Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian–human immunodeficiency virus infection

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Although maternal human immunodeficiency virus type 1 (HIV-1) transmission occurs during gestation, intrapartum and postpartum (by breast-feeding), 50–70% of all infected children seem to acquire HIV-1 shortly before or during delivery[1]. Epidemiological evidence indicates that mucosal exposure is an important aspect of intrapartum HIV transmission[2,3]. A simian immunodeficiency virus (SIV) macaque model has been developed[4] that mimics the mucosal exposure that can occur during intrapartum HIV-1 transmission. To develop immunoprophylaxis against intrapartum HIV-1 transmission, we used SHIV–vpu+ (refs. 5,6), a chimeric simian–human virus that encodes the env gene of HIV-IIIB. Several combinations of human monoclonal antibodies against HIV-1 have been identified that neutralize SHIV–vpu+ completely in vitro through synergistic interaction[7]. Here, we treated four pregnant macaques with a triple combination of the human IgG1 monoclonal antibodies F105, 2G12 and 2F5. All four macaques were protected against intravenous SHIV–vpu+ challenge after delivery. The infants received monoclonal antibodies after birth and were challenged orally with SHIV–vpu+ shortly thereafter. We found no evidence of infection in any infant during 6 months of follow-up. This demonstrates that IgG1 monoclonal antibodies protect against mucosal lentivirus challenge in neonates. We conclude that epitopes recognized by the three monoclonal antibodies are important determinants for achieving substantial protection, thus providing a rational basis for AIDS vaccine development.

The monoclonal antibody combination used for passive immunization was highly synergistic and targeted to conserved epitopes: F105 (a human IgG1k monoclonal antibody) recognizes a discontinuous epitope that overlaps the CD4 binding domain[8]; 2G12 (a human IgG1 monoclonal antibody) recognizes a conformationally sensitive, glycosylation-dependent gp120 epitope[9]; and 2F5 (a human monoclonal antibody against gp41) recognizes the sequence ELDKWA (ref. 10). All three monoclonal antibodies neutralize laboratory and primary HIV isolates[7–11], including the primary isolate HIV89.6 (ref. 12) (Table 1). Even though considerably higher concentrations were required to neutralize HIV89.6 by single-agent monoclonal antibodies than for SHIV–vpu+, the triple combination was so synergistic that the total monoclonal antibody dose required for 50% neutralization was the same for HIV89.6 as for SHIV–vpu+ (Table 1). The triple monoclonal antibody combination also completely neutralized both viruses in human and macaque peripheral blood mononuclear cells (PBMCs) (ref. 7 and data not shown).

Next, we infused a single dose of the triple combination of F105, 2G12 and 2F5 into pregnant macaque L923 5 days before cesarean section (Fig. 1) and assessed plasma levels serially, using assays specific for each of the three monoclonal antibodies. The

Table 1 Synergy of human IgG1 monoclonal antibodies F105, 2G12 and 2F5 in MT-2 cells

Virus	mAb	Ratio in combination[a]	Concentration (μg/ml) of single mAb for 50% neutralization	CI[b] at EC_{50}	DRI[c] at EC_{50}	Concentration (μg/ml) for synergistic neutralization at 50%
SHIV–vpu+	F105		1.5		7.5	0.2
	2G12		0.5		5.3	0.1
	2F5		0.5		5.3	0.1
		2:1:1	0.5[d]	0.5		
HIV89.6	F105		49.5		439	0.11
	2G12		52		462	0.11
	2F5		10.5		93	0.11
		1:1:1	0.34[d]	0.015		

The concentration of each monoclonal antibody needed to achieve 50% virus neutralization was determined for each monoclonal antibody singly and in combinations (adapted from ref. 7). [a]Ratios are F105:2G12:2F5. [b]CI, combination index: < 1, synergism; 1, additive effect; > 1, antagonism[7]. [c]DRI, dose reduction indices, computed by comparing the doses required to reach a given degree of neutralization (such as 50%) when the monoclonal antibody was used singly and in combination with the other two monoclonal antibodies. [d]Sum of the three monoclonal antibodies combined at the ratio indicated. mAb, monoclonal antibody; EC_{50}, effective concentration to reach 50% neutralization.

