 3 times with PBS/0.05% Tween 20, bound antibodies were detected as described above and specific antibodies concentrations calculated based on the PGT145 standard data.

### SIV challenge stocks and mucosal challenges

SIV challenge stocks were prepared by transfecting 293T cells with full-length infectious molecular clones of SIV<sub>mac239</sub> and SIV<sub>mac239</sub> K180S and then expanding the virus on activated rhesus macaque PBMCs. Cell culture supernatant was collected from infected rhesus macaque lymphocytes and virus yields were determined by SIV p27 ELISA and by qRT-PCR to determine SIV RNA copies/ml. Infectivity titers (TCID<sub>50</sub>/ml) were determined by limiting dilution on CEMx174 cells.

Rhesus macaques were inoculated intrarectally with SIV<sub>mac239</sub> or SIV<sub>mac239</sub> K180S. Thirty minutes prior to challenge, cryopreserved vials of each virus were thawed. Wild-type SIV<sub>mac239</sub> was diluted to 6,000 TCID<sub>50</sub> per ml and SIV<sub>mac239</sub> K180S was diluted to 30,000 TCID<sub>50</sub> per ml in serum-free RPMI and loaded into one ml tuberculin syringes. Animals were

anesthetized, and with their pelvises elevated, the syringe (with the needle removed) was gently inserted into the rectum about 4 cm, then slightly withdrawn. The virus inoculum was slowly delivered to the rectal mucosa over about 1 minute. After withdrawing the syringe, it was examined for blood to ensure that there was no trauma to the rectum. To prevent drainage from the site of inoculation, the pelvic region of the animals was kept elevated for up to 30 minutes before returning them to their cages.

### Neutralization assay

Virus stocks for neutralization assays were prepared by transfecting 293T cells with full-length infectious molecular clones for SIV<sub>mac239</sub> and SIV<sub>mac239</sub> K180S and stored at -80°C. Neutralization of viral infectivity was measured using a standard TZM-bl reporter assay as previously described [47,48]. TZM-bl cells were seeded at 5,000 cells per well (100 µl) in 96-well plates the day before the assay. SIV<sub>mac239</sub> (2 ng p27) or SIV<sub>mac239</sub> K180S (5 ng p27) were incubated with serial dilutions of antibody or serum (100 µl) for 1 h at 37°C before being added to TZM-bl reporter cells. After 3 days, luciferase activity in cell lysates was measured and virus neutralization was calculated from the reduction in RLU relative to cells incubated with virus but without antibody or serum. Additional wells plated with uninfected TZM-bl cells were also included to control for background luciferase activity.

### ADCC assay

To maximize infection of the target cells for measuring ADCC, VSV G-pseudotyped stocks of SIV<sub>mac239</sub>, SIV<sub>mac239</sub> K180S and SHIVAD8-EO were prepared by co-transfecting 293T cells with *vif*-deleted clones for each virus together with a VSV G-expression construct and collecting supernatant 48 hours later. ADCC was measured as previously described [34,49]. CEM.NKR-CCR5-sLTR-Luc cells, which express firefly luciferase under the control of the SIV LTR promoter, were inoculated with VSV G-pseudotyped SIV<sub>mac239</sub>, SIV<sub>mac239</sub> K180S and SHIVAD8-EO. Two days later, the virus-infected target cells were incubated in triplicate with serial dilutions of antibody or plasma and an NK cell line (KHYG-1 cells) expressing rhesus macaque CD16 at a 10:1 effector-to-target ratio. Infected target cells incubated with NK cells but without antibody or plasma were included to determine maximal luciferase activity and uninfected target cells incubated with NK cells were included to determine background luciferase activity. ADCC was calculated as the percent relative light units (RLU) of luciferase remaining after an 8 hour incubation [% RLU = (experimental-background)/(maximal-background) X 100].

### Plasma viral loads

Plasma samples were isolated from blood collected with EDTA as an anticoagulant, cryopreserved at -80°C and SIV RNA levels were determined using a real-time RT PCR assay based on amplification of a conserved sequence in the SIV *gag* gene [50].

### Generation of SIV<sub>mac239</sub> K180S mutants

Nucleotide substitutions were introduced into the p239SpE3':K180S hemiviral plasmid by site-directed mutagenesis (New England Biolabs). Mutated p239SpE3':K180S hemiviral plasmids were digested with SphI-XhoI and joined with the 5' half of SIV<sub>mac239</sub> SpX and SIV<sub>mac239</sub> SpX ΔVif. Reconstructed full-length infectious molecular clones were sequence confirmed by the UW-Madison Biotechnology Center. Proviral DNA without the ΔVif mutation was transfected into HEK293T cells using GenJet (SignaGen) to generate viruses for neutralization

assays. SIV<sub>mac239</sub> SpX ΔVif plasmids were co-transfected with VSV-G envelope (pHDM.NJ strain) in a 2:1 ratio to generate viruses for ADCC assays and flow cytometry analyses. Culture supernatants were collected 48 h post-transfection, cleared of cell debris by centrifugation and concentrated on 50K MWCO centrifugal filters (Millipore Sigma). Concentrated viruses were well-mixed, aliquoted, and stored at -80°C. Concentrations were determined by anti-p27 ELISA (ABL, Inc.).

