Microbicides and other topical strategies to prevent vaginal transmission of HIV

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Abstract | The HIV epidemic is, by many criteria, the worst outbreak of infectious disease in history. The rate of new infections is now ~5 million per year, mainly in the developing world, and is increasing. Women are now substantially more at risk of infection with HIV than men. With no cure or effective vaccine in sight, a huge effort is required to develop topical agents (often called microbicides) that, applied to the vaginal mucosa, would prevent infection of these high-risk individuals. We discuss the targets for topical agents that have been identified by studies of the biology of HIV infection and provide an overview of the progress towards the development of a usable agent.

According to the World Health Organization[1], almost 40 million people were living with HIV at the end of 2004, a year in which 4.9 million people were newly infected and 3.1 million died of AIDS-related diseases. Most new infections are occurring in the developing world, where women are most vulnerable. In sub-Saharan Africa, for example, 57% of people living with HIV are women, and young women between 15 and 24 years old are at least three times more likely to be HIV positive than young men[2].

The devastating effects of this epidemic have brought together governments, international agencies, clinicians and scientists to explore the widest possible range of approaches for bringing the epidemic under control. However, until recently, the mainstream pharmaceutical industry was strikingly underrepresented in this partnership. To the extent that this unacceptable weakness in the world's response to HIV is due to economic reasons, it is the urgent duty not only of the pharmaceutical industry but also of industrialized nations as a whole to find a way to remedy this. Political approaches to curbing the HIV epidemic in the developing world include encouraging safer behaviour, such as the use of condoms, circumcision[3] and increased HIV testing. Broader provision of antiretroviral drugs to people infected with HIV, with consequent reductions in their HIV levels, should also help limit transmission of the virus and thereby help limit the number of new infections.

There are no candidate vaccines in the pipeline that can induce sterilizing immunity and protect against infection with HIV. Although the search for the ideal vaccine continues, expectations have had to be lowered and many vaccine strategies are now aiming only for attenuation of HIV replication in immunized people. The hope is that lower concentrations of HIV in genital secretions of immunized, infected people will limit the transmission of HIV to others. Therefore, there is an urgent need for what have become known as 'microbicides' — topically applied agents that prevent HIV transmission from person to person[4]. A successful microbicide would help to contain the epidemic until an effective vaccine becomes available, and could be used in parallel with vaccines as they come into use. In this Review, we mostly use the term 'topical prevention strategies' because many of the most promising agents are not microbicidal in the literal sense (that is, they do not kill microorganisms but they might prevent HIV transmission). Hopes for a single, safe microbicide or topical prevention strategy that would protect against infection with HIV and other sexually transmissible pathogens seem unlikely to be realized, because such a broad spectrum of activity has been achieved only by membrane-disrupting detergents that are also toxic to host cells. In our view, the microbicides that are most likely to be effective in preventing HIV infection will probably have to target only HIV.

Condoms can provide excellent protection against HIV and other sexually transmissible infections. But, in many regions of high HIV incidence, men can be reluctant to use condoms with regular partners because condom use implies that one, or both, partner(s) is unfaithful[5]. Similarly, there is reluctance in many societies to adopt male circumcision even though there is

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Box 1 | **Models to evaluate candidate topical prevention strategies**

***In vitro* and *ex vivo* culture systems**

In vitro testing of potential agents for protection from productive HIV infection using cultures of human blood or epidermal Langerhans cells[120] is a reasonable screening strategy. By contrast, this might not be suitable for agents or formulations, such as gels or buffering agents, that are incompatible with cell-culture conditions. Ectocervical explant models[121,122], however, provide a surface on which gels can be applied and can help evaluate the interactions among different cell types mediating susceptibility to or protection from infection with HIV. Tonsil histocultures have also been used as a model for examining the effects of candidate agents on virus dissemination to local lymphoid tissue[123]. Nonetheless, these models have limitations[124], including variable non-physiological permeability to virus, loss of cell and tissue viability and function after excision, and lack of sustained interaction with systemic elements found *in vivo*.

Small-animal models

Some of the concerns regarding *in vitro* and *ex vivo* models can be addressed by *in vivo* vaginal HIV challenge of severe combined immunodeficient (SCID) mice reconstituted with human peripheral-blood mononuclear cells[125,126]. But uncertainty remains as to whether the mouse vaginal mucosal environment models the key elements that restrict or promote human vaginal HIV transmission. Recently, Kish *et al.* engrafted human vaginal tissue onto non-obese diabetic SCID mice reconstituted with human blood cells and found that cell-associated, but not cell-free, virus could infect these animals[127,128]. More work is needed on this model to assess its utility as a method to test the activity of candidate microbicides.

Non-human primates

Non-human primates have been used extensively to test the ability of candidate microbicides to protect against infection with simian immunodeficiency virus or a chimeric simian–human immunodeficiency virus[75,107,129]. This is the most similar model (but might not be identical) to the human system. Progestins are often given to the animals to thin the vaginal epithelium and sufficient quantities of virus are applied to assure that infection is nearly universal. This is a severe test, but given the probable importance of amplified transmission on the HIV/AIDS epidemic, and as discussed in the main text, challenge models should have a high probability of transmission, and strategies conferring high-level protection in such models should be the goal.

now increasing evidence to indicate that it reduces the probability of HIV transmission[6]. Therefore, these social issues provide another reason why the development of topical strategies that women can use to protect themselves from infection with HIV is such a high priority. In this Review, we discuss the rationale for developing topical prevention strategies, the potential targets for different strategies, the approaches that are currently under evaluation and future perspectives in the field. The situation is so desperate that no potentially promising solution should be discarded hastily, but this does not justify the consumption of large quantities of economic or human resources, or engaging populations in clinical trials when the chances of success are remote or there is an appreciable risk of causing harm.

How does HIV breach mucosal defences?

To develop effective topical prevention strategies, we must understand the biology of HIV transmission. Much of our understanding has come from HIV epidemiology, from studies of human tissue explants, from a few *in vivo* human studies and from studies on non-human primates challenged with either simian immunodeficiency virus (SIV) or a chimeric simian–human immunodeficiency virus (SHIV) — which in this context means SIV within the HIV envelope. Although studies using non-human primates have proven valuable, our incomplete understanding of HIV transmission and how to protect against infection means that there is no guarantee that lessons learned in these and other experimental systems will apply precisely to protective strategies in humans. Therefore, the concurrent exploitation of existing models of HIV infection (BOX 1), the development of newer models and, ultimately, clinical trials in humans are required to develop effective topical strategies to prevent HIV transmission.

Vaginal mucosal tissue and natural barriers to infection. Vaginal transmission of HIV through sex can involve transmission by vaginal or cervical routes (FIG. 1). The chance of this occurring is usually relatively low because both sites constitute natural obstacles to infection, but the risk of infection is markedly increased when these natural barriers are compromised. The intact vaginal mucosa is a multicellular layer of squamous epithelial cells, which has not been shown to be infected *in situ* and is unlikely to promote transcytosis of virus to submucosal sites after *in vivo* exposure (reviewed in REF. 7) (FIG. 1). However, HIV applied to ectocervical explants might permeate below the most superficial layer of epithelial cells[8]. Features of cervicovaginal secretions, such as their low pH and their hydrogen peroxide (H_2O_2) content, provide broad antimicrobial activity[9,10] (FIG. 2). Disruption of the integrity of the vaginal mucosa, such as occurs in dry[11] or traumatic sex[12,13], or if there is bacterial vaginosis[14,15], or inflammatory or ulcerative disease of the vagina[16], heightens the risk of HIV transmission (FIG. 2). Furthermore, although the squamous epithelium of the vagina can be thickened by oestrogens[17] it can be thinned by progestins[18], which decrease[17] and heighten[18] the risk of infection with SIV, respectively. Despite the presence of these natural barriers to infection, studies in macaques that have had their uterus and cervix removed indicate that SIV can be transmitted through the squamous epithelium of the vagina[19,20].

Although the columnar endocervical epithelium is thought to be able to support HIV transmission, the endocervix is protected by a thick mucous plug that provides a physical barrier to HIV transmission[20] (FIG. 2). However, cervical ectopy is associated with an increased risk of HIV transmission[21]. In this condition, which is most common in younger women, the transition between endocervical columnar epithelium and ectocervical squamous epithelium does not occur at the cervical orifice but is extended outward along the ectocervix beyond the mucous plug. The columnar epithelium is only one cell layer thick and is thought to be particularly susceptible to HIV transmission if unprotected by the mucous plug.

Target cells implicated in transmission. Semen-borne virus exists in both cell-free form and within infected leukocytes, but cell-free virus seems to be substantially more infectious[22]. Therefore, targeting intralumenal free virus or its cellular targets should be more effective clinically than the more complex strategies needed to eliminate virus contained within infected leukocytes in the semen. Within an hour of vaginal exposure, SIV enters the mucosa, where Langerhans cells are the main

Severe combined immunodeficienct mice
Mice with this defect in their immune system do not have B or T cells and can, therefore, accept cells from another species without rejection.

Squamous epithelial cell
A flattened epithelial cell that, in layers that are several cells thick, comprises the surface of the skin, vagina, penis, mouth and anus.

Transcytosis
The process of transport of material across an epithelium by uptake on one side of the cell into a coated vesicle, which can then be sorted through the *trans*-Golgi network and transported to the opposite side of the cell.

Progestins
Synthetic steroid hormones with progesterone-like activity. Progesterone is an ovarian hormone that prepares the endometrium (the lining of the uterus) to receive the fertilized egg and sustain pregnancy.

Endocervix
The internal canal and internal surfaces of the uterine cervix.

Discordant couple
Sexual partners in a long-term relationship wherein one is HIV infected and the other is not.