1:8 until postnatal day 4, after only one dose of the monoclonal antibody combination (Fig. 1). Infant PK1, treated with monoclonal antibodies prenatally and twice postnatally on days 0 and 8, maintained 100% virus neutralization at a plasma dilution of 1:8 until at least postnatal day 21. Cord blood of PK1 contained 12.8 μg/ml F105, 12.8 μg/ml 2G12 and 6 μg/ml 2F5, indicating that neutralizing levels had been reached by transport of human IgG1 monoclonal antibodies across the macaque placenta.

After the initial pharmacokinetic studies, we infused pregnant macaque dams L605, L943, J421 and L975 with the triple monoclonal antibody combination 5 days before cesarean section and again 3 days postpartum (Fig. 2a). We challenged the dams with intravenous SHIV–vpu+ 1 hour after the second infusion. We detected neutralizing levels of the three monoclonal antibodies in plasma of all dams (Fig. 2c, e and g). The monoclonal antibodies had the following mean plasma half-

dam's plasma, obtained at cesarean section (day 0), contained 52.3 μg/ml F105, 114.9 μg/ml 2G12 and 72.4 μg/ml 2F5, which greatly exceeded the levels required to neutralize SHIV–vpu+ *in vitro* by single agents or the triple combination (Table 1). The dam's plasma completely neutralized SHIV–vpu+ at a dilution of

Table 2 Virus load in PBMCs from juvenile and adult macaques exposed intravenously to SHIV–vpu+

Animal	0	1	2	4	6	(7), 8	12	13	(16) 17	21	(24) 25
Juvenile/Adult Controls											
Juvenile R1											
PBMC cx	neg	neg	+		26	26		13	(neg)		
DNA PCR	neg	neg	+		+	+					
Juvenile R3											
PBMC cx	neg	6	+		102	102		neg	(neg)		
DNA PCR	neg	+			+	neg					
Juvenile R4											
PBMC cx	neg	1	+		102	6		neg	(neg)		
DNA PCR	neg	neg	+		+	+					
Juvenile R5											
PBMC cx	neg	6	+		26	6	12	neg	(neg)		
DNA PCR	neg	neg	+		+	+					
Adult L927											
PBMC cx	neg		1640	6		(1), 1			neg	neg	
DNA PCR	neg		+	+		+					
mAb-treated Adults											
Adult L605											
PBMC cx	neg	neg	neg	neg	neg	neg	neg		neg		neg
DNA PCR	neg		neg	neg		neg					
Adult L943											
PBMC cx		neg	neg	neg	neg	neg		neg	(neg)		(neg)
DNA PCR		neg	neg	neg		neg					
Adult J421											
PBMC cx	neg		neg	neg		(neg), neg			neg	neg	neg
DNA PCR	neg		neg	neg		neg					
Adult L975											
PBMC cx	neg		neg	neg	neg			neg	neg	neg	
DNA PCR	neg		neg	neg		neg					

Weeks after exposure

For PBMC culture (cx), data represents number of infectious cells per 1×10^6 PBMCs; +, co-cultivation of 1×10^6–2×10^6 PBMCs with CEMx174 cells yielded detectable p27 Gag antigen in culture supernatants, as described[4]. For DNA PCR, 1 μg genomic PBMC DNA was analyzed as described[16]; +, positive results. mAb, monoclonal antibody.

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Table 3   Virus load in PBMCs from infant macaques exposed orally to SHIV–vpu[+]

Animal	Weeks after exposure											
	0	1	2	4	5	6	(7), 8	(10) 12	(13) 14	(16) 17	(20) 21	(24) 25
Control Infants												
Infant R9C												
PBMC cx	neg		+	+		410	8		26			
DNA PCR			+	+		+			+			
Infant R10C												
PBMC cx	neg		+	102			(+)	(102)	26			
DNA PCR			+	+			(+)	(+)	+			
Infant R11C												
PBMC cx	neg		1640		+		26	6				
DNA PCR			+		+		+	+				
Infant R16C												
PBMC cx		+					(+), +	26		(26)	(1)	
DNA PCR			neg				(+)					
mAb-treated Infants												
Infant R12M												
PBMC cx	neg	neg		neg		neg	neg	neg		neg		neg
DNA PCR	neg		neg									
Infant R13M												
PBMC cx		neg	neg	neg		neg	neg		(neg)	(neg)		(neg)
DNA PCR	neg					neg			(neg)			
Infant R14M												
PBMC cx	neg		neg				(neg), neg			neg	neg	neg
DNA PCR	neg	neg		neg			neg					
Infant R15 M												
PBMC cx	neg		neg	neg		neg			(neg)	neg	neg	
DNA PCR	neg		neg	neg		neg			(neg)			

