# Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1

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**How well antibodies can protect against disease due to HIV-1 infection remains a pivotal but unresolved issue with important implications for vaccine design and the use of prophylactic antibody to prevent infection after accidental exposure to the virus and to interrupt transmission of virus from mother to child. Strong doubts about the possible utility of antibodies *in vivo* have been raised because of the relative resistance of primary viruses to antibody neutralization *in vitro*. Primary viruses are likely to be close to the viruses transmitted during natural infection in humans. Vaccine studies have been of little value in assessing antibody efficacy *in vivo* because none of the strategies described to date have elicited significant neutralizing antibody responses to primary viruses (reviewed in ref. 1). Passive immunization studies are similarly hindered by the paucity of reagents able to neutralize primary viruses effectively[2] and a single study has suggested some benefit[3]. Here we describe experiments to explore the ability of passive antibody to protect against primary virus challenge in hu-PBL-SCID mice. In this model, severe combined immunodeficient (SCID) mice are populated with human peripheral blood mononuclear cells (PBMCs) and infected with HIV-1 (ref. 4). We find that the potent neutralizing human monoclonal antibody IgG1b12 at high dose is able to completely protect even when given several hours after viral challenge. The results are encouraging for antibody-based postexposure prophylaxis and support the notion that antibody induction could contribute to an effective vaccine.**

A variety of studies using viruses multiply passaged in T cell lines [so-called T-cell line-adapted (TCLA) viruses] have indicated a beneficial role for antibodies in protection against HIV-1 challenge. Vaccine studies in animal models show a good correlation between protection and serum neutralizing antibodies when the challenge virus is a TCLA virus, for example, HIV-$1_{IIIB}$ in the chimpanzee[5] or $SHIV_{IIIB}$ (ref. 2). Further, passive immunization of chimpanzees with an anti-V3 monoclonal antibody protects against challenge with a TCLA virus[6]. However, it is now well appreciated that TCLA viruses are relatively easily neutralized by antibodies, including serum antibodies from infected donors and monoclonal antibodies[7-9]. Primary isolates of HIV-1, which have not been multiply passaged, are relatively resistant to antibody neutralization *in vitro* and are expected to be more resistant to antibodies *in vivo*[10]. Because these viruses are presumably much closer to the viruses transmitted during natural infection in humans, the ability of antibodies to protect *in vivo* has been questioned. Passive immunization with specific monoclonal or polyclonal antibody is a classical approach to elucidation of the role of antibody in protection against viral challenge. In the single passive immunization study with a monoclonal antibody to protect against a primary isolate performed to date, chimpanzees were infused with the potent, neutralizing anti-gp41 monoclonal antibody 2F5 before infection. The antibody delayed but did not prevent seroconversion, and the peak of viral RNA was reduced in one animal[3].

We have concentrated on passive immunization in the hu-PBL-SCID mouse model. The model serves as far more than a "furry test tube" for propagation of HIV-1. The architecture of the peritoneal cavity and lymph nodes, the mixture of human cells that engraft, the density of human target cells that engraft, the density of human target cells for viral infection, and the presence of complement and NK cells that can interact with antibody preparations in blocking HIV-1 infection, all combine to create a valuable model[11]. Initially, we demonstrated that a murine monoclonal antibody directed against the V3 loop of HIV-$1_{LAI}$, BAT123, can protect hu-PBL-SCID mice from challenge with this virus strain[12]. This protection was apparent when the antibody was given before or up to 5 hours after virus inoculation[13]. However, primary isolates of HIV-1 were not sensitive to neutralization by BAT123 *in vitro*, and the antibody did not offer protection against primary isolates *in vivo* in hu-PBL-SCID mice[13].

We also showed that IgG1b12, a recombinant antibody directed to an epitope overlapping the CD4 binding site (CD4bs) of gp120, could protect hu-PBL-SCID mice from challenge with the TCLA strain HIV-$1_{SF2}$ (ref. 14). However, in contrast to BAT123, IgG1b12 showed potent neutralization of a broad range of primary isolates of HIV-1 in vitro and ex vivo[15,16]. Therefore, this molecule was an excellent candidate for evaluating protection against infection with primary isolates in passive immunization experiments in the hu-PBL-SCID mouse model. We further attempted to determine whether IgG1b12 could effectively block infection when administered after HIV-1 challenge, as this would have a profound impact on potential clinical usefulness.