At-risk exposure
Any exposure, either through sex or through contact with blood or blood products that places an uninfected person at risk of acquiring HIV infection.

High-risk seronegative individual
A person who remains uninfected despite numerous at-risk exposures.

cell type to become infected[23] (FIG. 1). Tears in the vaginal mucosa probably promote viral access to submucosal T cells, macrophages and dendritic cells (DCs) other than Langerhans cells, and therefore increase the chance of infection. Nonetheless, virus traversing the mucous layer can probably reach directly the dendritic projections of Langerhans cells that are thought to extend to, or near, the lumenal surface[7,22]. Thereafter, small foci of infected mucosal $CD4^+$ T cells can be found[20], and these cells are probably the source of viral dissemination following the initial infection[24].

Epidemiology: amplified transmission. The estimated risk of heterosexual HIV transmission in discordant couples is approximately 3–50 per 10,000 'at-risk' exposures[16]. This number seems too low to account for the rapid spread of the epidemic and has led to a model of 'amplified HIV transmission' being proposed[25]. In this model, transient periods in which high levels of viraemia occur in the infected partner, or compromised mucosal integrity and/or mucosal defences occur in either participant, contribute disproportionately to the risk of HIV transmission, and therefore to the spread of the epidemic. There is clinical evidence to support this model, with documentation of high-level viraemia[26] and high-frequency transmission[27] during acute HIV infection, and high frequencies of concurrent inflammatory or ulcerative sexually transmitted infections in high HIV incidence regions[26]. Amplified transmission might well be the factor driving the HIV/AIDS pandemic, and this should be taken into account when developing preventive strategies (see later).

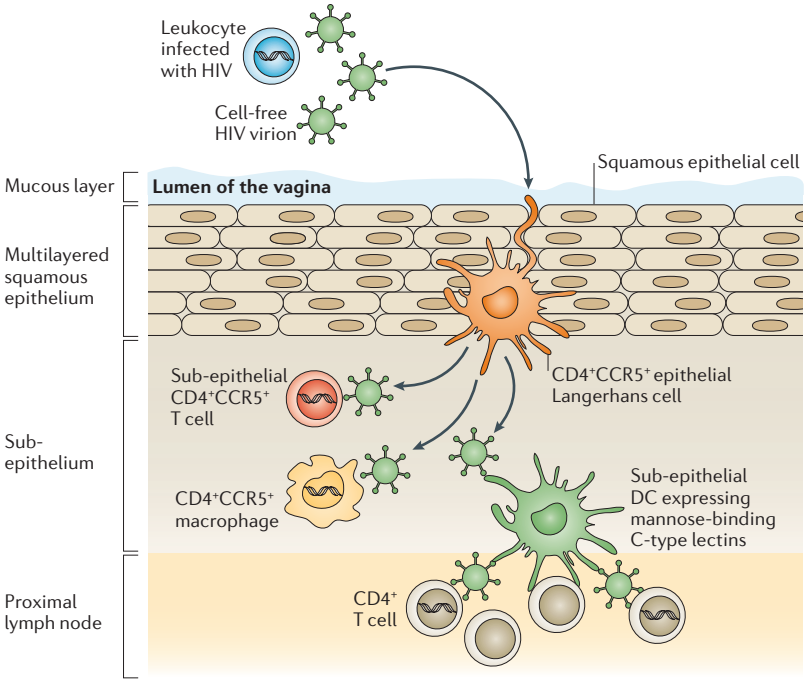

Figure 1 | **Vaginal acquisition of HIV infection.** The normal vaginal epithelium comprises a multi-cellular layer of stratified squamous epithelial cells. Cell-free virus can gain access to Langerhans cells (intra-epithelial $CD4^+$ CC-chemokine receptor 5 $(CCR5)^+$ dendritic cells (DCs)) with dendritic surfaces that might extend near to, or into, the mucosal lumen. It is more difficult for cell-associated virus breach this barrier. Within hours to a day after exposure, $CD4^+$ T cells in the sub-epithelium are infected as might be sub-epithelial macrophages and DCs expressing mannose-binding C-type lectins. Shortly thereafter, virus also can be found in nearby lymphoid tissues.

Removal or blockade of CCR5 protects against HIV infection. HIV isolates that use CC-chemokine receptor 5 (CCR5) as a co-receptor for entry into host cells are known as R5 isolates, those using CXC-chemokine receptor 4 (CXCR4) are known as X4 isolates, and those that use both are known as R5X4 isolates. All three isolates are found in people infected with HIV, but for reasons that are not clear, R5 isolates almost always predominate in early infection[28]. The availability of these co-receptors on different target cells might explain this phenomenon. For example, in the epidermis Langerhans cells characteristically express CCR5 but not CXCR4 (REF. 29). Another possible explanation for the predominance of R5 isolates in early infection is that locally expressed CXC-chemokine ligand 12 (CXCL12; also known as SDF1), which promotes CXCR4 internalization[30], and locally expressed β-defensins, which have also been reported in one study to induce CXCR4 internalization[31], might limit infection by X4 viruses (FIG. 2). Importantly, R5 viruses predominate in early infection irrespective of the route of transmission[28]. Therefore, there must be factors limiting infection by X4 viruses at all sites of HIV acquisition or replication or both; alternatively there might be other reasons to explain the dominance of R5 viruses in early infection.

The extent to which HIV depends on functional CCR5 for transmission is underlined by the experience of people with a 32-base-pair deletion in the coding region of *CCR5* (known as the $CCR5^{\Delta 32}$ allele), which results in a truncated protein that is not expressed on the cell surface (FIG. 2). Although this *CCR5* allele is almost absent among people of Asian or African origin[32], ~1% of Caucasians are homozygous for this allele ($CCR5^{\Delta 32/\Delta 32}$) and they are almost completely protected from infection with HIV[33,34]. Therefore, cell-surface expression of CCR5 seems to be necessary for HIV acquisition and a reduction in the availability of CCR5 should provide protection. In support of this, several studies among people who are at high risk of HIV infection but who remain uninfected, so-called high-risk seronegative (HRSN) individuals, found increased expression of one or more CCR5 ligand(s), CC-chemokine ligand 3 (CCL3; also known as MIP1α), CCL4 (also known as MIP1β) and CCL5 (also known as RANTES)[35–38]. Other similar studies did not find this increase [39–41], however a large cross-sectional study among several populations found that people with more copies of the *CCL3* gene were at lower risk for HIV infection[42].

Other potential protective mechanisms. Study of HRSN individuals[43], should help us to better understand the mechanisms of HIV transmission and also might help guide the design of prevention strategies. Although genetic factors that might limit viral access to CCR5 (see earlier) provide some explanation for the seronegative status of these individuals, it should be noted that for many people in HRSN cohorts[40,41], there is no identified

Cross-sectional study
A clinical study describing a group of people at a single time point or brief time period.

or even putative mechanism to explain this protection against HIV infection. Therefore, we anticipate that there are as-yet-unidentified factors that confer high-level protection, or that combinations of factors — each providing modest protection against infection — protect many HRSN individuals. Identification of these potentially protective factors is a priority as it is possible that they could be exploited to develop topical prevention strategies. Whereas some investigators have found HIV-specific IgA in cervicovaginal secretions of HRSN women[44–47] and HIV-specific $CD8^+$ and $CD4^+$ T cells in the circulation of such individuals, their role in mediating protection from infection with HIV (as opposed to reflecting exposure to HIV) is unclear. A number of antiviral mucosal factors, such as defensins[31,48], CXCL12 (REFS 49,50), secretory leukocyte protease inhibitor (SLPI)[51,52], and type I and type II interferons[53], have been proposed as potentially contributing to antiviral defences at mucosal sites, although confirmation of their roles is lacking (FIG. 2).

