Prevention of Vaginal SHIV Transmission in Rhesus Macaques Through Inhibition of CCR5

Michael M. Lederman,[1]*† Ronald S. Veazey,[2]* Robin Offord,[3]* Donald E. Mosier,[4] Jason Dufour,[2] Megan Mefford,[2] Michael Piatak Jr.,[5] Jeffrey D. Lifson,[5] Janelle R. Salkowitz,[1] Benigno Rodriguez,[1] Andrew Blauvelt,[6]‡ Oliver Hartley[3]

Topical agents, such as microbicides, that can protect against human immunodeficiency virus (HIV) transmission are urgently needed. Using a chimeric simian/human immunodeficiency virus (SHIV SF162), which is tropic for the chemokine receptor CCR5, we report that topical application of high doses of PSC-RANTES, an amino terminus–modified analog of the chemokine RANTES, provided potent protection against vaginal challenge in rhesus macaques. These experimental findings have potentially important implications for understanding vaginal transmission of HIV and the design of strategies for its prevention.

Because the vast majority of HIV infections are acquired via transmission across mucosal surfaces, strategies to prevent mucosal transmission are urgently needed. Unfortunately, the mechanisms whereby HIV gains entry at mucosal sites, especially vaginal sites of infection, are incompletely understood. Thus, there is no uniform agreement regarding the critical host cellular and molecular targets during infection after vaginal exposure, and resolution of these issues is needed for the design of plausible microbicide strategies to prevent mucosally acquired HIV infection.

The chemokine receptor CCR5 serves as an essential cofactor for HIV entry and acquisition of infection. Thus, persons whose cells lack surface CCR5 expression because of mutation are almost completely protected from acquiring HIV infection (*1*–*5*). Furthermore, viruses that utilize CCR5 predominate in early stages of mucosal transmission (*6*, *7*), which suggests that mucosal transmission may selectively involve CCR5. Hence, inhibition of CCR5 has been proposed as a possible "microbicide" strategy for prevention of HIV infection.

[1]Department of Medicine, Case Western Reserve University, University Hospitals, 2061 Cornell Road, Cleveland, OH 44106, USA. [2]Tulane National Primate Research Center, 18703 Three Rivers Road, Covington, LA 70433, USA. [3]Department of Structural Biology and Bioinformatics, Faculty of Medicine, University of Geneva, 1211 Geneva 4, Switzerland. [4]Scripps Research Institute, 10550 Torrey Pines Road, La Jolla, CA 92037, USA. [5]AIDS Vaccine Program, SAIC Frederick, Inc., National Cancer Institute, Frederick, MD 21702-1201, USA. [6]Dermatology Branch, National Cancer Institute, Bethesda, MD 20892–1908, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: MXL6@case.edu
‡Present address: Department of Dermatology, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA.

However, HIV is able to use other host cell factors that are present at mucosal sites to achieve or to facilitate infection (*6*–*12*). These findings have led to some debate about the importance of CCR5 for infection across mucosae, as well as concern that targeting CCR5 alone may be inadequate to prevent transvaginal HIV transmission (*13*).

We previously described the synthesis of an amino terminus–modified form of the chemokine RANTES, the aminooxypentane oxime of [glyoxylyl[1]]RANTES [2-68], known as AOP-RANTES (*14*). This compound is significantly more potent at inhibiting HIV-1 replication than the parent chemokine. Subsequently, a series of amino-terminally modified RANTES analogs have been developed and tested (*15*–*20*) in an effort to improve potency and durability of HIV inhibitory activity.

AOP-RANTES blocked in vitro propagation of multiple CCR5-using HIV isolates representing clades A to F (*17*) with inhibitory concentrations in the nanomolar range. Pretreatment of hu-PBL-SCID chimeras (mice with severe combined immunodeficiency disease reconstituted with human peripheral blood lymphocytes) with another more potent RANTES analog, N^{α}-(*n*-nonanoyl)-*des*-Ser[1]-RANTES (known as NNY-RANTES) protected animals from parenteral HIV challenge, although in some, escape with both R5- and X4-using viruses was demonstrated (*15*).