For PBMC culture (cx), data represents number of infectious cells per 1×10^6 PBMCs; +, co-cultivation of 1×10^6–2×10^6 PBMCs with CEMx174 cells yielded detectable p27 Gag antigen in culture supernatants, as described[4]. For DNA PCR, 1 µg genomic PBMC DNA was analyzed as described[16]; +, positive results. mAb, monoclonal antibody.

lives: F105, 7.2 ± 2.2 days; 2G12, 14.0 ± 7.9 days; 2F5, 4.2 ± 0.8 days, comparable to published data[13–15]. Serial blood samples remained negative for SHIV–vpu[+] by PBMC co-culture, DNA PCR (Table 2), RT–PCR (not shown) and serology, using either SIV or HIV antigens (Fig. 3*a*, *c*, and *e*). Necropsy tissues (PBMCs, mandibular, mesenteric and inguinal lymph nodes, spleen and thymus) obtained 6 months after challenge were negative for infection by DNA PCR (using 1 µg genomic DNA) and co-cultivation (using between 1×10^6 and 1×10^7 cells) (not shown). In contrast, all control juvenile/adult macaques (R1, R3, R4, R5 and

L927) became infected ($P = 0.016$) and tested positive by RT-PCR during peak viremia (not shown) and by DNA PCR, virus isolation (Table 2) and serology (Fig. 3*a*, *c*, and *e*). Systemic infection was confirmed in lymphoid tissues at necropsy by DNA PCR in all positive controls (not shown).

Oral exposure of neonatal macaques to cell-free SIV or SHIV–vpu[+] results in reproducible systemic infection[4,17]. We administered the three monoclonal antibodies intravenously to the four pregnant dams described above (Fig. 2*a*), delivered their offspring by cesarean section 5 days later, and then infused each infant intravenously with the three monoclonal antibodies (Fig. 2*b*. Then, 1–4 hours later, we challenged the neonates orally with SHIV–vpu[+]. At 8 days after this exposure, we administered a
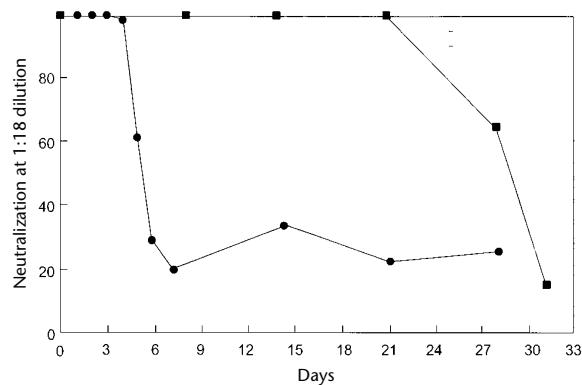
Fig. 1 Neutralizing activity of plasma obtained from an uninfected mother–infant pair. The dam (L923; ●), received an intravenous administration of monoclonal antibodies F105, 2G12 and 2F5 5 days before cesarean section on day 0. Daily blood samples were collected for the first postnatal week and weekly thereafter. The infant (PK1; ■) received an intravenous dose of the three monoclonal antibodies on day 0 and again on day 8. Because of its small size, only limited blood samples could be collected. The plasma samples of mother and infant were tested for their ability to neutralize SHIV–vpu[+] in an MT-2 assay. Data with 1:8 plasma dilutions are shown.

