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Early short-term treatment with neutralizing human monoclonal antibodies halts SHIV infection in infant macaques

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Prevention of mother-to-child transmission (MTCT) of HIV remains a major objective where antenatal care is not readily accessible. We tested HIV-1–specific human neutralizing monoclonal antibodies (NmAbs) as a post-exposure therapy in an infant macaque model for intrapartum MTCT. One-month-old rhesus macaques were inoculated orally with the simian-human immunodeficiency virus $SHIV_{SF162P3}$. On days 1, 4, 7 and 10 after virus exposure, we injected animals subcutaneously with NmAbs and quantified systemic distribution of NmAbs in multiple tissues within 24 h after antibody administration. Replicating virus was found in multiple tissues by day 1 in animals that were not treated. All NmAb-treated macaques were free of virus in blood and tissues at 6 months after exposure. We detected no anti-SHIV T cell responses in blood or tissues at necropsy, and no virus emerged after $CD8^+$ T cell depletion. These results suggest that early passive immunotherapy can eliminate early viral foci and thereby prevent the establishment of viral reservoirs.

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Recent advances in the discovery of human HIV NmAbs that have high potency and breadth of coverage have rekindled an interest in their use as pre-exposure prophylaxis, as well as therapeutic agents, including in the setting of MTCT, in which the time of exposure is known[1,2]. A combination of measures—including antiretroviral treatment (ART) of the mother and the infant, cesarean section and formula feeding—have diminished the rate of MTCT from 35% to less than 3% (ref. 3). Despite this reduction, HIV infects approximately 200,000 children yearly, primarily in places where ART is not available[4]. Treatment of babies with ART during both the early peripartum and the breast-feeding timeframes is recommended[5], but risks remain, including the toxicities associated with long-term use and the development of drug-resistant viral variants[6]. Therefore, discovering less toxic methods to limit transmission to newborns would be advantageous[2].

In mucosal HIV and SIV transmission, the virus establishes a small founder population of infected cells after it has traversed the vaginal mucosal barrier[7]. This localized infection rapidly expands and spreads to local draining lymph nodes (LN), before disseminating systemically by 1 week after exposure[8,9]. Similarly in nonhuman primate (NHP) models of oral SIV exposure, the oral and esophageal mucosa and the tonsils are sites of early viral infection within 1 d post-exposure (d.p.i.), with rapid systemic dissemination, via the regional lymphatics, occurring within 1 week after exposure[10,11]. Because IgG from the circulation contributes substantially to the immunoglobulin pool in tissue and genital tract secretions, passively transferred neutralizing antibodies (NAbs) may have a protective effect by interaction with the virus at the mucosal level[12], thus preventing systemic spread. In adult NHP models of mucosal SHIV transmission, there is abundant evidence for protective prophylactic efficacy with passively transferred human NmAbs[13–18]. *In vitro*, NmAbs have been shown to block HIV infection of dendritic cells and subsequent transmission to T cells[19]. Direct vaginal application of NAbs before challenge is protective in macaques[20], and in HIV-exposed but uninfected humans, mucosal IgA can block transcytosis *in vitro*[12]. Vaccine-induced protection from vaginal challenge correlates with levels of glycoprotein 41 (gp41)-specific cervicovaginal IgA and IgG that have antiviral and transcytosis-blocking activities[21]. However, the tissue localization and the kinetics of passively transferred antibodies are still not well defined[13,22].

There is evidence for an impact by NmAbs in lowering plasma virus levels in established infections in NHP models[23–25] and in humans[25,26]. In NHP models, post-exposure prophylaxis using cocktails of the first-generation human NmAbs b12, 2G12, 2F5 and 4E10 partially prevented oral SHIV infection in newborns[24]. A single dose

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combining the newer, more-potent NmAbs VRC07-523 and PGT121 delivered 10 d after intravenous SHIV infection suppressed acute viremia and limited seeding of viral reservoirs in adult macaques[27]. We have shown that neutralizing polyclonal IgG purified from SIV- or SHIV-infected macaques that are injected subcutaneously (s.c.) can effectively control viremia and accelerate B cell responses, resulting in reduced pathogenesis in SIV-infected adults[28] and in SHIV-infected newborn macaques[29,30]. We hypothesized that a cocktail of two potent and broadly cross-reactive NmAbs, VRC07-523 and PGT121, would slow the initial virus expansion and reduce the chance of rapid escape in infant macaques exposed to pathogenic SHIV. We show that combined doses as low as 10 mg per kg body weight (mg/kg) administered 24 h after exposure can intercept replicating viral foci established by day 1 and prevent orally administered virus from establishing permanent viral reservoirs.

RESULTS

Titration and biodistribution of subcutaneously administered antibodies in macaques

We initially conducted studies to define the protective dose and kinetics of the CD4-binding site–directed NmAb VRC01 in blocking newborn macaques from oral $SHIV_{SF162P3}$ infection after s.c injection and to determine the kinetics of passively transferred IgG in naive and infected macaques. First, we administered VRC01 to a total of seven male and female one-month-old macaques at 20 mg/kg ($n = 2$) or 5 mg/kg ($n = 5$) 24 h before SHIV exposure. We measured $SHIV_{SF162P3}$ envelope–specific binding and neutralizing antibody kinetics *in vivo*. The time to maximal concentration in the plasma was 24 h, independent of dose, and the serum (plasma) half-life of VRC01 was 3.9–4.2 d (**Supplementary Fig. 1**). Neither of the two macaques injected with the 20 mg/kg dose and only one of five macaques injected with the 5 mg/kg dose became infected. In this macaque, the magnitude and kinetics of virus in the plasma, termed the plasma virus load (PVL), were indistinguishable from that of control animals treated with IgG purified from naive macaques[30] (**Supplementary Fig. 1**). These data are consistent with results from passive protection studies using VRC01 in juvenile and adult macaques[18] and guided the therapeutic range we used for infant macaques.