A third analog, N^{α}-(*n*-nonanoyl)-*des*-Ser[1]-[L-thioproline[2], L-α-cyclohexyl-glycine[3]]RANTES (PSC-RANTES) represents a new RANTES analog chemically identical to native RANTES except for the substitution of a nonanoyl group, thioproline, and cyclohexylglycine for the first three N-terminal amino acids of the native protein (Fig. 1). PSC-RANTES has more potent in vitro antiviral activity than earlier analogs, with inhibitory concentrations for some HIV-1 isolates in the picomolar range (*21*). The induction of receptor internalization and down-modulation on binding by chemokines is believed to play a major role in their anti-HIV action (*22*), although some contribution from competitive binding cannot be excluded (*23*, *24*). Our RANTES analogs show a particularly enhanced capacity to induce such internalization and down-modulation, and this may be the basis of their potent anti-HIV activity (*18*, *25*, *26*).

We first confirmed that PSC-RANTES inhibited propagation of the SHIV SF162 R5-tropic virus in rhesus peripheral blood mononuclear cells (PBMCs) and completely blocked SHIV SF162 replication, with median inhibitory concentration (IC_{50}) values in the subnanomolar range [Fig. 2; (*27*)]. Furthermore, PSC-RANTES caused down-modulation of macaque CCR5 (Fig. 2). After only 15 min of exposure, the decrease in CCR5 levels on both $CD4^-$ and $CD4^+$ peripheral blood cells was already maximal [>90%; (*28*)].

To examine PSC-RANTES' ability to prevent acquisition of SHIV infection at a mucosal site, 30 progesterone-treated (*27*) adult female rhesus macaques were pretreated with 4 ml PSC-RANTES at the indicated concentrations or with phosphate-buffered saline (PBS). The animals were subsequently challenged with a high multiplicity [300 $TCID_{50}$ (median tissue culture infectious dose)] of SHIV SF162 and monitored for up to 24 weeks for the development of plasma viremia (*29*). All five animals treated with the highest dose (1 mM) of PSC-RANTES were protected from SHIV infection, with no detectable viremia for the entire duration of follow-up (Fig. 3). Lower doses also proved protective, with four out of five animals treated with 330 μM and three out of five treated with 100 μM PSC-RANTES also showing protection from infection. One of five animals treated with

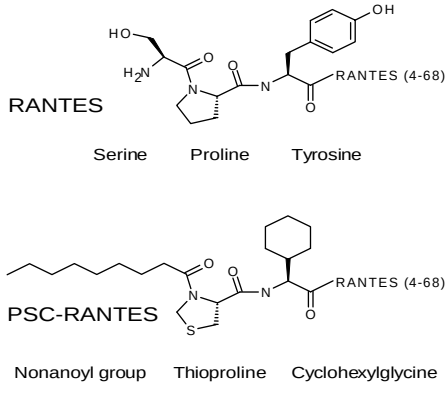

Fig. 1. Structure of native RANTES and PSC-RANTES.

33 μM PSC-RANTES and two of five animals treated with 10 μM or less were protected. Overall, 12 out of 15 animals (80%) pretreated with ≥100 μM were protected from infection, whereas only 4 out of 15 animals (27%) pretreated with <100 μM or placebo showed protection [P = 0.009, Fisher's exact test; risk ratio (RR) = 0.27, 95% confidence interval (CI), 0.09 to 0.78 (*27*)]. There was a significant dose-effect relationship when considering the whole range of dosing levels (P = 0.0048, Cochran-Armitage exact trend test). Based on exact logistic regression modeling, we estimated that in this system a 10-fold increase in PSC-RANTES dose was associated with an odds ratio (OR) of infection of 0.39 [95% CI, 0.17 to 0.82], (P = 0.035).