Table 4 Virus load in necropsy samples from infant macaques exposed orally to SHIV–vpu[+]

Infant	Plasma[a]	PBMC[b]	Mandibular LN	Mesenteric LN	Inguinal LN	Spleen	Thymus
Control Group							
R9C							
Culture	neg	1	1	6	1	26	neg
PCR	13,607	+	+	+	+	+	+
R10C							
Culture	neg	6	1	6	6	1	1
PCR	14,790	+	+	+	+	+	neg
R11C							
Culture	neg	6	410	6	+	1	neg
PCR	<2,721	neg	+	+	N.D.	+	+
R16C							
Culture	neg	1	1	26	6	neg	neg
PCR	68,036	+	+	+	+	+	+
Treatment Group							
R12M							
Culture	N.D.	neg[c]	neg[d]	neg[d]	neg[d]	neg[d]	neg[d]
PCR	<3,402	neg	neg	neg	neg	neg	neg
R13M							
Culture	neg	neg[e]	neg[e]	neg[e]	neg[e]	neg[e]	neg[e]
PCR	<3,092	neg	neg	neg	neg	neg	neg
R14M							
Culture	neg	neg[e]	neg[f]	neg[f]	neg[e]	neg[e]	neg[e]
PCR	<3,780	neg	neg	neg	neg	neg	neg
R15M							
Culture	neg	neg[g]	neg[g]	neg[g]	neg[g]	neg[g]	neg[g]
PCR	<3,093	neg	neg	neg	neg	neg	neg

[a]Quantitative competitive RT–PCR was done as described[16]. The lower limits of detection ranged between 2,721 and 3,780 RNA copies/ml. [b]Culture data reflect the number of infectious cells per 1×10^6 cells. +, co-cultivation of 1×10^6 cells; –, 2×10^6 PBMCs with CEMx174 cells yielded detectable p27 Gag antigen in culture supernatants, as described[4]. For DNA PCR, 1 µg of genomic DNA was analyzed as described[16]: +, positive results. [c]6×10^6 cells; [d]2×10^6 cells; [e]1×10^7 cells; [f]5×10^6 cells; [g]1×10^7 cells. N.D., not done.

final monoclonal antibody infusion to each neonate (Fig. 2b). As controls, we challenged four untreated neonates (R9C, R10C, R11C and R16C) orally with SHIV–vpu[+].

We detected neutralizing levels of each monoclonal antibody in cord blood samples. High levels of each monoclonal antibody were achieved in plasma obtained from each treated infant subsequently (Fig. 2d, f and h). During the next 6 months, we evaluated both groups of infants for evidence of infection. Each untreated neonate was positive for virus isolation throughout (Table 3). In contrast, the infants treated with monoclonal antibody (R12M, R13M, R14M, and R15M) were protected (P = 0.028). In these treated infants, we could not detect any plasma viral RNA during the time of peak viremia in untreated infants (not shown). PBMC co-cultures and DNA PCR of the four infants treated with monoclonal antibody remained negative throughout (Table 3). Serology at 6 months after challenge was negative (Fig. 3b, d and f). Furthermore, we found no evidence of infection in lymphoid tissues or plasma obtained at necropsy (Table 4).

As we found no evidence of infection by any test, our data indicate that the triple combination of human IgG1 monoclonal antibodies protected all macaques against either intravenous or mucosal challenge. We accomplished these 'proof-of-concept' experiments with SHIV–vpu[+], a chimeric virus that encodes env of the laboratory-adapted HIV-IIIB known to be sensitive to neutralization. Because the triple combination we studied in vivo was highly synergistic and also completely neutralized the primary isolate HIV89.6 in human PBMCs, this or similar combination regimens also hold promise for protection against primary isolates.