Next, in a separate study designed to determine whether the kinetics of passively transferred IgG is altered in the presence of viral antigen, we assessed the distribution of purified polyclonal Ig from SIV-infected macaques (referred to as SIVIG) in a total of four male and female macaques, and we compared SIVIG kinetics in SIV-infected macaques to that in naive macaques. SIVIG was rapidly distributed in the plasma and tissues of infected and naive animals (**Supplementary Fig. 2**). We used *in situ* hybridization to localize SIV in tissue samples collected at 24 h and at 2 weeks after oral challenge with SIV_{smE660}. SIV was undetectable in 24-h tissue samples but was detectable after 2 weeks in tissues both adjacent to and distant from the site of challenge (**Supplementary Fig. 3**). Thus, IgG delivered subcutaneously is rapidly and widely distributed, and is unimpeded by viral antigen.

NmAb cocktail immunotherapy in the presence of SHIV

We next assessed the effectiveness of HIV-1 NmAbs as post-exposure prophylaxis in one-month-old infant inoculated orally with $SHIV_{SF162P3}$. For *in vivo* therapy, we tested a cocktail of VRC07-523 and PGT121, two potent NmAbs that target different regions of the HIV-1 envelope and that have been shown to have additive effects *in vitro*[31]. VRC07-523 is an engineered clonal relative of VRC01 that shows increased neutralization of most HIV strains and improved *in vivo* protection capabilities[32]. Therefore we used VRC07 instead of VRC01 for these therapeutic studies. PGT121 interacts with the variable regions and glycans of HIV-1 gp120 (refs. 33,34) and protects adult macaques from mucosal challenge at very low plasma titers[35]. Cocktails of PGT121 and VRC07-523 were prepared at total doses of 10 mg/kg (5 mg/kg each antibody) and 40 mg/kg (20 mg/kg each antibody) and delivered subcutaneously.

We inoculated 20 one-month-old rhesus macaques orally with $SHIV_{SF162P3}$ on day 0 and followed them for up to 28 weeks to assess virological, immunological and disease outcomes, with or without NmAb treatment starting on day 1. Pairs of animals were killed at days 1, 2 or 14 after exposure to monitor the development of $SHIV_{SF162P3}$ infection in the blood and tissues of treated and untreated macaques (**Table 1**; groups 1–4). We delivered NmAbs on days 1, 4, 7 and 10 after SHIV exposure (**Fig. 1a** and **Table 1**; groups 4–6). $SHIV_{SF162P3}$ infection by the oral route in one-month-old macaques results in reproducible, sustained PVL at $>10^7$ copies/ml plasma and $\sim10^4$ copies per µg DNA for at least 24 weeks in all of the animals[30]. To conserve animals, historical controls were used as a comparison group for the 24-week follow-up (**Table 1**; group 7).

We evaluated the kinetics of the individual NmAbs and the cocktail in plasma from all 12 treated infants that were on the study for at least 2 weeks (**Table 1**; groups 4, 5 and 6). Peak NmAb cocktail concentrations in plasma occurred by 24 h after the s.c. injection in all animals at both NmAb doses. In the four macaques that received the 10 mg/kg dose, the average cocktail concentration during the first 2 weeks was 44 µg/ml, and in the eight macaques that received the 40 mg/kg dose, it was 113 µg/ml (**Fig. 1b**). By using reagents designed to specifically

Table 1 Experimental design for testing a human NmAb cocktail as a therapy

Group	Size	Animal identification number (ID)	Virus exposure (d)	NmAb treatment (d)	NmAb dose (mg/kg)	CD8 depletion (d)	Necropsy (d)
1	2	34342; 34337	0	–	–	–	1
2a	2	34263; 34290	0	1	10	–	2
2b	2	34365; 34345	–[a]	1	10	–	2
3	2	33172; 33186	0	–	–	–	14
4	2	33379; 33400	0	1, 4, 7, 10	40	–	14
5	6	33165; 33216; 33260; 33261; 33308; 33309	0	1, 4, 7, 10	40	–	168
6	4	33494; 33505; 33536; 33537	0	1, 4, 7, 10	10	168–196	196
7[b]	8	28792; 28785 29003; 29010 29012; 29077 29079; 29081	0	–	–	–	168

[a]Indicates none or not done. [b]Indicates published previously[30].

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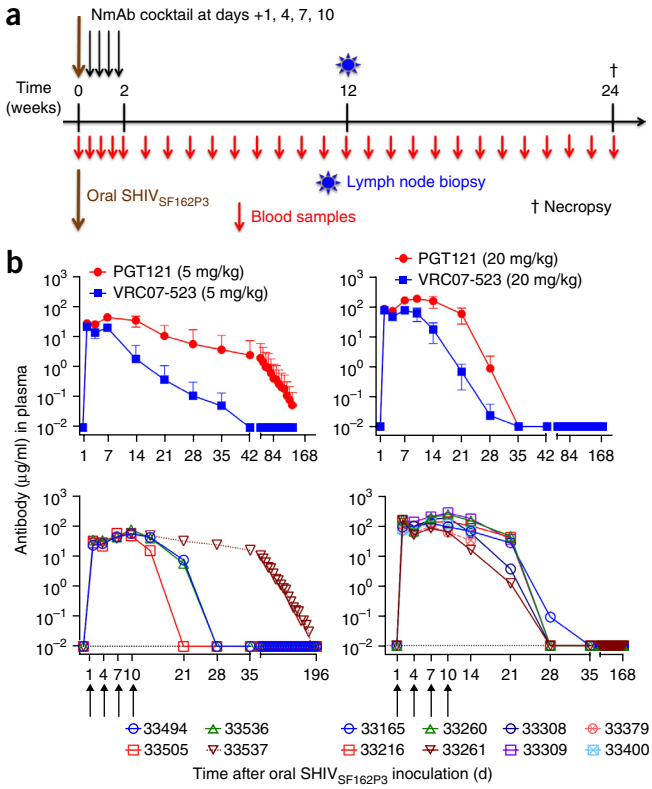