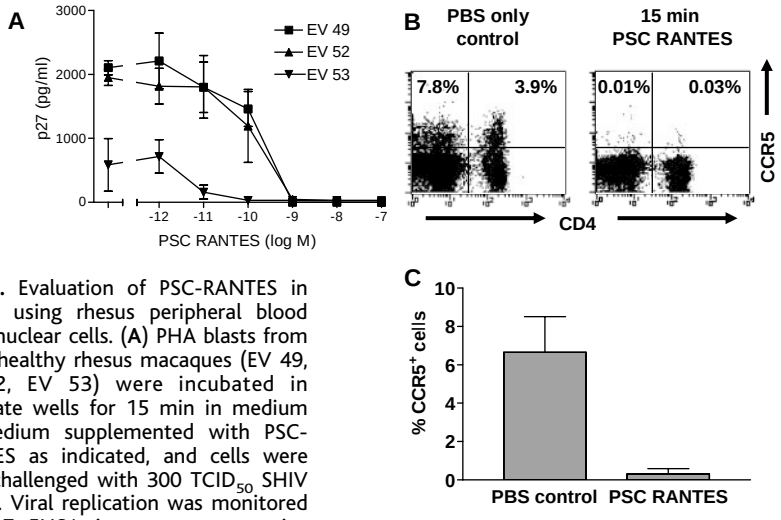

Fig. 2. Evaluation of PSC-RANTES in assays using rhesus peripheral blood mononuclear cells. (**A**) PHA blasts from three healthy rhesus macaques (EV 49, EV 52, EV 53) were incubated in triplicate wells for 15 min in medium or medium supplemented with PSC-RANTES as indicated, and cells were then challenged with 300 $TCID_{50}$ SHIV SF162. Viral replication was monitored by p27 ELISA in supernatant twice weekly and found to peak at day 11. Data shown are mean p27 levels at day 11. Error bars indicate SEM. (**B**) Whole blood was incubated with 10 μM PSC-RANTES in PBS or PBS control for 15 min. Surface expression of CCR5 was examined by flow cytometry on $CD4^-$ and $CD4^+$ peripheral blood cells. (**C**) Summary mean (standard deviation) percentages of peripheral blood cells expressing CCR5 in five separate experiments in the presence of PBS control or PSC-RANTES.

Plasma samples obtained at week 11 from all protected animals and four infected animals were tested for the presence of antibodies to simian immunodeficiency virus (SIV) proteins by Western blot. All four infected animal plasmas had strong bands corresponding to p17, p27, p55, and p66, whereas no positive bands were found in any of the protected animals (*27*). Plasma levels of PSC-RANTES were measured in samples obtained at intervals (1 hour, 4 hours, and 24 hours) after intravaginal administration of the maximum concentration used (1 mM). PSC-RANTES was undetectable (lower than the 300 pM limit of assay sensitivity) in all samples tested (*30*).

We have shown that PSC-RANTES, targeting CCR5 alone, protected rhesus macaques from intravaginal exposure to a chimeric SHIV containing an R5-tropic envelope of HIV-1. Thus, pursuing a strategy that targets this receptor seems reasonable for development. However, the concentrations used in the highest dose group (1 mM), exceed by orders of magnitude the subnanomolar IC_{50}

