Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys

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Virus-specific antibodies protect individuals against a wide variety of viral infections[1–7]. To assess whether human immunodeficiency virus type 1 (HIV-1) envelope-specific antibodies confer resistance against primate lentivirus infections, we purified immunoglobulin (IgG) from chimpanzees infected with several different HIV-1 isolates, and used this for passive immunization of pig-tailed macaques. These monkeys were subsequently challenged intravenously with a chimeric simian–human immunodeficiency virus (SHIV) bearing an envelope glycoprotein derived form HIV-1_{DH12}, a dual-tropic primary virus isolate. Here we show that anti-SHIV neutralizing activity, determined *in vitro* using an assay measuring loss of infectivity, is the absolute requirement for antibody-mediated protection *in vivo*. Using an assay that measures 100% neutralization, the titer in plasma for complete protection of the SHIV-challenged macaques was in the range of 1:5–1:8. The HIV-1-specific neutralizing antibodies studied are able to bind to native gp120 present on infectious virus particles. Administration of non-neutralizing anti-HIV IgG neither inhibited nor enhanced a subsequent SHIV infection.

Of the primate lentiviral vaccine approaches evaluated during the past decade, live attenuated vaccines have proven the most consistent for inducing resistance against subsequent virus challenges. However, because of concerns about the possible reversion to virulence and/or natural or induced fluctuations in immune function during the lifetime of the vaccinee that might lead to increased virus loads, other vaccine strategies are now being systematically assessed. Nonetheless, the delineation of protective mechanisms elicited by any vaccine against a primate lentiviral infection would be very useful for designing an effective prophylactic vaccine. Nonhuman primate model systems, such as HIV-1 infection in chimpanzees and SIV (simian immunodeficiency virus) or SHIV (simian–human chimeric immunodeficiency virus) infections in macaques have been widely used for this purpose[8]. Although live attenuated virus vaccines have been shown to confer resistance against HIV or SHIV by a mechanism(s) that does not involve neutralizing antibodies[9–11], chimpanzee studies, including passive immunization experiments, correlated protection from HIV-1 infection with levels of neutralizing antibody[12–16]. These seemingly conflicting results indicate that a diversity of immunological mechanisms have the potential to protect animals from a retrovirus challenge. Here we have focused on antibody-mediated protection and have addressed a fundamental question relevant to the development of a prophylactic vaccine: Can high-titer antibody, directed against the HIV-1 envelope and capable of completely neutralizing an HIV infection in a tissue culture system, block the establishment of a SHIV infection *in vivo* after its passive transfer to macaque monkeys?

We studied chimpanzees that were infected with a variety of HIV-1 isolates and subsequently developed high-titer neutralizing antibodies. Ch 4750 had been simultaneously co-infected with three different clade B primary isolates, each representing a different host cell-tropic phenotype (HIV-1_{DH12}, a T-cell line/macrophage dual-tropic primary virus isolate[17]; HIV-1_{DH20}, T-tropic[18]; and HIV-1_{DH29}, M-tropic[18]); Ch 1206 had been infected with HIV-1_{DH12} alone; Ch 911 had been infected with the T-tropic, clade B isolate, HIV-1_{IIIB} (ref. 19); and Ch 73 was uninfected (Table 1 and Fig. 1*a*). IgG was prepared as described[20] from these four chimpanzees and passively transferred to pig-tailed macaques. The macaques were subsequently challenged with the pathogenic MD14 derivative of SHIV-1_{DH12} (ref. 21)(Fig. 1*b*), a chimeric virus with envelope glycoproteins derived from HIV-1_{DH12}, a dual-tropic primary virus isolate[17]. The ability of HIV-1_{DH12} to be neutralized was compared with that of several laboratory adapted, T cell-tropic HIV-1 isolates; two different human sera, capable of neutralizing HIV-1_{IIIB} and HIV-1_{MN}, failed to neutralize HIV-1_{DH12} (data not shown). The broadly neutralizing monoclonal antibody b12 (ref. 22) neutralized HIV-1_{DH12} in peripheral blood mononuclear cells (PBMC) at 4.7 μg/ml (as measured by 90% p24 reduction assay), a similar concentration to that required to neutralize other primary isolates (Ref. 22 and R.S., unpublished observation).

To formally demonstrate that chimpanzee IgG capable of neutralizing HIV-1_{DH12} could also neutralize a SHIV (SHIV$_{DH12}$) bearing the HIV-1_{DH12} gp120, we did a neutralization assay using IgG purified from heparinized plasma of Ch 1206 (infected with HIV-1_{DH12}) and Ch 911 (infected with HIV-1_{IIIB})(Fig. 1*c*). Serial threefold dilutions of the IgG preparations were incubated with 100 $TCID_{50}$ (50% tissue culture infectious dose) of SHIV$_{DH12}$ for 1 hour at room temperature, and the mixture was then used as the inocula for quadruplicate, 2-week infections of MT-4 cells. Non-neutralized culture supernatants appear as black dots on the autoradiograms of these ^{32}P reverse transcriptase (RT) assays, reflecting the presence of (progeny) virus-associated RT activity in the medium. The absence of black dots indicates complete neutralization of virus