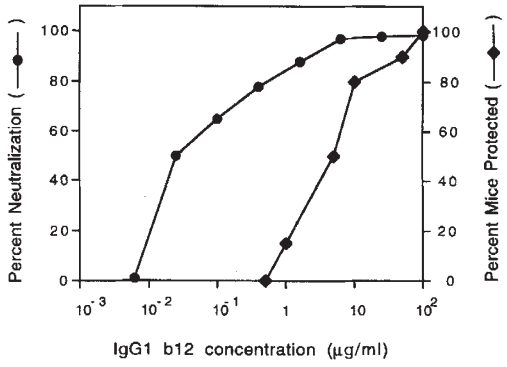


**Fig. 1** Comparison of protection of hu-PBL-SCID mice from HIV-$1_{LAI}$ infection and *in vitro* neutralization of HIV-$1_{LAI}$ by IgG1b12. IgG1b12 was injected i.p. into hu-PBL-SCID mice at various doses from 0.005 to 10 mg/kg 1 h before HIV-$1_{LAI}$ inoculation (10 $MID_{50}$). Protection against infection was assessed 3 weeks later. *In vitro* neutralization of HIV-$1_{LAI}$ was performed with PHA-activated PBMCs as indicator cells and was assessed by measuring p24 antigen in ELISA after 7 days of culture. Similar *in vitro* neutralization sensitivity of HIV-$1_{LAI}$ was observed in assays by using other indicator cells and reporter assays including: MT2 cells in a microplaque reduction assay[15], CEM-SS cells in an assay which enumerated syncytium formation[15], H9 cells and p24 ELISA, and a HeLa-CD4 cell assay in which viral infectivity is linked to β-galactosidase expression (not shown).

The results of the experiments illustrate that complete protection against HIV-1 primary isolate challenge can be achieved by using a potent neutralizing antibody at the appropriate dose. Furthermore, this protection is achieved even when antibody is administered several hours after viral exposure.

The pharmacokinetics of IgG1b12 were studied before performing HIV-1 challenge experiments. IgG1b12 was injected intraperitoneally (i.p.) into a group of four hu-PBL-SCID mice at a dose of 10 mg/kg, and serum concentrations were monitored over a period of 14 days. No toxicity was apparent in any of the animals. The serum levels of IgG1b12 were measured by enzyme-linked immunosorbent assay (ELISA; data not shown). A peak serum concentration of 100 μg/ml was obtained with an estimated half-life of approximately 7–14 days. This is in good agreement with an IgG1b12 half-life of 7.4 ± 0.7 days found in our previous study, in which the IgG1b12 half-life was determined with radiolabeled antibody[14], and with the range of IgG clearance rates in mice[17]. The favorable pharmacokinetics allowed an evaluation of IgG1b12 postexposure prophylaxis in the hu-PBL-SCID mouse model. It is further important to note that the half-life of human IgG1 in human (generally between 21 and 23 days[17]) is three times that found for the human IgG1 in the mouse model.

The TCLA strain HIV-$1_{LAI}$, as compared with primary isolates, is relatively sensitive to antibody neutralization. We used this strain to perform a more detailed comparison of *in vitro* neutralization and *in vivo* protection by IgG1b12 than is practicable with primary isolates. Further, a more quantitative comparison of protection by IgG1b12 and HIVIG could be made by using the TCLA virus (see below).

Hu-PBL-SCID mice were injected i.p. with IgG1b12 at various doses. One hour later, mice were challenged by i.p. injection of 10 median animal infectious doses ($MID_{50}$) of HIV-$1_{LAI}$. Mice were killed after 3 weeks, and the presence of HIV-1 infection was determined as described. The results are represented in Fig. 1. Protection was titratable in the range 0.005–10 mg/kg, corresponding to peak serum concentrations of 0.05–100 μg/ml. Complete protection was only observed at an IgG1b12 dose of 10 mg/kg corresponding to a peak serum concentration of 100 μg/ml. Fifty percent protection was achieved at a peak level of IgG1b12 (10 μg/ml), approximately two orders of magnitude greater than the 50% *in vitro* neutralization titer (50 ng/ml). Complete protection occurred at a peak serum level of IgG1b12, corresponding to a neutralization titer considerably greater than 90% and probably greater than 99%. The results provide confirmation of a trend suggested by our previous study on the TCLA strain HIV-$1_{SF2}$ (ref. 14).