Figure 1 NmAb cocktail dosing and kinetics in plasma. (**a**) Experimental design of the early NmAb therapy experiment. (**b**) ELISA assays using recombinant proteins RSC3 (resurfaced stabilized core gp120 protein)[48] and ST0A9 (scaffold protein displaying the V1V2 region and PGT121 epitope)[18] for the specific detection of VRC07-523 and PGT121, respectively (5 mg/kg and 20 mg/kg each NmAb, respectively) (top). VRC07-523 and PGT121 were combined in a 1:1 ratio by mass (μg/ml) to generate a cocktail for s.c. injection at doses of 10 mg/kg and 40 mg/kg (bottom). The NmAb cocktail was assayed by an SHIV$_{SF162}$ gp140–specific ELISA (bottom left, 10 mg/kg cocktail; bottom right, 40 mg/kg cocktail). Data shown are NmAb concentrations in the plasma of 12 macaques. The concentrations were determined using nonlinear regression and the half-maximal effective concentration (EC_{50}) of the NmAb cocktail or the individual NmAb, and were graphed in GraphPad Prism. Error bars indicate s.d. The individual NmAbs and the NmAb cocktail were used as standard curves. Pre-treatment plasma (day 0) was used as a negative control for the assay.

detect each NmAb independently, we found that PGT121 concentrations in the plasma were consistently higher at both doses than those of VRC07-523. Multiple dosing prevented us from calculating the *in vivo* half-lives of each NmAb, but PGT121 was detectable in plasma for 2 weeks longer than VRC07-523 in several macaques. PGT121, administered at 5 mg/kg, was maintained for >20 weeks in the plasma from a single macaque, 33537 (**Fig. 1b**, bottom left). An unusually slow antibody decay rate in the plasma of subjects that were passively infused with PGT121 has been recently reported, in which plasma concentrations of 5–20 μg/ml were still present after 10 weeks[27].

We assessed SHIV$_{SF162P3}$-neutralization activity in the plasma of all infant macaques and found that it decayed by 6–7 weeks in all of the animals except macaque 33537, in which declining neutralization of SHIV$_{SF162P3}$ was detected at titers of 10^2–10^3 before becoming undetectable at week 20 (**Supplementary Fig. 4**). Calculation of the average 50% inhibitory concentration in plasma (IC_{50}) of the NmAb cocktail during the first 2 weeks after SHIV$_{SF162P3}$ exposure was 0.0134 and 0.0120 μg/ml in the 10 mg/kg and 40 mg/kg groups, respectively, which is close to the IC_{50} (0.0128 μg/ml) obtained from purified NmAbs specific for SHIV$_{SF162P3}$ in the TZM-bl standardized cell line that expresses luciferase in the presence of HIV or SIV Tat and that is used to quantify NAbs *in vitro* (**Supplementary Fig. 4**).

To measure transudation of NmAb into tissues and to assess neutralization potency within tissues and organs during the first 2 weeks, we extracted specimens from six macaques at different necropsy time points. Analysis of the antibody extracted from two macaques (group 4) that were sacrificed at 14 d after four doses of NmAbs showed that NmAbs were systemically distributed at concentrations from 48–700 ng/ml of tissue lysate (**Supplementary Table 1**). Two macaques (group 2a) exposed to SHIV$_{SF162P3}$, treated once with a NmAb cocktail dose of 10 mg/kg 1 d later and sacrificed on day 2 had NmAbs in tissue lysates in concentrations up to 791 ng/ml. Two macaques (group 2b) treated once with 10 mg/kg, without SHIV exposure, had NmAbs in tissues at levels similar to those in macaques 34263 and 34290, which were pre-exposed to SHIV. We assessed the neutralizing activity to SHIV$_{SF162P3}$ in tissue homogenates from ~100 mg of necropsy samples from the animals sacrificed at 1, 2 and 14 d after exposure (groups 2 and 4). The 50% neutralization titers (ID_{50}) for SHIV$_{SF162P3}$ of tissue lysates averaged ~1:50 in the tested samples at 1 d after s.c. NmAb injections, increased to an average of ~1:100 by day 2 and were 1:150 at day 14, with good agreement between the titers observed for macaques that were sacrificed at the same time points (**Table 2**). However, NmAb titers in the colon and reproductive tract from macaque 34290 were about 3–5 times higher than those from macaque 34263, which was necropsied at the same time point. Tissue-associated IC_{50} concentrations of the NmAb cocktail in the samples tested ranged from 0.5–10.0 ng/ml, which is similar to the IC_{50} of the NmAb cocktail (**Supplementary Fig. 4**). We conclude that the presence of SHIV during the first day after oral exposure did not affect NmAb distribution or levels *in vivo* and that the NmAb cocktail was rapidly distributed to tissues.

SHIV$_{SF162P3}$ dissemination with and without NmAb therapy

To determine the *in vivo* transudation kinetics of SHIV$_{SF162P3}$ in blood and tissues early in infection with and without NmAb cocktail therapy after exposure, we quantified the amount of virus in blood from macaques killed on days 1, 2 or 14 after oral SHIV$_{SF162P3}$ exposure (**Table 1**, groups 1–4). The 2-week time point was anticipated to be nearest to the time of peak PVL. Plasma viremia was detected by day 4, increased rapidly and peaked between 1×10^8 to 5×10^8 copies/ml in macaques that were not treated with NmAbs and necropsied at day 14 (**Fig. 2a**), consistent with results from the VRC01 study (**Supplementary Fig. 1**) and prior studies[29,30]. In stark contrast, no virus was detected in plasma or peripheral blood mononuclear cells (PBMC) from NmAb-treated macaques killed at day 14 (**Fig. 2a**,**b**).

We collected multiple tissues from all of the macaques at necropsy (**Fig. 2c**), and in samples collected within 2 d of SHIV$_{SF162P3}$ exposure, we measured low levels of SHIV$_{SF162P3}$ DNA in mucosa and LN that were proximal and distal to the oral exposure site (**Fig. 2d**,**e**) in treated and untreated animals. In comparison to treated animals, the virus was widespread at day 14 in untreated animals (**Fig. 2f**) and peaked at >3,000 copies/μg of DNA throughout the LN and gut, consistent with levels of DNA in the tissues of adult and six-month-old *Macaca nemestrina* with high levels of plasma viremia[36]. As seen in the blood, following NmAb treatment on days 1, 4, 7 and 10 at 40 mg/kg, virus was not detectable in any tissue at day 14 (**Fig. 2g** and **Supplementary Table 2**).