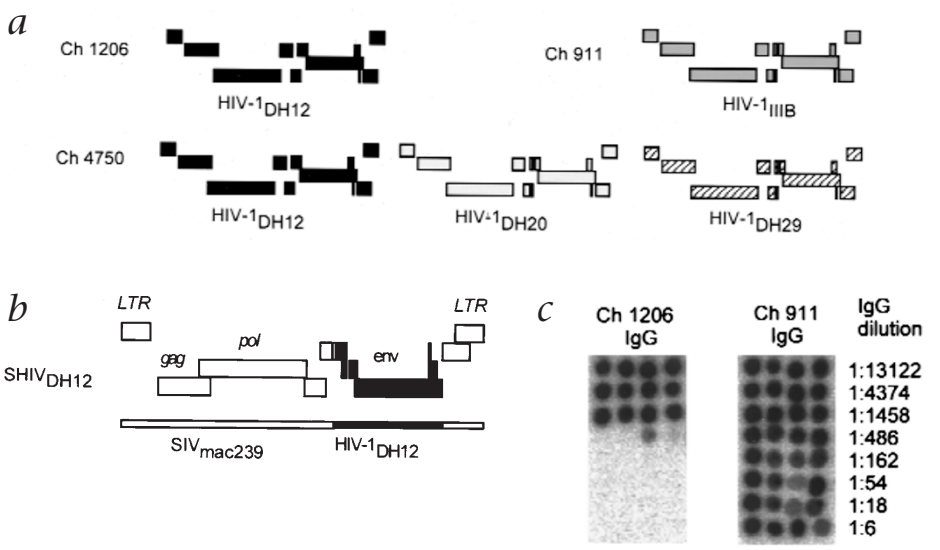

Fig. 1 ***a***, HIV-1 isolates used to infect the three IgG donor chimpanzees (Ch 1206, Ch 911 and Ch 4750). ***b***, Genomic organization of the $SHIV_{DH12}$ proviral DNA clone MD14YE (ref. 21), with the HIV-1_{DH12} *env* gene. ***c***, ^{32}P RT assays showing neutralization of 100 $TCID_{50}$ of $SHIV_{DH12}$ in quadruplicate cultures as described[11], with threefold serial dilutions of IgGs purified from chimpanzees chronically infected with HIV-1_{DH12} (Ch 1206) or HIV-1_{IIIB} (Ch 911).

infectivity, not merely the reduction of virus production. In the assay shown, $SHIV_{DH12}$ infectivity was blocked at the higher concentrations of IgG from Ch 1206. The calculated[23] neutralization titer (the dilution at which two of the quadruplicate infections would show neutralization, indicated by the absence of RT activity after 2 weeks of infection) of Ch 1206 IgG against 100 $TCID_{50}$ of $SHIV_{DH12}$ was 1:486 (Fig 1*c*). The neutralization titer of IgG from Ch 4750, the chimpanzee initially infected with three different HIV-1 isolates including HIV-1_{DH12}, was in a similar range (data not shown). In contrast, IgG from Ch 911 had no detectable neutralizing activity against 100 $TCID_{50}$ (Fig. 1*c*, right) or 10 $TCID_{50}$ (data not shown) of $SHIV_{DH12}$, although it was able to neutralize HIV-1_{IIIB} (data not shown). All of the neutralization assays used (for convenience) MT4 cells, although, as reported[24], high titers of neutralizing antibody directed against HIV-1_{DH12} are readily measured in chimpanzee serum using human PBMC.

The observed neutralization of $SHIV_{DH12}$ by chimpanzee IgG is most likely mediated by antibodies targeting the HIV-1 envelope glycoproteins. Sera from $SHIV_{DH12}$ infected rhesus monkeys, but not SIVΔ2- or SIVΔ3-infected macaques, readily neutralized $SHIV_{DH12}$ (ref. 11), indicating that Env- and not Gag-directed antibodies (both SIV and SHIV encode identical Gag proteins) were capable of blocking virus infectivity. Moreover, the IgG from chimpanzees 1206 and 4750, both previously infected with HIV-1_{DH12}, and not the IgG purified from chimpanzee 911, chronically infected with HIV-1_{IIIB}, neutralized the SHIV with the HIV-1_{DH12} envelope glycoprotein. This result is consistent with reports linking envelope sequence variability with differential susceptibilities to neutralizing antibodies[25]. Finally, serum from Ch 1206, which failed to neutralize the macrophage-tropic HIV-1_{Ada} isolate, was able to neutralize chimeric HIV-$1_{AD8/DH12}$, with gp160- (ref. 24) or gp120-coding sequences from HIV-1_{DH12}, but not a chimeric virus expressing only the HIV-1_{DH12} gp41 (M.W.C., unpublished observations).

Monkeys immunized with whole inactivated SIV prepared in human lymphocytes or T-cell lines resist a subsequent virus challenge when the SIV inoculum is derived from human but not macaque cells[26]. In those studies, the target molecules for neutralization were most likely human lymphocyte-derived cell surface antigens incorporated into progeny virions during the budding process[26]. Here, the $SHIV_{DH12}$ challenge stock was prepared in macaque PBMC, and none of the IgG donor chimpanzees had been previously exposed to macaque cells or proteins. Thus, the neutralizing activities associated with preparations of chimpanzee IgG target the HIV-1_{DH12} gp120 envelope glycoprotein. The neutralization assays here measure the 100% 'end points' of neutralization against a known titer of infectious virus. Unlike neutralization assays that measure 50% or 90% inhibition titers of virus-induced cytopathicity or the reduction of progeny virus produced from infected cells, the neutralization titers reported here represent the dilution of plasma that completely neutralizes the infectivity of input virus.

