

Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120

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A topical microbicide reduces the probability of virus transmission when applied to the vagina or rectum of a person at risk of sexually acquiring HIV-1 infection¹⁻³. An effective microbicide could significantly reduce the global spread of HIV-1, particularly if women were able to use it covertly to protect themselves. A microbicide could target the incoming virus and either permanently inactivate it or reduce its infectivity, or it could block receptors on susceptible cells near the sites of transmission¹⁻³. We describe here how vaginal administration of the broadly neutralizing human monoclonal antibody b12 can protect macaques from simian-human immunodeficiency virus (SHIV) infection through the vagina. Only 3 of 12 animals receiving 5 mg b12 vaginally in either saline or a gel and then challenged vaginally (up to 2 h later) with SHIV-162P4 became infected. In contrast, infection occurred in 12 of 13 animals given various control agents under similar conditions. Lower amounts of b12 were less effective, suggesting that protection was dose dependent. These observations support the concept that viral entry inhibitors can help prevent the sexual transmission of HIV-1 to humans.

To assess which entry inhibitors might be most effective for human use, we are performing a series of comparative experiments using vaginal infection of depo-medroxyprogesterone acetate (Depo-Provera)-treated rhesus macaques by SHIV-162P4 (refs. 4–7). This virus is derived from a subtype-B primary isolate that uses CCR5, the co-receptor most relevant to HIV-1 sexual transmission⁵⁻⁷. We first studied b12, a broadly neutralizing human monoclonal antibody to HIV-1 gp120 (ref. 8). *In vitro*, b12 inhibited SHIV-162P4 infection of macaque peripheral blood mononuclear cells, immature human dendritic cells derived from monocytes, and human cervical explants^{7,9,10}. The antibody also blocked uptake of infectious SHIV-162P4 through migratory immune cells that emigrate from cervical tissue exposed to the virus. In these experimental systems, the half-maximal inhibitory concentration (IC₅₀) ranged from 1 to 10 µg/ml (data not shown). *In vivo*, intravenously administered b12 can protect against vaginal challenge of macaques with SHIV-162P4 (ref. 11).

We first examined the infectivity of an *in vitro*-made mixture of 5 mg b12 and 300 TCID₅₀ (50% tissue culture infectious

doses) SHIV-162P4 in 1 ml of saline. None of four macaques tested became infected when challenged with the pre-formed immune complexes, whereas six of six animals became infected when challenged vaginally with the same inoculum but without b12 (Table 1, animals 2–11). Thus, an immune complex between b12 and SHIV-162P4 is not infectious ($P = 0.0048$, Fisher's exact test). We next studied whether vaginal administration of b12 could have a short-term protective effect. An otherwise effective inhibitor (including, but not limited to, b12) might be gradually diluted out as it dissipated into tissues, so that any virus following the same route could infect the animals. The infectious life span of a virus might thus exceed the duration of the action of a potential inhibitor, masking any short-term protective effect. We therefore lavaged the vagina with 200 ml of mild anti-viral soap 1 h after SHIV-162P4 treatment, to terminate the infectivity of any virus still present in the vagina at this time. A mixture of SHIV-162P4 and soap was non-infectious, confirming that this procedure did destroy the virus (Table 1, animal 1). Infection still occurred in two animals, however, when soap solution was applied 1 h after adding virus (Table 1, animals 10–11). Thus, 1 h is sufficient for SHIV-162P4 to move into cells and tissues—similar to the case for SIV¹²—where it is presumably beyond the reach of a topically applied inhibitor.

When b12 was added to the vagina before SHIV-162P4 vaginal challenge, it successfully protected most animals from infection. Overall, only two of eight animals receiving 5 mg b12 became infected when SHIV-162P4 was added 15 min later (Table 1, animals 19–26). In contrast, nine of ten animals given no antibody, irrelevant polyclonal human IgG or irrelevant human monoclonal antibody became infected (Table 1; animals 6–15; $P = 0.0088$). In addition, one of two macaques given 5 mg b12 formulated in 5 ml 2.5% hydroxymethyl cellulose (HMC) gel became infected, as did three of three animals given HMC alone (Table 1, animals 16–18 and 30–31). Whether b12 can be formulated in a gel such as HMC (which is itself inert) and still be consistently protective will require additional study.

