Published in final edited form as:
Virology. 2014 August ; 0: 115–125. doi:10.1016/j.virol.2014.05.036.

Efficacy of Broadly Neutralizing Monoclonal Antibody PG16 in HIV-infected Humanized Mice

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Abstract

Highly potent broadly neutralizing human monoclonal antibodies hold promise for HIV prophylaxis and treatment. We used the SCID-hu Thy/Liv and BLT humanized mouse models to study the efficacy of these antibodies, primarily PG16, against HIV-1 clade A, B, and C. PG16 targets a conserved epitope in the V1/V2 region of gp120 common to 70–80% of HIV-1 isolates from multiple clades and has extremely potent in vitro activity against HIV_{JR-CSF}. PG16 was highly efficacious in SCID-hu mice as a single intraperitoneal administration the day before inoculation of R5-tropic HIV-1 directly into their Thy/Liv implants and demonstrated even greater efficacy if PG16 administration was continued after Thy/Liv implant HIV-1 infection. However, PG16 as monotherapy had no activity in humanized mice with established R5-tropic HIV-1 infection. These results provide evidence of tissue penetration of the antibodies, which could aid in their ability to prevent infection if virus crosses the mucosal barrier.

Introduction

Human monoclonal antibodies that potently neutralize a broad range of HIV isolates hold promise for the prevention of HIV infection. The anti-gp120 broadly neutralizing monoclonal antibodies 2G12 and b12 and anti-gp41 antibodies 4E10 and 2F5 block diverse HIV variants because they target conserved, functionally important Env epitopes (Muster et al., 1994; Roben et al., 1994; Sagar et al., 2012; Stiegler et al., 2001; Trkola et al., 1996). Importantly, passive transfer of these antibodies can protect against intravenous (Mascola et al., 1999) and mucosal (Burton et al., 2011; Hessell et al., 2009a; Hessell et al., 2009b;

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Hessell et al., 2010; Mascola et al., 2000; Parren et al., 2001) challenge in macaque models of simian/HIV (SHIV) infection. In recent years, several extraordinarily potent neutralizing antibodies with activity against a wide range of HIV clades have been discovered, including the somatically related antibodies PG9 and PG16 (Davenport et al., 2011; Pancera et al., 2010; Walker et al., 2009); VRC01 and VRC07 (Wu et al., 2010; Zhou et al., 2010); CH01-CH04 (Bonsignori et al., 2011); and 3BNC117, NIH45–46, PGV04, and PGT121 and PGT128 (Diskin et al., 2013; Diskin et al., 2011; Falkowska et al., 2012; Scheid et al., 2011; Walker et al., 2011; Wu et al., 2011). Sterilizing protection against vaginal mucosal SHIV challenge has been achieved in macaques with PGT121 (IC_{50} of 0.005 μg/ml against $SHIV_{SF162P3}$) by passive intravenous transfer of as little as 0.2 mg/kg, corresponding to a "single-digit" serum concentration of 1.8 μg/ml at the time of virus challenge (Moldt et al., 2012).

Encouraged by the highly potent neutralizing activity of PG16 against HIV_{JR-CSF} in vitro (IC_{50} of 0.001 μg/ml), we sought to determine whether PG16 would be effective as a prophylactic modality against HIV challenge in humanized SCID-hu Thy/Liv mice. PG16 targets the V1/V2 loop region at residues 160 and 162, corresponding to a potential N-linked glycosylation site that may form the PG16 epitope (McLellan et al., 2011; Pejchal et al., 2010; Walker et al., 2009). The crystal structure of the antigen-binding fragment (Fab) of PG16 revealed that the antibody is sulfated and has a unique complementarity determining region (CDR) H3 subdomain structure with a stable stalk mediating extensive H3 protrusion from the combining site and two interconnected loops (Pejchal et al., 2010).

The SCID-hu Thy/Liv mouse model of HIV infection is a useful platform for the preclinical evaluation of antiviral efficacy in vivo. The human thymus implant in these mice supports long-term differentiation of human T cells, and the model has been standardized and validated with four classes of licensed antiretrovirals for the evaluation of antiviral drugs against HIV (Rabin et al., 1996; Stoddart et al., 2007). One important advantage of SCID-hu Thy/Liv mice for studies of HIV prophylaxis is their high (essentially 100%) susceptibility to HIV infection after injection of the virus directly into the thymus/liver implant. In previously reported humanized mouse studies, b12 antibody completely protected hu-PBL-SCID mice from intraperitoneal (i.p.) challenge with HIV_{JR-CSF} but only when administered at very high dosage levels (50 mg/kg) (Gauduin et al., 1997). We hypothesized that PG16 would protect against HIV_{JR-CSF} infection at much lower dosage levels because it is >200 times more potent than b12 (IC_{50} of 0.001 versus 0.210 μg/ml) (Walker et al., 2009), and higher in vitro neutralization potency of PGT-121 against $SHIV_{SF162P3}$ has been shown to translate into enhanced protection against virus challenge in macaques (Moldt et al., 2012). In addition to HIV_{JR-CSF}, we assessed the prophylactic activity of PG16 against four other clade B and non-clade B viruses in SCID-hu Thy/Liv mice and also explored the potential for PG16 in treating established HIV_{JR-CSF} infection.

Results

PG16 half-life in SCID-hu Thy/Liv mice

To determine the frequency of PG16 administration, we determined the half-life ($t_{1/2}$) of PG16 in a separate pharmacokinetics study performed in uninfected SCID-hu Thy/Liv mice.

Mice were treated with various doses of PG16 (5, 50, and 500 μg per mouse) by i.p. injection, and the level of human IgG was measured by ELISA in mouse serum collected 1, 3, and 6 days after treatment (Fig. 1A). When administered at the highest dose (500 μg), PG16 exhibited an initial rapid decline during the first 3 days, which could be the result of a combination of IgG concentration-dependent catabolism and distribution to extravascular spaces (Lobo et al., 2004). Consistent with this explanation, the more gradual decline from days 3 to 6 was similar for the 500-μg and 50-μg doses. The PG16 $t_{1/2}$ was 3.7 days for the 500-μg dose and 4.2 days for the 50-μg dose (Fig. 1B). Importantly, the day after PG16 administration (corresponding to the time of HIV challenge in the protection studies), the mean level of human IgG in mouse circulation was 78 μg/ml, 14 μg/ml, and <1.5 μg/ml for 500 μg, 50 μg and 5 μg PG16, respectively (Fig. 1A).

Untreated SCID-hu Thy/Liv mice (but not unengrafted CB17-*scid* mice) had low levels (mean of 0.6 μg/ml) of human IgG in their serum, likely resulting from the presence of small numbers of human B cells (0.2–2.5% of implant cells) in the implants of these mice (Namikawa, et al., 1990; Dittmer et al., 1999). On the day after treatment with 5 μg PG16, the mean human IgG concentration was 1.5 μg/ml, a portion of which (0.3–1.1 μg/ml) was nonspecific human IgG (Fig. 1C). Determination of the $t_{1/2}$ for the 5 μg PG16 dose was therefore not possible because the pan-human IgG ELISA cannot discriminate PG16 from endogenously produced human IgG. Taking into account the results of the pharmacokinetics experiments, we elected to give the antibody i.p. to the mice three times per week (i.e., every other day) for studies involving repeated administration of PG16.