We initially studied the binding properties of the three chimpanzee IgG preparations to individual $SHIV_{DH12}$ proteins by immunoblotting (Fig. 2). As expected, IgG from Ch 4750, originally infected with three HIV-1 isolates including HIV-1_{DH12}, bound strongly to the gp120 and gp41 envelope proteins of $SHIV_{DH12}$; cross-reactivity with the p27 Gag CA protein of $SHIV_{DH12}$, derived from SIV_{mac239}, was also seen. IgG from Ch 1206, despite having high titer neutralizing activity against $SHIV_{DH12}$, showed only weak binding to the immobilized $SHIV_{DH12}$ proteins at the two dilutions tested (Figs. 1*c* and 2). Weak binding of Ch 1206 IgG to $SHIV_{DH12}$ proteins was also detected by ELISA (data not shown). Finally, IgG from Ch 911 (infected with HIV-1_{IIIB}) showed binding activities to p27 CA similar to those of Ch 4750 IgG but less reactivity with the envelope proteins of $SHIV_{DH12}$. The properties of the chimpanzee IgG used for passive transfer into pig-tailed

Table 1 Properties of chimpanzee IgG used for passive immunization

Donor ID	HIV-1 infection	Duration of infection	IgG conc. (mg/ml)	Binding to DH12 envelope[a]	Neutralization of $SHIV_{DH12}$[b]
ch 73	None	None	18.1	– (<15)	–
ch 4750	HIV-1_{DH12}, HIV-1_{DH20}, HIV-1_{DH29}	3.0 yrs	15.0	++++ (1750)	+
ch 1206	HIV-1_{DH12}	2.8 yrs	23.0	+ (<15)	+
ch 911	HIV-1_{IIIB}	9.9 yrs	17.9	+++ (530)	–

[a]Assayed by immunoprecipitation, ELISA and immunoblotting. The values in parentheses indicate the relative radioactivities associated with the gp120/gp160 bands shown in Fig. 2 (1:200 dilution).
[b]See Fig. 1*c*.

macaques are summarized in Table 1.

In the initial *in vivo* challenge experiment, 100 $TCID_{50}$ of $SHIV_{DH12}$ was selected as the amount of virus to be intravenously inoculated after passive transfer of chimpanzee IgG. Based on a challenge dose of this size, we determined the amounts of IgG administered to macaque monkeys. The plasma volume of a 4-kg pig-tailed macaque is approximately 5% of its body weight, or 200 ml; therefore, the intravenous transfer of 0.5 ml of chimpanzee IgG would result in a 1:400 dilution during its distribution throughout the plasma. Based on the results of *in vitro* virus neutralization assays (Fig. 1*c*), the neutralizing activity present in 0.5 ml of Ch 1206 IgG or Ch 4750 IgG would be sufficient to neutralize 100 $TCID_{50}$ of $SHIV_{DH12}$ in plasma after passive transfer. However, because of uncertainties about further dilution of the administered IgG due to extravascular diffusion into other body compartments, we decided to administer tenfold more (5 ml) of Ch 1206 IgG and Ch 4750 IgG. The dose (17 ml) of IgG from the HIV-1_{IIIB}-infected chimpanzee (Ch 911), which demonstrated no detectable neutralizing activity against $SHIV_{DH12}$, was determined by normalizing its binding activity to SHIV proteins, deduced from quantitative ELISA and immunoblotting assays, to that of Ch 4750 IgG (Fig. 2 and Table 1). Finally, the total amount (306 mg) of IgG administered to each macaque was made equal by supplementation with IgG prepared from an uninfected chimpanzee (Ch 73). The half-life of chimpanzee IgG in pig-tailed macaques, determined by measuring the decay of anti-HIV gag antibody, was 10 to 14 days (data not shown).

Table 2 Passive immunization of pig-tailed macaques[a]

Recipient ID	Weight (kg)	HIV-1 (+) IgG (Donor, total mg)	HIV-1 (–) IgG (Donor, total mg)	ELISA titer[b]	Neutralization of $SHIV_{DH12}$[c]	Challenge dose ($TCID_{50}$)	Challenge result
EXPERIMENT 1							
pt 94P018	4.0	none	ch 73,306 mg	< 1:75	<1:2	100	Infected
pt 94P025	3.7	none	ch 73,306 mg	< 1:75	<1:2	100	Infected
pt 94P027	3.7	ch 4750,75 mg	ch 73,231 mg	1:1875	1:2.8	100	Infected[d]
pt 94P028	4.5	ch 4750,75 mg	ch 73,231 mg	1:1875	1:4	100	Infected
pt 94P029	4.3	ch 1206,115 mg	ch 73,191 mg	<1:75	1:2.5	100	Infected
pt 94P032	4.0	ch 1206,115 mg	ch 73,191 mg	<1:75	1:2.8	100	Infected
pt 94P033	4.1	ch 911,306 mg	none	1:1875	<1:2	100	Infected
pt 94P034	3.9	ch 911,306 mg	none	1:1875	<1:2	100	Infected
EXPERIMENT 2							
pt 94P010	4.6	ch 1206,115 mg	none	not tested	1:5	10	Protected
pt 94P011	5.0	ch 1206,1150 mg	none	not tested	1:8	100	Protected

[a]Plasma samples collected 24 h after passive immunization and before $SHIV_{DH12}$ challenge were used for the assays. [b]ELISA antibody titers in plasma were measured using a commercial assay kit. [c]Neutralizing antibody titers in plasma against the challenge dose (100 or 10 $TCID_{50}$ of $SHIV_{DH12}$). See Fig. 3. [d]Virus was detected only transiently. (+), -positive; (–), -negative.