We have shown previously that IgG1b12 can efficiently neutralize a range of HIV-1 TCLA strains and primary isolates *in vitro*[15,18]. Those HIV-1 isolates included the TCLA strain HIV-$1_{LAI}$, and primary isolates HIV-$1_{JR-CSF}$ (ref. 19) and HIV-$1_{AD6}$ (ref. 20, 21) used in this study. In confirmatory studies, IgG1b12 was found to neutralize (>90%) AD6 and JR-CSF at concentrations of approximately 10–25 μg/ml (ref. 16 and Fig. 3). Because this study was the first attempt to protect against these primary isolates of HIV-1 in hu-PBL-SCID mice, we chose to perform an experiment with two doses of IgG1b12 of 50 and 10 mg/kg. A dose of 5 mg/kg would provide a peak serum level of IgG1b12 of the order of 500 μg/ml which is a concentration more than 10 times that required to neutralize (>90%) HIV-$1_{JR-CSF}$ and HIV-$1_{AD6}$ *in vitro*.

Protection experiments were performed with groups of five or six mice that received either IgG1b12 or control antibody 1 hour before viral inoculation with 10 $MID_{50}$ of either HIV-$1_{AD6}$ or HIV-$1_{JR-CSF}$ (Fig. 2, *a* and *b*). None of the mice inoculated with HIV-$1_{AD6}$ or HIV-$1_{JR-CSF}$ that received the high dose of IgG1b12 (50 mg/kg) became infected, as indicated by the failure to recover HIV-1 from the spleen or peritoneal lavage cocultures (Fig. 2). In contrast, for the low dose of IgG1b12 (10 mg/kg), HIV-1 was recovered from five of six mice inoculated with HIV-$1_{AD6}$ and three of six mice inoculated with HIV-$1_{JR-CSF}$. IgG1b12 was therefore effective in protecting against both isolates at a dose of 50 mg/kg, but at 10 mg/kg was only partially effective against JR-CSF and relatively ineffective against AD6. Serum concentrations of IgG1b12 that were expected to be in the range required to achieve 90% neutralization *in vitro*, therefore, did not protect or only partially protected the mice. Good protection was observed when the serum concentrations were anticipated to correspond to those giving >99% neutralization *in vitro*, in line with the above findings by using a TCLA strain of HIV-1.

The acronym HIVIG is applied to HIV-1 immune globulin fractions prepared from the plasma of HIV-1 seropositive donors. In this experiment, we studied the ability of an HIVIG preparation at 150 mg/kg to protect hu-PBL-SCID mice against infection with HIV-$1_{LAI}$, $HIV_{JR-CSF}$ and HIV-$1_{AD6}$ by using the procedures described above. Partial protection was observed against HIV-$1_{LAI}$ but no significant protection against the two primary isolates (Fig. 2*c*). The HIVIG fraction used was enriched for HIV-1-neutralizing activity by including only plasma from donors with the top 12.5% of HIV-$1_{LAI}$-neutralizing antibody titers[22]. Another HIVIG preparation has recently been through phase I trials[23], during which it was proposed that a dose of 200 mg/kg could be used for prophylactic application in humans. The HIVIG used for the phase I trial, in contrast to the one in our study, was prepared from seropositive donors for whom the selection criteria included high serum titers to p24 and $CD4^+$ T-cell counts above 400/μl but did not include HIV-1 neutralizing activity in serum[23,24]. It is therefore very unlikely that this HIVIG preparation would have a greater activity in HIV-1 prophylaxis than the one studied here. Our data suggest that currently available preparations of HIVIG may be ineffective in the prophylaxis of HIV-1 infection in humans.