Selection of HIV for SCID-hu Thy/Liv mouse protection studies

Because protection in vivo is generally highly correlated with neutralization in vitro (Burton et al., 2011; Moldt et al., 2012), before initiating our studies we evaluated PG16 in both pseudovirus and PBMC neutralization assays against several HIV isolates that have been previously characterized in SCID-hu Thy/Liv mice (Stoddart, et. al., 2007 and unpublished observations). The data shown in Table 1 confirm the extreme sensitivity of $HIV_{JR\text{-}CSF}$ and lower susceptibility of $HIV_{NL4\text{-}3}$ to PG16 (Walker et al., 2009). Of the other six HIV clade B isolates in our SCID-hu Thy/Liv panel, HIV_{JD} was the most sensitive to PG16 neutralization with an IC_{50} of 0.008 μg/ml in the pseudovirus assay and 0.1 μg/ml with PBMC. The PG16 resistance exhibited by four of these six clade B HIV isolates in our SCID-hu panel (HIV_{PD}, HIV_{EW}, HIV_{EF}, and HIV_{GV}) was unexpected given the reported broadly neutralizing activity (~80% of 162 pseudoviruses with IC_{50} <50 μg/ml) of this antibody. We found that both HIV_{PD} and HIV_{EW} have the N160K mutation in gp120 (data not shown), which explains the PG16 resistance of these primary isolates. However, no known PG16-resistance mutations in the C1 through C2 regions of gp120 were identified for the other two PG16-resistant isolates (HIV_{EF} and HIV_{GV}). We also tested two non-clade B HIV isolates with the greatest reported sensitivity to PG16 neutralization in the Walker et al. (2009) pseudovirus assay, clade A $HIV_{92/RW/008}$ (IC_{50} 0.002 μg/ml) and clade C $HIV_{98/IN/022}$ (IC_{50} 0.003 μg/ml). Except for $HIV_{NL4\text{-}3}$, the IC_{50} values for all viruses were substantially higher in PBMC than in the pseudovirus assay. The two assays have previously been reported to differ in assay sensitivity attributable to greater envelope spike density and

stability of pseudoviruses compared to primary isolates, thus accounting for a higher sensitivity to neutralization by pseudoviruses (Fenyo et al., 2009; Heyndrickx et al., 2012).

Rationale and study design for in vivo protection studies

The first set of experiments was performed in mice inoculated with HIV_{JR-CSF}, a molecular clone reported by Walker et al. (2009) to be highly sensitive (IC_{50}: 0.001 μg/ml) to PG16 neutralization in vitro. The second set was performed with HIV_{JD}, a dual/mixed primary isolate in our SCID-hu mouse panel that is also highly sensitive to PG16 in vitro, and a third set with HIV_{NL4-3}, which is less sensitive to PG16 with a plateau in dose response at <100% neutralization. The fourth set of experiments was performed with clade A and clade C isolates, and a final set was carried out in mice with established HIV_{JR-CSF} infection to assess the potential of PG16 for HIV therapy. In each study, a range of PG16 dosage levels was used to establish a dose-response effect. The dosage range was very large (0.05–500 μg) across the studies for two main reasons: 1) very high doses were used in an attempt to produce sterilizing protection in the implants (which could rarely be achieved at 500 μg), and 2) very low doses were necessary to establish a no-effect level in the mice for this extremely potent antibody. We included in each study a positive control group treated with an antiretroviral regimen (either 3TC or Truvada) known to have reproducible efficacy in the model.

Highly potent protection by PG16 against challenge with HIV_{JR-CSF}

For studies with HIV_{JR-CSF}, implants from SCID-hu Thy/Liv mice were collected 42 days after inoculation, a time point when HIV_{JR-CSF} replication typically peaks in the implants, and assayed for cell count, HIV RNA, and p24. In the first study, mice were injected i.p. with varying doses of PG16 starting the day before inoculation and repeating every other day until Thy/Liv implant collection. Specifically, groups of 5 or 6 mice each were given a wide range of PG16 doses from 1.5 to 150 μg and challenged with 1,000 50% tissue culture infectious doses ($TCID_{50}$) of HIV_{JR-CSF} by direct injection of 50 μl virus into the implants of anesthetized mice. In mice treated with as little as 1.5 μg (0.05 mg/kg) PG16, we observed a 630-fold reduction in HIV RNA (from a mean of $10^{4.7}$ HIV RNA copies per 10^6 cells in untreated mice to $10^{1.9}$ copies in PG16-treated mice) (Fig. 2A, Supplementary Table 1). In fact, three of the five mice treated with 1.5 μg had no detectable viral RNA 42 days after inoculation, and all but one PG16-treated mouse (in the 5 μg group) had no detectable p24 (<5 pg per 10^6 cells) in their implants. Mice in the positive antiviral control group treated twice daily with 3TC (30 mg/kg/day) by i.p. injection had similarly large reductions in viral RNA (from a mean of $10^{4.7}$ to $10^{1.8}$ copies per 10^6 cells) relative to untreated mice.

In the second study, we treated groups of 6 mice each with a single prophylactic administration of 0.05, 0.5, or 5 μg PG16 or a single administration of oral Truvada (200 mg/kg tenofovir disoproxil fumarate [TDF] and 130 mg/kg emtricitabine [FTC] or 2,000 mg/kg TDF and 1,300 mg/kg FTC the day before HIV_{JR-CSF} challenge (Fig. 2B, Supplementary Table 2). In a previous report, we showed that a single administration of Truvada the day before inoculation had minimally protective activity against HIV_{NL4-3} challenge in the mice (Stoddart et al., 2012), unlike the much more potent activity we reported for multiple licensed antiretroviral drugs when administered continually once or

twice a day until implant collection (Stoddart et al., 2007). We found that 5 μg (0.18 mg/kg) PG16 reduced HIV RNA at 42 days by 79-fold (from a mean of $10^{5.0}$ to $10^{3.1}$ copies per 10^6 cells) with no statistically significant reductions at the lower doses (Fig. 2B, Supplementary Table 2). Despite the high dose, a single prophylactic administration of Truvada resulted in reductions in HIV RNA that were small (from $10^{5.0}$ to $10^{4.6}$ copies per 10^6 cells) but statistically significant at the lower dose and not statistically significant (because of higher sample variance) at the higher dose 42 days after inoculation (Fig. 2B). In the third study, we treated mice with a single administration of 5 μg PG16 at 1, 7, and 14 days before $HIV_{JR\text{-}CSF}$ inoculation and observed statistically significant reductions in HIV RNA for all three prophylactic time points (Fig. 2C, Supplementary Table 3).

Potent protection by PG16 against challenge with HIV_{JD}

Similar to the studies described above in SCID-hu Thy/Liv mice inoculated with $HIV_{JR\text{-}CSF}$, we found that PG16 also had potent activity in mice inoculated with HIV_{JD}. Mice were injected i.p. with varying doses of PG16 starting one day before HIV_{JD} injection and repeating three times per week until peak virus replication and implant collection 14 days after inoculation for cell count, HIV RNA, and p24. We observed a 1,600-fold reduction in HIV RNA in mice given 500 μg PG16, a 2,000-fold reduction in mice given 150 μg PG16, and a 630-fold reduction in those given 50 μg PG16 relative to untreated mice (Fig. 3A, Supplementary Table 4). A human IgG1 isotype control antibody had no activity at the highest dose of 500 μg given three times per week. In this same study (Fig. 3A), we compared the activity of PG9, a somatically related antibody, and PG16 at the 500-μg dose level and found somewhat less protective activity for PG9 (320-fold reduction in HIV RNA) compared to PG16 (1,600-fold reduction). This difference was also reflected in the lack of detectable p24 in PG16-treated mice while 2 of 7 PG9-treated mice had 38 and 42 pg p24 per 10^6 implant cells, respectively (Supplementary Table 4). The greater protective activity of PG16 compared to PG9 against HIV_{JD} challenge is also consistent with the 9-fold lower pseudovirus neutralization IC_{50} for PG16 (0.008 μg/ml) compared to PG9 (0.074 μg/ml) (Table 1).

We performed two additional studies with progressively lower doses to determine a minimally protective dose for PG16 against HIV_{JD} challenge. In the first study, administration of as little as 1.5 μg PG16 three times per week for 14 days beginning the day before virus inoculation resulted in a 1,600-fold reduction in HIV RNA (from a mean of $10^{5.9}$ to $10^{2.7}$ copies per 10^6 cells) and reduced HIV p24 to undetectable levels in 5 of 6 mice (Fig. 3B, Supplementary Table 5). In the subsequent study, the amount of antibody was further reduced to determine the dose at which PG16 had no measurable effect on HIV replication (Fig. 3C, Supplementary Table 6). Here we determined the no-effect level of PG16 to be 0.15 μg three times per week (Fig. 3C). When administered as a single prophylactic dose of 5 μg, PG16 was highly protective against HIV_{JD} challenge with a 1,600-fold reduction in HIV RNA (from a mean of $10^{4.9}$ to $10^{1.7}$ copies per 10^6 cells) (Fig. 3C), which was substantially more effective than the 79-fold reduction observed for $HIV_{JR\text{-}CSF}$ (Fig. 2B).