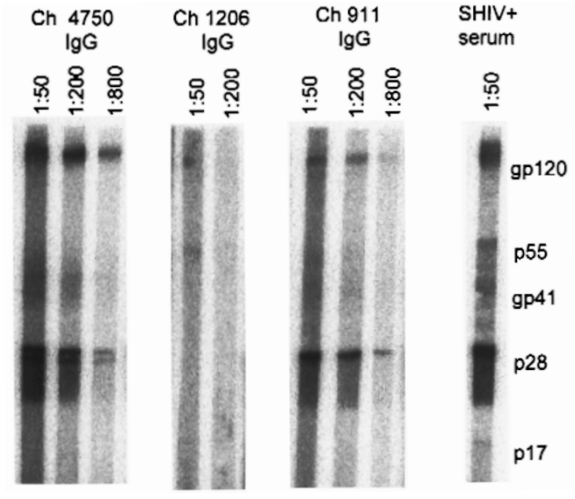

Fig. 2 Immunoblotting of SHIV proteins using three different chimpanzee IgGs. The $SHIV_{DH12}$ proteins in infected MT-4 cell lysates were resolved by SDS–PAGE and incubated with the indicated serially diluted chimpanzee IgG samples or the serum from a $SHIV_{DH12}$ infected macaque (positive control). Right margin, the positions of the HIV-1 gp120 and gp41 envelope glycoproteins and the SIV p55, p28 and p17 Gag proteins.

In experiment 1, chimpanzee IgG was passively transferred to four pairs of pig-tailed macaques (Table 2). Blood was collected from the eight monkeys 24 hours after intravenous IgG administration (and immediately before the $SHIV_{DH12}$ challenge), and plasma neutralization titers against 100 $TCID_{50}$ of virus were determined *ex vivo*. Plasma from the four macaque recipients of IgG from chimpanzees chronically infected with HIV-1_{DH12} was able to neutralize $SHIV_{DH12}$, with neutralizing titers ranging from 1:2.5 to 1:4 (Fig. 3*a*, top). In contrast, plasma from the two macaque recipients of IgG from Ch 73 (naive) and from the two monkeys given IgG from Ch 911 (HIV-1_{IIIB}) failed to neutralize 100 $TCID_{50}$ of $SHIV_{DH12}$. As expected, the binding antibody titers in the plasma of the four macaques receiving Ch 4750 IgG or Ch 911 IgG, as measured by ELISA, were similar (approximately 1:2,000; Fig.3*a*, middle, and Table 2), whereas ELISA binding activity was undetectable in monkeys passively immunized with Ch 1206 IgG or Ch 73 IgG.

The eight macaques were challenged intravenously with 100 $TCID_{50}$ of $SHIV_{DH12}$, and virus infectivity *in vivo* was monitored by quantitative DNA PCR of proviral DNA in PBMC. High-titer SHIV infections were established within 10 days of inoculation in recipients of either the naive or HIV-1_{IIIB} IgG preparations (Fig. 4*a*). In contrast, virus infection was considerably delayed during this early period in the four animals passively immunized with the neutralizing IgG preparations. The proviral DNA loads in these macaques also tended to be lower at later times compared with the levels measured in the recipients of the naive or IIIB IgG (Fig. 4*b*). Animal 94P027 became only transiently infected (that is, was positive on days 14 and 28 and remains virus negative at week 54) after challenge with $SHIV_{DH12}$ (Fig. 4*b*). These results indicate that passively transferred neutralizing IgG had some inhibitory effect on $SHIV_{DH12}$ infections *in vivo* and that the quantities of Ch 1206 IgG and Ch 4750 IgG administered might have been 'borderline' for complete protection from infection.

IgG from Ch 911 (HIV-1_{IIIB}) conferred no resistance against the initial $SHIV_{DH12}$ infection (compared with Ch 4750 IgG or Ch1206 IgG; Fig. 4a) nor did it enhance the subsequent SHIV infection (compared with the Ch 73 control IgG administered to macaques 94P018 and 94P025; Fig. 4*b*).

To determine whether the amount of the neutralizing IgG administered to the four monkeys was in fact 'borderline', we did a