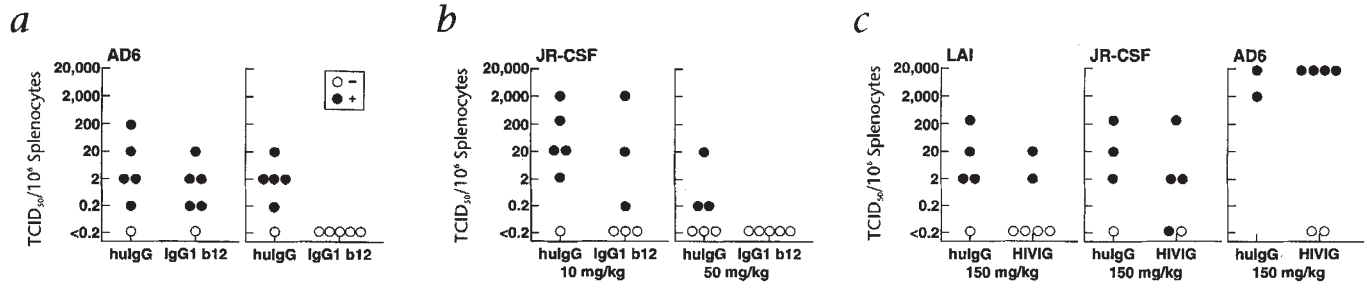


**Fig. 2** Protection by IgG1b12 and HIVIG against infection with primary isolates of HIV-1. Hu-PBL-SCID mice were injected i.p. with IgG1b12 at doses of 10 and 50 mg/kg in 0.5 ml PBS (*a* and *b*) or 150 mg/ml HIVIG (*c*). Human IgG from a seronegative donor at similar doses was used as a control. After 1 h, the mice were challenged i.p. with 10 $MID_{50}$ of HIV-1. The mice were killed after 3 weeks, and it was determined whether HIV-1 infection was present. Protection with IgG1b12 was assessed after challenge with primary isolates $HIV\text{-}1_{AD6}$ (*a*) or $HIV\text{-}1_{JR\text{-}CSF}$ (*b*). Protection with HIVIG was assessed after challenge with 10 $MID_{50}$ of HIV-1 isolates LAI, AD6 or JR-CSF (*c*). White circles indicate the mouse to be HIV-1 negative in both peritoneal lavage and spleen cell coculture assays; black circles indicate mice that were scored positive. Black circles at ≥0.2$TCID_{50}/10^6$ splenocytes indicate that HIV-1 was detected in both assays, whereas black circles at <0.2 $TCID_{50}/10^6$ splenocytes indicate mice that were negative by spleen cell coculture but positive by peritoneal lavage coculture. The differences between the hu-IgG and IgG1b12 assay groups at 50 mg/kg were significant ($P < 0.05$); the differences between hu-IgG and HIVIG were found not significant.

To determine the *in vitro* neutralization kinetics of the TCLA strain and the primary isolates by IgG1b12, the three HIV-1 isolates were coincubated over a period of 3 hours with phytohemagglutinin (PHA)-stimulated PBMCs from seronegative donors, and graded doses of monoclonal antibody were added to the culture at different time-points (at $t = 0$, 30, 60 and 120 minutes). Production of p24 was measured on day 7 and the percentage neutralization was calculated (Fig. 3).

At suboptimal antibody concentrations, much of the ability of IgG1b12 to neutralize $HIV\text{-}1_{LAI}$ *in vitro* was lost within the first 30 minutes. For the two primary isolates, the retention of neutralizing activity was more robust with relatively little diminution of activity at 1 hour and still considerable activity at 2 hours. The relatively strong overall maintenance of activity was encouraging for postexposure protection studies (see below) and suggests that IgG1b12, as well as interfering with the binding of gp120 to the primary HIV-1 receptor CD4, may also disrupt a phase of infection after binding of the virion to the $CD4^+$ cell. It has indeed been shown that IgG1b12 strongly inhibits the interaction between gp120 and the secondary HIV-1 receptor CCR5 following gp120–CD4 interaction[25]. Alternatively, however, the delayed addition of the antibody may be effective by preventing the formation of a critical number of HIV–cell receptor contacts necessary for viral entry[26].

To study postexposure protection by IgG1b12, the antibody (or control antibody) was given at various time points after challenge of hu-PBL-SCID mice with 10 $MID_{50}$ of $HIV\text{-}1_{LAI}$, $HIV\text{-}1_{JR\text{-}CSF}$ or $HIV\text{-}1_{AD6}$ at doses of 5 (LAI) or 50 mg/kg (primary isolates). Mice were killed 3 weeks later, and HIV-1 infection was assessed as described (Fig. 4).

