

# An African perspective on mucosal immunity and HIV-1

P Pala<sup>1</sup>, VR Gomez-Roman<sup>2</sup>, J Gilmour<sup>2</sup> and P Kaleebu<sup>1,3</sup>

HIV prevention mandates an understanding of the mechanisms of mucosal immunity with attention to some unique features of the epidemic and mucosal environment in the developing world. An effective vaccine will have to induce mucosal protection against a highly diverse virus, which is equipped with a number of immune evasion strategies. Its development will require assessment of mucosal immune responses, and it will have to protect a mucosal environment where inflammation and altered immune responses are common because of the presence of other mucosal infections, such as sexually transmitted infections and parasites, and where nutritional status may also be compromised. Ideally, not only prevention methods would protect adults but also provide cover against gastrointestinal transmission through maternal milk. Prevention might also be complemented by microbicides and circumcision, two alternative approaches to mucosal protection. It seems unlikely that a single solution will work in all instances and intervention might have to act at multiple levels and be tailored to local circumstances. We review here some of the mucosal events associated with HIV infection that are most relevant in an African setting.

## INTRODUCTION

The majority of the 33 million individuals currently infected with HIV-1 live in sub-Saharan Africa. The HIV-1 epidemic in this region has some unique features compared with other settings. HIV-1 transmission in Africa is mostly through heterosexual intercourse and mother to child transmission (intrauterine, perinatal, and through breast milk) is still an issue despite availability of anti-retroviral treatment. Other routes such as parenteral–intravenous needle sharing contribute marginally, and homosexual transmission is poorly documented and probably contributing less than elsewhere.<sup>1–3</sup>

Apart from mechanical barrier methods and circumcision, the preventive arsenal does not currently contain many effective weapons. Clinical trials using treatment of sexually transmitted infections (STIs) for prevention have been disappointing.<sup>4</sup> None of the microbicides so far tested for efficacy has yielded positive results and at least one has increased the risk of transmission through the disruption of the vaginal mucosa.<sup>5</sup> Similarly, vaccine candidates aiming to induce humoral or cell-mediated immunity have disappointed.<sup>6</sup>

A preventive **vaccine** would have to induce solid mucosal immunity capable of stopping a highly diverse, rapidly evolving

virus that targets some of the very effector cells of the immune system required to prevent or blunt the infection. Rational design of such a vaccine will require a thorough understanding of early transmission events at the mucosal interface as well as understanding of immune correlates of virus control in HIV-infected individuals who spontaneously control their infection. This will require development of new **methods and assays**—with particular attention to their suitability for vaccine trials in developing countries—to evaluate the correlates of mucosal immunogenicity and protection.

Although overall mortality is mainly affected by systemic TB co-infections, multiple pathogens are responsible for widespread background morbidity at mucosal level including **genital infections** and intestinal helminths. Their interactions with HIV-1 result in enhanced transmission and disease. Inflammation at mucosal sites increases the likelihood of HIV-1 acquisition and transmission.<sup>7</sup> Prevention efforts consequently focus on understanding the mechanisms of these interactions to find ways to prevent or limit mucosal transmission. An established HIV-1 infection may in turn affect the immune response to other mucosal pathogens—human papilloma virus (HPV) infection and cervical carcinoma being an important example

<sup>1</sup>Medical Research Council—Uganda Virus Research Institute, Uganda Research Unit on AIDS, Entebbe, Uganda; <sup>2</sup>International AIDS Vaccine Initiative, Core Laboratory, Imperial College London, London, UK; <sup>3</sup>Uganda Virus Research Institute—International AIDS Vaccine Initiative, HIV Vaccine Program, Entebbe, Uganda. Correspondence: P Kaleebu (pontiano.kaleebu@mrcuganda.org)

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in Africa. However, we will not consider the effect of HIV-1 on other pathogens in this review.

The connection between **breast-feeding** and HIV-1 transmission still has particularly severe consequences in a developing country setting where safe alternatives are not available. There is uncertainty on the exact mechanism used by HIV-1 to traverse the gastrointestinal mucosal barrier of the feeding baby. A pediatric vaccine capable of inducing mucosal protection is required.

HIV-1 entry through the mucosal surfaces of the penis and urethra is less characterized than entry through cervicovaginal epithelia; however, the protective effect of **circumcision** is clear. For a one-time intervention that could have a significant public health impact in developing countries—comparable to a moderately effective vaccine—there is however uncertainty on the mechanism of protection and the concern that ensuing behavioral changes might negate its benefits. Understanding the foreskin route of infection could guide decisions for large-scale interventions.

**Microbicides** aim to block HIV transmission through antiviral agents and/or enhancement of non-specific immunity mechanisms at the genital mucosa level. Their basic mechanisms of interference with viral entry could be a robust approach to the extreme viral diversity encountered in the African continent.

In this review, we will consider those aspects of mucosal immunology that are particularly relevant to control of HIV-1 in Africa and the developing world. The approach is multi-faceted because it is unlikely that a single type of intervention could achieve maximal control.

## MUCOSAL TRANSMISSION

Mucosal surfaces of the genital and intestinal systems are the entry point of most HIV-1 infections (reviewed by Hladik and McElrath<sup>8</sup>). The immunological subsystem of the gut and the lower genital tract is subsequently the main target organ for HIV-1 pathology (recently reviewed by Brenchley and Douek<sup>9</sup>). In simian immunodeficiency virus (SIV)-infected macaques the virus establishes foci of infection in the submucosa within 60 min of contacting the vaginal mucosa,<sup>10</sup> rapidly disseminates to the local draining lymph nodes and is detected systemically 6–25 days later.<sup>11,12</sup> Although HIV-1 can traverse epithelial cells and dendritic cells (DCs),<sup>13–16</sup> the first cells to become productively infected are probably the CCR5<sup>+</sup> CD4<sup>+</sup> T cells, DC, and macrophages; DCs and T-cell clusters then drive the infection, and DCs migrating to lymph nodes facilitate the dissemination of the virus and destruction of 30–60% of CD4<sup>+</sup> memory cells throughout the body within a few days, particularly in mucosal compartments, the most affected being the gut.<sup>17</sup> The SIV/macaque model further highlighted that both resting and activated CD4<sup>+</sup> T cells are susceptible to infection<sup>18</sup> and that gut pathology in early infection includes apoptosis of epithelial cells in the small and large intestine.<sup>19</sup>

HIV-1 transmission has features of limiting dilution cloning: in a study of acute infection in HIV-1 discordant couples in Zambia<sup>20</sup> it was shown that one or two viruses establish the infection despite exposure to a heterogeneous quasi-species, a

finding that has been confirmed.<sup>21,22</sup> This transmission bottleneck would appear to be at the mucosal surface<sup>7</sup> and could be a major opportunity for protective interventions.