Protection conferred by vaginally applied b12 was long lasting and occurred in most test animals whether soap solution was applied after 2 or 7 h or not at all (Table 1). We also stud-



ied temporal effects by adding 5 mg b12 to the vagina for 1, 2 or 6 h before adding SHIV-162P4 (Table 1, animals 27–29). The macaques challenged 1 or 2 h after b12 addition were not infected. Infection did occur in the one animal in which the challenge was delayed for 6 h, although its peak viral load was less than in most of the control animals (Table 1, animal 29). Additional experiments will be required to further study the time dependence of protection by b12.

In total, only 3 of 12 animals became infected when challenged with SHIV-162P4 within 2 h of receiving 5 mg b12 in either saline or HMC. Infection occurred in 12 of 13 control animals given various control agents under similar conditions (Table 1). The difference is statistically significant ($P = 0.00087$), indicating that b12 is strongly protective against vaginal SHIV-162P4 transmission.

The 12 infected control animals had similar levels of plasma viremia, which peaked on days 14 or 21, in the range of 2.1×10^5 – 3.4×10^6 RNA copies/ml (mean log value \pm s.d. of 5.86 ± 0.42). The four animals that became infected despite receiving 5 mg b12 for various times (animals 23, 24, 29 and 31) had

lower and possibly delayed (days 21–28) peak viral loads of 2.3×10^4 to 2.2×10^5 (mean log value \pm s.d. of 4.80 ± 0.44). The difference in viral loads between the two groups was significant ($P = 0.0090$, Student's *t*-test). Thus, a high dose of b12 may reduce the peak level of plasma viremia after infection.

All the above experiments used 5 mg b12 delivered to the vagina in 5 ml. This concentration (1 mg/ml) is much higher than that required to inhibit SHIV-162P4 infection *in vitro* (1–10 μ g/ml), so we assessed whether lower concentrations might be effective. In this study, no soap wash was used at any time. One of two macaques receiving 200 μ g/ml (1 mg total) b12 became infected upon challenge. Both animals treated with 40 μ g/ml (0.2 mg total) and all four control animals became infected (Table 1, animals 31–34, and Fig. 1). In contrast, only one of five macaques given b12 at 1 mg/ml (5 mg total) under the same experimental conditions became infected. Peak viremia in this animal was lower than in most controls, so the high b12 dose may have been partially protective. However, there was no evidence of partial protection in the infected animals that received lower b12 concentrations (Fig. 1).

We have shown that vaginal application of b12, the human monoclonal antibody against gp120, can protect macaques against vaginal transmission of SHIV-162P4. So far, we have challenged only with cell-free virus; whether vaginally applied b12 can inhibit the vaginal transmission of cell-associated virus remains to be addressed. The role of cell-associated virus in human HIV-1 transmission is controversial^{1,6–8,13}. However, topically applied monoclonal antibodies against the HIV envelope protein (Env) should be able to target any infectious virus released locally from infected cells. In addition, the opsonization of Env-expressing cells by such monoclonal antibodies could trigger immune responses that clear infected cells through effector mechanisms¹⁴. IgA or IgM versions of b12 might have additional advantages.

The effect of b12 within the vagina was long lasting; protection could be achieved without the need to inactivate residual, non-neutralized virus by soap lavage. Immune complexes formed *in vitro* between b12 and SHIV-162P4 were not infectious after vaginal inoculation, indicating that antibody coating of virions prevents the biological events involved in establishing infection. A neutralizing antibody could therefore inhibit transmission after virus has first been taken up by dendritic cells, whether virus-cell fusion events first occur at or close to the vaginal epithelium, or take place only some time later in a distal location^{6,8,9,15,16}.