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Table 2 Neutralizing activity in tissue homogenates of infant rhesus macaques colocalizes with virus

NmAb cocktail dose	10 mg/kg							No NmAb		40 mg/kg	
Time of sample collection (d)	1[a,b]				2[c]			14[d,e]			
Animal ID	34342	34337	34365	34345	34263		34290		33172	33186	33379	33400
	vRNA	vRNA	ID_{50}		ID_{50}	vRNA	ID_{50}	vRNA	vRNA	vRNA	ID_{50}	ID_{50}
Cerebellum									1,936.7	1,334.7		
Buccal mucosa			<20	<20	<20		32				72	88
Pharyngeal mucosa			<20	<20	<20		<20					54
Mixed mesenteric LNs	1.3	0.2	44	56	66		32	0	417,832.3	671,839.9	36	175
Spleen	0	0	74	34	71		52	0	887,585.7	392,031.7	145	157
Iliosacral LN	0.7	65.1										
Inguinal LN								0				
Colon	0.2	0	72	34	96		289	0	1,410,029.7	105,623.6	33	245
Rectum			<20	28	58		71				<20	42
Reproductive tract			71	82	79		386				69	110

[a]SHIV day 0; no NmAb treatment; necropsy on day 1. For 34342 and 34337. Quantified viral DNA (vDNA) (**Supplementary Table 2**). vRNA, viral RNA. [b]NmAbs day 0; no SHIV; necropsy on day 1. For 34365 and 34345. [c]Oral SHIV day 0 and s.c. NmAbs day 1; no vDNA (**Supplementary Table 2**); necropsy on day 2. For 34263 and 34290. [d]Oral SHIV day 0; no NmAb treatment; necropsy on day 14. For 33172 and 33186. [e]Oral SHIV day 0 with s.c. NmAbs day 1, 4, 7 and 10; No vDNA (**Supplementary Table 2**). For 33379 and 33400. ID_{50} = dilution of plasma that results in 50% neutralization of $SHIV_{SF162P3}$ in TZM-bl cells. SIV *gag* viral RNA (copies/1 × 10^6 cell equivalents); coded samples; ultrasensitive nested qPCR and RT-PCR in 12 replicates. Blank indicates no data.

To determine whether the viral DNA–positive tissues contained replicating SHIV, we measured viral RNA in several tissue samples taken from these same macaques that were sacrificed on 1, 2 or 14 d after virus exposure. Viral DNA and RNA levels, tested as blinded samples from these tissues, show that productive $SHIV_{SF162P3}$ infection had begun in multiple tissues by day 1, increasing exponentially by day 14 (**Table 2**). However, in two macaques that were treated with 10 mg/kg on day 1 (group 2a; sacrificed on day 2), there was no viral RNA detected in the samples tested. Notably, the NmAbs were colocalized in these virus-positive tissues, suggesting the potential for antibody effects as early as 1 d after treatment. Moreover, the results suggest that virus present early after exposure can be intercepted and cleared by NmAbs present in the same tissues (**Table 2**).

Prevention of productive infection, viral rebound and pathogenesis

To evaluate the effect of early short-term NmAb therapy on viral control, we monitored $SHIV_{SF162P3}$ in blood, LN and tissues in animals followed for 24–28 weeks. PVL in the controls routinely peaked at 2 weeks post-infection (w.p.i.) and persisted at levels that ranged from 10^6–10^8 copies/ml. In newborns that were treated with 10 mg/kg (**Fig. 3a**) or 40 mg/kg (**Fig. 3b**) NmAbs, there was no plasma viremia detected in any of the samples collected over the course of the study. A single time point in the 40 mg/kg group was positive for only one of two replicates, and additional material to retest this sample was not available. Longitudinal cell-associated viral loads (CAVL) in PBMC DNA were negative for each of the >300 samples tested from the ten macaques in groups 5 and 6 (**Fig. 3c**,**d**). In short, all of the NmAb-treated infants had undetectable PVL or CAVL in blood.

We also measured the levels of $SHIV_{SF162P3}$ DNA in >300 homogenized tissue samples obtained at 24–28 w.p.i. and in inguinal LNs collected 12 w.p.i. from all ten macaques, using ultrasensitive qPCR[37]. Tissue samples from all SHIV-exposed infants that received the 2-week course of NmAb cocktail were tested as coded samples and were negative for virus in both dosage groups (**Supplementary Table 2**). As discussed above, only very low levels of virus were detected in tissue specimens from the group 2a macaques 34263 and 34290,

