Topically Applied Recombinant Chemokine Analogues Fully Protect Macaques from Vaginal Simian-Human Immunodeficiency Virus Challenge

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Effective strategies for preventing human immunodeficiency virus infection are urgently needed, but recent failures in key clinical trials of vaccines and microbicides highlight the need for new approaches validated in relevant animal models. Here, we show that 2 new chemokine (C-C motif) receptor 5 inhibitors, 5P12-RANTES (regulated on activation, normal T cell expressed and secreted) and 6P4-RANTES, fully protect against infection in the rhesus vaginal challenge model. These highly potent molecules, which are amenable to low-cost production, represent promising new additions to the microbicides pipeline.

The HIV/AIDS epidemic produces ~2.5 million new infections per year [1], nearly all in the developing world, where women and young girls are most at risk. The spread of the epidemic must be slowed, and several prevention strategies are being explored.

Despite 25 years of heavy investment, there are currently no plausible HIV vaccine candidates. A key clinical trial recently failed, suggesting that a lengthy series of challenging scientific problems must be solved before promising vaccine strategies can emerge [2]. Topical prevention strategies have fewer conceptual problems and could reach the clinical proof-of-principle stage sooner than vaccines. Such strategies involve substances, generally known as microbicides, that have the potential to prevent or reduce the risk of HIV transmission when applied to the genital mucosa before intercourse [3]. However, disappointing results from recent large-scale microbicide trials [4, 5] have underlined the need for new, rationally designed microbicide candidates with in vivo activity validated in an appropriate animal model [3, 6].

The HIV coreceptor CCR5 is a logical target for such strategies [3, 7]. Entry inhibitors that block CCR5 have shown promise in the rhesus vaginal challenge model [7, 8], possibly the most relevant in vivo model for preclinical evaluation of topical prevention strategies [6]. In this model, PSC-RANTES, an N-terminally modified analogue of a natural chemokine ligand of CCR5 [9], has protected all animals studied (originally 5 of 5 [7] and now 12 of 12 [R.S.V., M.M.L., R.O., D.M., and O.H., additional unpublished data]) when used at a concentration of 1 mmol/L.

PSC-RANTES is a highly potent entry inhibitor for CCR5-using HIV. Its inhibitory mechanism involves the durable intracellular sequestration of CCR5 [9]. However, because it contains nonnatural, noncoded structures, its production requires expensive chemical synthesis steps, and, despite its promising potency and efficacy, it might be impossible to produce affordably for the developing world [10].

In the food and detergent industries, microbial fermentation readily yields multiton quantities of pure, food-grade, good-manufacturing-practice proteins [11], and a fully recombinant analogue of PSC-RANTES would be amenable to such production techniques. Using a modified phage display strategy, we successfully identified 2 such analogues, which we have called 5P12-RANTES (Q^0-[G^1-P^2-P^3-L^4-M^5-A^6-T^7-Q^8-S^9]RANTES/CCL5) and 6P4-RANTES (Q^0-[G^1-P^2-P^3-G^4-D^5-I^6-V^7-L^8-A^9]RANTES/CCL5) [12]. In vitro, both show picomolar anti-HIV potency indistinguishable from that of PSC-RANTES [12]. The aim of the present study was to compare the efficacy of these 2 new molecules with that of PSC-RANTES in a standard macaque vaginal challenge model.

Methods. PSC-RANTES was produced by total chemical synthesis, as described elsewhere [9]. 5P12-RANTES and 6P4-RANTES, prepared by total chemical synthesis, were produced

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Potential conflicts of interest: O.H. is listed as the inventor on a patent application covering the new chemokine analogues described in this article; the patent is held by the Mintaka Medical Research Foundation, a nonprofit foundation registered in Geneva, Switzerland. O.H. and R.O. are cofounders of the Mintaka Foundation and serve as its chief scientific officer and chief executive officer, respectively. All other authors report no potential conflicts.