Protection by PG16 against challenge with HIV_{NL4-3}

We next evaluated the prophylactic efficacy of PG16 against HIV_{NL4-3}, which is less susceptible to PG16 neutralization in vitro (Table 1). SCID-hu Thy/Liv mice were injected i.p. with 50, 150, or 500 μg PG16 starting one day before virus inoculation and repeating three times per week until peak virus replication and implant collection on day 21. In contrast to our findings with HIV_{JR-CSF} and HIV_{JD}, high-dose (500 μg) PG16 had very low (2-fold reduction in HIV RNA) protective activity against HIV_{NL4-3} (Fig. 4A, Supplementary Table 7), consistent with the less potent neutralization of HIV_{NL4-3} by PG16 observed in vitro (Table 1). In a separate study, treatment of the mice with PG9 showed somewhat higher protective activity (25-fold reduction in HIV RNA for 50 and 150 μg PG16) against HIV_{NL4-3} challenge (Fig. 4B, Supplementary Table 8).

Protective effects of a single administration of PG16 against challenge with clade A $HIV_{92/RW/008}$

The non-clade B HIV isolates reported by Walker et al. to have the greatest sensitivity to PG16 neutralization, clade A $HIV_{92/RW/008}$ and clade C $HIV_{98/IN/022}$, were also evaluated in SCID-hu Thy/Liv mice. There were statistically significant reductions (8–16-fold) in HIV RNA 42 days after inoculation in mice treated with a single prophylactic administration of 5, 15, and 50 μg PG16 the day before $HIV_{92/RW/008}$ inoculation, but no protective effect was detected for 1.5 μg (Fig. 5A, Supplementary Table 9). Similarly to what we observed for HIV_{JR-CSF} (Fig. 2A), there was no statistically significant protective effect of a very high single oral administration of Truvada given the day before $HIV_{92/RW/008}$ inoculation. In contrast to the moderate protective effects observed for $HIV_{92/RW/008}$, no significant protective effect was observed after PG16 treatment of mice inoculated with clade C $HIV_{98/IN/022}$ (Fig. 5B, Supplementary Table 10) despite the high in vitro sensitivity of this strain to PG16.

Substantially reduced activity of PG16 when administered after HIV_{JR-CSF} challenge

We next evaluated the therapeutic activity of PG16 in HIV_{JR-CSF}-inoculated mice. Mice were treated with 5 μg PG16 three times per week starting 1 day before or 8 or 15 days after HIV_{JR-CSF} challenge and with 50 μg PG16 three times per week starting 8, 15, or 22 days after HIV_{JR-CSf} challenge. In comparison to starting PG16 treatment the day before inoculation, which showed the expected protective effect with 5 μg PG16, delay of treatment initiation to 8 days after inoculation resulted in only 2.5–3-fold HIV-inhibitory activity (from a mean of $10^{5.3}$ HIV RNA copies per 10^6 cells in untreated mice to $10^{4.8–4.9}$ copies in all groups treated after inoculation (Fig. 6A, Supplementary Table 11). In a separate study where SCID-hu Thy/Liv mice with established HIV_{JR-CSF} infection were treated 17 weeks after inoculation with 500 μg PG16 administered three times per week for 3 weeks, no protection was observed (Fig. 6B, Supplementary Table 12).

It is difficult to achieve significant and sustained antiviral activity in SCID-hu Thy/Liv mice with established HIV infection even with high-dose combination therapy including a protease inhibitor (Amado, et al., 1999). Mindful of this potential limitation with the SCID-hu Thy/Liv model, we also treated NOD-*scid* IL-2R$\gamma^{-/-}$ (NSG) BLT mice (NSG-BLT) mice with established HIV infection and stable viremia. In the NSG-BLT model, Thy/Liv

implantation is supplemented by the injection of $CD34^+$ hematopoietic stem/progenitor cells (HSPC) isolated from the autologous fetal liver. The Thy/Liv implant allows for positive and negative selection of human T cells to occur in autologous human thymus tissue, while the injected HSPC populate the mouse bone marrow to reconstitute and maintain human hematopoiesis. This approach leads to the most comprehensive reconstitution of the human immune system in a mouse model yet reported, with high levels of multilineage human cell engraftment and sustained HIV plasma viremia after parenteral and mucosal HIV exposure.

We treated groups of 6–7 HIV-viremic NSG-BLT mice with a very high dose of PG16 (1.5 mg) or PBS vehicle 6 and 12 weeks after i.p. $HIV_{JR\text{-}CSF}$ inoculation and observed no reduction in plasma HIV RNA after the first administration and up to 7 days after the second administration (Fig. 6C, Supplementary Table 13). Plasma viral load increased dramatically in one mouse after the first PG16 treatment, but this mouse had evidence of graft-versus host disease necessitating euthanasia before the second PG16 treatment. To determine whether viral escape from PG16 had occurred in the mice, we sequenced gp120 RNA obtained from spleens 1 week after the second PG16 administration and observed a mutation at residue 162 (T162N) in two of the six treated mice. Outgrowth of T162N was also reported in the previous work in PG16-treated humanized NRG mice along with other substitutions at positions T162 and N160, and these mutants were found to be highly resistant to PG16 neutralization in vitro (Klein et al., 2012). It is unlikely, however, that the lack of protective activity we observed was the result of viral escape because we detected PG16-resistance mutations in only 2 of 6 mice with established $HIV_{JR\text{-}CSF}$ infection.

Discussion

The broadly HIV-neutralizing antibodies PG9 and PG16 were isolated from an African clade A-infected individual, who ranked in the top 5% of 1,800 HIV-infected donors screened for potent anti-HIV serum neutralizing activity in an international effort named Protocol G (Walker et al., 2009). From a panel of 162 HIV isolates, PG9 neutralized 127 and PG16 neutralized 119 of derived pseudoviruses with potencies ~1 $\log_{10}$ greater than broadly neutralizing antibodies 2G12, b12, 2F5, and 4E10 (Doores and Burton, 2010) and comparable to that of VRC01 (Wu et al., 2010).

In the present study, we evaluated both the prophylactic and therapeutic activities of PG16 against HIV challenge in humanized mice. We used five different challenge isolates that were sensitive to PG16 neutralization in vitro (Table 1), including a clade A ($HIV_{92/RW008}$) and a clade C ($HIV_{98/IN/022}$) isolate (Table 1). The IC_{50} values for PG16 ranged from 0.001 μg/ml for $HIV_{JR\text{-}CSF}$ to 0.23 μg/ml for $HIV_{NL4\text{-}3}$ in the in vitro pseudovirus assay. It is notable that four of our primary isolates (HIV_{PD}, HIV_{EW}, HIV_{EF}, and HIV_{GV}) were resistant to PG9 and PG16 (IC_{50} >50 μg/ml) and that they were all X4 tropic whereas the sensitive viruses were either R5 ($HIV_{JR\text{-}CSF}$, HIV_{JW}, $HIV_{92/RW008}$, and $HIV_{98/IN/022}$) or R5X4 (HIV_{JD}). This unusual pattern of neutralization sensitivity may be limited to our small sample size.

In our prophylaxis studies, we treated SCID-hu Thy/Liv mice with either a single prophylactic administration the day before HIV inoculation or repeated treatment three times

per week beginning the day before inoculation until implant collection 14–42 days after inoculation, depending on peak virus replication for the respective challenge virus (14 days for R5X4 HIV_{JD}, 21 days for X4 $HIV_{NL4–3}$, and 42 days for R5 strains HIV_{JR-CSF}, $HIV_{92/RW008}$, and $HIV_{98/IN/022}$). We chose the intrathymic HIV exposure route because injection of HIV directly into the human target tissue provides a more stringent assessment of the efficacy of the test agent under various study designs compared to the mucosal or intravenous routes, for which exposure of the virus to target organs is less direct.

In an initial dose-ranging study with the most PG16-sensitive isolate, HIV_{JR-CSF}, we observed a 630-fold reduction in HIV RNA in mice treated with the lowest PG16 dose evaluated, 1.5 μg (0.05 mg/kg), starting the day before virus inoculation and repeating three times per week for 42 days. In a second study, we gave the mice a single administration of PG16 the day before HIV_{JR-CSF} challenge and found that 5 μg (0.2 mg/kg) reduced HIV RNA by 79-fold. The latter results are comparable to those reported by Gauduin et al., (1997) where 80% (actual number not specified) of hu-PBL-SCID mice were protected by a single administration of 1 mg/kg b12 antibody 1 h before i.p. inoculation with HIV_{LAI}. In our SCID-hu Thy/Liv mouse model, mice treated with 5 μg PG16 had an antibody serum concentration of <1.5 μg/ml the day after treatment, indicating a protective serum concentration for PG16 that is in the single-digit μg/ml range, similar to that recently reported for PGT121 in macaques protected from mucosal SHIV challenge (Moldt et al., 2012). The $t_{1/2}$ of 3.7 days we obtained corresponds well to the 2.5 days reported for a 500-μg dose of PG16 by Klein et al. (2012) in humanized NOD $Rag1^{-/-}IL2R\gamma^{-/-}$ (NRG) mice reconstituted with human fetal liver-derived $CD34^{+}$ cells at birth.