## DESIGN AND ASSESSMENT OF AN EFFECTIVE PREVENTIVE HIV-1 VACCINE

The need for a safe, effective, preventive HIV-1 vaccine, as part of a comprehensive prevention package, is compelling. Although strides have been made in treatment and prevention programs these have yet to have an impact on transmission rates, particularly in Africa where the medical infrastructure to provide lifelong chemotherapy and follow-up simply does not exist in the majority of countries. HIV-1 represents a unique combination of formidable challenges to vaccinologists (**Table 1**).<sup>23–25</sup> Of particular note in Africa is the tremendous sequence diversity and rapid virus evolution. Currently, 39 various preventive AIDS vaccines are being tested in human trials. Of these, 17 are being tested in developing countries (<http://www.iavireport.org/specials/OngoingTrialsOfPreventiveHIVVaccines.pdf>). Although the end points for phase 1 (vaccine safety) and phase 3 (vaccine efficacy) trials are relatively straightforward to measure, the end points for phase 2 vaccine immunogenicity trials are proving challenging to define with certainty. Although definitive correlates of protection will remain elusive until a vaccine candidate shows some level of efficacy in the clinic, protection, or containment of the virus within the mucosa or draining lymph nodes is only likely to be achieved if both antibodies and T cells capable of recognizing a broad spectrum of viruses are induced at the local site of infection. Potent systemic anti-viral CD8<sup>+</sup> T cells and antibodies, particularly, in mucosal tissues such as in the gut, may also have an impact on virus replication and disease progression should infection occur. Three trials with efficacy end points have been conducted to date, and all failed; the first two used an antibody inducing gp120 candidate and the third a T-cell inducing, recombinant adeno-5 viral vector: the gp120 candidate failed to induce broadly cross-reactive-neutralizing antibodies and the adeno-5 failed to induce a broad T-cell response. The design of an immunogen capable of inducing potent broadly cross-reactive antibodies has proved to be a formidable scientific challenge.<sup>26</sup> The recent failure of the MRKAd5 HIV-1 Gag/Pol/Nef candidate vaccine to either protect from disease acquisition or progression has shown that elicitation of systemic T-cell immunity, with limited breadth as measured with the interferon (IFN- $\gamma$ ) enzyme linked immuno spot assay, is not sufficient to confer protection against a mucosally acquired HIV-1 infection in men.<sup>27</sup> Although mucosal immunity was not directly evaluated, a trend toward an increased number of HIV-1 infections was observed in uncircumcised vaccine recipients,<sup>6,28</sup> suggesting that either no protective responses or even detrimental responses might have been induced in the foreskin. The majority of T-cell vaccine candidates have not been designed specifically to induce mucosal T-cell responses<sup>29</sup> and with very few exceptions vaccines are being administered parentally, with immunogenicity assessments and conclusions being drawn from peripheral blood samples in nearly all vaccine trials to date.

**Table 1 Challenges in the development of an HIV-1 vaccine**

Challenge	
Sequence diversity	Rapid mutation rate, RNA genome highly mutable and RT error prone, immune selection pressure, recombination
Integration of viral genome establishes latent viral pool	
No small animal model	
Correlates of protection unknown	
Mucosal transmission	Mucosal immune system compartmentalized and will require protection at multiple mucosal surface
Immune destruction in mucosal tissues, most notably the gut	Rapid T-cell depletion in mucosal tissues, immune dysregulation
Immune evasion of antibody and T-cell responses	Broadly cross-reactive neutralizing antibody is rarely produced because of masking of key epitopes  T-cell epitopes readily mutate and escape T-cell response, HIV-1 proteins modulate the host immune response
Assessing mucosal immune responses	

Clearly, mucosal correlates of protection need to be better defined and novel mucosal immunogenicity assays need to be implemented as part of vaccine evaluation and development. Vaccine discovery and development need to focus on strategies that may specifically induce mucosal immunity. These are among the priorities for mucosal immunity research recently proposed by the Global HIV-1 Vaccine Enterprise.<sup>30</sup>

#### ADDRESSING THE NEUTRALIZING ANTIBODY PROBLEM USING MUCOSAL SPECIMENS FROM DEVELOPING COUNTRIES AND A REPERTOIRE OF ASSAYS

For the purposes of standardization and high throughput, current assays to assess HIV-neutralizing antibodies use sera and cells such as the TZM-bl cell line, an epithelial, HeLa-derived cell line expressing artificially high levels of CCR5, or in peripheral blood mononuclear cell-based HIV-1-neutralization assays.<sup>31</sup> Thus, our knowledge of HIV-1-neutralizing antibody biology is still limited to the context of non-mucosal cells and non-mucosal fluids. It was recently shown that the broadly neutralizing antibodies 2F5, 2G12, and IgG1b12 lack the ability to inhibit transcytosis of cell-free and cell-associated HIV-1 across a tight monolayer of human endometrial carcinoma-1 cells. In contrast, IgG and S-IgA purified from colostrum samples collected from four HIV-infected women in Bangui (Central African Republic) were able to inhibit transcytosis of the X4-tropic HIV-LAI strain.<sup>32</sup> This suggests conventional HIV-1 neutralization and transcytosis, across a mucosal cell layer, are two distinct mechanisms and it remains

to be established which of the two will be more relevant in the context of a mucosal HIV-1 vaccine.

Rare and broadly neutralizing monoclonal antibodies such as 2F5, 2G12, and 4E10 have been isolated from the blood of clade-B HIV-1-infected patients. These antibodies are often used as the gold standards for the broad neutralization profile that one would desire to elicit with an HIV-1 vaccine. However, it is still unclear whether these antibodies can effectively neutralize a broad panel of HIV-1 strains isolated from semen and cervicovaginal fluids collected from developing countries. Panels of viruses representing founder viruses from around the world are beginning to be established, but there is yet to be a standard panel of HIV-1 strains purely derived from mucosal fluids. Conversely, broadly neutralizing monoclonal antibodies have not been isolated from mucosal compartments of non-clade-B HIV-1-infected individuals. Efforts are now underway to identify novel broadly neutralizing monoclonal antibodies from multiple clades and countries.<sup>33</sup>

It is also worth noting that sIgA plasma cells are found in mucosal tissues and humans secrete gram quantities of sIgA each day, making sIgA the most abundant immunoglobulin class in secretions, and undoubtedly important (reviewed by Macpherson *et al.*<sup>34</sup>). Although IgG antibodies transudate across mucosal surfaces and have certainly conferred protection against cervical cancer in the case of HPV vaccination,<sup>35</sup> they are rapidly degraded and unlike sIgA are also affected by the menstrual cycle,<sup>36</sup> which may have an impact on efficacy over time. Non-neutralizing sIgA could also have an impact on transmission by (a) immune exclusion (cross-linking and entrapment in mucus) to prevent adherence, (b) antibody-dependent cellular cytotoxicity destruction of infected cells, (c) blocking transcytosis, and (d) mediating transport back across the epithelial barrier by the polymeric immunoglobulin receptor. Neutralizing IgAs have also been shown to prevent R5 HIV-1 infection of both CD4<sup>+</sup> T cells and macrophages.<sup>37</sup> The role of mucosal IgA vs. serum IgG in HIV-exposed, persistently uninfected individuals was reviewed by Alexander and Mestecky.<sup>38</sup> Conversion of the 2F5 IgG monoclonal antibody into an IgA Fab increased its neutralizing potency and conferred the antibody with transcytosis-blocking activity,<sup>39</sup> suggesting that vaccine-induced IgA may have a more potent effect than IgG alone. To induce IgA, vaccines must be delivered through the mucosal route, for example mucosal delivery of HPV vaccine did induce IgA responses<sup>40</sup> and failure of candidate HIV-1 vaccine to elicit IgA thus far can be attributed to the almost exclusive use of parenteral vaccinations.