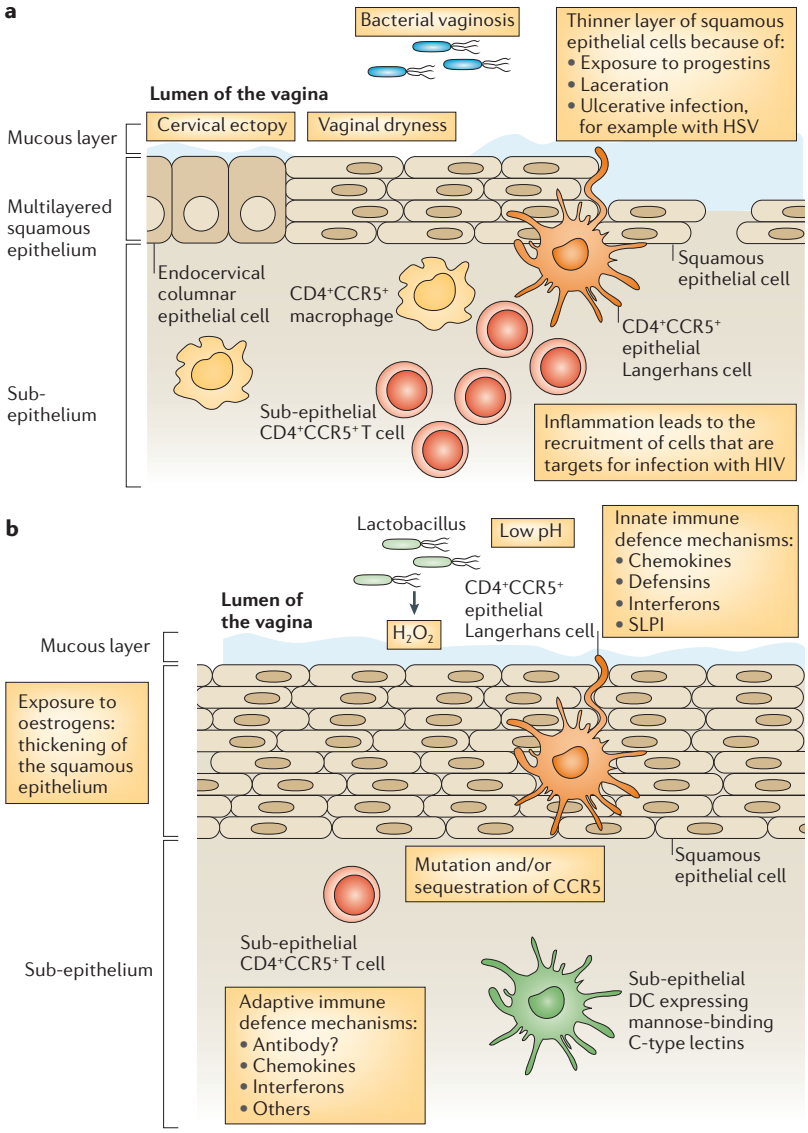

Figure 2 | **Factors that can increase or decrease risks for vaginal acquisition of HIV infection. a** | Factors that can increase the risk of infection with HIV include: ectopic protrusion of endocervical columnar epithelium into the ectocervix; thinning of the squamous epithelial layer; bacterial vaginosis; vaginal drying; inflammation, which leads to an increase in the number of target cells in the submucosa; trauma; ulcerative infections that might allow more ready access of virus to sub-epithelial dendritic cells (DCs) expressing C-type lectins, such as DC-specific intercellular adhesion molecule 3 (ICAM3)-grabbing non-integrin (DC-SIGN), and $CD4^+$ T cells. **b** | Factors that might decrease the risk of infection with HIV include: a thicker squamous epithelium; a mucous layer that physically traps virus; physical properties leading to HIV inactivation such as low pH and peroxides (for example, hydrogen peroxide (H_2O_2)) from lactobacilli; innate immune defense molecules, such as defensins, interferons, secretory leukocyte protease inhibitor (SLPI); absence of CC-chemokine receptor 5 (CCR5), either genetically (through homozygosity for a 32-base-pair deletion in the coding region of *CCR5* (known as the $CCR5^{\Delta 32}$ allele)) or induced (by endogenous chemokine ligands).

Where and how can topical strategies intervene?

So, what constitutes an ideal topical strategy to prevent HIV transmission? Apart from being safe, effective and affordable by people living in the poorest countries of the world, an ideal agent would be colourless, odourless and confer sustained protection from HIV after a single dose, giving women who use it the chance to do so without the knowledge of their partner. In the following section, we discuss the types of topical prevention strategies currently in development, together with newer strategies that might have promise, and these are summarized in (FIG. 3) and (TABLE 1).

Agents that inactivate HIV directly: detergents and agents that modify pH. The earliest microbicidal agents had detergent activities that disrupted the membranes of a number of microbial pathogens. The hope was to provide protection against HIV as well as other sexually transmissible infections, and in some cases that they would be spermicides[54]. Despite the risks of using detergents as microbicides that were identified in clinical trials with the first-generation detergent Nonoxynol-9 (N-9) (see later), a number of second-generation detergents are currently in clinical development. One such substance, Savvy (a 1% C31G vaginal gel; Cellegy Pharmaceuticals), which has a better (but in light of some reports not perfect, see later) toxicity profile than N-9 (REFS 55,56), is currently in Phase III clinical trials.

Because HIV is readily inactivated below pH4.5, a number of acidifying agents are in development for use as topical prevention strategies. Approaches as simple as using lime juice have been proposed, but the most advanced product is a buffered gel formulation (BufferGel; ReProtect)[57], currently in Phase III clinical trials. Although low pH buffering agents are inexpensive and easy to produce, it should be noted that semen is alkaline and that even a small rise in pH from the lowest possible value that BufferGel can maintain (~pH4) might allow infection[58].

Agents that target viral replication. Some registered antiretroviral drugs that block the HIV-1 reverse transcriptase, including non-nucleoside reverse transcriptase inhibitors such as UC-781 (Cellegy Pharmaceuticals) and TMC120 (Dapivirine; Tibotec Pharmaceuticals), as well as the nucleotide analogue tenofovir (Viread; Gilead Sciences), have been proposed as candidates for topical prevention of infection with HIV. Drugs belonging to

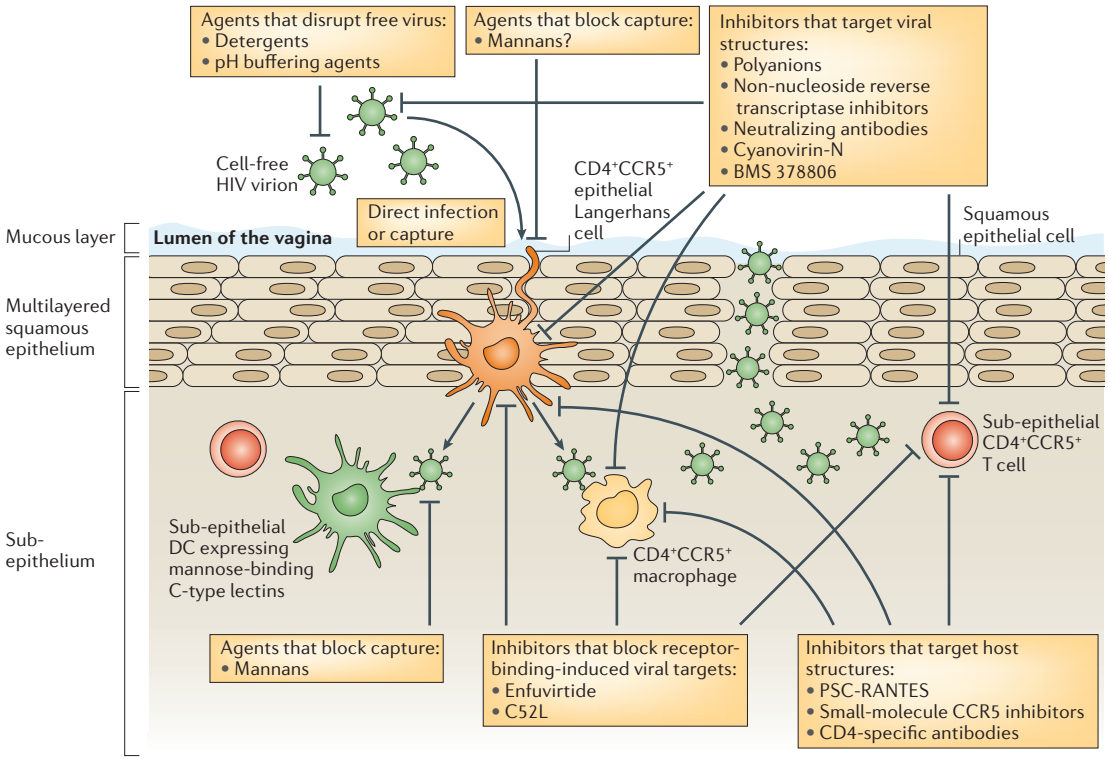

Figure 3 | **Topical strategies to prevent vaginal HIV transmission.** Intralumenal strategies include those that target free virus, such as detergents, polyanionic inhibitors, possibly some non-nucleoside reverse-transcriptase inhibitors (such as UC-781) and agents that target HIV envelope structures (such as cyanovirin-N and the envelope-protein-complex-specific antibodies b12 and 2G12). Strategies targeting host cells include mannans, CD4 inhibitors and CC-chemokine receptor 5 (CCR5) inhibitors, although all these agents probably function deeper within and/or below the mucosal surface. There are also strategies that are designed to target conformations of the envelope induced by binding to the target cell (such as the peptide C52L) and antiviral agents that function within infected cells (such as nucleoside and non-nucleoside reverse-transcriptase inhibitors) and both these types of agent might need to penetrate the mucosa to be functional.

these classes have the advantage of having succeeded as therapeutics, have been shown to prevent maternal to infant transmission[59,60] and have been applied systemically as pre- and post-exposure prophylaxis strategies[61,62]. In addition, their safety after systemic administration can accelerate the pace of their development, and UC-781 (which has no activity against HIV-2), tenofovir and TMC120 are already in clinical trials. However, concurrent use of the same antiviral strategy for treatment and prevention is not without risk (see later).

Agents that target viral entry. Virions accumulate on cell surfaces through interactions between positive charges on the viral envelope and negative charges on the cell surface[63]. Polyanionic substances, such as PRO 2000 (Indevus Pharmaceuticals), cellulose sulphate (Ushercell; Polydex Pharmaceuticals) and carrageenan (Carraguard; Population Council), probably mimic this charged interaction, coating the viral envelope and masking structures necessary for attachment to the cell surface and therefore entry into the cell. The polyanion binding site of gp120 consists of the V3 region and the CD4-induced co-receptor binding site[64]. In the absence of CD4 binding, polyanions only bind effectively to envelopes of X4 viruses, which have highly positively charged V3 regions[65]. Polyanions are active against R5 viruses, but only after CD4-induced exposure of the co-receptor-binding site. Therefore, these substances might prove to be less effective inhibitors of the R5 viruses at the centre of the HIV/AIDS pandemic.

More than half the mass of the HIV envelope-protein complex is comprised of sugars that have important roles in protecting viral envelope proteins from host-defence mechanisms. Mannose-rich glycans on the surface of the envelope are therefore the targets of a number of lectins with anti-HIV activity[66–68], as well as an unusual human monoclonal antibody (2G12), which neutralizes a broad range of HIV-1 strains[69]. The best known among the lectins is Cyanovirin-N (CV-N; Cellegy Pharmaceuticals), an 11 kDa protein originally isolated from the cyanobacterium *Nostoc ellipsosporum*[70], which potently inhibits entry of a broad range of R5 and X4 HIV, as well as SIV, isolates. Both topically applied CV-N[71] and systemically infused 2G12, administered in combination with other HIV-specific antibodies[72], showed efficacy in macaque vaginal models of SHIV challenge. However, viral escape mutants can arise: virus mutants lacking various N-linked glycan structures that are resistant to both 2G12 and CV-N have been found to be selected after *in vitro* culture in the presence of CV-N[73].