Encouraging data have been obtained in recent passive immunization experiments. Although monoclonal antibody 2F5 used singly did not prevent infection after intravenous HIV-1 challenge in chimpanzees, even though plasma 2F5 levels at the time of challenge were more than twice those in the adult macaques in our study, peak viremia was delayed in some or significantly decreased in other animals[13]. More recently, 2F5 and 2G12 were infused in combination with immunoglobins against HIV (HIVIG) before intravenous challenge of adult rhesus macaques with the pathogenic SHIV–89.6PD variant[15]; this combination prevented infection in some macaques and decreased virus burdens in the others. Direct comparisons with these and other studies are difficult to make, as either route of virus exposure, virus strain (and thus sensitivity to neutralization) or choice of antibodies differ. Moreover, a wide spectrum of pathogenicity exists among viral strains, including the various SHIVs generated from different HIV-1 clade B clones. In general, HIV-1 challenge of chimpanzees is characterized by low-level replication without disease. In contrast, infection of macaques with SHIV89.6PD (ref. 15) or SHIV$_{DH12}$ (ref. 18) results in CD4^{+} T-cell depletion. Similarly, two SHIV variants derived from the HXBc2 molecular clone of the laboratory-adapted HIV-IIIB also vary in pathogenicity. Although no disease was reported in SHIV–vpu^{+}-infected adult macaques[5,6], one macaque inoculated as neonate with SHIV–vpu^{+} has developed persistent inversion of CD4^{+}/CD8^{+} T-cell ratios, depletion of CD4^{+}CD29^{+} T-cell subsets, and depletion of CD4^{+} T cells to less than 300 cells/µl after 4 years of observation[17], consistent with the belief that the variant used in our studies has low pathogenicity. In contrast, SHIV$_{KU-1}$, a variant derived by serial passage of this strain, is virulent after oral, intravenous or vaginal exposure[19]. Passively administered polyclonal neutralizing serum or serum from naive macaques did not protect macaques against oral SHIV$_{KU-1}$ challenge[19]. Passive transfer of 'pooled', unfractionated serum from chronically SIV-infected rhesus macaques has been shown to protect rhesus macaques from oral exposure to cell-free SIV$_{mac251}$, but control serum from naive macaques did not. Unexpectedly, the passively administered protective serum had no detectable neutralizing activity against the challenge virus in vitro but contained IgG, IgA and IgM, in addition to high levels of chemokines[20]. Consequently, the protective component(s) could not be established. In contrast, our study used only well-characterized, neutralizing human IgG1 monoclonal anti-
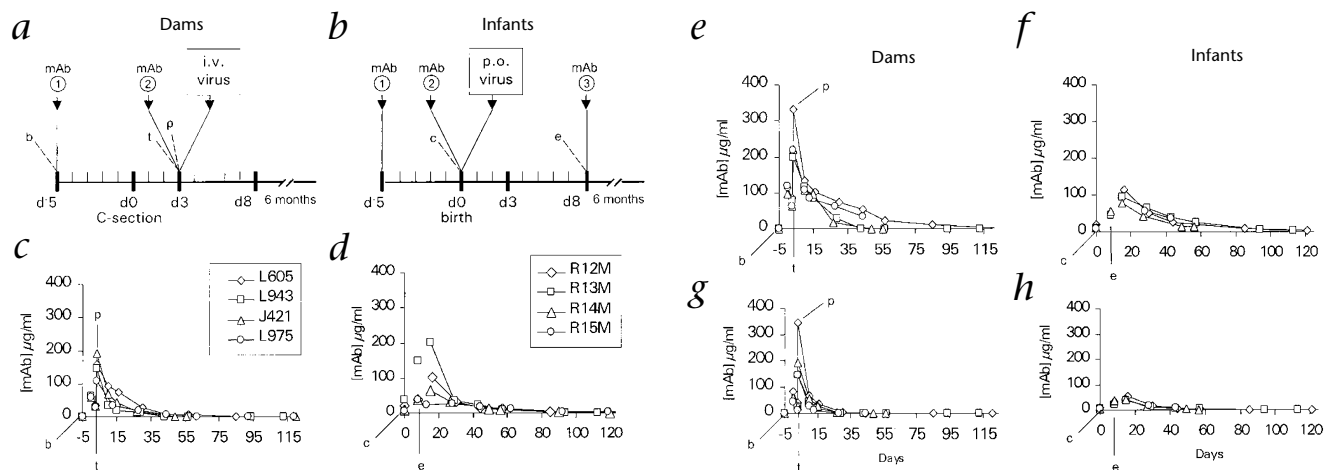
Fig. 2 Monoclonal antibody pharmacokinetics and viral challenge. **a** and **b**, Time course of intravenous (i.v.) infusion of the triple monoclonal antibody (mAb) combination, cesarean section delivery and SHIV–vpu⁺ inoculation of rhesus macaque dams (*a*) and their infants (*b*). *c*–*h*, Pharmacokinetics of monoclonal antibodies F105 (*c* and *d*), 2G12 (*e* and *f*) and 2F5 (*g* and *h*) in dams (*c,e,g*) and neonates (*d,f,h*). Symbols (keys) identify different macaques. Plasma samples collected from the dams before the first monoclonal antibody infusion (d-5; sample *a, b, c, e* and *g*) contained no detectable monoclonal antibodies. Plasma samples