We still observed protection by b12 when it was added to the vagina of one macaque 2 h, but not 6 h, before adding SHIV-162P4 (Table 1), although this will need to be confirmed using additional animals. The antibody probably dissipates gradually into the

Table 1 Effect of b12 treatment on SHIV transmission in macaques

	Animal no.	Treatment/Conditions	Peak Viral Load (PVL)	Day of PVL
Infectivity tests	1	SHIV+ soap (pre-mix)	<500	—
	2	SHIV+ b12 (pre-mix)	<500	—
	3	SHIV+ b12 (pre-mix)	<500	—
	4	SHIV+ b12 (pre-mix), soap at 1 h	<500	—
	5	SHIV+ b12 (pre-mix), soap at 1 h	<500	—
Controls	6	PBS + SHIV (15 min)	4.8×10^5	21
	7	PBS + SHIV (15 min)	9.3×10^5	28
	8	PBS + SHIV (15 min)	2.1×10^5	21
	9	PBS + SHIV (15 min)	2.3×10^6	21
	10	SHIV only, soap @ 1h	6.2×10^5	21
	11	SHIV only, soap @ 1h	3.7×10^5	21
	12	Irrel. MAb + SHIV (15 min), soap at 1 h	3.4×10^6	14
	13	Irrel. MAb + SHIV (15 min), soap at 7 h	2.5×10^5	14
	14	Irrel. IgG + SHIV (15 min)	2.5×10^5	14
	15	Irrel. IgG + SHIV (15 min)	<500	—
	16	HMC + SHIV (15 min)	5.5×10^5	14
	17	HMC + SHIV (15 min)	2.5×10^6	14
	18	HMC + SHIV (15 min)	1.5×10^6	21
High-dose b12	19	5 mg b12 in PBS + SHIV (15 min)	<500	—
	20	5 mg b12 in PBS + SHIV (15 min)	<500	—
	21	5 mg b12 in PBS + SHIV (15 min)	<500	—
	22	5 mg b12 in PBS + SHIV (15 min)	<500	—
	23	5 mg b12 in PBS + SHIV (15 min)	2.2×10^5	21
	24	5 mg b12 in PBS + SHIV (15 min), soap at 1 h	3.4×10^4	21
	25	5 mg b12 in PBS + SHIV (15 min), soap at 2 h	<500	—
	26	5 mg b12 in PBS + SHIV (15 min), soap at 7 h	<500	—
	27	5 mg b12 in PBS + SHIV @ 1h delay	<500	—
	28	5 mg b12 in PBS + SHIV @ 2h delay	<500	—
	29	5 mg b12 in PBS + SHIV @ 6h delay	2.3×10^4	21
	30	5 mg b12 in HMC + SHIV (15 min)	<500	—
Low-dose b12	31	5 mg b12 in HMC + SHIV (15 min)	9.6×10^4	28
	32	0.2 mg b12 in PBS + SHIV (15 min)	3.0×10^6	21
	33	0.2 mg b12 in PBS + SHIV (15 min)	2.3×10^6	21
	34	1 mg b12 in PBS + SHIV (15 min)	2.4×10^6	21
	35	1 mg b12 in PBS + SHIV (15 min)	<500	—

Except where indicated, all macaques were vaginally inoculated with SHIV-162P4. 'Soap at 1 h', '2 h' and '7 h' indicate that the vagina was washed out with 200 ml soap solution at those times after SHIV-162P4 inoculation. The term '15 min' indicates that viral challenge followed the administration of test substance by 15 min, whereas the terms '1 h', '2 h' and '6 h delay' indicate that the virus challenge was delayed proportionately longer ('1 h', for example, means 1 h 15 min after test substance). The highest plasma viremia value and the day on which it was measured are recorded. The data were generated in 3 separate experiments using the same virus and b12 stocks.