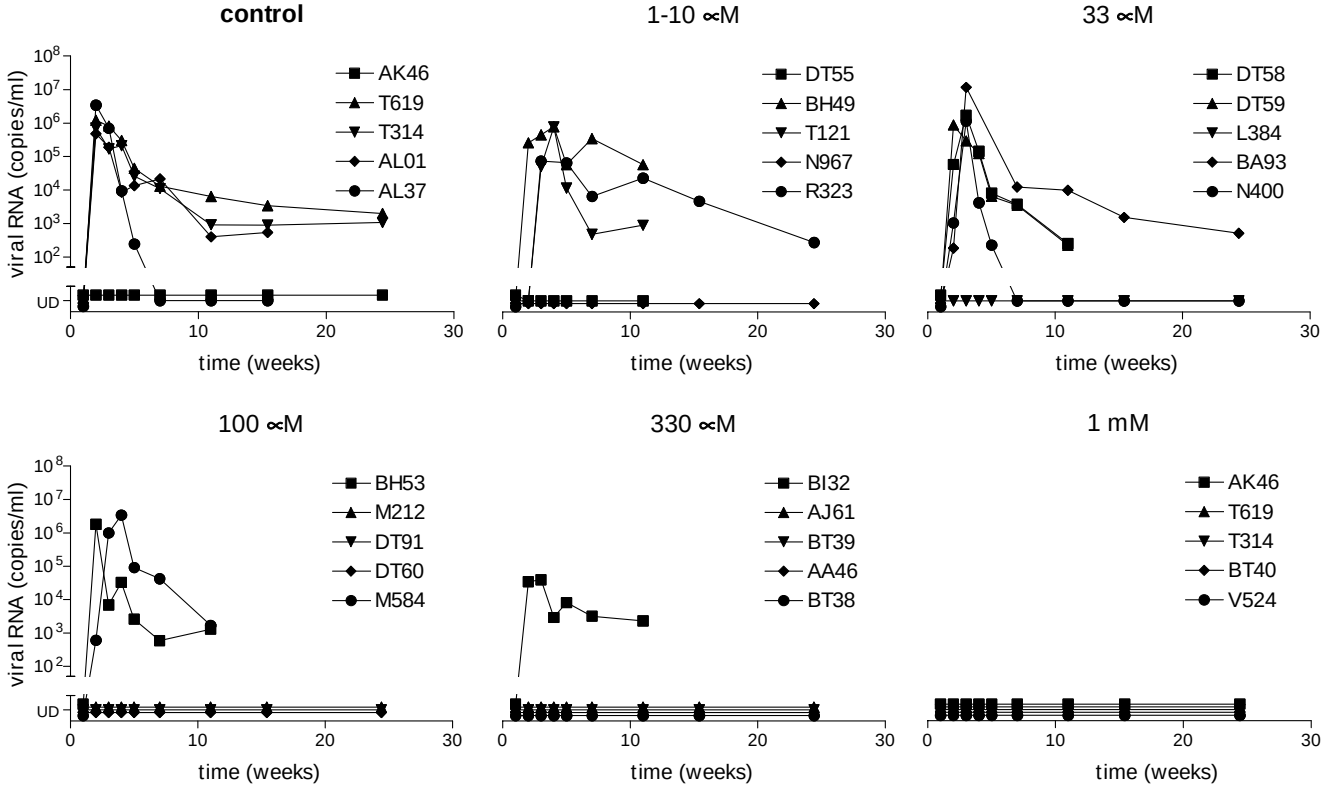

Fig. 3. Topical application of PSC-RANTES blocks infection by SHIV SF162. Six groups of five progesterone-treated rhesus macaques were anesthetized, treated intravaginally with 4 ml PBS control or PSC-RANTES at concentrations from 1 μM to 1 mM in 4 ml PBS, and then challenged 15 min later with 300 $TCID_{50}$ of SHIV SF162. Plasma was monitored as indicated for SHIV RNA by real-time reverse transcription polymerase chain reaction (RT-PCR). UD, undetectable (i.e., below 60 copies per ml).

of our agent against SHIV SF162. Recently, in the same animal model, the neutralizing antibody IgG1-b12 (*31*) gave partial protection against vaginal transmission of SHIV, also at concentrations vastly in excess of those needed in vitro. A small-molecule CCR5 inhibitor, highly potent in vitro, gave only minimal protection in the animal system used here, even as a virtually saturated solution (*32*). Cyanovirin partially inhibited vaginal transmission of a SHIV isolate that also targets CXCR4 but only at concentrations ~10,000 times those required for full inhibition in vitro (*33*). Possible explanations for these dose disparities include incomplete distribution, failure to penetrate to hypothetical submucosal target sites, nonspecific adsorption to mucosal surfaces, or degradation or inhibition by vaginal factors. But conceivably, the explanation might simply be that the progesterone treatment and the dose of SHIV that we and others use to ensure near-universal infection of control macaques [28 of 31 in this system (*28*, *31*, *32*)] constitute an extraordinary challenge. In the natural human setting, risk for acquisition of HIV infection after sexual exposure, although probably not uniform, is on average markedly lower than that shown by unprotected controls in this animal system (*34*). Given the discrepancy between in vitro and in vivo potency, we offer our findings as a proof of principle and as a direction for attempts to render the approach economically acceptable.