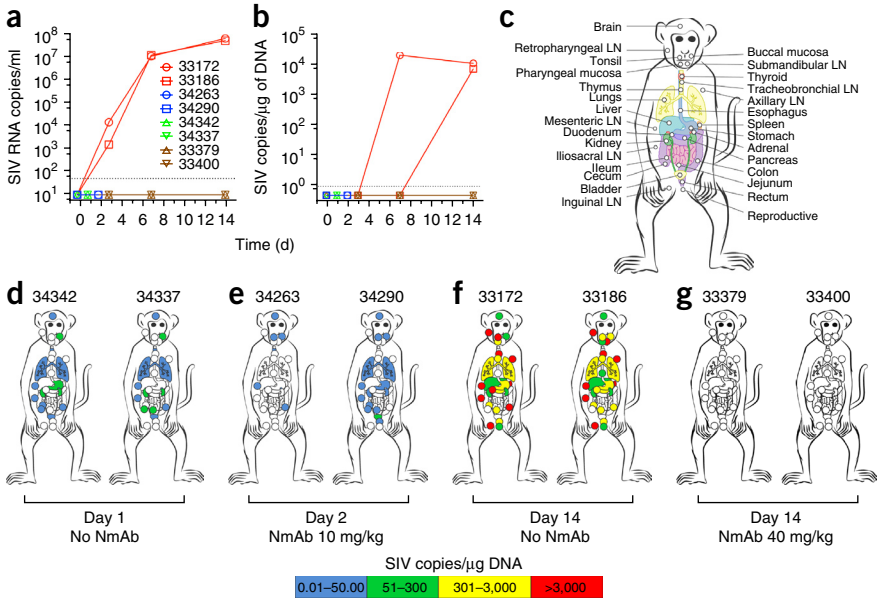

Figure 2 Viral kinetics and tissue distribution during the first 2 weeks after oral SHIV exposure. $SHIV_{SF162P3}$ viremia was quantified in eight male and female macaques that were either treated or untreated with NmAbs. (**a**,**b**) PVLs (as assessed by measurements of SIV viral RNA in blood using a qRT-PCR) (**a**) or CAVLs in PBMC (as assessed by qPCR) (**b**). (**c**) Anatomic locations of tissues collected at necropsy following oral inoculation. (**d**–**g**) Viral DNA in tissues of untreated macaques (**d**,**f**) or in macaques treated with 10 mg/kg (**e**) or 40 mg/kg (**g**) NmAb cocktails and killed at the indicated times, as detected by an ultrasensitive nested qPCR and RT-PCR[37] assay targeting a highly conserved region in SIV *gag* encoded in the SHIV. Each sample was assayed in 12 replicates (5 µg each). Virus copy numbers were derived from the frequency of positive replicates using the Poisson distribution and calculated as copies per µg of DNA or copies per 10^6 cell equivalents using the input nucleic acid mass and by assuming a DNA content of 6.5 µg per million cells. Infected tissues are colored to indicate virus amounts, quantified as SIV *gag* copies/µg of DNA, according to the scale shown at the bottom.

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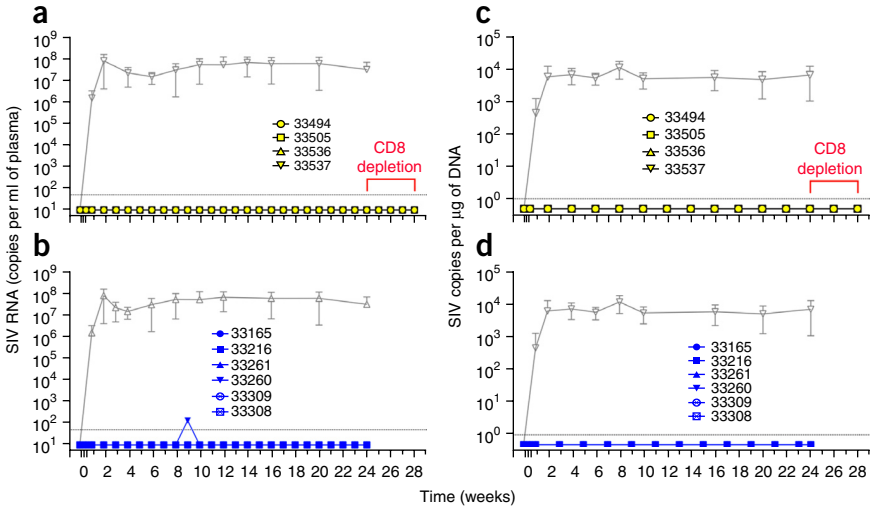
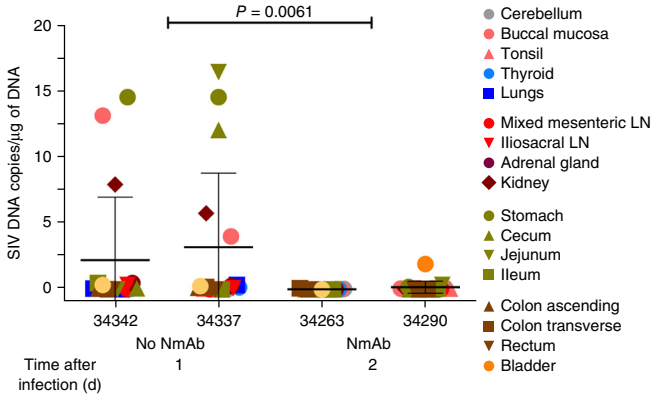


Figure 3 $SHIV_{SF162P3}$-associated viremia is not established in plasma or PBMC of NmAb-treated infants. (**a**–**d**) Quantification of virus in blood (**a**,**b**) and in peripheral blood cells (**c**,**d**) in both NmAb dosing groups of male and female infant rhesus macaques ($n = 10$). Plasma viral loads were assessed by measurements of SIV viral RNA in blood using a qRT-PCR assay (**a**,**b**) and in PBMC by qPCR (**c**,**d**). $CD8^+$ T cell–depletion study timeline is shown in red. Data shown in gray indicate mean levels of virus in plasma (±s.d.) from eight historical controls from an earlier study[18,30].

which were sacrificed at 2 d.p.i. after a single NmAb dose (**Fig. 2e**). Similarly to that seen in blood, virus was widespread in tissues at 14 d.p.i. in group 3 control macaques that did not receive NmAbs (**Fig. 2f**). As early as 1 d.p.i., tissue-associated virus in mucosal tissue adjacent to the exposure site, the draining LN and the gut tissue was evident in macaques from group 1 (untreated controls) (**Fig. 2d** and **Supplementary Table 2**). As compared to the untreated (group 1) macaques killed at 1 d.p.i., NmAb-treated (group 2a) macaques that were sacrificed 2 d.p.i had significantly lower amounts of tissue-associated virus ($P = 0.0061$; **Fig. 4**). The discovery of traces of virus at 2 d.p.i (**Fig. 2e**) and none at 14 d.p.i. (**Fig. 2g**) implies that NmAbs intercepted and neutralized $SHIV_{SF162P3}$ replication, cleared infected cells and halted the spread of infection in these macaques and in all ten NmAb-treated infants that were followed for 6 months. Consistent with these data, pathology results also showed the absence of organ or tissue pathology (**Supplementary Fig. 5**).