Other types of non-conventional HIV-1 neutralization in mucosal compartments could be examined in more detail. This would include mucosal antibody-dependent cellular cytotoxicity of HIV-infected cells, the lysis and opsonization of antibody-coated HIV-infected cells by complement deposition in the mucosa and the inhibition of HIV-1 infection mediated by natural antibodies (Nabs). For example, healthy and HIV-infected women in Bangui were recently shown to have natural anti-CCR5 antibodies in sera, breast milk, and cervicovaginal lavage. These natural antibodies have the capacity to inhibit the infection of macrophages and DCs with R5-tropic HIV-1

strains. Natural antibodies also have the capacity to inhibit the transfer of R5-tropic HIV-1 from DCs to T cells.<sup>41</sup> Harnessing the activity of natural antibodies present in mucosal secretions may prove to be beneficial in the development of microbicides and vaccines.

### T-CELL ISSUES AND MUCOSAL VACCINE DEVELOPMENT

Probably the most significant challenge to developing effective CD8<sup>+</sup> T-cell immunogens—particularly in Africa—is the incredible sequence diversity of the virus, its ability to recombine, and its rapid escape under immune selection pressure. This is coupled with the need to have these cells present in the mucosal tissue at the time of exposure to contain the infection locally, or in the gut shortly thereafter to impact viremia and disease progression.<sup>42</sup> Efforts are now starting to focus on the design of T-cell immunogens based on highly conserved regions of the viruses mosaic, ancestral, or conserved sequences<sup>43–45</sup> that might induce responses to a broad and diverse range of viruses.<sup>42</sup> T-cell assays used in vaccine trials and HIV-1 pathogenesis studies often rely on the use of high concentrations of exogenously added peptides bypassing natural antigen processing pathways, and measuring both high and low avidity cells, or irrelevant target cells such as B cells. Not all T-cell epitopes are equivalent, and not all T cells will have potent or broad antiviral activity. ELISPOT, flow cytometry, or tetramer assays are akin to binding antibody assays; however, the 1st generation of T-cell neutralization assays<sup>25</sup> for use in HIV-1 vaccine trials, using replication competent panels of viruses and autologous target cells, are in qualification stages. They correlate with *in vivo* virus control in HIV-1-infected individuals and show excellent specificity and precision in vaccines (Gilmour *et al.*, unpublished).

### DELIVERY ROUTES

Mucosal vaccine development presents a unique set of challenges and issues. The mucosal immune system is highly compartmentalized and the administration route, adjuvant and antigen delivery system may be as important as the immunogen design itself. This has been extensively reviewed recently.<sup>31,46–48</sup> A central role of certain mucosal immune compartments such as the gastrointestinal tract is to induce tolerance, which may make induction of mucosal immune responses through these routes challenging. However, it is also becoming apparent that to elicit specific mucosal immune responses priming may need to be through a mucosal surface (reviewed in refs.<sup>46,47,49</sup>).

**Oral vaccination** has proved effective with a number of vaccines including live polio, inactivated oral cholera, live typhoid fever, and rotavirus. These vaccines have tropism for cells in the intestine, and induce good antibody and T-cell responses both systemically and in the upper intestine, but not in the lower intestine or reproductive tract. Common nutritional deficiencies and intestinal infections such as helminths may significantly alter the effectiveness of oral vaccination in Africa.

**Rectal immunization** has resulted in rectal, lower intestinal, and systemic immune responses without significant responses in the genital tract. Rectal immunization of macaques induces

anti-viral T cells in the rectal mucosa and induction of protective immunity after rectal challenge with pathogenic simian human immunodeficiency virus-1.<sup>50,51</sup>

Mechanisms of immune induction in the male and female **genital tract** are poorly understood. M cells or organized lymphoid follicles have not been identified. Local immunization in the vagina with replicating and non-replicating vaccine antigens in mice<sup>52</sup> non human primate<sup>53</sup> and humans<sup>54–56</sup> induces local CD8<sup>+</sup> T cell, IgG, and IgA responses and could be affected by the menstrual cycle.<sup>56</sup> Very little is known about vaccination of the male urethra and penile foreskin; however, the majority of men in Africa will be infected through this route. Significant gender differences in mucosal immunity and vaccine efficacy might exist, as suggested by a phase 3 herpes simplex type-2 (HSV-2) vaccine trial<sup>57</sup> where protection could be achieved in 74% of seronegative women but 0% of men or HSV-1 seropositive women. For this reason it will be essential that vaccine candidates be tested in trials enrolling both men and women.

**Intranasal (IN) vaccination** offers an attractive route of vaccine administration and resulted in good antibody and T-cell responses in the female genital tract, urethra, saliva, intestine systemically, but not in the rectum. Responses in the female genital tract following intranasal immunization were better than after systemic immunization, required 1/10 the dose and aerosolization may not be required.<sup>58,59</sup> Perhaps an optimal regimen might have to consider priming both intranasally and rectally, followed by systemic boosting.