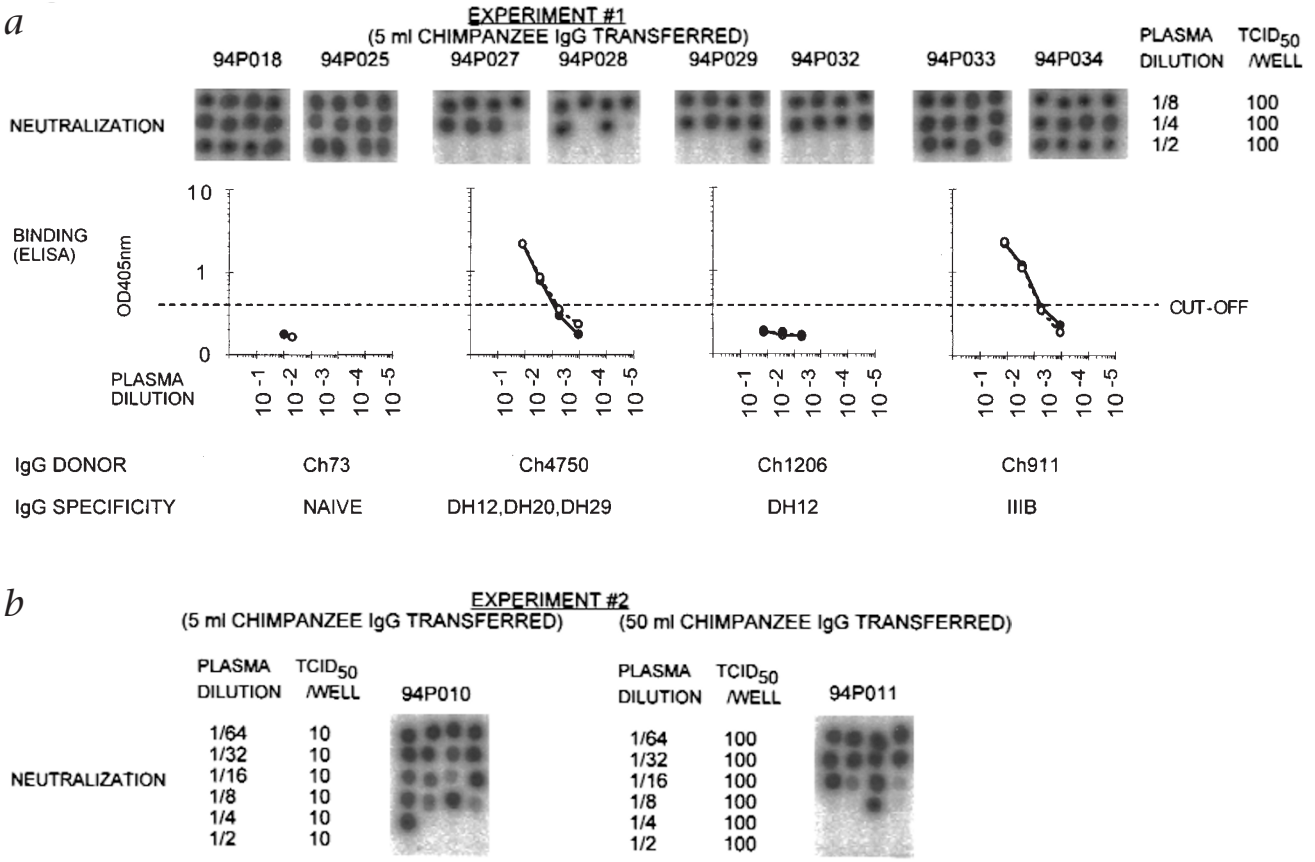

Fig. 3 Neutralizing and binding activities in the plasma of passively immunized pig-tailed macaques before $SHIV_{DH12}$ challenge (24 h after IgG administration). ***a*** (experiment 1), Serially diluted plasma samples were tested for neutralizing activity (top) against 100 $TCID_{50}$ of $SHIV_{DH12}$ and binding activity (middle), using an HIV-1 ELISA plate. Bottom, Donor chimpanzee ID numbers and IgG specificities. ***b*** (experiment 2), the neutralizing activities in serially diluted plasma samples from animals 94P010 and 94P011, challenged with 10 and 100 $TCID_{50}$ of $SHIV_{DH12}$, respectively.

second passive transfer experiment involving two additional pig-tailed macaques (Table 2). One animal (94P011) was given tenfold more Ch 1206 IgG (50 ml) than that used previously and was challenged with 100 $TCID_{50}$ of $SHIV_{DH12}$. The second monkey (94P010) received the same amount (5 ml) of the Ch 1206 IgG administered in experiment 1, but was inoculated with tenfold less (10 $TCID_{50}$) $SHIV_{DH12}$. Chronic infections have been established infections in two of two pig-tailed macaques parenterally inoculated with 0.8 $TCID_{50}$ of the $SHIV_{DH12}$ stock (intravenously[21] or after submucosal injection, R.O., unpublished data).

Ex vivo assays of samples collected 24 hours after IgG transfer had neutralization titers of 1:8 and 1:5 against 100 and 10 $TCID_{50}$ of $SHIV_{DH12}$, respectively, in the plasma of the two passively immunized monkeys studied in experiment 2 (Fig. 3*b* and Table 2). Complete protection from infection was achieved after subsequent $SHIV_{DH12}$ challenge in both of these macaques (Fig. 4*c*): no PBMC-associated proviral DNA was detected in ten specimens analyzed over a 1-year period (the first 4 months after challenge are shown in Fig. 4*c*) and lymph nodes, biopsied in weeks 1 and 3, were negative by DNA PCR. Infectious virus could not be isolated from either PBMC or lymph node samples.

To reconcile the apparent discrepant neutralizing and immunoblotting properties of the Ch 1206 and Ch 911 IgGs (Table 1), we measured the direct binding of antibodies to ^{35}S-methionine-labeled particles bearing the $HIV\text{-}1_{DH12}$ envelope glycoprotein[24,27,28]. Radiolabeled virions were incubated under non-denaturing conditions with the non-neutralizing Ch 73 IgG and Ch 911 IgG or with the neutralizing Ch 4750 IgG and Ch 1206 IgG. The virion–IgG complex was then separated from any remaining free IgG and non-virion associated proteins by centrifugation[24,27]. The virion pellet was treated with 0.1% Triton X-100 and the HIV-1 proteins bound to chimpanzee IgGs were immunoprecipitated using protein A-agarose. The remaining supernatant, containing virion-associated proteins that had not bound to chimpanzee IgGs, were subsequently immunoprecipitated using AIDS patients' sera and protein A-agarose. The neutralizing IgGs (from Ch 4750 and Ch 1206) reacted with virion associated gp120 (Fig. 5*a*, left lanes), whereas the non-neutralizing IgGs from the naive and $HIV\text{-}1_{IIIB}$ infected chimpanzees did not (Fig. 5*b*, left lanes). The protein gp120 is in fact present in the non-binding fractions of HIV-1 proteins incubated with the non-neutralizing IgGs (Fig. 5*b*, right lanes). These results indicate that anti HIV-1 specific neutralizing antibodies are able to recognize and bind to native gp120 present on virus particles.