No evidence for T cell immunity or viral rebound

To evaluate T cell immunity in the SHIV-exposed macaques, we used intracellular cytokine staining (ICS) to measure PBMC, spleen and mesenteric LN responses specific for the SIV_{mac239} proteins Gag and Vif (present in the SHIV chimera) in the ten macaques that were studied for 6 months. No SHIV-specific T cell responses were detected in PBMC at week 20 or in tissue samples after necropsy (**Supplementary Fig. 6**). To determine whether there were any reservoirs controlled by $CD8^+$ T cell–mediated suppression, we depleted $CD8^+$ cells to undetectable levels in the four animals in the 10 mg/kg group and monitored them for viremia for 4 weeks (**Supplementary Fig. 6**). There was no evidence of virus rebound in the plasma of these animals during the $CD8^+$ depletion phase (**Fig. 3a**,**c**), further supporting the concept that early passive NmAb therapy with the cocktail of VRC07-523 and PGT121 disrupted establishment of virus reservoirs, thereby preventing exposure to antigen and development of cellular immunity.



DISCUSSION

Pre-exposure prophylaxis (PrEP) with ART is effective in limiting transmission in the setting of MTCT, as well as in healthy adults[38]. One of the major goals in treating HIV-1 infection is to discover methods that can clear the established viral reservoir[39]. To date, only a single case of a 'functional cure' has been documented following bone marrow transplantation[40]. Vaccine-induced, persistent T cell responses cannot prevent infection but can reduce established SIV reservoirs to undetectable levels in about half of vaccinated macaques[41]. NHP studies with ART suggest that treatment as early as 3 d.p.i. is too late to prevent the establishment of the reservoir, as the virus rebounded after cessation of drug treatment[42]. These data are consistent with the case of the 'Mississippi baby', in which ART therapy was started within 30 h of birth but did not prevent HIV infection[43,44]. Thus, the time for intervention is extremely limited, and ART alone may not be effective at eliminating a founder infection.

Viral RNA has been detected in macaques as early as 1 d following vaginal exposure to SIV[45], but there is a common view that HIV and SIV may have a short 'eclipse phase' of limited, localized viral replication that lasts a number of days, in which the spread of the founder infection is dependent upon target cell availability to spread to lymphatic tissues[45]. Our data show that, at least in this model system of oral SHIV exposure in infant macaques, virus replication is detected in lymphatic tissues at 24 h after infection and is not locally restricted. Here we found evidence of an immediate impact of a single dose of passively transferred NmAbs on seeding of the virus, with a significant difference in early tissue-associated viral RNA and DNA in treated versus nontreated infants. Early post-exposure, short-term administration of powerful NmAbs effectively cleared the virus *in vivo* by 14 d and prevented viral rebound after decay of the passive NmAbs. We present three lines of evidence that the ten macaques studied for

Figure 4 NmAb cocktail lowers tissue-associated viremia within 24 h after s.c. delivery. SHIV DNA quantified by ultrasensitive nested qPCR and RT-PCR[37] in each tissue sample shown from four control animals (**Table 1**, groups 1 and 2a) at either 1 d after SHIV exposure with no NmAb treatment or 1 d after s.c. injection with 10 mg/kg NmAb cocktail and 2 d after SHIV inoculation. $P = 0.0061$; by Wilcoxon-signed rank test (statistics performed in SAS 9.4 software).

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>24 weeks were clear of virus. Firstly, all of the macaques failed to develop adaptive immune responses. Secondly, we showed that >300 coded tissue samples from these macaques were virus negative, using an ultrasensitive PCR methodology based on detection of the SIV *gag* gene. Thirdly, we depleted the $CD8^+$ T cells in the four lower-dose macaques (group 6) and observed no viral rebound.

These experiments show that NmAbs delivered subcutaneously are swiftly distributed to blood and tissues and that they maintain neutralizing activity at distal sites. They further indicate that NmAbs are effective at clearing viral foci in blood and tissues during the earliest stages of HIV penetration of the tissues, a different mechanism from that of ART. We hypothesize that antibody-mediated effector functions, including cytotoxicity and phagocytosis, are required for killing infected cells that express HIV envelope gp160 on their surface[46]. If so, then NmAbs present for an extended period of time after exposure would have the capability to destroy infected cells and neutralize virus particles emanating from cells in which infection was established within the first few hours following an exposure event. In this setting, post-exposure therapy, using a NmAb cocktail administered 24 h after SHIV exposure and continuing for 2 weeks, resulted in the maintenance of high NmAb concentrations *in vivo* for at least 2 weeks. The importance of repeated dosing is not known, but in the case of breast-feeding mothers, there is continued opportunity for transmission of HIV-1. It will be important to understand whether repeated NmAb dosing in babies could expand the protective window.

Several relevant questions remain unanswered for the treatment of HIV-infected newborns and children born to HIV-positive mothers, including the practical and cultural issues of treating breast-feeding mothers and babies, as well as a determination of optimal antibody cocktail formulations. Any future use of human NmAbs in the clinic will presumably require several antibodies, or engineered antibodies with multiple specificities, to avoid the potential for the emergence of viral escape mutants. Because ART has a short half-life and requires strict adherence to the drug regimen to be effective, supplementation with passive NmAbs with relatively long half-lives may widen the therapeutic window. Identifying human NmAbs that can impact infection in a macaque model for MTCT can provide a proof of principle for the value of using antibodies to augment ART. In fact, safety trials in HIV-exposed newborns for treatment with the NmAb VRC01 have begun in the USA and South Africa (http://impaactnetwork.org/studies/P1112.asp), following a safety trial in adults[47]. Our findings begin to define the window of opportunity for effective treatment after intrapartum exposure. If these results can be applied to clinical settings, then there is optimism that early passive immunotherapy may provide protection from HIV infection, even in the absence of ART.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Studies were designed and planned by N.L.H. and A.J.H.; experimental work was done by A.J.H., J.P.J., E.E., K.M., S.P., J.R., W.F.S., K.B.H., T.A.C., P.T.B., A.W.L., S.P., X.C., K.W., D.S., D.B.; pathology was described by A.W.L.; veterinary care was provided by J.J.S.; A.J.H., N.L.H., J.B.S., J.R.M. and B.S.G. wrote the manuscript; A.J.H., J.P.J., E.E., C.K., M.K.A., V.M.H., A.P., J.B.S. and N.L.H. analyzed the data; B.S.P. performed statistical analyses.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animal models and humane-care guidelines. The Oregon Health and Science University West Campus Institutional Animal Care and Use Committee approved all macaque studies. Studies were performed at the Oregon National Primate Research Center in Beaverton, Oregon, USA (ONPRC). The ONPRC is accredited by the American Association for the Accreditation of Laboratory Animal Care International and adheres to the Guide for the Care and Use of Laboratory Animals[49] and the United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/references/phspol.htm). The initial study with SIVIG used four *M. mulatta* (male and female) of varying ages that were obtained from the breeding colony. For the studies in one-month-old macaques, 27 7-d-old *M. mulatta* (rhesus macaques, male and female) were obtained from the breeding colony and raised for 3 weeks in the animal biosafety level (ABSL)-2 infant nursery. Time of birth determined animal allocation, so that animals were randomly assigned to the study groups as they accrued. Protection studies with VRC01 ($n = 7$ infants) were pilot studies and were not designed for statistical analyses (all or none effects of virus acquisition). Group sizes of six had been previously shown to allow statistically distinguishable measurements in plasma and cell-associated virus loads at 6 months as the primary study outcome for antibody treatment. Serial sacrifice studies included groups of two animals each, and viral quantification analyses included ~30 tissue samples per animal. Infants were excluded if the sire or dam could not be confirmed to be absent of *M. mulatta* B*08 and B*17 major histocompatibility complex (MHC) class I alleles. At 1 month of age, after adaptation to formula feeding, animals were transferred to ABSL-2+ containment for study procedures. Infants were paired with another macaque of the same age for nursery care and containment housing. In all studies, infants were monitored for clinical signs of disease. Clinical evaluation included measurement of peripheral LN size and body weight, as well as evaluation of appetite, attitude and stool quality. All animals were euthanized under IACUC guidelines and consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia[50].