Low-absorption efficiency at mucosal surfaces and suboptimal mucosal immune responses have often meant mucosal adjuvants are required.<sup>60</sup> There has been a growing trend in vaccine development toward non-replicating vaccine modalities for safety reasons; however, the efficacy of live-attenuated SIV in non-human primates is consistent with the efficacy observed with some of the most successful vaccines licensed against viral infections (measles, polio, mumps, rubella), which are all live-attenuated vaccines. Given the unacceptable safety concerns with an attenuated replication competent HIV-1 vaccine, an alternative strategy might be intranasal delivery of a mucosal tropic, replication competent vector carrying the HIV-1 target antigens. This strategy is likely to overcome the need for adjuvants and could also provide similar activation of the inflammasome and innate pathways thought to be required for an effective vaccine.<sup>61</sup> This is a strategy being pursued by the IAVI and DNAVec using the Sendai vector.<sup>62,63</sup>

### MUCOSAL ASSAY DEVELOPMENT AND IMPLEMENTATION

Low cell yields and the need for frequent and reliable access to mucosal samples are the major hurdles to developing robust and relevant mucosal assays. Methods to assess T cells and antibody responses in blood in HIV-1 vaccine trials have been implemented according to good clinical laboratory practice across Africa<sup>64,65</sup> and a number of centers have also expert flow-cytometry facilities. In preparation for HIV-1 vaccine immunogenicity trials using cervical specimens, methods are being developed in many countries using cervical cytobrushes from HIV-negative and HIV-positive women. A cervical cytobrush yields an average

**Table 2 Methods to collect mucosal specimens for immunogenicity studies and HIV-1 vaccine trials**

Collection device	Collection method	Mucosal sites that can be accessed	Mucosal specimens that can be collected	Advantages	Disadvantages
Digene cervical brush	Speculum examination	Cervix Rectum	Cellular material from cervical os	Provides material for cellular assays	Limited sample volume Cannot be self-administered
Cytobrush	Speculum examination	Cervix Rectum	Cells lining the rectal epithelium		
Tampon	Insertion for 10 min	Cervix Rectum	Primarily cell-free material (secreted antibody and local cytokines)	Can be self-collected Non-invasive	Small sample volume
Swab	Self-collection by swirling	Cervix Rectum			
Cervicovaginal lavage	Speculum examination followed by flushing cervical os with sterile saline	Cervix and vaginal walls	Cellular material and cell-free material (secreted antibody and local cytokines)	Allows collection of larger volume Easy to perform in clinic Moderately invasive	Lack of standardization of volume collected Collected material is diluted Cannot be self-administered
Absorption wicks, including the Sno Strip filter-paper wicks	Speculum examination followed by insertion of paper wick into endocervix	Endocervix	Primarily cell-free material (antibodies, local cytokines and HIV-1 shed at the cervix)	Precision of mucosal site from which sample is being collected Allows for standardization of HIV-1 viral load measurements	Cannot be self-administered
Ophthalmic sponges, including the Weck-Cel, Ultracell, and MeroCell sponges	Adapted ophthalmic fluid collection devices	Cervix Rectum	Primarily cell-free material (antibodies and local cytokines)	Allows for non-traumatic collection of cervical and rectal secretions	Small sample volume
Surgical forceps	Oesophagogastroduodenoscopy for upper GI tract Flexible sigmoidoscopy for lower GI tract	Duodenum Sigmoid colon Rectum	Cellular material	Yields sufficient mucosal mononuclear cells for cellular assays	Invasive Risk of colon perforation

GI, gastrointestinal

of half a million mucosal mononuclear cells that can be directly stimulated with HIV-1 peptides to assess production of multiple cytokines by flow cytometry. Thus, Gag-specific cervical T-cell responses have been assessed in women attending community clinics in Cape Town, South Africa.<sup>66</sup> Genital responses did not correlate with those detected in blood and T-cell yields were often low in the absence of inflammation. Systems biology approaches requiring low cell numbers, such as micro-arrays, are becoming more robust and affordable, and could become part of a comprehensive immune monitoring panel in vaccine trials for both blood and mucosal tissues.<sup>61</sup> However, given the complex bio-informatics associated with such approaches, collaboration with partners outside Africa will be required initially.

**Table 2** outlines a number of non-invasive methods commonly used for collecting mucosal secretions. The absorbent Weck-Cel (Medtronic Xomed, Jacksonville, FL) sponge appears to be more reproducible and effective than lavage.<sup>67</sup> Such non-

invasive sampling could be deployed relatively easily at HIV-1 vaccine trial sites and research centers in Africa, where many teams routinely carry out smear tests and have taken part in microbicide trials. Genital fluids can also be used in relatively simple assays to assess mucosal antibodies, viral RNA or p24 antigen, and multiple cytokines. Collection of secretions from the female reproductive tract could be implemented in HIV-1 vaccine trials; however, the feasibility of sampling the male genital tract and rectum needs to be addressed also.

The gut is the initial site of HIV-1 replication and massive CD4<sup>+</sup> T-cell depletion irrespective of the transmission route. It is conceivable, therefore, that an efficacious HIV-1 vaccine could be one that prevents high-level virus replication and CD4<sup>+</sup> T-cell depletion in the gut during this so-called initial “window” of opportunity. One would expect HIV-specific CD8<sup>+</sup> T cells residing in the gut with potent and broadly cross-reactive anti-viral responses to control viral replication. Their elicitation during

vaccination is, therefore, highly desirable. Consistent with this hypothesis, the magnitude and the complexity of rectal HIV-1 gag-specific gut CD8<sup>+</sup> T-cell responses were shown to correlate with lower viral loads and with elite-controller clinical status in subtype-B, chronically infected cohorts in the United States.<sup>68,69</sup> A major challenge to HIV vaccinologists will be to elicit and assess such a potentially protective response in the gut.

In a recent phase 1 safety and immunogenicity trial using the vaccinia-based TBC-3B HIV-1 vaccine given subcutaneously, participants consented to donating rectal biopsies by undergoing flexible sigmoidoscopy. Expanded cells did not produce significant levels of IFN- $\gamma$  on stimulation with HIV-1 peptides.<sup>70</sup> This study shows that invasive sampling can be performed in phase 1 studies; however, this vaccine delivered intramuscularly did not induce rectal responses.

Would gut sampling be ethical and feasible across HIV-1 vaccine clinical trial sites in the developing world? In terms of safety, rectal and colonic biopsies are considered invasive and may pose a minor risk of colon perforation to the patient, making them relatively unappealing for wide use. Tearing the rectal lining during flexible sigmoidoscopy could also increase the risk of the donor to rectally acquired HIV-1 infection, raising ethical concerns about its feasibility in clinical trials with volunteers who may be at risk of HIV-1 exposure through the same surface. Assessment of T-cell responses from mucosal tissues (reproductive tract and gut) should become part of the immunogenicity assessment in early product development through small phase 1 studies, with volunteers who are at very low risk of HIV-1 acquisition.

## GENITAL INFECTIONS

STIs are among the most common reasons for seeking medical care in many parts of Africa and other developing world and their contribution to HIV-1 infection has been shown in many studies (reviewed in ref.<sup>71</sup>). The non-specific or innate immune mechanisms, which include the skin, secretions, opsonins and phagocytes, are important first lines of defence.<sup>72</sup> In fact an intact genital epithelium is an effective barrier, infections that disrupt the mucosal surfaces i.e., the genital ulcerative diseases and non-ulcerative STIs that cause inflammation can greatly increase the chances of HIV-1 infection (reviewed in ref.<sup>73</sup>) and facilitate infection by multiple genetic variants,<sup>74</sup> relieving the transmission bottleneck<sup>7</sup> and—presumably—ultimately accelerating viral evolution through recombination.