V3 region
The third variable (V3) region of the HIV envelope glycoprotein gp120. The amino-acid sequence in the V3 region determines whether the virus uses CXC-chemokine receptor 4 (CXCR4) or CC-chemokine receptor 5 (CCR5) as its co-receptor.

Table 1 | **Development status of current and potential topical strategies to prevent infection with HIV**

Inhibitor	Additional information	Product developer or source	Status
Miscellaneous			
Praneem Polyherbal	*Azadirachta indica* leaves, *Sapindus mukerossi* and *Mentha citrata* oil	Talwar Research Foundation	Phase II clinical trials for use as a microbicide
Inner Confidence	Contains protected lactobacilli and benzalkonium chloride	Biofem	Phase II trials clinical for use as a microbicide
Detergents			
Nonoxynol-9	NA	NA	Failed clinical trial for use as a microbicide
Savvy	1% C31G vaginal gel	Cellegy Pharmaceuticals	Phase III clinical trials for use as a microbicide
Invisible Condom	Sodium lauryl sulphate	Laval University	Phase I/II clinical trials for use as a microbicide
Agents that modify pH			
Buffergel	Carbopol 974P gel	ReProtect	Phase II/III clinical trials for use as a microbicide
ACIDFORM	NA	Topcad and Cemicamp	Phase I clinical trials for use as a microbicide
Lime juice	NA	CONRAD and USAID	Phase I clinical trials for use as a microbicide
Replication inhibitors			
UC-781	NA	Cellegy Pharmaceuticals	Phase I clinical trials for use as a microbicide
Viread	Tenofovir	Gilead Sciences	Phase II clinical trials for use as a microbicide
Dapivirine	TMC120	Tibotec Pharmaceuticals and IPM	Phase I clinical trials for use as a microbicide
Polyanions			
Ushercell	Cellulose sulphate	Polydex Pharmaceuticals	Phase III clinical trials for use as a microbicide
Cellacefate	Cellulose acetate 1,2-benzenedi-carboxylate	Lindsley F. Kimball Research Institute	Phase I clinical trials for use as a microbicide
Carraguard	Carrageenan, also known as PC-515	Population Council	Phase III clinical trials for use as a microbicide
Pro 2000	Also known as PRO 2000/5	Indevus Pharmaceuticals	Phase III clinical trials for use as a microbicide
Vivagel	SPL7013	Starpharma Holdings	Phase I clinical trials for use as a microbicide
Agents that bind glycans			
Cyanovirin-N	11 kDa *Nostoc ellipsosporum* protein	Cellegy Pharmaceuticals	Tested in primate SHIV vaginal challenge model
2G12	Antibody specific for glycans on gp120	University of Vienna	Tested together with other antibodies in primate SHIV vaginal challenge model
Agents that interact with the CD4-binding site of HIV gp120			
b12	Antibody specific for a conformation-dependent pocket of gp120	Scripps Research Institute	Tested in combination with other antibodies in primate model
PRO 542	NA	Progenics Pharmaceuticals	Tested in a cervical explant model
BMS 378806	NA	Bristol–Myers Squibb	Tested in combination with other molecules in primate SHIV vaginal challenge model
Agents that bind to gp41			
2F5	Antibody specific for the membrane-proximal region of gp41	University of Vienna	Tested in combination with other antibodies in primate SHIV vaginal challenge model
Fuzeon	Enfuviritide, also known as T-20	F. Hoffmann–La Roche and Trimeris	Registered for use as anti-HIV therapeutic
C52L	NA	Weill Medical College of Cornell University	Tested in combination with other molecules in primate SHIV vaginal challenge model
Agents that target glycan receptors			
Soluble mannan	NA	NA	Tested in combination with other substances in primate SHIV vaginal challenge model
Agents that bind to CD4			
TNX-355	NA	Tanox and Biogen	In clinical trials for use as anti-HIV therapeutic
Agents that target CC-chemokine receptor 5 (CCR5)			
CMPD-167	NA	Merck & Co.	Tested in combination with other molecules in primate SHIV vaginal challenge model
Vicriviroc	SCH-D	Schering–Plough Corporation	In clinical trials for use as anti-HIV therapeutic
Maraviroc	UK-427,857	Pfizer	In clinical trials for use as anti-HIV therapeutic
Aplaviroc	GSK-873140	GlaxoSmithKline	Ceased development as anti-HIV therapeutic
PRO 140	Antibody specific for CCR5	Progenics Pharmaceuticals	In clinical trials for use as anti-HIV therapeutic
CCR5mab004	Antibody specific for CCR5	Abgenix and HGS	In clinical trials for use as anti-HIV therapeutic
PSC-RANTES	NA	Gryphon Therapuetics	Tested in primate SHIV vaginal challenge model

HGS, Human Genome Sciences; IPM, the International Partnership for Microbicides; NA, not applicable; SHIV, simian–human immunodeficiency virus.

Binding to CD4 is essential to the HIV infection process because it induces conformational changes in the envelope that are necessary for membrane fusion and viral entry[74]. The highly conserved CD4-binding site, located in a conformation-dependent pocket of gp120, is a promising target for inhibitors of viral entry, and a monoclonal antibody (b12) specific for this region showed efficacy when applied topically in a macaque vaginal SHIV challenge model[75]. Similarly, an engineered polyvalent CD4–immunoglobulin fusion protein (PRO 542; Progenics Pharmaceuticals)[76], has protective activity in a cervical explant model[77]. Another promising inhibitor, BMS 378806 (Bristol–Myers Squibb)[78,79], is a small-molecule allosteric modulator that locks the envelope-protein complex in a conformation that might, or might not, allow it to bind CD4 and the co-receptor but nonetheless prevents key conformational changes that are required for viral entry[78].

Conformational changes in the envelope-protein complex that unmask conserved structures are induced by binding to CD4, these structures could prove useful targets for entry inhibitors. Whereas intact immunglobulins specific for these structures show relatively poor neutralizing activity against primary HIV isolates, smaller antibody fragments, such as Fabs or single chain Fvs, are much more effective[80], presumably for steric reasons.

Among the CD4-induced conformational changes in the envelope-protein complex are those that expose parts of the gp41 trimer. Subsequent co-receptor binding induces further conformational changes, leading to the formation of the pre-fusion complex, in which the gp41 trimer is in an extended form that bridges the host-cell and viral membranes. Conformational rearrangement of this structure into a six-helix bundle brings the two membranes together, driving the fusion process. Fusion can be blocked by antibodies (such as 2F5) specific for the membrane-proximal region of the gp41 ectodomain, and when systemically administered in combination with 2G12 (which is specific for glycans on gp120), macaques can be protected from vaginal SHIV challenge[72]. Similarly, fusion of the host-cell and viral membranes is blocked by peptides such as enfuvirtide (Fuzeon; F. Hoffmann–La Roche and Trimeris), a synthetic 36-amino-acid polypeptide derived from the carboxy terminus of the gp41 ectodomain that binds sites at the amino terminus of the gp41 trimer and prevents gp41 from folding into the six-helix bundle. It should be noted, however, that escape mutants arise readily when enfuvirtide is administered as an injectable therapeutic to people infected with HIV[81]. A peptide closely related to enfuvirtide, C52L, showed substantial protective activity when used topically in combination with small-molecule fusion inhibitors, BMS 378806 and CMPD167 (Merck & Co.) in macaque SHIV vaginal challenge experiments[79].

Agents that target host-cell structures. Currently, all registered anti-HIV therapeutics target viral elements: the HIV protease, reverse transcriptase and, more recently, the gp41 envelope protein. Each round of viral replication results in variant progeny on which selection pressures can act, allowing the evolution of better-adapted strains in an individual host. More than two decades of treatment experience has shown that targeting virally encoded elements results in the rapid generation of escape mutants, unless viral replication is profoundly suppressed. By contrast, host-encoded proteins essential for the viral life cycle cannot be influenced by viral evolution, and any structural variation in the virus is constrained by its need to continue to interact with them. From this perspective, these host factors might provide better therapeutic targets[82], as long as they can be blocked without compromising host-cell function. A large number of candidate entry inhibitors based on several of these targets have now been described.

The monoclonal antibody TNX-355 (Tanox and Biogen) is specific for CD4, preventing it from interacting with the HIV envelope, but not blocking it from interacting with MHC class II molecules, and has been engineered to minimize toxicity to cells expressing CD4 (REF. 83). It is currently in development as an injectable therapeutic, and molecules with similar properties could potentially be developed as topical prevention strategies.

Different DC subsets express distinct sets of mannose-binding C-type lectins, such as DC-specific intercellular adhesion molecule 3 (ICAM3)-grabbing non-integrin (DC-SIGN), which bind mannose residues on the HIV envelope[84]. As a result of this mannose-residue binding, these C-type lectins trap HIV in a compartment from which it can be transferred efficiently to infect $CD4^+$ T cells[85,86]. Mannan, a mannose-rich oligosaccharide derived from yeast, and antibodies specific for DC-SIGN can block DC uptake of HIV *in vitro*[85], but mannan was unable to protect macaques against SHIV challenge after topical application[79]. By contrast, mannan, when applied together with an antibody specific for CD4, brought about a modest decrease in the quantity of virus in DCs emigrating from cervical explants in a histoculture system[77]. Therefore, it is not yet clear whether blocking C-type lectins will be a useful topical strategy for prevention of HIV infection.