The IgG1b12 effectively protected hu-PBL-SCID mice against HIV-1 infection when given within 6 to 24 hours of HIV-1 inoculation. Only 2 out of 22 animals were infected with $HIV\text{-}1_{LAI}$ when IgG1b12 was given within 8 hours after exposure, and only 1 out of 27 animals became infected with $HIV\text{-}1_{JR\text{-}CSF}$ when IgG1b12 was given within 24 hours after exposure. Protection against infection with $HIV\text{-}1_{AD6}$ was somewhat less dramatic but significantly, IgG1b12 induced complete protection in all animals if administered within 6 hours after exposure.

The time intervals after which IgG1b12 was able to protect the hu-PBL-SCID mice from infection are significant when compared with the rapid HIV-1 replication and turnover rates demonstrated in peripheral blood lymphocytes of HIV-1 seropositive individuals[27–29]. It is therefore likely that the virus went through several or at least a single round of replication during the 6- to 24-hour window for successful postprophylactic intervention. This indicates that protection by IgG1b12 may not only be accomplished by interfering with the primary infection but that the antibody may also be able to disrupt the establishment of infection early after exposure when only low numbers of cells are affected. When breakthrough occurred, the titer of the infectious virus in the spleens was as high as in control animals, indicating that IgG1b12 had little effect on the acute virologic course if the establishment of infection was not blocked.

In summary, the results presented here show that IgG1b12 can protect hu-PBL-SCID mice against challenge with primary isolates and TCLA strains of HIV-1. There is a rough qualitative correlation between *in vitro* neutralization activity and *in vivo* efficacy of protection. In quantitative terms, the dose required to achieve complete protection in the mouse model corresponded to a serum

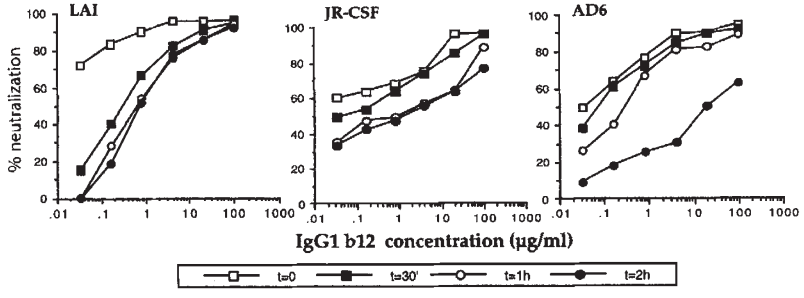


**Fig. 3** In vitro kinetics of neutralization by IgG1b12. The three isolates of HIV-1 (LAI, AD6 and JR-CSF) were coincubated over a period of 3 h with PHA-stimulated PBMCs, and IgG1b12 was added to the mixture at the beginning of the culture or after 30, 60 or 120 min, and then washed extensively. Culture supernatants were assayed for virus by p24 antigen ELISA on day 5–8 of culture. Neutralization was defined as the percent reduction in the amount of p24 antigen released into the culture supernatants from wells treated with antibody as compared with controls without antibody. The results shown represent the mean of two to three independent experiments with s.e.m. <3%.

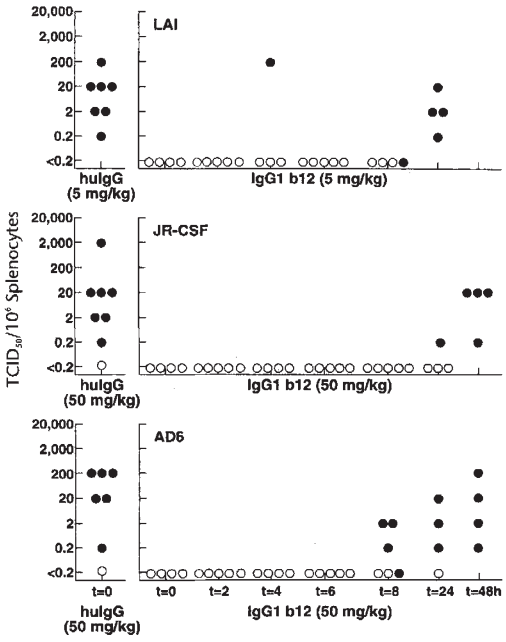


**Fig. 4** Postexposure protection by IgG1b12. Hu-PBL-SCID mice were injected with 10 $MID_{50}$ of HIV-$1_{LAI}$, HIV-$1_{JR-CSF}$ or HIV-$1_{AD6}$, and IgG1b12 [5 mg/kg (LAI) or 50 mg/kg (primary isolates)] was given at various time intervals after exposure as indicated. Mice were killed after 3 weeks, and HIV-1 infection was assessed as described.

level of reagent considerably in excess of that required to achieve 90% neutralization in a typical *in vitro* assay. Therefore there is no indication from these studies that current neutralization assays underestimate the efficacy of antibody activity *in vivo*[30]; rather, the model suggests that only very high levels of *in vitro* neutralization (>99%) correlate with *in vivo* protection.