Compared to a single administration of 0.2 mg/kg (5 μg) PG16, a single very large dose of Truvada (2,000 mg/kg TDF and 1,300 mg/kg FTC) resulted in only relatively small reductions in HIV RNA in HIV_{JR-CSF}-challenged mice (Fig. 2B). We previously reported similarly small reductions in HIV RNA after a single preexposure administration of Truvada in SCID-hu Thy/Liv mice inoculated with $HIV_{NL4–3}$ (Stoddart et al., 2012). The potent activity of a single administration of PG16 observed in the present study is reminiscent of the sustained activity obtained for an albumin-conjugated C34 peptide fusion inhibitor with prolonged plasma half-life (~20 h rats) in that previous report (Stoddart et al., 2012). Moreover, we showed that a single treatment with PG16 had sustained, although lower, activity when HIV_{JR-CSF} challenge was delayed by up to 7 or 14 days.

PG16 was also highly protective against HIV_{JD} challenge, with a 1,600-fold reduction in HIV RNA and lack of detectable p24 in 5 of 6 mice treated with 1.5 μg three times per week for 14 days and a 1,300-fold reduction in HIV RNA after a single prophylactic administration of 5 μg. In contrast, the somatically related PG9 antibody was somewhat less protective than PG16 at the 500-μg dose level. The greater protective activity of PG16 compared to PG9 against HIV_{JD} challenge is consistent with the 9-fold lower pseudovirus neutralization IC_{50} for PG16 (0.008 μg/ml) compared to PG9 (0.074 μg/ml).

In contrast to our findings with HIV_{JR-CSF} and HIV_{JD}, high-dose (500 μg) PG16 had minimal protective activity against HIV_{NL4-3} when administered three times per week, which is consistent with the less potent neutralization of HIV_{NL4-3} by PG16 observed in

vitro. It should be noted that, unlike for other viruses, the PG9 and PG16 pseudovirus neutralization curves for HIV_{NL4-3} plateaued at <100% neutralization (Walker et al., 2009), and this was confirmed in our study. The PG16 dose-response curves for HIV_{NL4-3} in the PBMC assay did not plateau with a relatively low IC_{90} value of 0.7 μg/ml (Table 1). This incomplete in vitro neutralization appears to be reflected in the plateaued dose responses we obtained for PG9 and PG16 in HIV_{NL4-3}-challenged mice.

Contrary to predictions from in vitro neutralization potency, a single prophylactic administration of up to 50 μg PG16 had 1 $\log_{10}$ lower protective activity against challenge with clade A $HIV_{92/RW/008}$ than against HIV_{JR-CSF}. No detectable activity against clade C $HIV_{98/IN/022}$ was observed. Similar to HIV_{JR-CSF}, both of these isolates have the greatest in vitro sensitivity to PG16 neutralization (IC_{50} 0.002–0.003 μg/ml), so the difference in in vivo protection against these non-clade B isolates was unexpected. While a higher dosage of antibody or repeated treatment during the infection period may have resulted in more potent protection from $HIV_{92/RW/008}$ and $HIV_{98/IN/022}$ challenge, it remains unclear whether the lack of greater protection with a single administration is associated with differences in their infection behavior in SCID-hu Thy/Liv mice despite the fact that they have the same tropism.

We compared the prophylactic and therapeutic activities of PG16 in a series of experiments. First, we treated SCID-hu Thy/Liv mice with 5 μg PG16 three times per week starting 1 day before or 8 or 15 days after HIV_{JR-CSF} challenge and with 50 μg PG16 three times per week starting 8, 15, or 22 days after HIV_{JR-CSF} challenge. Although repeated dosing of 5 μg PG16 starting the day before inoculation had the expected protective effect, delay of treatment initiation after inoculation resulted in little protective activity. Since it remained possible that a higher repeated dosage of PG16 would lead to reductions in HIV RNA in the implants, we further evaluated the therapeutic activity of high-dose PG16 in established HIV infection in two separate studies. In one study, a high repeat-dose PG16 treatment of SCID-hu Thy/Liv mice at 500 μg (18 mg/kg) for 3 weeks starting 17 weeks after HIV_{JR-CSF} inoculation had no effect on HIV RNA levels in the implants 3 weeks after treatment. This limited efficacy in established HIV infection is consistent with results reported previously for b12, 2G12, 2F5, or their combination using hu-PBL-SCID mice (Poignard et al., 1999).

In the second study using HIV-viremic NSG-BLT mice, a very high dose of PG16 (1.5 mg or 54 mg/kg) at 6 and 12 weeks after i.p. HIV_{JR-CSF} challenge resulted in no reduction in plasma HIV RNA measured 2 and 4 weeks after the first treatment and 1 week after the second treatment. In both of these models, the lack of therapeutic efficacy by PG16 might be the result of using antibody monotherapy. This possibility is supported by the results from a recent report where PG16 was evaluated in an established infection model in humanized NOD $Rag1^{-/-}Il2r\gamma^{null}$ (NRG) mice that were reconstituted with human fetal liver-derived $CD34^{+}$ cells at birth (Klein et al., 2012). In that report, mice were given 500 μg (20 mg/kg) PG16 once or twice a week after infection was established by i.p. challenge with HIV_{YU-2}, a clone of HIV_{NL4-3} carrying the envelope of YU-2, and only a transient reduction of HIV RNA was detected before virus rebound. Moreover, unlike the NRG mice, in which nearly all rebound virus contained escape mutations at N160 or N162, we detected viral escape in the rebound virus population after two administrations of 1.5 mg (54 mg/kg) PG16 in only

two of the six SCID-hu Thy/Liv mice. Overall, the observed effect of PG16 treatment in these two models of established JR-CSF infection was limited by the antibody monotherapy regimen we used. Recently, a combination of PG16 with an anti-CD4 binding sites and an anti-V1/V2 loop antibody administered at 1 mg each (40 mg/kg) twice a week rapidly suppressed plasma viral RNA in NRG mice with established HIV_{YU-2} infection and demonstrated the protective activity of PG16 and its therapeutic potential in the context of combination therapy (Horwitz et al., 2013).

The current study confirms the usefulness of the SCID-hu Thy/Liv mouse model for evaluation of in vivo preexposure prophylaxis of human HIV-specific monoclonal antibodies and demonstrates the utility of in vitro characterization of challenge viruses prior to in vivo experimentation. The high (essentially 100%) HIV susceptibility of SCID-hu Thy/Liv mice across many cohorts makes such prophylaxis experiments feasible because it increases confidence that the observed protection is not the result of poor susceptibility to infection.

A major advantage of the BLT mouse model is the establishment of systemic HIV infection and plasma viremia after HIV challenge by multiple routes; the model's major drawbacks are variability between mice in HIV susceptibility (Long and Stoddart, 2012) and a high incidence (35% by 22 weeks) of GvHD (Greenblatt et al., 2012; Covassin et al., 2013), which might have perturbed the efficacy of PG16 in the BLT mice. Indeed, we show in Fig. 6C a spike in HIV viremia in a PG16-treated mouse experiencing signs of GvHD and surmise that systemic immune activation driven by the GvHD disease process may have led to greater HIV expression. According to Greenblatt et al., GvHD in BLT mice is associated with the infiltration of human $CD4^+$ T cells into the skin and a shift towards Th1 cytokine production. GvHD also induced a mixed M1/M2 polarization phenotype in a dermal murine macrophage population that is $CD11b^+$ and MHC class II^+. GVHD mice displayed robust expression of human IFNγ and the profibrotic mediators human IL13 and human CCL2. The presence of xenogeneic GvHD in BLT mice presents both a major obstacle in the use of humanized mice and an opportunity to conduct preclinical studies on GvHD in a humanized model.