IgG and NmAb preparations. Normal IgG was purified from 1 liter of pooled plasma from simian retrovirus (SRV)-negative and SIV-negative adult rhesus macaques as previously described[30]. VRC01, VRC07-523 and PGT121 were expressed as IgG1 antibodies by transient transfection of Expi293F cells (ThermoFisher Scientific Inc.) and purified over protein A columns[32,51]. The V_H and V_L regions of PGT121 were synthesized based on the published sequence[33]. Purified polyclonal anti-SIV_{smE660} IgG (SIVIG) was pooled from two SIV_{smE660}-infected animals from a prior experiment[28]. All antibody preparations were delivered subcutaneously at multiple sites around the dorsal cervical and thoracic regions of the animals in the doses described in the text.

Virus inoculations. Infant macaques were administered a 50% animal infectious dose (AID_{50}) (~7×10^8 viral RNA copies) of a macaque cell–grown stock of $SHIV_{SF162P3}$ (ref. 52), divided into two 1-ml oral doses given ~15 min apart. AID_{50} was determined in a titration experiment described previously[36].

Virus detection in plasma, PBMC and tissue homogenates. Nucleic acid from plasma, cell culture supernatant or PBMC was purified using a Maxwell 16 instrument (Promega, Madison, WI) according to the manufacturer's protocol, using the LEV Viral Nucleic Acid Kit and the LEV Whole-Blood Nucleic Acid Kit, respectively. SHIV viral loads in plasma and cell culture supernatant were determined by quantitative RT-PCR using the methods developed by Piatak *et al.*[53], except for a slightly modified master mix to increase sample input per reaction. SHIV viral loads in PBMC DNA were determined by quantitative PCR using Fast Advanced Mastermix on an Applied Biosystems QuantStudio 6 Flex instrument (Life Technologies, Carlsbad, CA). Reactions were performed with 2 µg nucleic acid input for 45 cycles using the FAST cycling protocol (95 °C for 1 s, 60 °C for 20 s) in a 30-µl reaction volume. Virus copy numbers were estimated by comparison to a linearized pBSII-SIV*gag* standard curve and calculated per cell equivalent using the input nucleic acid mass and by assuming a DNA content of 6.5 µg per million cells. Primers and probe used for plasma and PBMC assays were those described by Piatak *et al.*[51]: SGAG21 forward (GTCTGCGTCATPTGGTGCATTC), SGAG22 reverse (CACTAGKTGTCTCTGCACTATPTGTTTTG), and pSGAG23 (5′-(FAM)-CTTCPTCAGTKTGTTTCACTTTCTCTTCTGCG-(BHQ1)-3′).

For viral RNA and DNA reservoir detection in tissues, a recently developed ultrasensitive nested quantitative PCR and RT-PCR approach[37] targeting a highly conserved region in SIV and SHIV *gag* was used. Primers used for DNA pre-amplification were SIVnestF01 (GATTTGGATTAGCAGAAAGCCTGTTG) and SIVnestR01 (GTTGGTCTACTTGTTTTTGGCATAGTTTC). The reverse-transcription step in the RNA assay used the SIVnestR01 primer instead of random hexamers, in order to facilitate priming of specific target sequences. Primers used for quantitative PCR were SGAG21 forward, SGAG22 reverse, and pSGAG23 as described above. PCR reaction conditions for both rounds were as described with minor modifications[54]. Briefly, samples were heated at 95 °C for 5 min and then put on ice. Each sample was assayed in 12 replicates (5 µg each), with two of the reactions including a spike of 10 or 20 copies of DNA or RNA, respectively, containing the SIV *gag* target sequence in order to assess PCR reaction efficiency. None of the tested RNA and DNA samples showed significant amplification inhibition, which was defined as a 5-cycle amplification delay as compared to the amplification kinetics of reactions containing solely 10 copies of standard. First-round amplification involved 12 cycles (95 °C for 30 s and 60 °C for 1 min) in 50-µl reactions. Then, 5 µl of each pre-amplified replicate was assayed by quantitative PCR using Fast Advanced Mastermix in a 30-µl reaction volume in the QuantStudio 6 Flex instrument. Reactions were performed for 45 cycles using the FAST cycling protocol. Virus copy numbers were derived from the frequency of positive replicates using the Poisson distribution and calculated as copies per µg of DNA. Staff members performing the RNA and DNA assays were blinded to the plasma and tissue samples that were being tested for virus.