were collected from the dams during cesarean section (d0) and on day 3 (d3) before the second monoclonal antibody infusion (trough, t). Peak levels were determined 30 min after this infusion (p; *c, e* and *g*). SHIV–vpu⁺ challenge followed 1 h after the completion of the monoclonal antibody infusion. Because of their small size, only cord blood (*c; b, d, f* and *h*) and one set of samples before the third monoclonal antibody infusion on day 8 (d8; *e*) could be collected from the infants challenged orally (p.o.) with SHIV–vpu⁺ 1–4 h after the completion of the second monoclonal antibody infusion.

bodies, and provides evidence that secretory IgA or cytokines are not necessary for protection from mucosal lentivirus exposure, even though it has been generally believed that mucosal immunity, particularly secretory IgA, is required to protect against mucosal virus exposure.

The mechanism involved in the protection against mucosal SHIV–vpu⁺ is unknown. The triple combination of F105, 2G12 and 2F5 could have protected simply by blocking or altering virus binding to cell surface CD4 and chemokine co-receptors.

However, it is possible that the challenge virus SHIV–vpu⁺ was not effectively neutralized by saliva, crossed the mucosal barrier by mechanisms that are unknown, and infected the first set of target cells. In neutralization studies, saliva samples collected from the monoclonal antibody-treated infants before virus challenge were not more effective than control saliva samples collected from naive infants (not shown).

Human IgG1 can facilitate other protective mechanisms, such as complement activation or deposition, which could directly
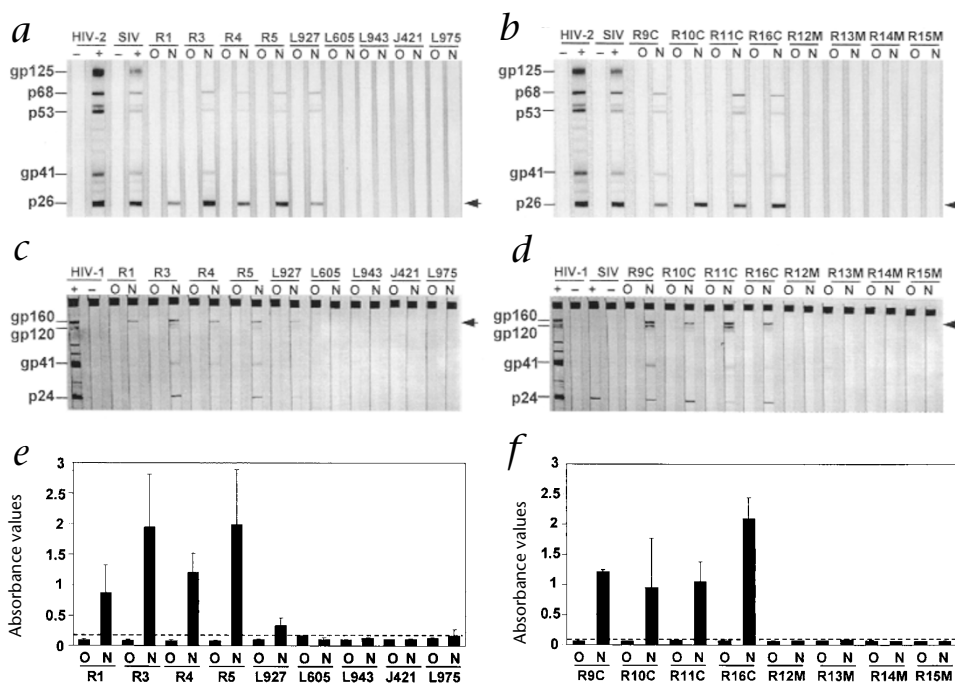
Fig. 3 Western blot analysis and ELISA of plasma samples. *a*–*d*, Plasma samples from adults (*a* and *c*) and infants (*b* and *d*). *a* and *b*, Western blot analysis with HIV-2 strips routinely used to monitor anti-SIV antibody responses. *c* and *d*, Western blot analysis with HIV-1 strips that could be used because the human monoclonal antibodies had been eliminated from the monkeys plasma by day 120 (Fig. 2). Left margins, migration of viral proteins; arrows (right margins), migration of Gag antigen (*a* and *b*) or envelope glycoproteins (*c* and *d*). *e* and *f*, Antibody responses detected by anti-SIV Gag ELISA in plasma samples from adults (*e*) and infants (*f*). O, samples collected before challenge on day 0; N, plasma samples collected on the day of necropsy, 6 months after challenge. Macaque names, above blots and below graphs.