Genital ulcerative and non-ulcerative infections cause inflammatory cell infiltration at the mucosal site of infection, and hence increase the number of potential target cells for HIV-1 infection.<sup>75</sup> DCs in the normal uninfected genital mucosa reside immediately beneath the epithelium, i.e., the Langerhans cells and in the submucosa (submucosal DCs).<sup>76</sup> However, during infections or inflammations, additional myeloid DCs and IFN- $\alpha$ -producing plasmacytoid DCs are recruited from the peripheral blood. The role of DCs in HIV-1 transmission has been recently reviewed.<sup>77</sup> As pro-inflammatory cytokines and chemokines are produced locally, more macrophages, and later CD4<sup>+</sup> and CD8<sup>+</sup> T cells are recruited.<sup>78,79</sup> There is, as a result, an increase in DC-SIGN<sup>+</sup> DC and CCR5<sup>+</sup>

CD4<sup>+</sup> T cells, which are targets for HIV-1. These early events have been reviewed.<sup>80</sup>

**Table 3** summarizes the features of common mucosal infections that influence HIV acquisition in Africa.

## HERPES SIMPLEX TYPE 2

HSV-2 is the leading cause of genital ulcerative diseases in both developing and developed countries,<sup>81</sup> with nearly 90% prevalence in HIV-1-positive persons. It is believed that HSV-2 plays a major role in the spread of HIV-1<sup>82</sup> increasing the infectiousness of an HIV-1-positive person through increased HIV-1 shedding,<sup>83,84</sup> viral replication<sup>85,86</sup> and increasing acquisition in the HIV-1 uninfected people. A recent analysis of data from a male circumcision trial in Orange Farm, South Africa reported a population fraction of incident HIV infection attributable to HSV-2 of 27.8%.<sup>87</sup> The possible mechanisms of how HSV-2 can lead to increased acquisition of HIV-1 include not only disruption of the mucosal membrane, but also HSV-2 has been implicated in the increase in the HIV-1 target cells in the genital mucosa as described above and transactivation of the HIV-1 long terminal repeat *in vitro*.<sup>88</sup>

HIV-1 prevention strategies would therefore include treatment of HSV-2. Unfortunately, the results of clinical trials using treatment of HSV-2 for prevention have been disappointing.<sup>89-92</sup> HSV-2 suppressive therapy with valacyclovir or acyclovir can lower the HIV-1 viral load in the blood, rectal secretions,<sup>93</sup> and seminal fluid<sup>94</sup> of HIV-1 and HSV-2 co-infected men and genital shedding in women,<sup>95</sup> which might have a public health benefit of limiting transmission as well as improving HIV-1 suppression in the infected individual. Conversely, two recent trials reported no efficacy of HSV-2 suppressive therapy on reducing HIV-1 acquisition.<sup>4,96</sup> The reasons for this failure are not yet clear but taken together these results suggest that HSV-2 suppressive therapy might be more effective in reducing shedding from HSV-2/HIV-1 co-infected individuals than in preventing HIV-1 acquisition in HSV-2-infected individuals.

The effect of HSV-2 in modulating mucosal responses and HIV-1 transmission will have to be considered in the evaluation of future mucosal vaccines.

## GONORRHOEA

Non-ulcerative STIs such as *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* infections may also recruit and activate HIV-susceptible cells as described above.<sup>75,97</sup> Genital infections can also influence the adaptive immune responses. The interaction between gonorrhoea and HIV-1 goes in opposite directions depending on the stage of infection. In heterosexual HIV-1 acquisition, genital *Neisseria gonorrhoeae* has recently been associated with enhanced HIV-1-specific CD8<sup>+</sup> T-cell responses though not with differences in viral load set point.<sup>98</sup> However, in chronic HIV-1 infection, incident infection with *Neisseria gonorrhoeae* was associated with transient increases in blood HIV-1 viral load and decreased absolute CD4<sup>+</sup> T-cell counts,<sup>99</sup> an effect that might be related to impairment of HIV-1-specific CD8<sup>+</sup> T-cell responses<sup>100</sup> and CD4<sup>+</sup> T-cell function.<sup>101</sup> *Neisseria gonorrhoeae* has also been shown to

**Table 3 Common genital diseases in Africa and their effect on HIV-1 acquisition**

Category of genital disease	Infectious agent or disease	Possible mechanisms responsible for increased susceptibility to HIV-1 infection	Effects on the innate and adaptive immune response to HIV	References
Genital ulcerative diseases	HSV-2	Increased shedding and replication of HIV-1 observed in HSV-infected individuals. Disruption of the mucosal membrane. Activation and recruitment of HIV-susceptible cells	Increase in immature dendritic cells expressing DC-SIGN Increased expression of CCR5 on CD4 <sup>+</sup> cells	83–86,194
Non-ulcerative sexually transmitted infections	<i>Neisseria gonorrhoeae</i>	Inflammation, recruitment, and activation of HIV-susceptible cells	Enhancement of HIV-specific CD8 <sup>+</sup> T cell responses Reduced systemic CD8 <sup>+</sup> T cell responses Impaired CD4 T cell function Inhibition of dendritic cells maturation	75,97–102
	<i>Chlamydia trachomatis</i> (LGV)	Inflammation, recruitment, and activation of HIV-susceptible cells	Increased production of pro-inflammatory cytokines	73,75,97
Non-sexually transmitted condition	Bacterial vaginosis <i>Gardnerella vaginalis</i> and various anaerobic species	Dysfunction of innate vaginal mucosa Inflammation, recruitment, and activation of HIV-susceptible cells	Reduced concentration of vaginal lactobacilli Increase in vaginal pH Reduced concentration of antimicrobial polypeptides, defensins, and lactoferrin Reduced concentration of secretory leukocyte protease inhibitors Increased production of pro-inflammatory cytokines	106–116,194
Female genital schistosomiasis	<i>Schistosoma haematobium</i>	Genital inflammation and lesions resulting in recruitment and activation of HIV-1-susceptible cells	Unknown	122

inhibit maturation of DCs and their ability to prime HIV-specific immune responses.<sup>102</sup> Whether these events start by modulating the innate and adaptive responses at the mucosal level is not clear as gonococcal lipo-oligosaccharide can induce anti-HIV-1 innate immunity in primary human macrophages through IFN- $\beta$  release induced by toll-like receptor-4 signaling.<sup>103</sup> The related *N. meningitidis* polysaccharides are now being developed for use as adjuvants in mucosal vaccine research.<sup>104,105</sup>