In summary, our results demonstrate the ability of PG16 to penetrate and protect primary lymphoid tissues from HIV infection and that antibodies can work in central immune sites, not just at the mucosal surface. This feature could add to the broadly neutralizing monoclonal antibodies' ability to prevent infection if HIV crosses the mucosal barrier. Overall, these findings suggest that this antibody or similar agents with high potency and sustained activity may hold promise as a single intervention modality or in cocktail combinations (to prevent viral escape) for targeting early infection events after HIV exposure. The potent protective efficacy we observed for a single preexposure administration supports further preclinical and clinical evaluation of this promising passive immunization strategy.

Materials and methods

Viruses

The following reagents were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV molecular clones pYK-JRCSF (R5) from Dr. Irvin SY Chen and Dr. Yoshio Koyanagi (Cann et al., 1990; Haltiner et al., 1985; Koyanagi et al., 1987), pNL4-3 (X4) from Dr. Malcolm Martin (Adachi et al., 1986), and HIV-1 92RW008 (clade A) and 98IN022 (clade C) (from The UNAIDS Network for HIV Isolation and Characterization). Primary HIV isolates HIV_{JD} (Kovalev et al., 1999; Stoddart et al., 2007; Su et al., 1995), HIV_{EW} (Kovalev et al., 1999; Rabin et al., 1996; Su et al., 1995), HIV_{PD}, HIV_{EF}, HIV_{JW}, and HIV_{GV} were obtained from Dr. J. M. McCune. Working stocks of the molecular clones were prepared in HEK 293T cells by lipofectamine 2000 transfection, and primary isolates were expanded in phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells. Stock virus titers (50% tissue culture infectious doses; $TCID_{50}$) were determined in PHA-activated PBMC by 50% endpoint dilution and assessment of supernatant p24 by ELISA after 7 days.

Antibodies and drugs

PG16 and PG9 were provided by Theraclone Sciences and were purified chromatographically from cultures of CHO-S1 cells cotransduced with PG16 or PG9 heavy and light chain genes (Bleck et al., 2012). Lamivudine (3TC), tenofovir disoproxil fumarate (TDF), and emtricitabine (FTC) were kindly provided by the NIH AIDS Research and Reference Reagent Program. PG16 in mouse serum was measured by ELISA for human IgG (Bethyl Laboratories).

In vitro neutralization assays

Pseudoviruses were produced by cotransfection of HEK 293 cells with a subgenomic plasmid, pHIV-1lucΔu3, that incorporates a firefly luciferase indicator gene and a second plasmid, pCXAS, that expresses HIV-1 Env libraries or clones. Following transfection, pseudoviruses were harvested and used to infect U87 cell lines expressing either CCR5 or CXCR4 (Richman et al., 2003).

PHA-activated PBMCs pooled from six donors were inoculated with HIV-1 at an MOI of 0.001 for 2 h at 37°C, and triplicate wells of round-bottom 96-well plates containing 100,000 cells in 100 μl were treated with 100 μl of serially diluted antibody or medium alone and cultured for 7 days. Supernatants were collected and assayed for p24 antigen at 1:800 dilution in HIV p24 antibody-coated microplates (Perkin-Elmer) by quantitative ELISA using the p24 standard supplied by the manufacturer. IC_{50} values were determined by a 4-parameter fit model (SOFTmax PRO 3.0, Molecular Devices). At day 7, untreated virus control wells had mean p24 concentrations of 5–20 ng/ml.

SCID-hu Thy/Liv mice

Male C.B-17 SCID (model #CB17SC-M, homozygous, C.B-*Igh-1*$^{b/\mathrm{IcrTac}}$-*Prkdc*^{scid}) mice were obtained at 6–8 weeks of age from Taconic and coimplanted with 1-mm^3 pieces of human fetal thymus and liver under the kidney capsule to generate SCID-hu Thy/Liv mice

as described previously (Rabin et al., 1996; Stoddart et al., 2007). Cohorts of 50–60 mice each were generated from the tissues of one donor, and implants were inoculated 18 weeks after implantation with 50 μl of stock virus (1,000 $TCID_{50}$) or RPMI 1640 medium (mock infection) by direct injection into the implants of anesthetized mice. Each experiment was performed in a separate SCID-hu Thy/Liv mouse cohort, and details for the twelve cohorts are shown in Supplementary Tables 1–12. Of the 559 mice included in the studies, 20 (3.6%) mice died during the course of the experiment, and 22 (3.9%) mice had abnormal implants and were excluded from analysis.

Antibodies were administered i.p. to the mice (5–7 mice per group) at the indicated dosages beginning, in most experiments, the day before inoculation of the Thy/Liv implants. Thy/Liv implants were collected from euthanized mice 14 days after inoculation with HIV_{JD} inoculation, 21 days after HIV_{NL4-3}, and 42 days after HIV_{JR-CSF}, $HIV_{92RW008}$, and $HIV_{98IN022}$ when virus replication peaks in the implants with these isolates. Animal protocols were approved by the UCSF Institutional Animal Care and Use Committee.

NSG-BLT mice

One cohort of humanized NOD-*scid* IL-2R$\gamma^{-/-}$ (NSG) BLT mice (NSG-BLT) mice was used to study PG16 treatment of established HIV infection. NSG-BLT mice were produced as described previously (Lan et al., 2006; Long and Stoddart, 2012; Melkus et al., 2006) by coimplanting human fetal liver and thymus under the kidney capsule of NSG mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ; Jackson Laboratories). Human $CD34^+$ hematopoietic stem progenitor cells were purified from fetal liver by magnetic bead selection and cryopreserved until tail vein injection (815,000 cells per mouse) 3 weeks after Thy/Liv implantation and 30 h after conditioning with 225 cGy gamma irradiation. Of the cells injected, 917 were $CD45^+$, $CD34^+$, Lin-1^{neg}, $CD38^{neg}$, C-kit+, CD90+, and $CD45RA^{neg}$ human hematopoietic stem cells (HSC) (Long and Stoddart, 2012). NSG-BLT mice were inoculated intravaginally with HIV_{JR-CSF} (8,000 $TCID_{50}$) 12 weeks after $CD34^+$ cell injection.

Thy/Liv implant processing and assay

Single-cell suspensions were made by placing the implant into a sterile nylon mesh bag, submerging the bag in phosphate-buffered saline (PBS)/2% fetal bovine serum (FBS) in a 60-mm tissue culture dish, and dispersing the tissue between the nylon layers with forceps, as described previously (Rabin et al., 1996; Stoddart et al., 2007; Stoddart et al., 2000). The cells were counted with a Coulter counter to determine total implant cellularity. For the bDNA assay, dry pellets of 5×10^6 implant cells were frozen and stored at −80°C. Cells were disrupted with sterile disposable pestles and a cordless motor grinder (Kontes) in 8 M guanidine HCl with 0.5% sodium N-lauroylsarcosine. The RNA was extracted with 0.5 ml 100% ethanol and pelleted at 12,000 × g for 20 min at 4°C. Supernatants were aspirated to remove DNA, and RNA pellets were washed with 0.5 ml 70% ethanol, placed on dry ice, and digested with reagents supplied by the manufacturer (VERSANT™ HIV-1 RNA 3.0 Assay, Siemens Healthcare Diagnostics). Implant HIV RNA is expressed as copies per 10^6 implant thymocytes, and the $\log_{10}$ values were used for calculation of geometric means. The limit of detection was $10^{1.48}$ RNA copies per 10^6 cells, and this lower-limit value was used

for calculation of means for implants with undetectable viral RNA. For p24 ELISA, pellets of 2.5×10^6 cells were resuspended in 400 μl of p24 lysing buffer (1% Triton X-100, 0.5% sodium deoxycholate, 5 mM EDTA, 25 mM Tris Cl, 250 mM NaCl, and 1% aprotinin), rotated overnight at 4°C, and stored at −20°C. Thawed samples were transferred into HIV p24 antibody-coated microplates (PerkinElmer Life Sciences) for quantitative ELISA. A standard curve was generated with the kit-supplied standards, and the results were calculated as pg p24 per 10^6 cells. Implant cells were also stained with antibodies to CD3, CD4, and CD8 for analysis of T-cell subsets by multiparameter flow cytometry (Supplementary Material).

Statistical analysis

Results are expressed as the mean ± SEM for each mouse group. Nonparametric statistical analyses were performed by use of the Mann-Whitney U test. Data for mice in each group were compared to those for untreated infected mice, and P values <0.05 were considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by Federal funds from NIAID, NIH, under Contract no. HHSN266200700002C/N01-AI-70002 (Contracting Officer Representative: Dr. Brigitte Sanders). This work was also supported in part by the AIDS Research Institute at UCSF and the Harvey V. Berneking Living Trust. We dedicate this work in memory of Dr. Paul L. Black (DAIDS, NIAID, NIH), who provided key input into the design of the PG16 efficacy studies.