lyse virions or infected cells[21], and antibody-dependent cell-mediated cytotoxicity[22]. Potent antibody-dependent cell-mediated cytotoxicity activity of monoclonal antibodies 2G12 and F105 has been found *in vitro*[9,22]. These mechanisms could maintain the viral load well below our level of detection until additional virus-specific, MHC class I-restricted, cellular immune host defenses could develop and eliminate a small number of cells infected when the virus initially crossed the mucosal surface. The high level of neutralizing monoclonal antibodies would have prevented further 'waves' of viral spread from these initial target cells. At present, we cannot discount this possibility; however, using sensitive tissue culture, molecular and immunologic methods of detection, it seems that solid protection, as defined by the threshold of detection for our assays, was achieved. No virus could be detected either by sensitive co-culture or DNA PCR methods in any of the infants treated with monoclonal antibody that were challenged orally. Furthermore, no evidence of a humoral antibody response to infection (that is, no seroconversion) was detected in any of the monoclonal antibody recipients. Clearly, none of the macaques treated with monoclonal antibody was persistently infected, even if the possibility of low-level, transient local infection at the level of the mucosa cannot be ruled out.

In conclusion, we have demonstrated that passive immunity, consisting solely of well-defined, broadly neutralizing human IgG1 monoclonal antibodies, can protect primates against both parenteral and mucosal lentivirus exposure. We conclude that the broadly neutralizing human monoclonal antibodies used in our study react with three epitopes that are essential determinants for achieving protection. Efforts to understand how these human IgG1 antibodies protect against mucosal lentivirus infection could yield new insights and provide focus for future efforts directed at the rational development of a safe, effective AIDS vaccine. Furthermore, our passive immunoprophylaxis approach may represent a new approach for preventing mother-to-infant transmission of HIV.

Methods

Animals. Rhesus monkeys (*Macaca mulatta*) from a specific pathogen-free colony were used according to National Institutes of Health Guidelines on the Care and Use of Laboratory Animals at The University of Texas, M. D. Anderson Cancer Center, a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The experiments were approved by the Animal Care and Use Committees at The University of Texas, M.D. Anderson Cancer Center and at the Dana-Farber Cancer Institute. Fetal ultrasound determinations and cesarean section deliveries were done on anesthetized macaques as described[4].

Administration of human monoclonal antibodies. All monoclonal antibody preparations were of clinical grade purity and were endotoxin-free. Polymune Scientific produced monoclonal antibodies 2G12 and 2F5. The macaques (dams or neonates) were treated intravenously with 10 mg/kg of each of the three monoclonal antibodies in combination.

Virus stocks, detection of virus infection of SHIV–vpu+-exposed macaques, and statistical analysis. Both SHIV–vpu+ virus stocks used for either oral or intravenous challenge were propagated in rhesus monkey PBMC cultures with human interleukin (IL-2). Supernatants were clarified by centrifugation, filtered, and stored in vapor-phase liquid nitrogen. Both SHIV–vpu+ stocks were titrated in CEMx174 cells. The oral SHIV–vpu+ stock contained 2.078×10^5 50% tissue culture infectious doses ($TCID_{50}$)/ml. To determine the 50% animal infectious dose (AID_{50}) for the oral route, seven neonatal rhesus monkeys were exposed orally to serial dilutions of cell-free SHIV–vpu+. This virus stock contained 3.79 oral AID_{50}/ml (ref. 23). For oral challenge, 10 AID_{50} (determined for the oral route in

neonates) were given in a non-traumatic manner. This dose yields a 99% probability of infection[23].