An effective prophylactic vaccine for HIV-1 may have to elicit multiple immune responses (for example, CTLs, cytokine production, neutralizing antibodies) capable of interfering with different steps of the virus life cycle *in vivo*. Here we provide proof of the principle that anti-HIV-1 antibodies, capable of blocking virus infections *in vitro*, are by themselves protective *in vivo*. To achieve *in vivo* protection from infection by antibodies alone,

the plasma of passively immunized animals had to be capable of completely neutralizing the intravenous challenge dose (measured in these experiment at a dilution of 1:5 to 1:8).Our end-point neutralization assay imposes a much more stringent readout than many assays commonly used, in which 90% reduction of p24 production on day 7 of infection is used to define the neutralization titer. Our results are consistent with a passive immunization study in which the protective titer in the hu-PBL-SCID mouse model corresponded to the 99% not the 90% p24 reduction titer measured *in vitro*[22].

A chief shortcoming of our study is that we assessed only homologous combinations of challenge virus ($SHIV_{DH12}$) and passively transferred IgGs (prepared from HIV-1_{DH12} infected chimpanzees). In addition, the molecularly cloned, dual-tropic HIV-1_{DH12} envelope target of the neutralization antibodies analyzed here may not be representative of a strict CCR5 chemokine coreceptor using gp120 associated with viral strains that initiate most acute infections in humans. We have identified a few sera from HIV-1 infected individuals and some broadly anti-HIV-1 neutralizing monoclonal antibodies that can neutralize HIV-1_{DH12} *in vitro*. A passive immunization study using 'heterologous' (cross-neutralizing) combinations might be useful. The passive transfer of a broadly neutralizing monoclonal antibody considerably delayed the establishment of an HIV-1 infection and reduced virus loads in two chimpanzees inoculated with a primary HIV-1 isolate[30].

Passive immunization studies using animal challenge models have provided evidence that sufficient pre-existing titers of neutralizing antibody can completely block infection initiated by primary HIV-1 isolates (or SHIVs with a primary HIV-1 envelope). Although the experiments reported here must be replicated in a larger number of macaques using a variety of neutralizing IgG combinations and challenge virus strains, our results indicate that a vaccine capable of inducing sufficient levels of neutralizing antibody against HIV-1 clinical isolates could prevent the establishment of a virus infection.

Despite the promising results obtained and their implications for HIV-1 vaccine development, several essential questions remain. Can neutralizing antibodies confer resistance against a cell-associated virus challenge and/or mucosal challenge, and what neutralization titer is sufficient for protection? What is the size of a 'physiological' HIV-1 challenge dose, transmitted from an infected person to a naive recipient, that must be blocked by an effective prophylactic vaccine? It is certainly much less than 10–100 AID_{50}, the dose commonly used in animal model studies like the one described here. Thus, the measurement of end-point neutralizing titers of human vaccinees against such a 'physiological' challenge dose may not be technically feasible. And finally, how does one formulate a vaccine that can induce sufficient neutralizing titers against a broad range of HIV-1 clinical isolates?

Methods

Animal experiments. Pig-tailed macaques (*Macaca nemestrina*) and chimpanzees (*Pan troglodytes*) were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*[31] and were housed in a biosafety level 2 facility; biosafety level 3 practices were followed. Animals were anesthetized with intramuscular injections of Tiletmine-HCl and Zolazepam-HCl