### Env staining

CEM.NKR-CCR5-sLTR-Luc cells were infected with *vif*-deleted SIV<sub>mac239</sub>, with or without Env mutations and pseudotyped with VSV G by spinoculation at 1200 g for 1 hour in the presence of 40 µg/ml Polybrene. Antibody binding to Env was evaluated by staining the cells 3 days post-infection. The cells were treated with Live/Dead NEAR IR viability dye (Invitrogen), washed in staining buffer (PBS with 1% FBS), and then incubated on ice for 30 minutes with 10 µg/ml of Env-specific antibody or a 1:32 dilution of SIV<sup>+</sup> plasma pool from chronically infected rhesus macaques used in other studies. A dengue virus-specific antibody (DEN3) was used as a negative control. An AF647-conjugated goat anti-human F(ab')<sub>2</sub> (Jackson ImmunoResearch) and PE-Cy7-conjugated anti-CD4 (Clone OKT4, Biolegend) were used for subsequent staining on ice. For intracellular p27 staining, cells were fixed in PBS with 2% paraformaldehyde, washed in staining buffer, and stained with FITC-conjugated anti-SIV Gag antibody (clone 552F12) in Perm/Fix Medium B (Invitrogen). After washing, the cells were fixed in PBS with 2% paraformaldehyde and analyzed using a BD FACS Symphony flow cytometer. Antibody binding to Env was assessed by Env staining on the surface of SIV-infected (CD4<sub>low</sub>Gag<sup>+</sup>) cells.

### SIV sequencing

Replicating SIV was sequenced, as previously described [51]. Briefly, Qiagen MinElute virus spin kits were used to isolate vRNA. We then generated cDNA and amplified it using the SuperScript III one-step reverse-transcription (RT)-PCR with Platinum Taq High Fidelity, along with four different primer pairs: SIV-ampA-F (5' TGTCTTTTATCCAGGAAGGGTA 3'), SIV-ampA-R (5' CTCTAATTAACCTACAGAGATGTTTGG 3'), SIV-ampB-F (5' AAAATTGAAGCAGTGGCCATTAT 3'), SIV-ampB-R (5' TACTTATGAGCTCTCGG-GAACCT 3'), SIV-ampC-F (5' GCTTTACAGCGGGAGAAGTG 3'), SIV-ampC-R (5' TGCCAAGTGTGATTATTTGTC 3'), SIV-ampD-F (5' GGTGTTGGTTTGGAGGAAAA 3'), SIV-ampD-R (5' GAATACAGAGCGAAATGCAGTG 3'). The four amplicons were purified and then quantified using a Quant-IT double-stranded DNA (dsDNA) HS kit (Invitrogen). The four amplicons were pooled to a total of 1 ng and then the Nextera XT kit (Illumina) was used to fragment the DNA and generate uniquely tagged libraries. The tagged libraries were quantified and fragment size distribution was determined with a high-sensitivity Agilent Bioanalyzer chip. Libraries were pooled and sequenced on an Illumina MiSeq. The sequence confirmation of the SIV<sub>mac239</sub> K180S challenge stock, *env* sequences in plasma collected at weeks 4 and 8 post-infection in macaques challenged with SIV<sub>mac239</sub> K180S, and *env* sequences in plasma collected at week 3 post-infection in macaques challenged with SIV<sub>mac239</sub> are available at SRA BioProject PRJNA1031618.

### Statistical analysis

Macaques were randomly assigned to treatment or control groups that received the Env-specific antibody PGT145 or the control antibody DEN3. In two independent experiments, the animals were challenged intrarectally with SIV<sub>mac239</sub> or SIV<sub>mac239</sub> K180S. Statistical



analyses were performed separately for each challenge experiment. Viral loads were log<sub>10</sub> transformed prior to statistical modelling to create more normally distributed datasets. Mean log<sub>10</sub> peak viral loads and time to peak viremia were compared between antibody treatment and control groups by linear regression. Differences in chronic phase viral loads (8–24 weeks) between antibody treatment and control groups were compared using linear mixed models adjusted for repeated measurements on an animal.

## Supporting information

**S1 Fig. PGT145 does not exert detectable selective pressure on Env after challenge with wild-type SIV<sub>mac</sub>239.** Viral RNA was isolated from plasma at week 3 post-infection and sequenced. The predicted amino acid sequences in Env from PGT145-treated animals (top, bold) and from DEN3-treated animals (bottom, plain text) were aligned to SIV<sub>mac</sub>239 Env. Regions of Env with substitutions in multiple animals are shown. Positions of identity are indicated by periods and amino acid differences are identified by their single letter code. Amino acid ambiguities are indicated with non-standard letters as follows: U = D/V, J = N/V. The frequencies of each substitution within the virus population are indicated by color. (TIF)

**S2 Fig. Resistance of the SIV<sub>mac</sub>239 K180S challenge virus to neutralization by PGT145.** Two-fold dilutions of the SIV<sub>mac</sub>239 K180S challenge virus (starting at 5.56 ng/ml p27) were incubated in the presence of a constant amount of PGT145 and K11 (50 µg/ml) for one hour before the addition of TZM-bl cells. Luciferase activity in the TZM-bl cells as an indicator of SIV infectivity was measured after a 3-day incubation. The percentage of residual infectivity was calculated from the luciferase activity in presence of each antibody relative to maximal luciferase activity in the absence of antibody after subtracting background luciferase in uninfected cells. (TIF)

**S3 Fig. Serum concentrations of PGT145 decline after passive administration.** Serum concentrations of PGT145 in animals challenged with SIV<sub>mac</sub>239 K180S were measured by ELISA on plates coated with an anti-His antibody and captured 6-His-tagged HIV-1 BG505 SOSIP trimers. Error bars indicate standard deviation of the mean. The data was analyzed by nonlinear regression. The best-fit curve is shown in black with an R<sup>2</sup>-value of 0.9698 and an estimate antibody half-life of 8.7 days. (TIF)

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## Author Contributions

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