PSC-RANTES protected macaques from intravaginal challenge without detectable toxicity or histological changes. Consequently, further development of this and related compounds, either alone or in combination with other agents, and improvement of their formulation are reasonable subjects for further study as an approach to the prevention of sexual transmission of HIV.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5695/485/DC1
Materials and Methods
References and Notes

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Cleavage of proBDNF by tPA/Plasmin Is Essential for Long-Term Hippocampal Plasticity

Petti T. Pang,[1,3] Henry K. Teng,[2] Eugene Zaitsev,[1] Newton T. Woo,[1] Kazuko Sakata,[1] Shushuang Zhen,[2] Kenneth K. Teng,[2] Wing-Ho Yung,[3] Barbara L. Hempstead,[2] Bai Lu[1]*

Long-term memory is thought to be mediated by protein synthesis–dependent, late-phase long-term potentiation (L-LTP). Two secretory proteins, tissue plasminogen activator (tPA) and brain-derived neurotrophic factor (BDNF), have been implicated in this process, but their relationship is unclear. Here we report that tPA, by activating the extracellular protease plasmin, converts the precursor proBDNF to the mature BDNF (mBDNF), and that such conversion is critical for L-LTP expression in mouse hippocampus. Moreover, application of mBDNF is sufficient to rescue L-LTP when protein synthesis is inhibited, which suggests that mBDNF is a key protein synthesis product for L-LTP expression.

Long-lasting changes in synaptic efficacy are thought to mediate long-term memory (*1*, *2*). A well-studied model system is the late phase of long-term potentiation (L-LTP) in the hippocampus. Unlike the early phase of LTP (E-LTP), L-LTP requires new protein synthesis and involves synaptic growth (*2*). A key molecule implicated in L-LTP is the secretory protein BDNF. Hippocampal slices from BDNF heterozygous ($BDNF^{+/-}$) mice fail to exhibit L-LTP (*3*). Inhibition of BDNF signaling by blocking its receptor TrkB with antibody to TrkB or with BDNF scavenger TrkB–immunoglobulin G also inhibits L-LTP (*4*, *5*). In the mammalian brain, BDNF is synthesized as a precursor called proBDNF, which is proteolytically cleaved to generate mature BDNF (mBDNF), the form of BDNF that binds to and activates TrkB (*6*, *7*). In cultured hippocampal neurons and in heterologous cells, proBDNF accounts for a substantial proportion of total BDNF secreted extracellularly (*8*–*11*). A recent study suggests that the precursor and mature forms of neurotrophins interact with very different receptor/signaling systems to induce opposing biological effects (*12*). If a similar principle could be applied to the hippocampus, extracellular cleavage of proBDNF at synapses may have profound implications for synaptic modulation (*7*).

One molecule that may play a role in the conversion of proBDNF to its mature form is the extracellular serine protease tPA. Several studies have implicated tPA in the expression of long-lasting forms of synaptic plasticity: Induction of L-LTP enhances the expression of tPA in the hippocampus (*13*), tPA can be secreted from neuronal growth cones and

[1]Section on Neural Development and Plasticity, Laboratory of Cellular and Synaptic Neurophysiology, National Institute of Child Health and Human Development (NICHD), Bethesda, MD 20892, USA. [2]Division of Hematology, Department of Medicine, Weill Medical College of Cornell University, New York, NY 10021, USA. [3]Department of Physiology, Faculty of Medicine, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

*To whom correspondence should be addressed. E-mail: bailu@mail.nih.gov