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Highlights

- We tested potent broadly HIV-neutralizing human monoclonal antibodies in humanized mice.
- We studied PG16 in the SCID-hu Thy/Liv and BLT models against HIV clade A, B, and C.
- PG16 was efficacious in SCID-hu mice as a single dose the day before inoculation.
- PG16 as monotherapy had no activity in humanized mice with established HIV infection.
- These results show tissue penetration of the antibodies, which could prevent infection.

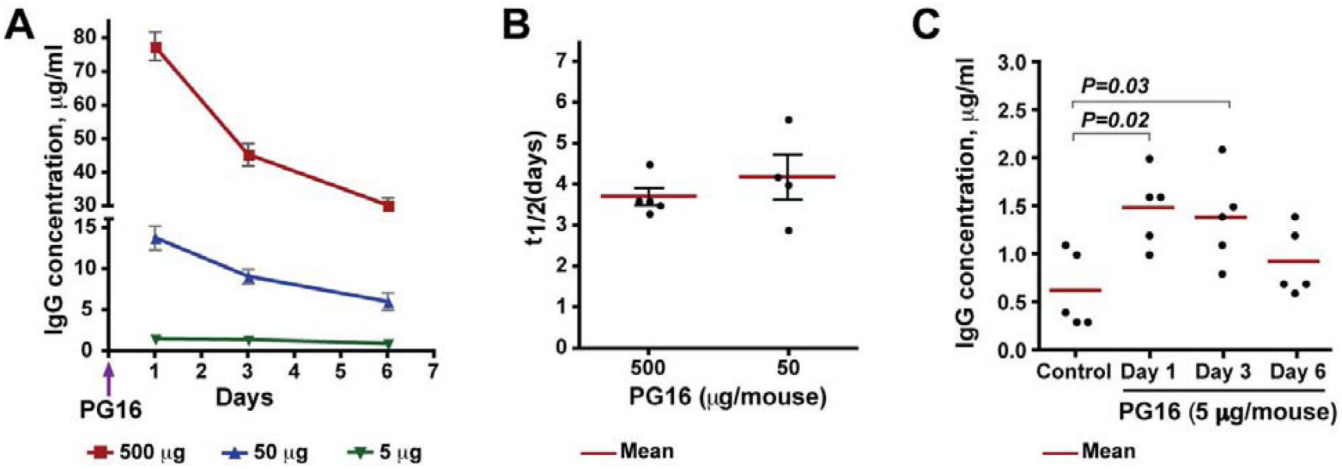

Fig. 1. PG16 serum half-life after a single administration of 5, 50, or 500 μg in SCID-hu Thy/Liv mice

(A) Mice were treated with PG16 by i.p. injection, and the level of human IgG was measured by pan-human IgG ELISA in mouse serum collected 1, 3, and 6 days after treatment. (B) PG16 mean $t_{1/2}$ was 3.7 days for the 500-μg dose and 4.2 days for the 50-μg dose. (C) Untreated SCID-hu Thy/Liv mice (control) had low levels (mean of 0.6 μg/ml) of human IgG in their serum, so the $t_{1/2}$ for the 5-μg PG16 dose could not be accurately determined. On the day after treatment with 5 μg PG16, the mean human IgG concentration was 1.5 μg/ml, a portion of which (0.3–1.1 μg/ml) was nonspecific human IgG, as demonstrated by the low levels in serum from untreated control SCID-hu Thy/Liv mice.

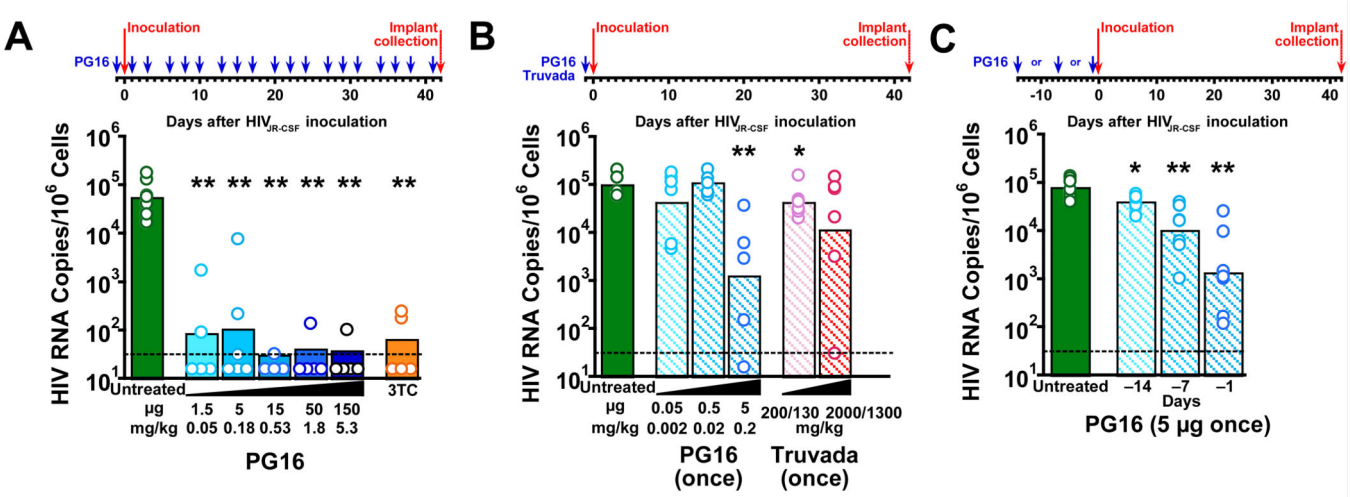

Fig. 2. PG16 protected SCID-hu Thy/Liv mice from infection with HIV$_{JR-CSF}$ in three independent challenge studies

(A) HIV RNA was reduced to $<10^{2.0}$ copies per 10^6 implant cells in mice treated i.p. with 1.5–150 µg PG16 (blue arrows) three times per week beginning the day before inoculation and continuing until implant collection at 42 days. Similar reductions in HIV RNA were observed in mice treated i.p. with 30 mg/kg 3TC once daily beginning the day before inoculation until implant collection. (B) HIV RNA was reduced to a mean of $10^{3.0}$ copies per 10^6 cells in mice treated with a single administration of 5 µg PG16 the day before inoculation, which was a greater reduction than observed in mice treated by oral gavage with a single administration of high doses of Truvada (200 mg/kg TDF plus 130 mg/kg FTC or 2,000 mg/kg TDF plus 1,300 mg/kg FTC). (C) Statistically significant reductions in HIV RNA occurred in mice treated with a single administration of 5 µg PG16 at 1, 7, and 14 days before inoculation. The columns represent means, and the open circles represent individual mice. **

P<0.01 and **P*<0.05 compared to untreated HIV-infected mice by the Mann-Whitney U test. The dotted line indicates the HIV RNA detection limit. ($10^{1.5}$ copies per 10^6 implant cells).