The ability of IgG1b12 to protect against HIV-1 primary isolate challenge completely lends support to attempts to develop a vaccine incorporating a potent humoral response to the virus[31]. In particular, the CD4 binding site region provides important overlapping epitopes with conserved elements in a high proportion of primary isolates[32]. Finally, the favorable pharmacokinetic profile and excellent postexposure protective activity of IgG1b12 suggest that this antibody may be useful to protect against infection following exposure to HIV-1 in humans, for example, as could arise from needle stick injuries.

## Methods

**Monoclonal antibodies.** A human monoclonal antibody (mAb) IgG1b12 produced from an immune phage display library has been described previously[15,18,33–35]. IgG1b12 recognizes a discontinuous epitope overlapping the CD4bs on HIV-1 gp120 (ref. 33, 34, 36). HIVIG is an HIV-1 immune globulin fraction prepared from human plasma selected for high titers of HIV-1 neutralizing antibodies and provided by Linda Andrus (New York Blood Center, New York, NY)[22]. The control hu-IgG is a polyclonal human IgG fraction purified from an HIV-1 seronegative donor. All antibodies were given to hu-PBL-SCID mice by i.p. injection.

**SCID mouse reconstitution.** A breeding colony of homozygous SCID ($CB.17^{scid/scid}$) mice, maintained under specific pathogen-free conditions at the Aaron Diamond AIDS Research Center, served as source of animals for these studies. Non-leaky phenotype mice were reconstituted by i.p. injection of $20 \times 10^6$ freshly isolated normal human PBMCs suspended in 0.5 ml of phosphate-buffered saline (PBS) for each mouse[37,38]. Two weeks after PBMC transfer, reconstitution was confirmed by analysis of the mouse sera for the presence of human immunoglobulin by ELISA (SangStat, Menlo Park, CA)[38]. Only human immunoglobulin-positive mice were used for pharmacokinetics and protection studies. All procedures for injection and maintenance of the hu-PBL-SCID mice were performed in a biosafety level 3 facility.

**Serum antibody concentration.** Blood was obtained by bleeding from the tail vein of the mice. The serum concentration of the injected antibodies was determined by ELISA. Pharmacokinetics for IgG1b12 were determined by using a gp120 ELISA. Immulon-2 microdilution plates (Dynatech Laboratories, Chantilly, VA) were coated overnight at room temperature with sheep anti-gp120 C5 region antibody D7324 (Aalto Bioreagents, Dublin, Ireland) diluted 1:200 in $NaHCO_3$ pH = 8.6. The plates were washed two times with Tris-buffered saline (TBS) and treated with 2% nonfat dry milk in PBS for 5 min at room temperature. After washing, HIV-$1_{MN}$ gp120 was added to the plates at 20 ng/ml in TBS/10% fetal calf serum (FCS) and incubated for 1 h. Plates were washed twice, and the IgG1b12-containing mouse serum was added to the plates. After washing, bound antibody was detected by addition of goat anti-human IgG-alkaline phosphatase conjugate diluted 1:10,000 (Accurate Chemicals, Westbury, NY). The IgG1b12 concentration was determined by comparing absorbance of sample dilutions with standards measured at 492 nm by using an ELISA plate reader.

**Virus stocks.** Virus stocks were prepared from the supernatants of infected PBMCs. Briefly, cell-free virus was harvested on day 5 to 7 from acutely infected PHA-stimulated PBMCs and titrated by serial dilutions on uninfected PHA-stimulated PBMCs. The infectious dose of each virus stock was expressed as the median tissue culture infective dose per milliliter ($TCID_{50}$/ml) by using the method of Reed and Muench[39,40]. Virus stocks were titrated for infectivity in hu-PBL-SCID mice and similarly expressed as the 50% mouse infective dose per milliliter ($MID_{50}$/ml). The following HIV-1 isolates were used in this study: HIV-$1_{LAI}$, a laboratory strain of HIV-1 adapted to grow in transformed T-cell lines[41]; HIV-$1_{JR-CSF}$, a molecularly cloned primary HIV-1 isolate[19]; and HIV-$1_{AD6}$, a primary isolate from an acute seroconvertor[42] that has been passaged twice in mitogen-stimulated PBMCs (ref. 20). Neither HIV-$1_{JR-CSF}$ nor HIV-$1_{AD6}$ grows in transformed T-cell lines.