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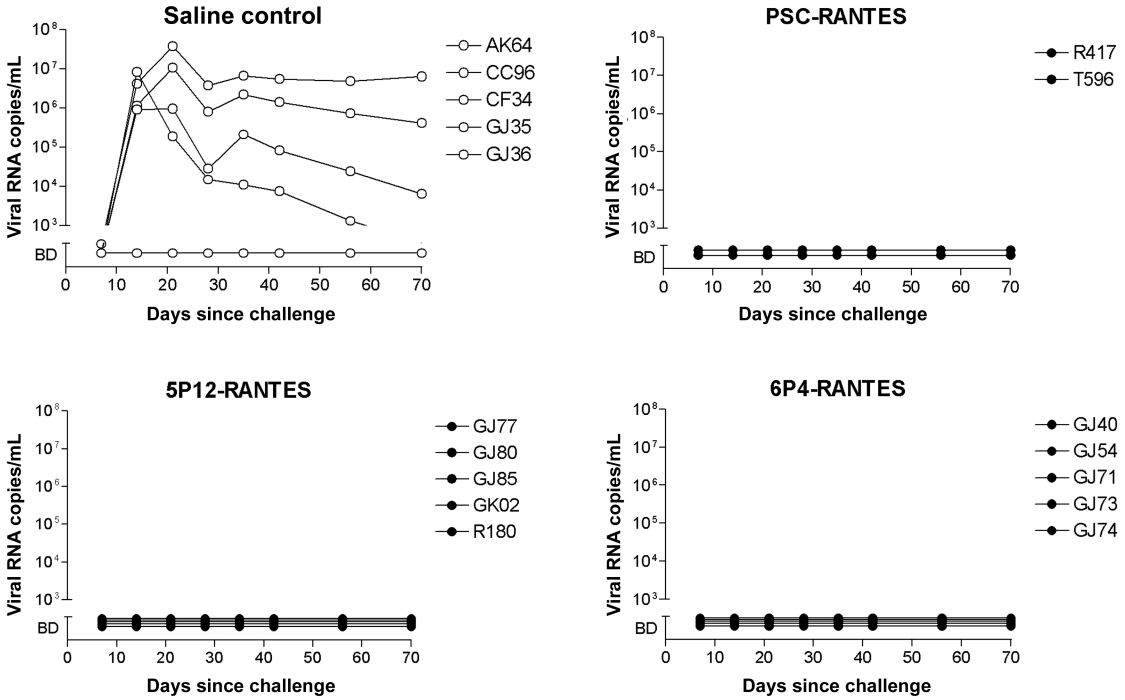

Figure 1. Full protection of macaques against vaginal simian-human immunodeficiency virus (SHIV) challenge by intravaginal pretreatment with recombinant chemokine analogues. Thirty minutes before challenge with 300 $TCID_{50}$ of SHIV162P3, animals were treated with either PBS or RANTES analogues at 1 mmol/L. They were then monitored regularly for plasma viremia for 10 weeks.

by Bachem. Before administration, analogues were made up as 1 mmol/L solutions in PBS.

Challenge experiments were performed under conditions similar to those used in other studies [7, 8, 13]. All studies adhered to the guidelines given in the *Guide for the Care and Use of Laboratory Animals* [14] and to the guidelines of the Tulane National Primate Research Center Institutional Animal Care and Use Committee. Normal-cycling adult female rhesus macaques *(Macacca mulatta)* were treated with a single 30-mg intramuscular injection of depot medroxyprogesterone acetate (Depo-Provera). After 30–33 days, they were sedated with Telazol (tiletamine plus zolazepam; Fort Dodge Animal Health) and placed in ventral recumbency with hips elevated; 4 mL of either PBS or 1 mmol/L solutions of chemokine analogues in PBS was introduced without trauma into the vaginal vault, using a pliable French catheter. The animals were challenged 30 min later with 300 $TCID_{50}$ of simian-human immunodeficiency virus (SHIV) SF162P3, obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, in 1 mL of RPMI 1640.