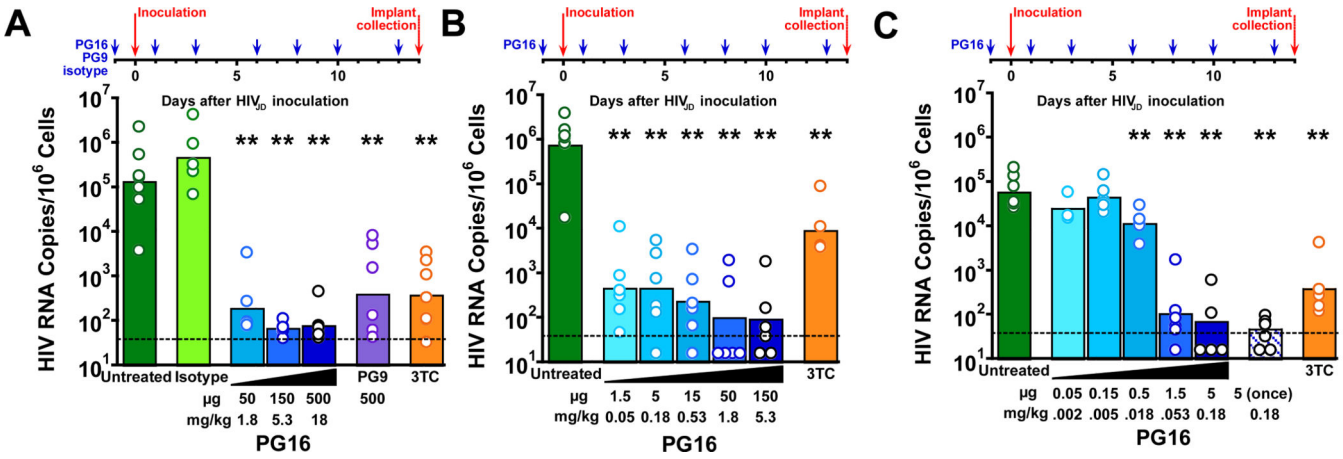

Fig. 3. PG16 protected SCID-hu Thy/Liv mice from infection with HIV_{JD} in three independent challenge studies with progressively lower antibody dose ranges

(A) Mean HIV RNA was reduced to $<10^{2.5}$ copies per 10^6 implant cells in mice treated i.p. with 50–500 μg PG16 three times per week beginning the day before inoculation and continuing until implant collection at 14 days. Similar reductions in HIV RNA were observed in mice treated i.p. with 500 μg PG9 under the same regimen as well as treatment with 30 mg/kg 3TC once daily beginning the day before inoculation until implant collection. No reductions occurred in mice treated with 500 μg isotype control mAb under the same regimen as PG16 and PG9. (B) Mean HIV RNA was reduced to $\leq 10^{2.5}$ copies per 10^6 implant cells in mice treated i.p. with 1.5–150 μg PG16 three times per week beginning the day before inoculation and continuing until implant collection at 14 days. (C) Statistically significant reductions in HIV RNA occurred in mice starting with a dose of 0.5 μg PG16 three times per week beginning the day before inoculation, and HIV RNA was undetectable in 2 of 5 mice treated with a single administration of 5 μg PG16 the day before inoculation. The columns represent means, and the open circles represent individual mice. $^{**}P<0.01$ compared to untreated HIV-infected mice by the Mann-Whitney U test. The dotted line indicates the HIV RNA detection limit. ($10^{1.5}$ copies per 10^6 implant cells).

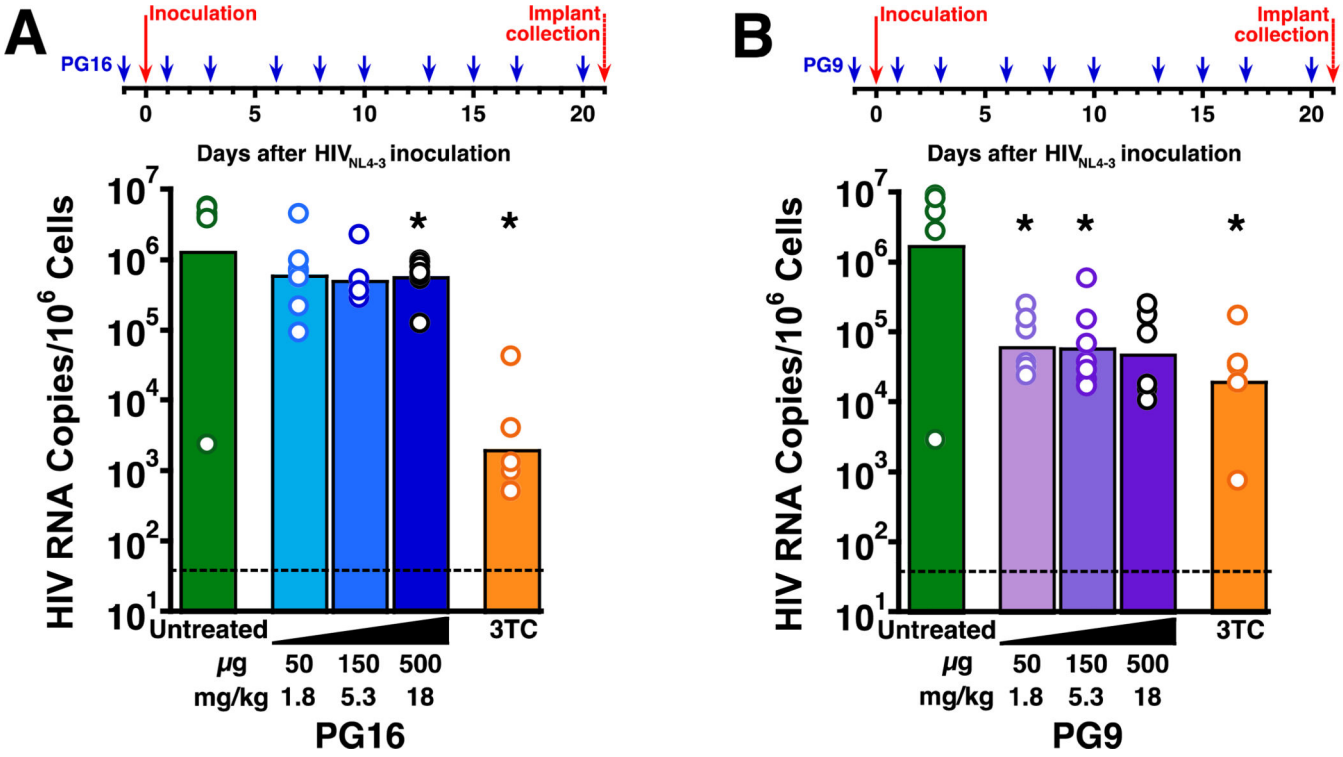

Fig. 4. PG16 and PG9 exhibited minimal protective activity in SCID-hu Thy/Liv mice challenged with HIV$_{NL4-3}$

(A) Statistically significant reductions in HIV RNA occurred in mice treated i.p. with 500 μg PG16 three times per week beginning the day before inoculation and continuing until implant collection at 21 days. Much larger (~3 $\log_{10}$) reductions in HIV RNA were observed in mice treated i.p. with 30 mg/kg 3TC once daily beginning the day before inoculation until implant collection. (B) Statistically significant reductions of >1 $\log_{10}$ in HIV RNA occurred in mice treated i.p. with 50 and 150 μg PG9 three times per week beginning the day before inoculation and continuing until implant collection at 21 days (P=0.055 for 500 μg PG9). Comparable reductions in HIV RNA were observed in mice treated i.p. with 30 mg/kg/day 3TC once daily beginning the day before inoculation until implant collection. The columns represent means, and the open circles represent individual mice. *P<0.05 compared to untreated HIV-infected mice by the Mann-Whitney U test. The dotted line indicates the HIV RNA detection limit. ($10^{1.5}$ copies per 10^6 implant cells).