***In vitro* neutralization assays.** Virus neutralization was assessed as described[43–45]. Briefly, culture medium [RPMI 1640 (50 µl) supplemented with 10% FCS, 10 U/ml recombinant human IL-2, and antibiotics] containing 150–200 $TCID_{50}$ HIV-1 was mixed in quadruplicate with 100 µl of serial twofold dilutions of test antibody or control. Virus/antibody mixture was then incubated with $5 \times 10^6$ PHA-stimulated PBMCs in culture medium (100 µl) over a period of 3 h, washed extensively and cultured for 5 to 8 days. Culture supernatants were assayed for viral expression by p24 antigen by using a commercial ELISA kit (Abbott Laboratories, Chicago, IL) on day 5–8 of culture. Neutralization was defined as the percent reduction in the amount of p24 antigen released into the culture supernatants from wells treated with antibody as compared with control wells not treated with antibody. For the kinetics of neutralization experiments, virus and cells were incubated for 3 h, and antibody was added to the mixture at the time of the incubation ($t = 0$), or after 30 min, 1 h or 2 h of the incubation.

**Viral challenge in hu-PBL-SCID mice.** All procedures for infection and maintenance of the hu-PBL-SCID mice were performed in a biosafety level 3 animal facility. The infection of hu-PBL-SCID mice was carried out 2 weeks after PBMC reconstitution. Hu-PBL-SCID mice were injected i.p. with 10 $MID_{50}$ of cell-free HIV-1 in 0.5 ml (ref. 46). The virus inoculates were previously determined by titration in hu-PBL-SCID mice and were shown to infect at least 80% of hu-PBL-SCID mice[46]. For protection experiments each antibody was administered i.p. in 0.5 ml of PBS, at various time points before or after HIV-1 inoculation. Mice were killed 3 to 4 weeks later, and the presence of HIV-1 was determined by end-point dilution coculture of the spleen cells and bulk culture of cells recovered from peritoneal lavage.

**Detection of HIV-1 by coculture.** Quantitative culture assays (end-point dilution culture system) were performed for the detection of HIV-1 in the spleen cells of the SCID mice in order to measure the level of infectious virus. Infection of peritoneal lavage cells was determined by bulk coculture. Mice were killed 3 weeks after viral challenge, and cells were recovered from peritoneal lavage and spleens as previously described[12,46]. In order to detect HIV-1

by coculture, peritoneal lavage cells ($2 \times 10^5$) were cultured in bulk with $2 \times 10^6$ PHA-activated PBL, and spleen cells ($5 \times 10^6$) were incubated with $2 \times 10^6$ PHA-activated PBL from HIV-1 seronegative donors[12] in an end-point dilution culture (10-fold serial dilutions)[12,39,40]. Cocultures were monitored weekly, over a period of 4 weeks, for the presence of HIV-1 p24 core antigen in the culture supernatant by using a commercial ELISA (Abbott). Cultures were considered positive for HIV-1 if a single sample contained >1,000 pg/ml or if two consecutive samples contained > 200 pg/ml of p24. The peritoneal lavage cultures were either scored as negative or positive. For the spleen cell cultures, the positive well containing the fewest spleen cells was taken as the end point, and the viral titers were expressed as $TCID_{50}$ per $10^6$ cells.

**Statistical analysis.** *In vitro* neutralization assays were performed in quadruplicate, and the results were calculated as the mean percent reduction in the amount of p24 antigen released into the culture supernatants from wells treated with antibody as compared with untreated control wells. All measures of variance are given as s.e.m. When necessary, the differences between experimental results were calculated by using the paired Student's *t*-test (Wilcoxon signed-ranks tests) or the two-sample (independent-groups) Student's *t*-test (Wilcoxon rank-sum tests). The experiments were performed at least twice with similar results.

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