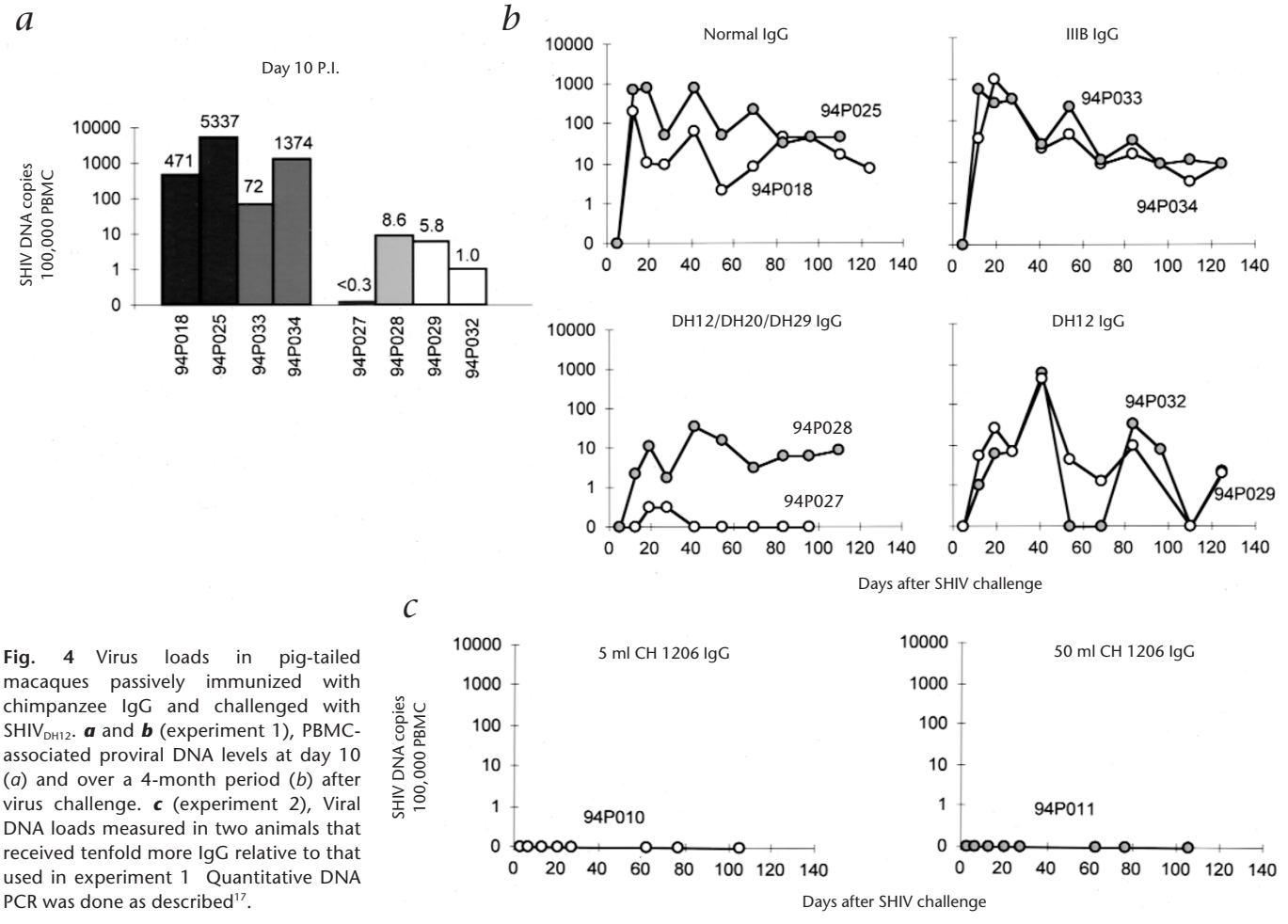

Fig. 4 Virus loads in pig-tailed macaques passively immunized with chimpanzee IgG and challenged with $SHIV_{DH12}$. ***a*** and ***b*** (experiment 1), PBMC-associated proviral DNA levels at day 10 (*a*) and over a 4-month period (*b*) after virus challenge. ***c*** (experiment 2), Viral DNA loads measured in two animals that received tenfold more IgG relative to that used in experiment 1 Quantitative DNA PCR was done as described[17].

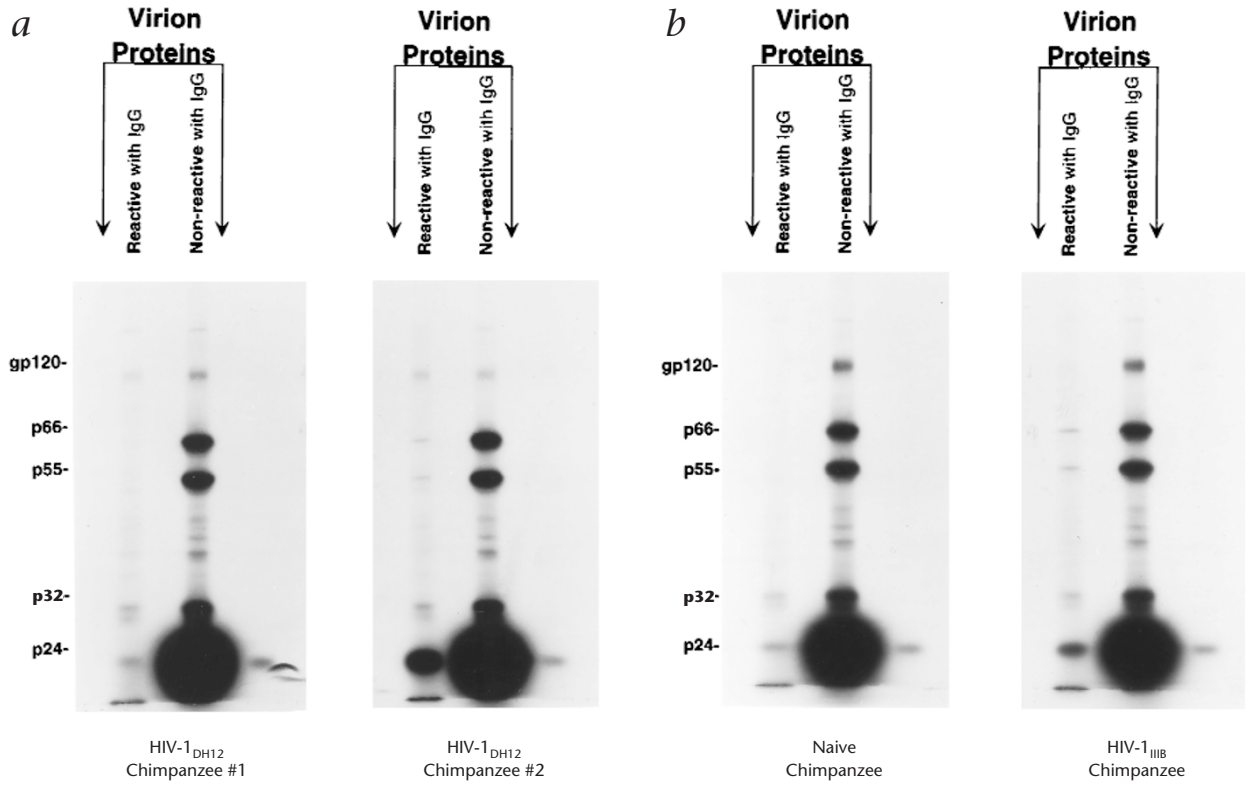

Fig. 5 Neutralizing antibodies bind directly to HIV-1 particles. Radiolabeled HIV-1 virions were incubated with chimpanzee IgG and virion-associated proteins reactive with the various IgGs were immunoprecipitated. IgGs from HIV-1_{DH12} chimpanzee #1 (Ch 1206), HIV-1_{DH12} chimpanzee 2 (Ch 4750), HIV-1_{IIIB} chimpanzee (Ch 911) and a naive chimpanzee (Ch 73). Left margin, the positions of the HIV-1 gp120 Env, p66 RT, p55 and p24 Gag, and p32 IN proteins.