Antibody detection in tissues and secretions. Tissue samples were sectioned and transferred into radio-immunoprecipitation assay (RIPA) buffer (PI89900, Thermo Fisher Scientific) with protease inhibitor cocktail (P8340, Sigma-Aldrich). Tissue disruption was accomplished with zirconia-silica beads (1.0 mm, Biospec Products) in a Beadbeater (Biospec Products) device with two cycles of 2-min intervals with brief incubations on ice between each cycle. Supernatants were aspirated and centrifuged for 5 min to pellet residual debris. Mucosal secretions were collected on Weck-cel spears and extracted as previously described[55]. Secretions were stored at −80 °C until assayed. Homogenates and secretions containing transudated antibody were used in ELISA and neutralization assays as described below.

$CD8^+$ T cell depletion and staining. Four animals were given the CD8-α–depleting antibody M-T807R1 (US NIH, Nonhuman Primate Reagent Resource). Peripheral blood was monitored for the presence of $CD8^+$ T cells for 4 weeks. 1×10^5 PBMC were stained with anti-CD3–AlexaFluor 700, anti-CD8–Pacific Blue (BD Biosciences), and anti-CD4–PE-Cy7 (Biolegend).

Intracellular cytokine staining (ICS). $CD4^+$ and $CD8^+$ T cell responses were measured from blood and tissues by flow cytometric ICS, as previously described[56]. Briefly, 1×10^6 mononuclear cells were incubated with Gag or Vif open-reading-frame pools and the co-stimulatory molecules CD28 and CD49d (BD Biosciences) for 1 h, followed by addition of brefeldin A (Sigma-Aldrich) for an additional 8 h. Co-stimulation without antigen served as a background control, while incubation with staphylococcal enterotoxin B (Toxin Technology) served as the positive control. The cells were then labeled with anti-CD4–PE-Cy7 (Biolegend) and anti-CD8–PerCP-Cy5.5 (BD Biosciences) and fixed with 2% paraformaldehyde. After permeabilization, the cells were stained with anti-CD3–Pacific Blue, anti–IFN-γ–APC, anti–TNF-α–FITC (BD Biosciences, all), and anti-CD6– PE-Texas Red (Beckman Coulter). The cells were fixed and flow cytometric analysis was performed on an LSR-II instrument (BD Biosciences). Analysis was done using FlowJo software (Tree Star, Ashland, OR). In some cases, cells were CD25-depleted before setting up the ICS experiment to remove T_{reg} cells (Miltenyi Biotec).

***In situ* hybridization (ISH).** In the SIVIG transudation experiments, measurement of *in-situ* hybridization for SIV antigen was performed on tissues collected

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from the two animals that underwent oral SIV challenge. Formalin-fixed, paraffin-embedded tissues were assayed for SIV viral RNA expression by ISH as previously described[57]. Briefly, following deparaffinization, the sections were hybridized overnight at 45 °C with either a sense or an antisense SIVmac239 digoxigenin-UTP–labeled riboprobe. The hybridized sections were blocked with 3% normal sheep and horse serum in 0.1 M Tris, pH 7.4, and then incubated with sheep anti-digoxigenin–alkaline phosphatase (Roche Molecular Biochemicals) and nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (BCIP; Vector Labs). ISH-stained tissues from submandibular LNs, tonsils and the ileum were visualized and photographed with a Zeiss Axiophot microscope.

Enzyme-linked immunosorbent assays (ELISAs). ELISA was used to detect total IgG and SIV gp130–specific antibodies in the SIVIG transudation experiments. Briefly, half-well EIA plates (Costar) were coated with either a goat anti–rhesus IgG (H+L) (unlabeled; Southern Biotech) (for total IgG ELISA) or recombinant SIV_{smE660} gp130, purified as described[28] (for gp130 ELISA), at 2 μg/ml in carbonate-bicarbonate buffer and incubated overnight. Plates were washed three times (0.1% Triton X-100 in 1× PBS) and blocked with 1% normal goat serum and 5% nonfat dried milk in PBS for 1 h at RT. SIVIG standards and homogenates were diluted in 1% Triton X-100, 2% bovine serum albumin, 5% FBS in PBS. After washing, a 1:4,000 dilution of goat anti–rhesus IgG (H+L)-horseradish peroxidase (HRP) (Southern Biotech) was added and incubated for 1 h at RT followed by TMB substrate (Southern Biotech). Plates were read on a SpectraMax 190 at an absorbance wavelength of 650 nm. Data were reported as the slope of absorbance over time. Concentrations of SIVIG in tissue disruption supernatants were calculated by comparing the average slope numbers to those from the SIVIG standard curve. ELISA was used to assess for the presence of gp140-specific antibodies as previously described[56] in plasma and tissue homogenates. Plasma NmAb levels were quantified using plates coated with either RSC3 (re-surfaced stabilized core gp120 protein)[48] (VRC07-523) or ST0A9 (scaffold protein displaying the V1V2-region and PGT121 epitope) (PGT121)[58]. Briefly, Nunc MaxiSorp (Thermo Fisher) plates were coated overnight with 200 ng/well of RSC3 in PBS, washed with PBST five times, and blocked with TBST with 5% milk and 2% BSA for 1 h at RT. Serial dilutions of all samples were plated in duplicate. Each NmAb (for standard curves) and positive and negative controls were included on each plate. Plasma was incubated for 1 h at RT, followed by a PBS–Tween 20 wash. Bound NmAbs were probed with a HRP-labeled goat anti–human IgG (1:5,000 dilution; Jackson Laboratories) for 30 min at RT. The plate was washed and TMB (Pierce) substrate was added. Once color was developed, stopping buffer was added and the optical density at 450 nm was read. GraphPad Prism and Microsoft Office software was used to calculate NmAb concentrations.

TZM-bl neutralization assay. Plasma samples from each animal were tested at all available time points for neutralizing activity using the 96-well TZM-bl neutralization assay described previously[59].

Statistics. The data in **Figure 4** shows SIV DNA copies measured in 17 anatomical sites in two paired groups of four control animals. A two-stage sequential approach was used to calculate the averages of SIV DNA copies of two biological replicates for each site in each group and apply a Wilcoxon-signed rank test to account for the matched pair nature of anatomical sites.

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