The demonstration that certain chemokines can inhibit HIV replication[87] contributed to the identification of CXCR4 and CCR5 as the co-receptors for HIV entry[88–93]. Subsequently, analogues of the natural chemokine ligands of CXCR4 and CCR5 with optimized anti-HIV activity were developed[94–99]. The more potent of these analogues share two common features: they feature a short, hydrophobic extension at the N terminus of the chemokine and they can prolong the sequestration of the co-receptor from the cell surface[100]. The prototype potent anti-HIV chemokine analogue was an analogue of CCL5, the aminooxypentane oxime of [glyoxylyl[1]]RANTES(2–68), known as AOP-RANTES (Gryphon Therapeutics)[99]. This CCR5 agonist is several orders of magnitude more active than CCL5 (RANTES). It rapidly induces profound and prolonged internalization of CCR5 (REF. 101). This is an attractive mechanism for topical prevention strategies for two reasons: first, this might provide protection of long duration after a single dose; and second, by removing an essential host-cell protein from the site of virus–cell interaction, the chance of generating viral

Six-helix bundle
A structure resulting from the interaction among three HIV envelope gp41 molecules, wherein two discontinuous repeating seven-residue sequences ('heptad repeats') on each gp41 molecule 'zip' together to form coiled coils with three molecules thereby forming a bundle of six helices. This coiling is thought to bring the virus membrane and cell membrane into close contact thereby facilitating fusion.

C-type lectin
Lectins are carbohydrate-binding molecules, and C-type lectins were named because their binding is dependent on the presence of calcium. C-type lectins on the surface of human cells have important roles in cellular adhesion and also in the uptake of microorganisms and microbial antigens.

escape mutants is presumably reduced. A more recent analogue, PSC-RANTES (Gryphon Therapeutics) is 50-fold more active in blocking cell fusion than AOP-RANTES, probably because of its increased capacity to promote CCR5 sequestration[98]. Topical application of PSC-RANTES has provided rhesus macaques with a high level of protection from vaginal challenge with SHIV 162P3 (REF. 102). The resistance to HIV infection and general good health of *CCR5*$^{\Delta 32/\Delta 32}$ individuals also makes CCR5 sequestration an attractive strategy.

Small-molecule compounds and monoclonal antibodies can also prevent HIV from using CCR5. A number of CCR5-specific antibodies have been shown to block HIV entry[103] and at least two, PRO 140 (Progenics Pharmaceuticals)[104] and CCR5mAb004 (Abgenix and Human Genome Sciences) are in clinical trials as injectable therapeutics. The first small-molecule CCR5 inhibitor described was TAK-779 (Takeda Pharmaceuticals North America)[105]. Since then, several other promising molecules have been described, and three of these have proceeded to the late stages of clinical evaluation as oral anti-HIV medicines[106]. Another small-molecule inhibitor of CCR5, CMPD 167, has been found to protect macaques from vaginal SHIV challenge[79,107]. The small-molecule CCR5 inhibitors described so far seem to be allosteric modulators that lock CCR5 into conformations that render it unusable as a co-receptor by the HIV envelope-protein complex. These agents might all share a common docking site on CCR5 (REF. 108). Accordingly, some escape mutants, generated against one inhibitor, that retain the use of CCR5 as a co-receptor showed cross-resistance to the others[109]. CCR5 blockade by one inhibitor, aplaviroc (GlaxoSmithKline) was shown to be extremely persistent (it has a binding half life of 136 hours) in *in vitro* drug wash-out experiments[108]. However, development of aplaviroc as a systemic agent has been stopped because of liver toxicity and clinical trials of another CCR5 inhibitor (vicriviroc; Schering–Plough Corporation) have been halted because of treatment failures in treatment-naive people or unblinded because of the unexpected occurrence of malignancies in people undergoing salvage therapy.

A question that remains is will blockade of CCR5 at mucosal sites be sufficient to provide protection from HIV transmission, or will other receptors, such as C-type lectins, be able to transport virus to CCR5^{+} target cells at deeper sites (that is, away from the sites protected by topical prevention strategies), such as cells in lymphoid tissues[110]? Recent results show that topically applied CCR5 inhibitors can provide macaques with partial[79,107] or high-level[79,102] protection from vaginal challenge with R5-tropic SHIV, indicating that topical inhibition of CCR5 alone is a valid prevention strategy. However, there is a striking discrepancy between the potency of these molecules *in vitro* and the high concentrations required for efficacy *in vivo*[102,107]. This marked difference might reflect a need for CCR5 inhibitors to be present at active concentrations at deeper sites, below the epithelium, for efficient protection. Alternatively, the multiple rugosities of the vaginal mucosa might present a far greater surface area that must be covered by the agent *in vivo* and might also lead to substantially greater adsorption losses. Although rare, people who are homozygous for the 32-base-pair deletion in CCR5 have acquired HIV infection with isolates that can use CXCR4 for entry. Therefore, topical agents that are also active against X4 isolates might provide some advantage in this setting. By contrast, the rarity of these occurrences might have only a small affect on the effectiveness of population-based prevention strategies that target CCR5 alone.

Formulation and delivery. Although identifying the best biological target(s) for topical agents is clearly central to the development of topical prevention strategies, it is also necessary to develop formulation and delivery tools that will allow successful application, distribution and retention of the agent where it is needed. Careful formulation studies are needed to optimize the viscosity and other physical characteristics of topically administered products to ensure the most favourable antiviral activity, good coverage of the vaginal surface, sufficient tissue penetration if necessary, and a product that is as undetectable as possible. Longer-acting agents, if tolerated, might allow less-frequent administration, possibly with depot strategies using vaginal rings[111]. Therefore, effective formulation could have a positive affect on both the efficacy of a topical prevention strategy and the likelihood of it being used. Strategies for introducing inhibitory RNAs are improving and results of experimental models[112] indicate that these approaches might, in time, be useful for topical inhibition of crucial host HIV receptors or co-receptors.

A recent report[79] indicates that combinations of entry inhibitors with different sites of action might provide additive protection when applied topically in the rhesus model of SHIV infection. Combination strategies revolutionized HIV treatment and might offer promise for effective topical prevention as well.

Safety

Topical strategies to prevent HIV infection must have safety profiles that justify their application to otherwise healthy people and tolerance profiles that assure that they are used. Moreover, safety issues for some of these strategies have implications not only for the individual but also for the entire community that is at risk from infection with HIV.

Damage to mucosal integrity. Safety concerns regarding detergent-based microbicides have emerged because, similar to pathogens that cause ulcerative sexually transmitted disease, they have the capacity to disrupt mucosal integrity. Although N-9 was thought to be sufficiently non-toxic and safe to allow its registration as a spermicide, clinical trials to investigate its potential as a prophylactic for HIV infection indicated that N-9 increased the risk of HIV transmission[113]. Furthermore, although Savvy has been shown to produce less irritation than N-9 at the concentration proposed for topical prevention, it is important to note that 'less' irritation does not always mean 'none'. For example, in one study genital irritation was seen in 44% of the subjects after 7 days administration (compared with 87% in people receiving N-9)[56]. The possibility that

Drug wash-out experiment
An experiment where exposure to drug is terminated by washing out free drug. This allows measurement of the duration of initial drug effect or binding.

long-term use might bring an increase in infection, even though perhaps at a lower level than for N-9, seems difficult to discount at this stage[58]. Underscoring the risks of surfactants, a recent prospective cohort study found that vaginal washing was associated with a heightened risk of infection with HIV and that rates of infection with HIV were greater among women who washed with soaps than among those who washed with water[114]. Low pH buffering agents might also affect mucosal integrity. Although BufferGel was relatively well tolerated, two-thirds of clinical-trial participants reported mild or moderate adverse experiences, mostly irritation of the genitourinary tract, and 3 out of 27 had colposcopic abnormalities[57]. Similarly, to the extent that an anionic polymer has detergent activity, BufferGel might impair mucosal integrity. Also, some anionic polymers have anticoagulant activity[58], which might exacerbate the effects of ulceration.

Inflammation. Induction of inflammation might increase local levels of interferons and antiviral chemokines such as CCL3, CCL4 and CCL5, and alloantigen-induced inflammation has been proposed as a potential mechanism for mucosal protection against HIV infection[115]. By contrast, inducing mucosal inflammation is unquestionably dangerous under some circumstances, as infection with sexually transmitted pathogens that cause inflammation is a significant risk factor for HIV infection[26] and induction of local inflammation is a possible factor in the increased risk of HIV infection seen in the N-9 clinical trial[113]. In rhesus macaques, vaginal application of agonists of Toll-like receptor 7 (TLR7) or TLR9 resulted in increased inflammatory responses and did not protect animals from infection but instead increased the magnitude of SIV replication after challenge[116]. As chemokines can induce inflammation and recruit target cells for HIV infection, CCL5 analogues with signalling activity need to be closely monitored for the induction of local inflammation. In this regard, current preclinical toxicity models might not suffice. For example, although the rabbit vaginal irritation assay, which is recommended for preclinical evaluation of topical prevention strategies[117], can provide a useful readout of chemical irritation, it might not detect biological outcomes such as inflammation induced by molecules such as PSC-RANTES, which are active on human receptors but not necessarily on the rabbit homologues. Therefore, as topical prevention strategies with greater specificity and selectivity emerge, the development of safety models of appropriate relevance is needed.