### BACTERIAL VAGINOSIS

Bacterial vaginosis (BV), a non-sexually transmitted condition, has been associated with increased risk of HIV-1 acquisition<sup>106,107</sup> possibly through a dysfunction in the innate immunity of the vaginal mucosa.<sup>108</sup> BV results from the abnormal outgrowth of several species of anaerobic bacteria outcompeting the normal vaginal lactobacilli flora and resulting in an elevation of vaginal fluid pH. Cervical lavage fluid from women with BV was found to be deficient in antimicrobial polypeptides such as lysozymes, lactoferrins, defensins, and in antibacterial activity compared with healthy women or other infections<sup>109</sup> though

the mechanism was unclear. These polypeptides are small, amphiphilic cationic molecules with broad-spectrum activity against bacteria, fungi, and virus and are increasingly considered to be major effectors of the innate immune system.<sup>110</sup> Others have also indicated a reduction in secretory leukocyte protease inhibitors (SLPIs) in women with BV and other STIs. These antimicrobial peptides and proteins have been shown to have some effect on HIV-1<sup>108,111,112</sup> contributing to the innate host defense of the vagina.<sup>113</sup> Although the association between the SLPI levels and reduced sexual HIV-1 acquisition is less clear, higher SPLI concentrations in vaginal secretions have been correlated with reduced perinatal transmission.<sup>114</sup> SLPIs have *in vitro* anti-HIV-1 activity by blocking macrophage infection at physiological concentrations<sup>115</sup> possibly through interaction with annexin-II.<sup>116</sup>

Similar to HSV-2 and other STIs, BV recruits inflammatory cells to the vaginal mucosa. Pro-inflammatory cytokines such as interleukin-1- $\beta$  (IL-1- $\beta$ ) and tumor necrosis factor- $\alpha$ , which can upregulate HIV-1 *in vitro*, are increased in genital secretions during BV infection.<sup>117</sup> Vaginal lavage fluids of women with BV were also found with low neutrophil-attracting chemokine IL-8

and  $\beta$ -defensins,<sup>109</sup> whereas others found increased IL-8 and pro-inflammatory chemokines but not neutrophils.<sup>118,119</sup> Other mechanisms for synergy between BV and HIV-1 include stimulation of HIV-1 expression through toll-like receptor-2 receptors (reviewed in ref.<sup>120</sup>) or effects on HIV-1 uptake and processing by DCs.<sup>119</sup> BV can induce IL-12, IL-23, and p40 secretion and induce activation markers CD40, CD83, CD86, and HLA-DR on DCs; however, the enhancement of HIV-1 infection is not mediated by trans-infection.<sup>119</sup>

## HELMINTHS

Helminths have been implicated in impairment of the immune response to HIV-1 leading to greater susceptibility to HIV-1 acquisition and more rapid HIV-1 progression (review in ref.<sup>121</sup>). This hypothesis was based on the concept that a T helper-2 (Th2) bias induced by helminths could be detrimental by suppressing protective Th1 responses to HIV-1 and expanding Th2 lymphocytes considered more susceptible to HIV-1 infection; also the immune activation might increase HIV-1 co-receptor expression on susceptible cells.

Early observations gave inconclusive results regarding associations between helminth infection and susceptibility to HIV-1 or HIV-1 disease progression. Cross-sectional studies of co-infection are handicapped by confounding factors and intervention studies have been hampered by ethical issues around withholding anti-helminths. No studies have directly addressed the effects of helminths on susceptibility to HIV-1 infection or effects of helminths on HIV-1 incidence except in the special case of female genital schistosomiasis, where genital inflammation and lesions are associated with increased risk of HIV-1 infection.<sup>122</sup> There is, however, one study in rhesus macaques where acute schistosoma mansonii infection was associated with susceptibility to systemic SHIV-1 clade-C infection after mucosal virus exposure.<sup>123</sup> Although several studies have shown in patients and experimental animal models that helminth infections significantly impair Th1-type vaccine-specific immune responses to bacterial and viral vaccines,<sup>124–129</sup> it is only recently that the effect on HIV-1 vaccines was also shown in a mice model, where HIV-1 DNA T-cell responses were suppressed in mice infected with human helminth schistosoma mansonii compared with controls with no such infection.<sup>130</sup>

Understanding the impact of helminths on vaccinations through modulation of innate and adaptive, systemic, and mucosal immune responses remains essential for the developing world, where co-infestations are common. A few phase 1b studies are planned in East Africa to investigate the impact of worm infections on Malaria, TB, and HIV-1 vaccine immunogenicity (Giuseppe Pantaleo, personal communication).

## BREAST-FEEDING

Transmission of HIV-1 through the oral route has been difficult to ascertain in adults engaging in oral sex, because of the frequent coincidence of multiple risky sexual practices (reviewed in ref.<sup>131</sup>). Despite the effectiveness of antiretroviral treatment

administered perinatally to mother and baby, in sub-Saharan Africa the late postnatal transmission by breast-feeding HIV-1<sup>+</sup> mothers to a suckling baby was about 6.9% (excluding sero-conversions occurring in the first six weeks after birth. Overall transmission rate at 12 months was 20.4%).<sup>132</sup> In developed countries, transmission is simply avoided by replacement of breast milk feeding with artificial formula milk but this approach is problematic in sub-Saharan Africa, where risks associated with formula milk feeding without adequate safe water provisions outweigh those of HIV-1 transmission. In addition, formula feeding may be viewed as a hallmark for HIV-1 infection and lead to social stigmatization, discrimination, and even violence and abandonment of the woman and her infant. The WHO recommends that HIV-1-infected mothers should breast-feed if safe formula milk feeding is not possible.<sup>133,134</sup> Mortality from any cause was higher with formula feeding than with breast-feeding in infants of HIV-1-positive mothers in rural Uganda.<sup>135</sup> The timing of weaning is important, as the risk of transmission increases with the duration of exposure. Interestingly, exclusive breast milk feeding is less risky than mixed breast milk and solid food feeding. This might be because of inflammatory effects of solid food-associated pathogens at the weaning transition, continuity breaches, or increased intestinal epithelium permeability; however, studies to substantiate such mechanism through the measurement of inflammation marker calprotectin in feces have yielded the unexpected finding of higher levels of calprotectin in, exclusively, breast-fed infants.<sup>136</sup> It is not clear where the portal of entry is: susceptible cells exist in tonsillar tissue but the saliva bathing the oral cavity also contains multiple anti HIV-1 factors including defensins, lysozyme, thrombospondin, and SLPI (reviewed in ref.<sup>137</sup>). Mucosal damage could facilitate entry of HIV-1 into sites where inflammation increases the influx of susceptible target cells and the secretion of inflammatory factors that increase expression of HIV-1 receptors and facilitate viral replication. With an intact mucosal surface, other mechanisms include transcytosis through epithelial cells, M cells in Peyer's patches or enterocytes-expressing galactosyl ceramide or Fc receptors, and DCs.<sup>138,139</sup>