Blood was collected in EDTA tubes every week after challenge for 70 days. Plasma viral levels were determined by quantifying simian immunodeficiency virus (SIV) *gag* RNA with a real-time reverse-transcription polymerase chain reaction (PCR) assay, as described elsewhere [15]. The assay has a sensitivity threshold of 5 RNA copies per PCR, or 30 RNA copies/mL of plasma for the standard volume tested (0.5 mL). Infection-free status was defined as a consistently undetectable plasma viremia for all of the analyses, and it was confirmed by monitoring for antibody seroconversion with Western blot analysis (ZeptoMetrix SIV Western Blot Kit) [16]. In addition, a PCR assay was performed on peripheral blood mononuclear cells (PBMCs) to detect proviral genomes. Genomic DNA was extracted from PBMCs isolated from EDTA-anticoagulated blood. SIV proviral DNA was detected by nested PCR using SIVmac-specific *gag* primers, as follows: for the first round, PF1 (5'-AGGAACCAACCACGACGGAG-3') and PR1 (5'-AAAGGGATTGGCACTGGTGCGAGG-3'; for the second round, PF2 (5'-TCCGTCTTGTCAGGGAAGAAAGCA-3') and PR2 (5'-ATGCACCAGATGACGCAGACAGTA-3'). First-round PCR was performed using ~0.5–1 μg of genomic DNA, with one-tenth of the product used for the second round.

Results. We compared the in vivo efficacy of 5P12-RANTES and 6P4-RANTES with that of PSC-RANTES in a standard macaque vaginal challenge model [7, 8, 13]. Animals received either PBS or RANTES analogues at 1 mmol/L in PBS, 30 min before challenge with 300 $TCID_{50}$ of SHIV162P3. They were then monitored weekly for plasma viremia for 10 weeks (figure 1). Although 4 of 5 control macaques became infected, all of the treated macaques were completely protected. For each analogue, protection was significant ($P < .05$; Fisher's exact test). Viremia was not detected at any time; no antiviral antibodies were found in serum by Western blot analysis 70 days after challenge, and no proviral DNA was detected in PBMCs by PCR 320 days after

challenge. Hence, 5P12-RANTES and 6P4-RANTES fully match the efficacy of PSC-RANTES in vivo.

Discussion. In addition to being potent, being effective in a relevant animal model, and presenting no obvious safety issues, candidate microbicides must also show adequate stability and be suitable for manufacture at a cost and scale appropriate for worldwide use [3, 10]. 5P12-RANTES and 6P4-RANTES show promising stability at elevated temperatures and low pH and after incubation with human cervicovaginal lavage samples [17]. Importantly, taking 5P12-RANTES as an example, we have achieved successful production of pure, authentic material via microbial fermentation (R.O., O.H., H. Gaertner, and F. Cerini, unpublished data).

We chose to test both 5P12-RANTES and 6P4-RANTES, because they differ slightly in their pharmacological properties and it is not yet clear which profile would be best for a candidate microbicide. Although 6P4-RANTES resembles PSC-RANTES in that it is a CCR5 agonist that induces intracellular sequestration of the receptor, 5P12-RANTES neither internalizes nor (as judged by calcium flux measurements) activates CCR5 [12]. Receptor internalization may afford prolonged protection of target cells after a single dose and provide a strong barrier to generating resistant escape mutants. However, CCR5 activation could induce inflammation [18], a known risk factor for HIV transmission.

More work will now be required to determine which of these new molecules is most suitable for further development. These could include safety studies in macaques involving chronic exposure of the vaginal lumen and further efficacy studies using more-virulent SIVmac isolates. The conclusion of this preliminary study is that both molecules meet the criteria for addition to the microbicide pipeline, and priority should be given to evaluating them as promising topical strategies for the prevention of HIV infection.

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