Effect on vaginal ecology. Topical agents that disturb the protective vaginal ecology could counterbalance their antiviral effect by undermining this natural barrier to HIV propagation, reducing their acceptability. On this point, it has been noted that although BufferGel did not deplete vaginal colonization by *Lactobacillus* spp. in one study, it resulted in an 80% reduction in levels of the strains of *Lactobacillus* that produce H_2O_2 (REF. 118) peroxide[118]. Because H_2O_2 functions as an intrinsic defence against HIV and other mucosal pathogens (FIG. 2), the reduction in this H_2O_2-producing strain of *Lactobacillus* might heighten the risk of infection. Topical agents might also undergo unfavourable interactions with resident vaginal microbes. For example, some anionic polymers and carageenan might have the potential to be metabolized by vaginal microbes into toxic metabolites and therefore might have the potential to damage the vaginal epithelial cells, increasing the risk of infection[58].

Immunogenicity. Any non-human agent that is applied topically to mucosal tissue on a regular basis risks being immunogenic. The more a protein structure differs from those found in humans, the more this is likely to be a problem. CV-N is a completely non-human, microbial protein and so individuals receiving such treatment must be carefully monitored for any CV-N-specific immune response. The lectin activity of CV-N should also be monitored carefully because some lectins can activate the immune system directly. The RANTES analogues mentioned earlier differ from the human RANTES precursor at a few amino-acid residues. Although, perhaps, presenting less of a concern than the microbial protein CV-N, their use must, nonetheless, be monitored just as carefully for any treatment-specific immune response.

Concurrent use of related inhibitors as therapeutics and as topical prophylactics. As antiretroviral therapies reach more people throughout the world, we must consider the potential dangers of using a substance both as a therapeutic agent for infected people and for the topical protection of uninfected people living alongside them. When escape mutants arise during therapy, the related topical prevention strategy might no longer provide protection. For example, the increasing therapeutic use of nucleoside and nucleotide analogue reverse transcriptase inhibitors (such as tenofovir) and the widespread use of non-nucleoside analogues in the developing world might limit the development of these agents as topical prophylactic strategies. When they come into general use as therapeutics, similar concerns will arise for entry inhibitors and drugs targeting other viral elements. The situation could be worsened if the prophylaxis is given orally[119] or is systemically absorbed after topical application at levels sufficient to select for escape mutation (but at levels insufficient to suppress HIV replication completely) when used by people who are infected but do not know it.

Future perspectives

In only a few years, topical strategies for the prevention of HIV infection have expanded from a field with only a few nonspecific microbicide candidates to a dynamic arena that includes a spectrum of promising agents with selective activity against viral and host elements needed for HIV replication. As this field is maturing, we propose that the development of safe and clinically effective strategies to prevent HIV transmission is theoretically plausible and practically possible.

So far, experience with detergent microbicides mandates caution and the need for careful safety monitoring as they are developed. It is very challenging to design an

agent that disrupts the viral membrane in a single exposure but does not disrupt human-cell membranes, from which the viral membrane is derived, even on chronic exposure.

Many small-molecule inhibitors can plausibly be used in topical prevention strategies, are relatively inexpensive to manufacture and are known to be safe when taken systemically. By contrast, if these agents are to be used both to treat and to prevent infection, or if they share resistance profiles with therapeutics in common use, the spread of escape mutants as a result of therapeutic use will, in the end, compromise the protective role of the agent. In the same context, it is important that the topical agents are not systemically absorbed to an extent that would favour the generation of escape mutants in the event of infection.

It is a misconception that proteins, such as chemokine analogues and CV-N, are too expensive for use in developing countries. Large-scale production of proteins by microbial fermentation is now a mature technology and the most expensive component of the growth media is often water. Transgenic plants can be designed to produce recombinant proteins on a large scale and on site in the developing world if the associated political issues can be addressed satisfactorily. Molecules that require at least some degree of chemical synthesis, such as PSC-RANTES, provide more of a challenge but no longer an unthinkable one. To the extent that producing monoclonal antibodies implies mammalian-cell culture, the economics are less favourable. Mammalian cells give lower protein yields and media are costly. But if a monoclonal antibody was found to be the best solution to the problem, it is difficult to imagine that the biotechnology industry, with all its achievements over the past 25 years, would fail to rise to the challenge of cheaper and easier production.

Conclusions

Recent strategies targeting specific elements of the HIV infection pathway have provided new and promising leads for the topical inhibition of infection with HIV. Targeted experimental approaches will also provide information necessary to direct the further development and refinement of strategies that will ultimately provide effective protection against mucosal HIV transmission. Continued testing of these strategies is a priority for government and not-for-profit institutions but not yet for industry, as the main market for these agents is likely to be the developing world. Because pharmaceutical companies generally excel at product development, every effort should be made to draw them into this vital effort and recent developments in this regard are encouraging (see the International Partnership for Microbicides).

1. Joint United Nations Programme on HIV/AIDS & World Health Organization. *AIDS Epidemic Update: 2005*. (Joint United Nations Programme on HIV/AIDS, Geneva, 2005).
2. Quinn, T. C. & Overbaugh, J. HIV/AIDS in women: an expanding epidemic. *Science* **308**, 1582–1583 (2005).
3. Cohen, J. AIDS research. Male circumcision thwarts HIV infection. *Science* **309**, 860 (2005).
4. Piot, P. Message on the occasion of World AIDS Day. *UNAIDS* [online], http://data.unaids.org/Media/Speeches02/SP_Piot_WAD2004_01Dec04_en.pdf (2004).
5. Pool, R. *et al.* Men's attitudes to condoms and female controlled means of protection against HIV and STDs in south-western Uganda. *Cult. Health Sex.* **2**, 197–211 (2000).
6. Auvert, B. *et al.* Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. *PLoS Med.* **2**, e298 (2005).
7. Miller, C. J. & Shattock, R. J. Target cells in vaginal HIV transmission. *Microbes Infect.* **5**, 59–67 (2003).
8. Maher, D., Wu, X., Schacker, T., Horbul, J. & Southern, P. HIV binding, penetration, and primary infection in human cervicovaginal tissue. *Proc. Natl Acad. Sci. USA* **102**, 11504–11509 (2005).
9. Quayle, A. J. The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. *J. Reprod. Immunol.* **57**, 61–79 (2002).
10. Klebanoff, S. J. & Coombs, R. W. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. *J. Exp. Med.* **174**, 289–292 (1991).
11. Baleta, A. Concern voiced over "dry sex" practices in South Africa. *Lancet* **352**, 1292 (1998).
12. Padian, N. S., Shiboski, S. C. & Jewell, N. P. The effect of number of exposures on the risk of heterosexual HIV transmission. *J. Infect. Dis.* **161**, 883–887 (1990).
13. Guimaraes, M. D., Vlahov, D. & Castilho, E. A. Postcoital vaginal bleeding as a risk factor for transmission of the human immunodeficiency virus in a heterosexual partner study in Brazil. Rio de Janeiro Heterosexual Study Group. *Arch. Intern. Med.* **157**, 1362–1368 (1997).
14. Sewankambo, N. *et al.* HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. *Lancet* **350**, 546–550 (1997).
15. Myer, L. *et al.* Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. *J. Infect. Dis.* **192**, 1372–1380 (2005).
16. Galvin, S. R. & Cohen, M. S. The role of sexually transmitted diseases in HIV transmission. *Nature Rev. Microbiol.* **2**, 33–42 (2004).
17. Smith, S. M., Baskin, G. B. & Marx, P. A. Estrogen protects against vaginal transmission of simian immunodeficiency virus. *J. Infect. Dis.* **182**, 708–715 (2000).
18. Marx, P. A. *et al.* Progesterone implants enhance SIV vaginal transmission and early virus load. *Nature Med.* **2**, 1084–1089 (1996).
19. Miller, C. J., Alexander, N. J., Vogel, P., Anderson, J. & Marx, P. A. Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques. *J. Med. Primatol.* **21**, 64–68 (1992).
20. Miller, C. J. *et al.* Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J. Virol.* **79**, 9217–9227 (2005).
This paper describes the kinetics and dissemination of SIV infection after vaginal challenge in the rhesus model.
21. Plourde, P. J. *et al.* Human immunodeficiency virus type 1 seroconversion in women with genital ulcers. *J. Infect. Dis.* **170**, 313–317 (1994).
22. Sodora, D. L., Gettie, A., Miller, C. J. & Marx, P. A. Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. *AIDS Res. Hum. Retroviruses* **14** (Suppl. 1), S119–S123 (1998).
23. Hu, J., Gardner, M. B. & Miller, C. J. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. *J. Virol.* **74**, 6087–6095 (2000).
24. Haase, A. T. Perils at mucosal front lines for HIV and SIV and their hosts. *Nature Rev. Immunol.* **5**, 783–792 (2005).
25. Cohen, M. S. & Pilcher, C. D. Amplified HIV transmission and new approaches to HIV prevention. *J. Infect. Dis.* **191**, 1391–1393 (2005).
This paper outlines a model of amplified HIV transmission. In this model, risks for HIV transmission are amplified in certain settings such as during acute infection and in the presence of other sexually transmitted infections.
26. Pilcher, C. D. *et al.* Frequent detection of acute primary HIV infection in men in Malawi. *AIDS* **18**, 517–524 (2004).
27. Wawer, M. J. *et al.* Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. *J. Infect. Dis.* **191**, 1403–1409 (2005).
28. Moore, J. P., Kitchen, S. G., Pugach, P. & Zack, J. A. The CCR5 and CXCR4 coreceptors — central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res. Hum. Retroviruses* **20**, 111–126 (2004).
29. Zaitseva, M. *et al.* Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. *Nature Med.* **3**, 1369–1375 (1997).
30. Amara, A. *et al.* HIV coreceptor downregulation as antiviral principle: SDF-1α- dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J. Exp. Med.* **186**, 139–146 (1997).
31. Quinones-Mateu, M. E. *et al.* Human epithelial β-defensins 2 and 3 inhibit HIV-1 replication. *AIDS* **17**, F39–F48 (2003).
32. Stephens, J. C. *et al.* Dating the origin of the CCR5-Δ32 AIDS-resistance allele by the coalescence of haplotypes. *Am. J. Hum. Genet.* **62**, 1507–1515 (1998).
33. Liu, R. *et al.* Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86**, 367–377 (1996).
This work first identified a 32-base-pair deletion in the open reading frame of the gene encoding the HIV co-receptor CCR5 (known as the *CCR5*$^{\Delta 32}$ allele) that results in a truncated protein and renders CD4$^+$ T cells of two HRSN people resistant to infection by R5-tropic HIV isolates.