The intravenous SHIV–vpu+ stock contains 4,600 $TCID_{50}$/ml and 4,600 intravenous AID_{50}/ml (ref. 6). Therefore, the intravenous SHIV–vpu+ stock contains 1,214-fold more AID_{50}/ml yet 45-fold less $TCID_{50}$/ml than the oral SHIV–vpu+ stock. These results are consistent with the fact that the intravenous route of exposure requires considerably less virus than the oral or other mucosal routes[24]. All intravenous challenges used 10 AID_{50} as determined by intravenous titration in juvenile macaques[6]. This dose yields a 99% chance of systemic infection[23].

Methods for PBMC cell culture, DNA PCR, and QC RT–PCR have been described[4,16]. Necropsy samples from lymphoid organs were minced. Cells were layered onto Ficoll and centrifuged to obtain the mononuclear cells. Cells were co-cultivated with CEMx174 cells and titrated by end-point dilution in quadruplicate wells. Culture supernatants were analyzed for p27 antigen as described[4]. Plasma samples were analyzed with commercially available western blot strips prepared from HIV-2 antigens as described[4]. HIV-1 western blot analyses were done using kits (Epitope, Beaverton, Oregon) according to the manufacturer's instructions. Statistically significant differences between the positive control and the monoclonal antibody treatment groups were determined by Fisher's exact test.

Anti-SIV Gag enzyme-linked immunosorbent assay (ELISA). 96-well plates were coated overnight at room temperature with recombinant Gag p27 SIVmac251 (ImunoDiagnostics, Bedford, Massachusetts) (3 µg/ml 0.05 M Na_2CO_3, pH 9.6), washed three times in PTA buffer (0.05 M K_2HPO_4/KH_2PO_4, 0.1% Tween 20, 0.02% sodium azide, pH 7.4) and blocked at room temperature for 1 h with 0.2% nonfat dry milk in PTA buffer. Then the plates were incubated at room temperature for 2 h with plasma samples (1:50 dilution), washed three times as described above, and incubated at 4 °C overnight with a conjugate of antibody against monkey IgG and alkaline phosphatase (Sigma) and washed as described above. Alkaline phosphatase activity was detected using a p-nitrophenyl phosphate system (Sigma). Absorbance was measured after 50 min of incubation with reconstituted p-nitrophenyl phosphate at a wavelength of 410 nm. Each sample was measured in triplicate. Data represent mean values.

Plasma monoclonal antibody concentration and pharmacokinetic analysis. Assays to quantify levels of F105, 2G12 and 2F5 have been described[15,25]. Anti-idiotype, ELISA-based assays were used to measure monoclonal antibodies F105 and 2G12. A peptide-binding assay was used to quantify monoclonal antibody 2F5. To determine the half-lives of the three monoclonal antibodies, natural logs of plasma monoclonal antibody levels were plotted as a function of time from the end of infusion. Slopes (m) of the linear graphs were determined by least-squares analysis. Correlation coefficients (R^2) ranged from 0.981 to 0.9996. Half-lives were calculated as $t_{1/2} = -(\ln 2)/m$.

Saliva collection and virus neutralization assay. Saliva was collected from the oral cavities of neonatal macaques using Weck-cel sponges (Xomed Surgical Products, Jacksonville, Florida). Each sponge was placed in 200 µl elution buffer containing 10 mM phosphate-buffered saline, protease inhibitor, bestatin, 100 U/ml penicillin/streptomycin, 100 µg/ml gentamicin sulfate and 600 U/ml mycostatin. Sponges were centrifuged in 0.45-µm centrifuge filters (Fisher Scientific) at 15,000g for 10 min at 4 °C. Neutralization activity was compared in saliva samples obtained at birth from neonates treated with monoclonal antibody or untreated control neonates. Serial dilutions of salivary filtrate were assayed in duplicate, as measured in a standard MT-2 cell infection assay with SHIV–vpu+ (ref. 7).

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