(Telazol; Fort Dodge Laboratories, Fort Dodge, Iowa) during passive immunization, phlebotomy or virus inoculation.

Immunoglobulin fractions were purified from HIV-1 infected chimpanzees as described[20]. IgGs were administered intravenously 24 h before virus challenge. A portion (1 ml) of the appropriately diluted $SHIV_{DH12}$ challenge stock was inoculated intravenously.

Virus load measurements were determined by quantitative DNA PCR analyses of PBMC and lymph node specimens from infected animals as described[11].

The challenge virus. An SHIV (ref. 21) derived from HIV-1_{DH12} was used for animal challenge experiments. HIV-1_{DH12} is a clade B, primary isolate capable of infecting and inducing syncytia in human T-cell lines and primary human macrophages[17] and uses either CCR5 or CXCR4 as the co-receptor for infection[28]. Its derivative $SHIV_{DH12}$ molecular clone, MD14YE (ref. 21), contains the LTR, gag, pol, vif, vpx and nef genes, and the first 17 codons of the vpr gene of SIV_{mac239}. The tat, rev and env genes and the remainder of the vpr gene were derived mostly from HIV-1_{DH12}, except for a small segment (145 bp) at the SIV/HIV-1 junction in vpr) that is of HIV-1_{NL4-3} origin. The $SHIV_{DH12}$ MD14YE animal challenge stock (3×10^5 $TCID_{50}$/ml; 86 ng p27 per ml), prepared in cultured macaque monkey PBMC, was diluted in RPMI medium to 100 $TCID_{50}$/ml (or 10$TCID_{50}$/ml), and 1 ml was used for intravenous inoculation of macaques. The *in vivo* titration of this $SHIV_{DH12}$ MD14YE stock in pig-tailed macaques has been reported[21].

Antibody assays. Neutralizing activities in the plasma of passively immunized monkeys were titered in an assay that measures 100% neutralization against known amounts of virus[11]. Here, the same amounts of $SHIV_{DH12}$ were used for *in vivo* challenge (100 $TCID_{50}$ or 10 $TCID_{50}$) and for *in vitro* neutralization assays. The $SHIV_{DH12}$ MD14YE animal challenge stock was initially diluted to 20,000 $TCID_{50}$/ml (or to 2,000 $TCID_{50}$/ml for neutralization assays in which 10 $TCID_{50}$ of virus was used). Plasma samples were serially diluted (twofold or threefold, starting at a dilution of 1:2 or 1:6) in a mixture of prepassive immunization plasma from the pig-tailed macaques. A 20-µl aliquot of each plasma dilution was incubated with 20 µl of $SHIV_{DH12}$ at room temperature for 1 h and was then used to infect MT-4 cells in quadruplicate; each culture (5×10^4 MT-4 cells in 0.25 ml volume) received 10 µl of the virus/plasma mixture, which contained 100 (or 10) $TCID_{50}$ of $SHIV_{DH12}$. Infected cultures were maintained for 2 weeks and virus replication was monitored by ^{32}P-reverse transcriptase assay[29]. After the 2-week incubation in MT-4 cells, any infectious $SHIV_{DH12}$ would be amplified to levels detectable by the assay. In the experiment shown in Fig. 3*b* (left panel), three of four cultures at the 1:4 plasma dilution failed to produce detectable reverse transcriptase activitiy (complete neutralization). The plasma dilution at which two of the four cultures would be RT-negative (neutralization positive) was calculated to be 1:5, using the method of Reed and Munch[23].

Binding antibodies to denatured HIV proteins were detected by a commercial ELISA kit (Vironostika HIV-1 Microelisa system; Organon Teknika, Durham, North Carolina). Immunoblots were analyzed using a Fuji phosphoimager (Fuji Medical System, Stamford, Connecticut) after incubation with ^{125}I-labeled protein A (ICN) as described[11].

To measure antibodies binding to native HIV-1_{DH12} virions, ^{35}S-methionine-labeled particles, released from transfected HeLa cells[24,27,28], were pelleted in an ultracentrifuge to remove non-particle associated viral proteins, resuspended in 100 µl of RPMI medium as described[27,29] and incubated with 20 µg of the chimpanzee IgGs for 2 h at 37 °C. The HIV-1/IgG mixture was centrifuged[27], the pellets were treated with 0.1% Triton X-100, and protein A-agarose was added to immunoprecipitate virion-associated proteins associated with the chimpanzee IgG. Supernatants from this immunoprecipitation were transferred to tubes containing protein A-agarose that had been pretreated with AIDS patients' sera for a second round of immunoprecipitation to assess the virion-associated proteins that did not bind to the chimpanzee IgG. The immunoprecipitated proteins were resolved by SDS–PAGE (ref. 27).

For immunoblotting of SHIV proteins using three different chimpanzee IgGs, the $SHIV_{DH12}$ proteins present in infected MT-4 cell lysates were resolved by SDS–PAGE, transferred to nitrocellulose membranes, incubated with the indicated serially diluted chimpanzee IgG samples or the serum

from a $SHIV_{DH12}$ infected macaque (positive control) and visualized with a Fuji phosphoimager after incubation with ^{125}I-protein A (ICN).

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