34. Dean, M. *et al.* Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **273**, 1856–1862 (1996).
35. Zagury, D. *et al.* C-C chemokines, pivotal in protection against HIV type 1 infection. *Proc. Natl Acad. Sci. USA* **95**, 3857–3861 (1998).
36. Koning, F. A. *et al.* Correlates of resistance to HIV-1 infection in homosexual men with high-risk sexual behaviour. *AIDS* **18**, 1117–1126 (2004).
37. Shieh, B. *et al.* Detection of elevated serum β-chemokine levels in seronegative Chinese individuals exposed to human immunodeficiency virus type 1. *Clin. Infect. Dis.* **33**, 273–279 (2001).
38. Iqbal, S. M. *et al.* Elevated T cell counts and RANTES expression in the genital mucosa of HIV-1-resistant Kenyan commercial sex workers. *J. Infect. Dis.* **192**, 728–738 (2005).
39. Fowke, K. R. *et al.* HIV type 1 resistance in Kenyan sex workers is not associated with altered cellular susceptibility to HIV type 1 infection or enhanced β-chemokine production. *AIDS Res. Hum. Retroviruses* **14**, 1521–1530 (1998).
40. Salkowitz, J. R. *et al.* Characterization of high-risk HIV-1 seronegative hemophiliacs. *Clin. Immunol.* **98**, 200–211 (2001).
Among haemophiliacs at 95% risk for HIV infection through parenteral exposure but who remained HIV uninfected, 16% were homozygous for the *CCR5*$^{\Delta 32}$ allele. However, no clear mechanism could be identified that protected the remainder.
41. Messele, T. *et al.* No difference in *in vitro* susceptibility to HIV type 1 between high-risk HIV-negative Ethiopian commercial sex workers and low-risk control subjects. *AIDS Res. Hum. Retroviruses* **17**, 433–441 (2001).
42. Gonzalez, E. *et al.* The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* **307**, 1434–1440 (2005).
43. Kulkarni, P. S., Butera, S. T. & Duerr, A. C. Resistance to HIV-1 infection: lessons learned from studies of highly exposed persistently seronegative (HEPS) individuals. *AIDS Rev.* **5**, 87–103 (2003).
44. Kaul, R. *et al.* HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. *AIDS* **13**, 23–29 (1999).
45. Mazzoli, S. *et al.* HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nature Med.* **3**, 1250–1257 (1997).
46. Mazzoli, S. *et al.* Human immunodeficiency virus (HIV)-specific IgA and HIV neutralizing activity in the serum of exposed seronegative partners of HIV-seropositive persons. *J. Infect. Dis.* **180**, 871–875 (1999).
47. Beyrer, C. *et al.* Epidemiologic and biologic characterization of a cohort of human immunodeficiency virus type 1 highly exposed, persistently seronegative female sex workers in northern Thailand. Chiang Mai HEPS Working Group. *J. Infect. Dis.* **179**, 59–67 (1999).
48. Chang, T. L. & Klotman, M. E. Defensins: natural anti-HIV peptides. *AIDS Rev.* **6**, 161–168 (2004).
49. Agace, W. W. *et al.* Constitutive expression of stromal derived factor-1 by mucosal epithelia and its role in HIV transmission and propagation. *Curr. Biol.* **10**, 325–328 (2000).
50. Spear, G. T. *et al.* Chemokines are present in the genital tract of HIV-seropositive and HIV-seronegative women: correlation with other immune mediators. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **18**, 454–459 (1998).
51. Moriyama, A. *et al.* Secretory leukocyte protease inhibitor (SLPI) concentrations in cervical mucus of women with normal menstrual cycle. *Mol. Hum. Reprod.* **5**, 656–661 (1999).
52. Jana, N. K., Gray, L. R. & Shugars, D. C. Human immunodeficiency virus type 1 stimulates the expression and production of secretory leukocyte protease inhibitor (SLPI) in oral epithelial cells: a role for SLPI in innate mucosal immunity. *J. Virol.* **79**, 6432–6440 (2005).
53. Abel, K., Rocke, D. M., Chohan, B., Fritts, L. & Miller, C. J. Temporal and anatomic relationship between virus replication and cytokine gene expression after vaginal simian immunodeficiency virus infection. *J. Virol.* **79**, 12164–12172 (2005).
54. Lange, J. M., Karam, M. & Piot, P. Boost for vaginal microbicides against HIV. *Lancet* **342**, 1356 (1993).
55. Bax, R. *et al.* Microbicides--evaluating multiple formulations of C31G. *Contraception* **66**, 365–368 (2002).
56. Mauck, C. K. *et al.* A randomized Phase I vaginal safety study of three concentrations of C31G vs. Extra Strength Gynol II. *Contraception* **70**, 233–240 (2004).
57. Mayer, K. H. *et al.* Safety and tolerability of BufferGel, a novel vaginal microbicide, in women in the United States. *Clin. Infect. Dis.* **32**, 476–482 (2001).
58. D'Cruz, O. J. & Uckun, F. M. Clinical development of microbicides for the prevention of HIV infection. *Curr. Pharm. Des.* **10**, 315–336 (2004).
An in-depth analysis of the strengths and weaknesses of a number of topical strategies for HIV prevention
59. Connor, E. M. *et al.* Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N. Engl. J. Med.* **331**, 1173–1180 (1994).
60. Guay, L. A. *et al.* Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. *Lancet* **354**, 795–802 (1999).
61. Updated U. S. Public Health Service guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. *MMWR Recomm. Rep.* **51**, 1–52 (2001).
62. Grant, R. M. *et al.* AIDS. Promote HIV chemoprophylaxis research, don't prevent it. *Science* **309**, 2170–2171 (2005).
63. Ugolini, S., Mondor, I. & Sattentau, Q. J. HIV-1 attachment: another look. *Trends Microbiol.* **7**, 144–149 (1999).
64. Vives, R. R., Imberty, A., Sattentau, Q. J. & Lortat-Jacob, H. Heparan sulfate targets the HIV-1 envelope glycoprotein gp120 coreceptor binding site. *J. Biol. Chem.* **280**, 21353–21357 (2005).
65. Moulard, M. *et al.* Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. *J. Virol.* **74**, 1948–1960 (2000).
66. Balzarini, J. *et al.* The mannose-specific plant lectins from *Cymbidium* hybrid and *Epipactis helleborine* and the (*N*-acetylglucosamine)$_n$-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication *in vitro*. *Antiviral Res.* **18**, 191–207 (1992).
67. Balzarini, J. *et al.* α-(1–3)- and α-(1–6)-D-mannose-specific plant lectins are markedly inhibitory to human immunodeficiency virus and cytomegalovirus infections *in vitro*. *Antimicrob. Agents Chemother.* **35**, 410–416 (1991).
68. Hansen, J. E. *et al.* Correlation between carbohydrate structures on the envelope glycoprotein gp120 of HIV-1 and HIV-2 and syncytium inhibition with lectins. *AIDS* **3**, 635–641 (1989).
69. Calarese, D. A. *et al.* Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. *Science* **300**, 2065–2071 (2003).
70. Boyd, M. R. *et al.* Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. *Antimicrob. Agents Chemother.* **41**, 1521–1530 (1997).
71. Tsai, C. C. *et al.* Cyanovirin-N inhibits AIDS virus infections in vaginal transmission models. *AIDS Res. Hum. Retroviruses* **20**, 11–18 (2004).
72. Mascola, J. R. *et al.* Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nature Med.* **6**, 207–210 (2000).
Intravenous administration of combinations of HIV neutralizing antibodies protected macaques from vaginal challenge with SHIV, although intravaginal concentrations of antibodies were relatively low. This indicates that systemic or intramucosal levels of neutralizing antibodies can protect against vaginal SHIV transmission.
73. Witvrouw, M. *et al.* Resistance of human immunodeficiency virus type 1 to the high-mannose binding agents cyanovirin N and concanavalin A. *J. Virol.* **79**, 7777–7784 (2005).
74. Moore, J. P. & Stevenson, M. New targets for inhibitors of HIV-1 replication. *Nature Rev. Mol. Cell Biol.* **1**, 40–49. (2000).
75. Veazey, R. S. *et al.* Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nature Med.* **9**, 343–346 (2003).
Topical application of a neutralizing antibody to HIV can protect some non-human primates from acquisition of SHIV after vaginal challenge and concentrations needed for protection were high.
76. Zhu, P., Olson, W. C. & Roux, K. H. Structural flexibility and functional valence of CD4-IgG2 (PRO 542): potential for cross-linking human immunodeficiency virus type 1 envelope spikes. *J. Virol.* **75**, 6682–6686 (2001).
77. Hu, Q. *et al.* Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J. Exp. Med.* **199**, 1065–1075 (2004).
Topical blockade of the HIV receptor CD4 or the co-receptors CXCR4 or CCR5 could protect ectocervical explants from mucosal infection. However, in this model, migratory cells could disseminate infection that could be blocked by targeting CD4 and C-type lectins. Targeting virus directly with neutralizing antibodies blocked both infection pathways.
78. Si, Z. *et al.* Small-molecule inhibitors of HIV-1 entry block receptor-induced conformational changes in the viral envelope glycoproteins. *Proc. Natl Acad. Sci. USA* **101**, 5036–5041 (2004).
79. Veazey, R. S. *et al.* Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* **438**, 99–102 (2005).
Combinations of inhibitors of HIV–cell fusion can provide high-level protection against vaginal acquisition of SHIV in rhesus macaques.
80. Labrijn, A. F. *et al.* Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1. *J. Virol.* **77**, 10557–10565 (2003).
81. Greenberg, M. L. & Cammack, N. Resistance to enfuvirtide, the first HIV fusion inhibitor. *J. Antimicrob. Chemother.* **54**, 333–340 (2004).
82. Lederman, M. M. Host-directed and immune-based therapies for human immunodeficiency virus infection. *Ann. Intern. Med.* **122**, 218–222 (1995).
83. Kuritzkes, D. R. *et al.* Antiretroviral activity of the anti-CD4 monoclonal antibody TNX-355 in patients infected with HIV type 1. *J. Infect. Dis.* **189**, 286–2891 (2004).
84. Turville, S. G. *et al.* Diversity of receptors binding HIV on dendritic cell subsets. *Nature Immunol.* **3**, 975–983 (2002).
85. Geijtenbeek, T. B. *et al.* DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **100**, 587–597 (2000).
86. McDonald, D. *et al.* Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* **300**, 1295–1297 (2003).
87. Cocchi, F. *et al.* Identification of RANTES, MIP-1α, and MIP-1β as the major HIV- suppressive factors produced by CD8$^+$ T cells. *Science* **270**, 1811–1815 (1995).
88. Feng, Y., Broder, C. C., Kennedy, P. E. & Berger, E. A. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**, 872–877 (1996).
89. Alkhatib, G. *et al.* CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**, 1955–1958 (1996).
90. Bleul, C. C. *et al.* The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **382**, 829–833 (1996).
91. Choe, H. *et al.* The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135–1148. (1996).
92. Deng, H. *et al.* Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661–666 (1996).
93. Dragic, T. *et al.* HIV-1 entry into CD4$^+$ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381**, 667–673 (1996).
94. Arenzana-Seisdedos, F. *et al.* HIV blocked by chemokine antagonist. *Nature* **383**, 400 (1996).
95. Yang, O. O. *et al.* Enhanced inhibition of human immunodeficiency virus type 1 by met- stromal-derived factor 1β correlates with down-modulation of CXCR4. *J. Virol.* **73**, 4582–4589 (1999).
96. Townson, J. R., Graham, G. J., Landau, N. R., Rasala, B. & Nibbs, R. J. Aminooxypentane addition to the chemokine macrophage inflammatory protein-1αP increases receptor affinities and HIV inhibition. *J. Biol. Chem.* **275**, 39254–39261 (2000).