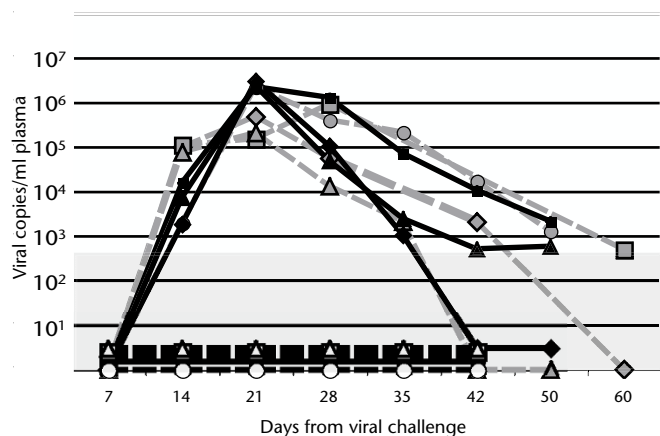


Fig. 1 Effect of vaginal b12 concentration on postinfection viral load in SHIV-162P4-challenged macaques. A 5-ml volume of either b12 in PBS or PBS only was added to the vagina for 15 min before challenge with SHIV-162P4. Plasma viremia was determined at 7, 14, 21, 28 and 42 d after infection in all animals, and through day 60 in some animals. The shaded area indicates the assay sensitivity limit (500 RNA copies/ml). Animals no. 6 (◆), no. 7 (■), no. 8 (●) and no. 9 (▲) received PBS only; animals no. 32 (◆) and no. 33 (■) received 40 µg/ml of b12 (0.2 mg total); animals no. 34 (●) and no. 35 (▲) received 200 µg/ml of b12 (1 mg total); and animals no. 19 (◇), no. 20 (□), no. 21 (△) and no. 22 (○) received 1 mg/ml (5 mg total) of b12 (see Table 1). The data in this figure were derived from 3 different experiments using the same challenge virus and b12 stocks.

vaginal tissues over a period of hours, eventually reducing its local concentration below the critical level. The half-life of IgG added passively to the mouse vagina is ~3–5 h, if losses by leakage are ignored^{17,18}. Whether longer-lasting protection can be achieved by formulating b12 differently, perhaps in an inert gel such as HMC, requires further study. The neutralizing activity of b12 *in vitro* is unaffected by the presence of 50% human seminal plasma or by exposure to pH 5.0 for several hours (R.J.S and D.R.B., unpublished observations).

Protection by b12 against vaginal transmission was dose dependent (Fig. 1; Table 1). Protection of 50% and 90% of macaques from SHIV-162P4 may require ~1 mg and >5 mg b12, respectively, delivered in 5 ml. The number of animals treated with intermediate concentrations of b12 was small, however, so the uncertainty is considerable. The 0.1% (1 mg/ml) b12 concentration is lower than the 0.5–5% solutions of microbicide candidates (Pro2000, Dextrin-2-sulphate and Carraguard) now in human efficacy trials^{1–3}. It is, however, 100- to 1,000-fold greater than is required *in vitro* to inhibit SHIV-162P4 infection of various target cells. The discrepancy may reflect, in part, the complexity of delivering a monoclonal antibody topically; a large quantity may be needed to achieve a sufficient local concentration throughout the entire lower genital tract and to penetrate fully between the numerous mucosal invaginations. Similar quantitative considerations would probably apply to various other inhibitors of Env-receptor interactions now under evaluation as microbicide candidates.

The macaques were given Depo-Provera to thin the vaginal epithelium and allow a consistently high rate of infection at a much-reduced viral dose⁴. Although this method may seem artificial, the events involved in human vaginal HIV-1 transmission are not known; it is possible that transmission occurs most often when the vaginal mucosa is naturally thin, as it is

around the luteal stage (during menses). Alternatively (but not exclusively), vaginal transmission may be associated with exposure to high viral loads in ejaculate. The SHIV-162P4 inoculum (300 TCID₅₀) corresponds to 208 infectious units *in vitro* and contains 8.6×10^7 RNA copies. The ~2 ng (1.5×10^{-14} mol) gp120 present in the inoculum can be contrasted with the 5 mg (3.3×10^{-8} mol) b12 required for consistent protection; b12 is in vast (>10⁶-fold) molar excess over virus. For any intervention strategy to be successful in humans, it should be able to counter the potential range of infectious HIV-1 challenge. The infectious inoculum to which women are exposed during heterosexual contact is highly variable, determined by various factors influencing seminal viral load^{19,20}. Most heterosexual encounters probably involve the deposition of small amounts of virus with a low probability of transmission, while relatively rare exposures to larger inocula presumably carry the greatest risk²¹. Viral loads in semen can reach as high as 1×10^7 RNA copies/ml²², although virus titers do not exceed 1×10^4 infectious units per ejaculation²³. The threshold for HIV-1 infection of a woman is also highly variable, influenced by various factors affecting epithelial integrity and inflammation²¹. However, even the high infection rates reported in Africa (transmission probability of up to 0.0041 per coital exposure)²⁴ are far lower than those in the macaque model. Information generated from macaques challenged with high (but

not absurdly so) viral inocula and a relatively low infection threshold may therefore be particularly informative for designing effective intervention strategies for human use. A candidate microbicide potent enough to work well in the macaque model, yet demonstrably safe, would be worth evaluating in humans.