It is generally agreed that IgA antibodies are not transported across the placenta and maternal milk provides IgA cover in the first months of an infant's life. However, in the case of HIV, specific IgAs present in the milk of infected mothers are not associated with protection.<sup>140</sup> Although *in vitro* studies have shown that secretory IgA or IgM may inhibit transcytosis of HIV-1 across enterocytes.<sup>15,141</sup> Infants may generate IgA responses under specific circumstances as shown in the replication-defective recombinant canarypox virus HIV-1 pediatric vaccination trial.<sup>142,143</sup> In a recent study conducted in Kenya, 8% uninfected infants exposed to milk from HIV-1-infected mothers have been shown to have HIV-1 gp160-specific IgA antibodies in salivary secretions<sup>144</sup> at a median time of one month after birth (13% of infants that instead became infected had also HIV-1-specific IgAs). All the exposed uninfected infants with IgAs remained uninfected over 12 months of follow-up, suggesting a possible protective mechanism (although not necessarily IgA mediated) despite exposure sufficient to induce a humoral

response. Unexposed uninfected controls had no IgA response. The finding parallels that of HIV-1-specific IgA1-neutralizing activity in IgG seronegative exposed uninfected men who engage in oral sex with men<sup>145</sup> as well as older reports of IgA-neutralizing antibodies in plasma and mucosal compartments of highly exposed persistently seronegative sex workers and members of discordant couples.<sup>138,139,146–148</sup>

CD8<sup>+</sup> T-cell responses have been detected in infants exposed to HIV-1-infected maternal milk, including some who did not become infected.<sup>149,150</sup> Initial findings were, recently, confirmed in a large prospective cohort study,<sup>151</sup> showing IFN- $\gamma$  responses to HIV-1 peptides in 47% of exposed uninfected infants during the first year of life. It is not clear whether such responses are protective or merely an indicator of exposure to defective virus or virus that is restricted in replication to mucosal or peripheral lymphoid tissues, as suggested by primate models.<sup>152</sup>

HIV-1 RNA viral load of breast milk, particularly cell-associated virus,<sup>153</sup> is a determinant of transmission<sup>154</sup> and HIV-1 and cytomegalovirus and Epstein–Barr virus shedding in milk from co-infected mothers are correlated;<sup>155</sup> conversely, mastitis is not a good predictor of HIV-1 RNA viral load in milk.<sup>156</sup>  $\alpha$ -Defensins levels in breast milk from HIV-1-infected women correlated with viral load or subclinical mastitis, but the correlation with HIV-1 transmission to the baby was not confirmed in various studies.<sup>157,158</sup> Breast milk levels of chemo-kines macrophage inflammatory protein-1 $\beta$  and regulated upon activation, normal T-cell expressed, and secreted were found to be higher in HIV-1-infected than uninfected mothers, and among HIV-1-infected women the transmitters had higher breast milk RANTES levels than non-transmitters.<sup>159</sup> Intriguingly, low levels of IL-7—a stromal/epithelial cell-derived factor with anti-apoptotic and growth activity for peripheral T lymphocytes—in the milk of HIV-1-infected mothers have been associated with decreased transmission to babies,<sup>160</sup> suggesting a possible role of milk CD4<sup>+</sup> T cells in infant infection.

An understanding of the mucosal innate and adaptive immunological events associated with HIV-1 transmission in breast-feeding and the immunogenicity correlates of pediatric exposure to antigens through the oral route will be required to develop interventions including a mucosal vaccine. Currently, some of the authors and others are involved in perinatal HIV-1 vaccine trials ([http://www.hptn.org/research\\_studies/HPTN027StudyDocuments.asp#Protocol](http://www.hptn.org/research_studies/HPTN027StudyDocuments.asp#Protocol)), but we still lack a candidate that can induce mucosal immune responses.

### MALE CIRCUMCISION

Apart from mechanical barriers such as condoms, the most effective way to prevent HIV-1 transmission through sexual intercourse is currently male circumcision, which can reduce female to male transmission by 60%.<sup>161–163</sup> Details of the mechanism of protection are still being explored and are likely to pivot on the reduction in the infection targets in the male partner. Circumcision might simply be reducing the chances that any one exposure to the virus will result in infection. Although the surface area of the prepuce is small, under a thin layer of

keratin the human foreskin mucosa contains CD4<sup>+</sup> T cells, macrophages, and Langerhans cells (a subset of DCs) expressing the CCR5 HIV-1 co-receptor and is more susceptible to infection than the external foreskin.<sup>164–166</sup> In an *in vitro* model of male mucosal infection, explanted foreskin, glans and urethral mucosa obtained from circumcision and gender reassignment surgery could be used to show HIV-1 protective effects of microbicides.<sup>167</sup> As discussed above, the number of macrophages, DCs, and Langerhans cells increases with other genital infections,<sup>165</sup> a possible basis for synergistic effects. Langerhans cells do not express the DC-SIGN receptor that mediates binding of HIV-1 to other DCs and their product langerin has been shown to inhibit HIV-1;<sup>168</sup> however, they have membrane processes that sample the extra-mucosal milieu<sup>169,170</sup> (although *in vivo* uptake of HIV-1 virions has not been formally shown) and could mediate trans-infection. *In vitro* studies of CD34<sup>+</sup> hemopoietic progenitor derived, activated, langerin<sup>+</sup>, Birbeck granule<sup>+</sup> Langerhans cells<sup>171</sup> show clustering of infectious HIV-1 virions within an intracellular multi-vesicular compartment with tetraspanin markers, CD1a and langerin, suggesting that transcytosis might also occur *in vivo*.

Although randomized clinical trials support male circumcision's effectiveness in preventing heterosexual transmission, a recent meta-analysis of studies of male circumcision and homosexual transmission of HIV-1 did not find evidence for a protective effect of circumcision, even when the analysis was restricted to HIV-1-negative men engaging primarily in insertive anal sex.<sup>172</sup> To our knowledge, the infectivity of HIV-1 present in rectal vs. vaginal mucosal secretions has not been compared, an association between circumcision and protection from homosexual transmission was, however, found for studies conducted before highly active anti-retroviral therapy availability, suggesting that the protective effect of circumcision seen in heterosexual transmission might be overcome by higher sexual risk behavior in men who have sex with men.<sup>172</sup> Circumcision was also shown to be associated with lower HIV-1 prevalence in a recent study of heterosexual transmission in African American men with known exposure to HIV-1, but the association was not significant for men whose exposure was not known.<sup>173</sup>

The acceptability of male circumcision varies with cultures and individuals, stimulating the search for non-surgical alternatives. It has been observed that estrogens cause an increase in keratinization and thickening of human vaginal epithelium and intravaginal estriol can increase resistance to SIV infection in ovariectomized rhesus macaques.<sup>174</sup> A similar increase in keratinization occurs when estriol is applied to the foreskin and could be an alternative or complementary to circumcision.<sup>175</sup> Understanding the mechanism of protection through circumcision could lead to new interventions.