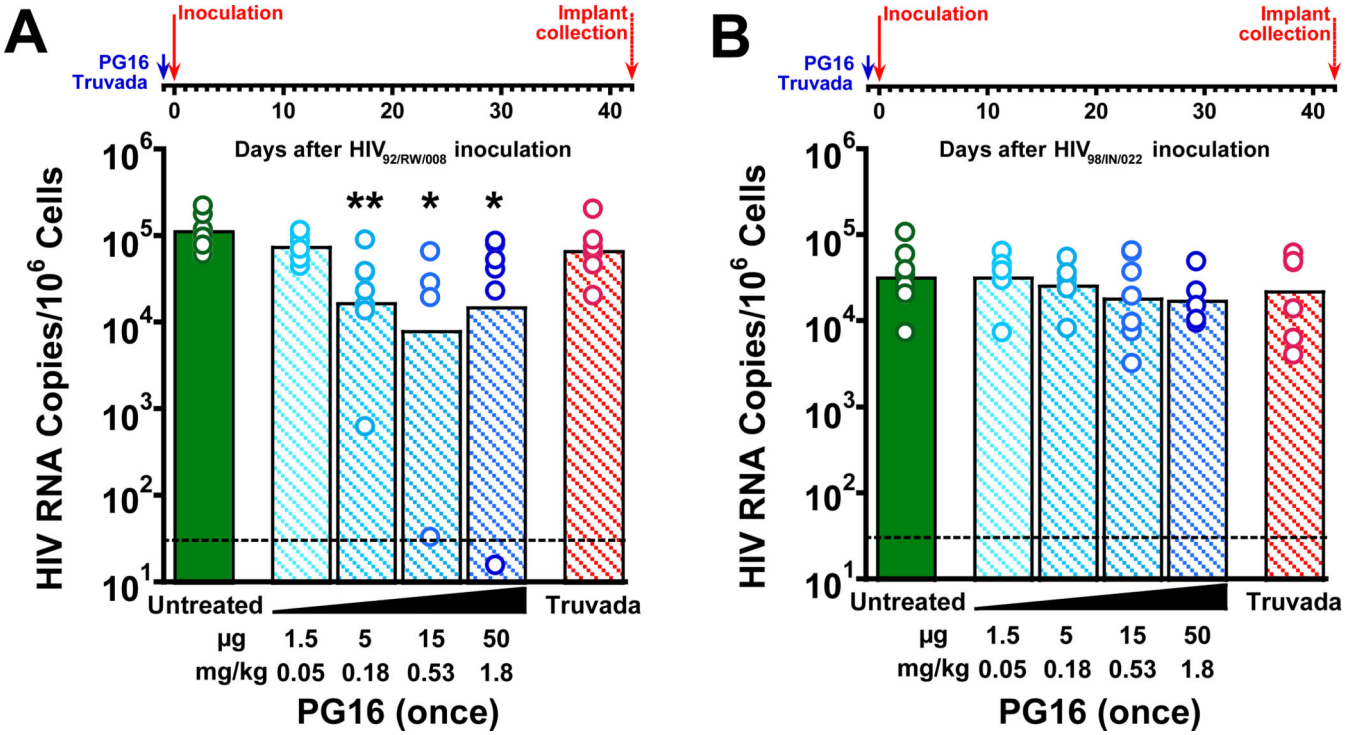

Fig. 5. A single administration of PG16 protected SCID-hu Thy/Liv mice from challenge with clade A $HIV_{92/RW/008}$ but not clade C $HIV_{98/IN/022}$

(A) HIV RNA was reduced by ~1 $\log_{10}$ in mice treated with a single administration of 5–50 µg PG16 the day before inoculation with $HIV_{92/RW/008}$, unlike mice treated once by oral gavage with high-dose Truvada (2,000 mg/kg TDF plus 1,300 mg/kg FTC), which had no reductions in viral RNA 42 days after inoculation. (B) No reductions in HIV RNA were observed in mice treated with a single administration of 1.5–50 µg PG16 the day before inoculation with $HIV_{98/IN/022}$. The columns represent means, and the open circles represent individual mice. $^{**}P<0.01$, $^{*}P<0.05$ compared to untreated HIV-infected mice by the Mann-Whitney U test. The dotted line indicates the HIV RNA detection limit. ($10^{1.5}$ copies per 10^6 implant cells).

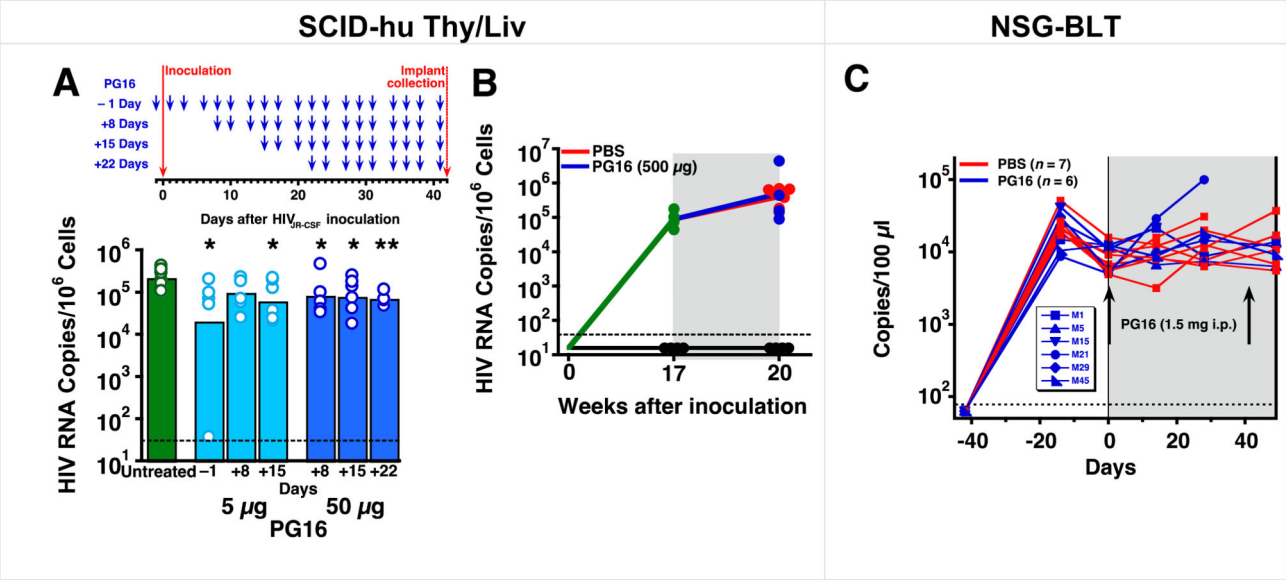

Fig. 6. PG16 had substantially reduced activity in SCID-hu Thy/Liv mice when treatment was initiated 8 days or more after HIV$_{JR-CSF}$ inoculation and had no significant activity in both SCID-hu Thy/Liv mice and NSG-BLT mice with established HIV$_{JR-CSF}$ infection

(A) HIV RNA was reduced by 1 $\log_{10}$ in SCID-hu Thy/Liv mice treated i.p. with 5 μg PG16 three times per week beginning the day before inoculation and continuing until implant collection at 42 days. Smaller reductions in HIV RNA were observed when treatment was delayed until 8 or more days after inoculation. The columns represent means, and the open circles represent individual mice. $^{**}P<0.01$ and $^{*}P<0.05$ compared to untreated HIV-infected mice by the Mann-Whitney U test. (B) No reduction in HIV RNA in SCID-hu Thy/Liv mice treated i.p. with high-dose (500 μg) PG16 or PBS three times per week for 3 weeks beginning 17 weeks after HIV$_{JR-CSF}$ inoculation. The dotted line indicates the HIV RNA detection limit. ($10^{1.5}$ copies per 10^6 implant cells). (C) Viremic NSG-BLT mice were treated with 1.5 mg PG16 at 6 and 12 weeks after intravaginal HIV$_{JR-CSF}$ inoculation. Each line represents an individual mouse, and sequence analysis of viral RNA from the spleens of PG16-treated mouse #5 and #29 (Supplementary Table 13) revealed Env mutation T162N (data not shown). Mouse #15 died and mouse #21 was euthanized with clinical signs consistent with graft-versus-host disease.

Table 1

HIV neutralization by P16 and PG9 in a pseudovirus reporter gene assay and with PBMC

Virus	Coreceptor usage	Antibody	Pseudovirus (luciferase) assay		PBMC assay	
			IC_{50} (μg/ml)	IC_{90} (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)
HIV_{JR-CSF}	R5	PG16	0.001	0.012	0.049	0.249
		PG9	0.003	0.025	ND[c]	ND
HIV_{JD}	R5X4	PG16	0.008	0.490	0.105	2.3
		PG9	0.074	2.3	ND	ND
HIVPD (N160K)	X4	PG16	>50	>50	2.3	>10
		PG9	>50	>50	ND	ND
HIV_{EW}(N160K)	X4	PG16	>50	>50	>10	>10
		PG9	>50	>50	ND	ND
HIV_{F}	X4	PG16	>50	>50	>10	>10
		PG9	>50	>50	ND	ND
HIV_{JW}	R5	PG16	0.12	>50	>10	>10
		PG9	11	>50	ND	ND
HIV_{GV}	X4	PG16	>50	>50	>10	>10
		PG9	>50	>50	ND	ND
HIV_{NL4-3}	X4	PG16	0.23	>50	0.093	0.714
		PG9	8.9	>50	ND	ND
$HIV_{92/RW/008}$(clade A)	R5	PG16	0.002[a]	NR[b]	0.046	0.520
		PG9	0.01[a]	NR	ND	ND
$HIV_{98/IN/022}$(clade C) [a]	R5	PG16	0.003[a]	NR	0.071	0.443
		PG9	0.006[a]	NR	ND	ND

[a] Data from Walker et al. (2009). Number of replicates not specified.

[b] Not reported.

[c] Not determined.

IC_{50} and IC_{90} values represent the average of two separate assays for both pseudovirus and PBMC assays.