For b12 to be developed as a microbicide, it would be essential, in practice, to combine it with other broadly neutralizing monoclonal antibodies. Although b12 can neutralize many primary HIV-1 isolates from multiple genetic subtypes, it is not pan-reactive^{7,14,25}. The combination of b12 with other monoclonal antibodies such as 2G12, 2F5, 4E10 and Z13, however, is likely to neutralize most known HIV-1 strains^{14,25}. We are now evaluating some of these antibodies in macaques. The practical development of monoclonal antibody combinations may be facilitated by new, much cheaper technologies for producing human proteins (as 'plantibodies' in plants, for example)²⁶. A particular advantage of neutralizing monoclonal antibodies is that they might not only reduce male-to-female HIV-1 transmission, but could also neutralize virus within vaginal secretions of an infected woman. Hence, with regular use, this approach might also reduce HIV-1 transmission from an unknowingly infected woman to her male sex partners. In addition, because monoclonal antibodies against Env will be highly unlikely to affect fertility, their use by discordant couples seeking a natural pregnancy is conceivable.

New approaches to microbicides will benefit from a more complete knowledge of the biology and virology of HIV-1 transmission. Our results support the concept of specific entry inhibitors as microbicides and provide a frame of reference for the evaluation of other such inhibitors in the macaque model. Identifying the compounds that succeed or fail in comparative studies in macaques may help identify the most plausible candidates for clinical development.

Methods

Challenge of rhesus macaques with SHIV-162P4. All studies adhered to the Guide for the Care and Use of Laboratory Animals, prepared by the US National Research Council, and were approved by the Institutional Animal Care and Use Committees of the Tulane National Primate Research Center and the Weill Medical College of Cornell University. Normal cycling, adult female rhesus macaques (*Macacca mulatta*) ranging from 4.2 to 19 years of age were used. To synchronize menstrual cycles and induce changes in the vaginal epithelium mimicking those of the luteal phase, animals were treated with a single 30 mg intramuscular injection of Depo-Provera (Pharmacia Upjohn, Kalamazoo, Michigan) for 30–33 days⁴. The macaques were then sedated with ketamine and placed in ventral recumbency with the hips slightly elevated. Five milliliters of b12 or control antibody in PBS were introduced atraumatically into the vaginal vault using a pliable French catheter. This volume provides the most effective coverage of the vaginal vault walls without undue leakage (R.S.V., unpublished observations). Except where specifically indicated, the animals were challenged 15 min later with 300 TCID₅₀ of SHIV-162P4 in 1 ml RPMI 1640 medium. In some cases, the virus was inactivated at various times after challenge by continuously lavaging the vaginal vault with 200 ml of a 10% aqueous solution of Nolvasan Surgical Scrub Solution (Fort Dodge Animal Health, Fort Dodge, Iowa), a mild antiviral soap. Blood was collected in EDTA-coated tubes every week after challenge. Plasma viremia was analyzed using the quantitative bDNA assay⁴. Infection-free status was defined as an undetectable viral load (<500 RNA copies/ml in this assay) and an inability to detect antibodies against p27 in plasma, by western blotting, throughout days 42–90 after challenge. Both parameters were always correlated; no animal with undetectable plasma viremia detectably seroconverted to p27.

Reagents. The irrelevant immunoglobulin was a pool of polyclonal human immunoglobulin prepared from the plasma of HIV-1 seronegative individuals; the irrelevant monoclonal antibody was KZ52, a human IgG1 to the Ebola virus GP glycoprotein (Scripps Research Institute). Each was added at 1 mg/ml (5 mg total).

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Competing interests statement

The authors declare competing financial interests: see the Nature Medicine website (<http://www.nature.com/naturemedicine>) for details.

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