97. Hartley, O. *et al.* Human immunodeficiency virus type 1 entry inhibitors selected on living cells from a library of phage chemokines. *J. Virol.* **77**, 6637–6644 (2003).
98. Hartley, O. *et al.* Medicinal chemistry applied to a synthetic protein: development of highly potent HIV entry inhibitors. *Proc. Natl Acad. Sci. USA* **101**, 16460–16465 (2004).
Through rational incorporation of non-natural amino acids and 'intelligent design', it was possible to apply techniques of medicinal chemistry to develop modified chemokine analogues with potency exceeding, by several logs, that of the original native ligand.
99. Simmons, G. *et al.* Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* **276**, 276–279 (1997).
100. Hartley, O. & Offord, R. E. Engineering chemokines to develop optimized HIV inhibitors. *Curr. Protein Pept. Sci.* **6**, 207–219 (2005).
101. Mack, M. *et al.* Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J. Exp. Med.* **187**, 1215–1224 (1998).
102. Lederman, M. M. *et al.* Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science* **306**, 485–487 (2004).
Blockade of the HIV co-receptor CCR5 by topical application of high concentrations of a modified RANTES analogue is sufficient to provide high-level protection against SHIV in the rhesus model.
103. Olson, W. C. *et al.* Differential inhibition of human immunodeficiency virus type 1 fusion, gp120 binding, and CC-chemokine activity by monoclonal antibodies to CCR5. *J. Virol.* **73**, 4145–4155 (1999).
104. Trkola, A. *et al.* Potent, broad-spectrum inhibition of human immunodeficiency virus type 1 by the CCR5 monoclonal antibody PRO 140. *J. Virol.* **75**, 579–588 (2001).
105. Baba, M. *et al.* A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl Acad. Sci. USA* **96**, 5698–5703 (1999).
106. Reeves, J. D. & Piefer, A. J. Emerging drug targets for antiretroviral therapy. *Drugs* **65**, 1747–1766 (2005).
107. Veazey, R. S. *et al.* Use of a small molecule CCR5 inhibitor in macaques to treat simian immunodeficiency virus infection or prevent simian–human immunodeficiency virus infection. *J. Exp. Med.* **198**, 1551–1562 (2003).
108. Watson, C., Jenkinson, S., Kazmierski, W. & Kenakin, T. The CCR5 receptor-based mechanism of action of 873140, a potent allosteric noncompetitive HIV entry inhibitor. *Mol. Pharmacol.* **67**, 1268–1282 (2005).
109. Marozsan, A. J. *et al.* Generation and properties of a human immunodeficiency virus type 1 isolate resistant to the small molecule CCR5 inhibitor, SCH-417690 (SCH-D). *Virology* **338**, 182–199 (2005).
110. Davis, C. W. & Doms, R. W. HIV transmission: closing all the doors. *J. Exp. Med.* **199**, 1037–1040 (2004).
111. Malcolm, R. K., Woolfson, A. D., Toner, C. F., Morrow, R. J. & McCullagh, S. D. Long-term, controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings. *J. Antimicrob. Chemother.* **56**, 954–956 (2005).
112. Palliser, D. *et al.* An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. *Nature* **439**, 89–94 (2006).
Durable gene silencing using a mucosal targeted small interfering RNA strategy holds promise as a topical HIV prevention strategy.
113. Hillier, S. L. *et al. In vitro* and *in vivo*: the story of nonoxynol 9. *J. Acquir. Immune Defic. Syndr.* **39**, 1–8 (2005).
A detailed account of why a topical surfactant strategy failed to protect women from HIV infection and actually increased the risk of HIV acquisition.
114. McClelland, R. S. *et al.* Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study. *AIDS* **20**, 269–273 (2006).
A prospective cohort study indicating that vaginal washing with water is associated with an increased risk of HIV infection and washing with soaps is associated with even greater risk.
115. Peters, B. *et al.* Effect of heterosexual intercourse on mucosal alloimmunisation and resistance to HIV-1 infection. *Lancet* **363**, 518–5124 (2004).
116. Wang, Y. *et al.* The Toll-like receptor 7 (TLR7) agonist, imiquimod, and the TLR9 agonist, CpG ODN, induce antiviral cytokines and chemokines but do not prevent vaginal transmission of simian immunodeficiency virus when applied intravaginally to rhesus macaques. *J. Virol.* **79**, 14355–14370 (2005).
Topical application of TLR agonists was not protective but instead increased the magnitude of SIV replication in infected animals. Robust inflammation was seen in tissues of pretreated animals.
117. Lard-Whiteford, S. L. *et al.* Recommendations for the nonclinical development of topical microbicides for prevention of HIV transmission: an update. *J. Acquir. Immune Defic. Syndr.* **36**, 541–552 (2004).
118. Clarke, J. G. *et al.* Microflora changes with the use of a vaginal microbicide. *Sex. Transm. Dis.* **29**, 288–293 (2002).
119. Veazey, R. S. *et al.* Protection of macaques from vaginal SHIV challenge by an orally delivered CCR5 inhibitor. *Nature Med.* **11**, 1293–1294 (2005).
120. Kawamura, T. *et al.* Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. *J. Exp. Med.* **192**, 1491–1500 (2000).
121. Collins, K. B., Patterson, B. K., Naus, G. J., Landers, D. V. & Gupta, P. Development of an *in vitro* organ culture model to study transmission of HIV-1 in the female genital tract. *Nature Med.* **6**, 475–479 (2000).
122. Greenhead, P. *et al.* Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. *J. Virol.* **74**, 5577–5786 (2000).
123. Fletcher, P. *et al.* The nonnucleoside reverse transcriptase inhibitor UC-781 inhibits human immunodeficiency virus type 1 infection of human cervical tissue and dissemination by migratory cells. *J. Virol.* **79**, 11179–11186 (2005).
124. Shattock, R. J., Griffin, G. E. & Gorodeski, G. I. *In vitro* models of mucosal HIV transmission. *Nature Med.* **6**, 607–608 (2000).
125. Di Fabio, S. *et al.* Vaginal transmission of HIV-1 in hu-SCID mice: a new model for the evaluation of vaginal microbicides. *AIDS* **15**, 2231–2238 (2001).
126. Khanna, K. V. *et al.* Vaginal transmission of cell-associated HIV-1 in the mouse is blocked by a topical, membrane-modifying agent. *J. Clin. Invest.* **109**, 205–211 (2002).
127. Kish, T. M., Budgeon, L. R., Welsh, P. A. & Howett, M. K. Immunological characterization of human vaginal xenografts in immunocompromised mice: development of a small animal model for the study of human immunodeficiency virus-1 infection. *Am. J. Pathol.* **159**, 2331–2345 (2001).
128. Kish, T. M. *et al.* HIV-1 infection in a small animal human vaginal xenograft model. *J. Acquir. Immune Defic. Syndr.* **34**, 454–460 (2003).
129. Manson, K. H., Wyand, M. S., Miller, C. & Neurath, A. R. Effect of a cellulose acetate phthalate topical cream on vaginal transmission of simian immunodeficiency virus in rhesus monkeys. *Antimicrob. Agents Chemother.* **44**, 3199–3202 (2000).

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Competing interests statement
The author declares competing financial interests: see web version for details.

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