### INNATE IMMUNITY IN THE GENITAL TRACT AND MICROBICIDES

HIV-1 can travel by diffusion in interstitial spaces between stratified squamous epithelium cells lining the female genital tract<sup>176</sup> and contact immature DCs, leading to uptake, DC maturation,

and dissemination of the virions to draining lymph nodes and infection of susceptible CD4<sup>+</sup> CCR5<sup>+</sup> T cells. However, the heterosexual transmission rate of HIV-1 is lower than expected on the basis of viral loads in genital secretions and multiple mucosal innate protective mechanisms are probably involved. In the female genital tract, cervical vestibular glands secrete mucus that covers the cervical and vaginal epithelia and incorporates anti-microbial peptides. Neutrophils, submucosal serous cells, vaginal epithelial cells, and cervical glands contribute anti-HIV-1 factors lactoferrin and lysozyme, SLPI,  $\alpha$ - and  $\beta$ -defensins. Combinations of these factors at physiological concentration can inhibit HIV-1 entry *in vitro*,<sup>113</sup> whereas individual factors may need to be used at pharmacological concentrations to achieve the same effect. Human defensin genes exhibit copy number polymorphism and single nucleotide polymorphisms in the  $\beta$ -defensin-1 gene, which are associated with resistance to infections; homozygosity for the A69G polymorphism being overrepresented in HIV-1-exposed seronegative individuals compared with seropositives.<sup>177</sup> Because of a stop mutation in the signal peptide sequence, humans do not produce  $\theta$ -defensin peptides, unlike other primates. However, synthetic  $\theta$ -defensin is remarkably active *in vitro*, protecting T cells from infection by clinical isolates of HIV-1.<sup>178</sup>

The menstrual cycle-related fluctuations in estradiol and progesterone hormones affect the thickness of the vaginal epithelium, pH of vaginal secretions, amount of mucus and levels of defensins, and SLPI in the endometrium, so that the protection afforded by these mechanisms is not constant. Innate non-specific protection can also be compromised by common conditions such as BV as mentioned earlier.

The use of microbicides represents a logical step to enhance the mucosal protection afforded by innate defenses. Early microbicide concepts involved potentiating the adsorbing capacity of mucus, pH control, or disruption of virions by detergents—an extension of non-specific natural protective mechanisms. The first surfactant-based microbicide—Nonoxynol-9—inactivates HIV-1 *in vitro* by disrupting the envelope lipid layer. It is also effective as a spermicide, used on condoms. However, it took the failure of efficacy trials to realize that regular Nonoxynol-9 use damages the vaginal epithelial layer, causing inflammation and paradoxically increasing the susceptibility to HIV-1.<sup>5</sup> In the same microbicide category Savvy (C31G cetyl betaine and myristamine oxide) had inconclusive phase 3 trials because of lower than expected HIV-1 infection incidence,<sup>179</sup> and sodium lauryl sulphate is scheduled for phase 3 trials. “Vaginal protectors” microbicides currently in clinical trials in Africa (Buffergel (<http://www.microbicide.org/galleries/clinical-trials/MicrobicideOngoingClinicalTrials.3Nov08.pdf>), Amphora (<http://www.microbicide.org/galleries/clinical-trials/MicrobicidePlannedFundedClinicalTrials.3Nov08.pdf>)) work by acidifying the vaginal milieu, maintaining mucosal conditions that are unfavorable to HIV. As they counteract the pH-raising effect of semen, these are also spermicidal agents. Another approach involves lactobacilli of exogenous origin to supplement the natural vaginal flora. Lactobacilli are also being promoted as vectors of bio-engineered proteins that act as HIV-1 entry inhibitors (CD4,

gp41, cyanovirin).<sup>180–182</sup> Cyanovirin-N is a fusion inhibiting lectin that binds high mannose residues in gp120 and has been effective *in vitro* on organ cultures and in macaque models with SHIV-1 challenges.<sup>183,184</sup>

Anionic polymer microbicides interfere with virus entry by having a negative electric charge complementary to the viral envelope protein. Carrageenan-based Carraguard was safe and well tolerated but ineffective;<sup>185</sup> naphthalene sulfonate-based PRO2000 is still being evaluated, although one arm of the study was stopped early for futility. Recently, an interim report on the continuing arm revealed a 30% protective effect, just below statistical significance.<sup>186</sup> Cellulose sulphate (Ushercell, Polydex Pharmaceuticals Limited, Toronto, Canada) binds to gp120 and is inhibitory *in vitro*. However, phase 3 trials were stopped when an interim analysis showed higher incidence in HIV-1 infections in the cellulose sulphate group. It has, recently, been appreciated that cellulose sulphate (and other polyanions such as dextrin sulphate and PRO2000) have a biphasic effect, inhibiting HIV-1 infection at high concentration and enhancing it at low concentration.<sup>187</sup> Synthetic PSC-RANTES<sup>188</sup> and CMPD167<sup>189</sup> bind to the CCR5 co-receptor and prevent virus attachment. Protective effect was shown in macaque models with SHIV-1 challenge and combination with synthetic peptides that bind to gp120 and gp41 can provide additional protection.<sup>190</sup> These microbicides do not affect the minority of HIV-1 strains that use the CXCR4 co-receptor—this loophole might have to be addressed if successful usage of CCR5 blockers resulted in selection of CXCR4 strains, for instance, by inclusion of fusion inhibitory peptide T-1249.<sup>191</sup>

A new generation of microbicide gels capitalizes on the effectiveness of antiretroviral compounds with specific anti-HIV-1 activity by incorporating reverse transcriptase inhibitors such as tenofovir in a form of pre-exposure prophylaxis. Low levels of systemic absorption occur and resistance mutations have not been observed except in those individuals already on anti retroviral therapy at enrollment. A phase 2 B trial of 1% tenofovir gel is ongoing—having just completed enrollment—in South Africa. (<http://www.microbicide.org/galleries/clinical-trials/MicrobicideOngoingClinicalTrials.3Nov08.pdf>). Other microbicides under evaluation incorporate non-nucleoside reverse transcriptase inhibitors TMC120 (Dapivirine, Tibotec Pharmaceuticals Limited, Mechelen, Belgium) and UC781.<sup>192</sup> This generation of microbicides including antiretrovirals has the potential to keep working even if the first mucosal barrier—the epithelial layer—is breached and the virus infects CD4<sup>+</sup> T cells, macrophages, or DCs present in the vaginal or rectal submucosa. This could actually have a beneficial effect, slowing viral replication, preventing depletion of CD4<sup>+</sup> T cells in the intestinal lamina propria and allowing acquired immune responses to develop, effectively turning an abortive infection into an immunization. Protection and priming of SIV-specific T-cell responses was indeed observed in a rectal transmission model of macaques treated with tenofovir gel.<sup>193</sup>

In summary, the microbicide field is using the known viral entry and immune mechanisms to find alternative approaches to prevention; at this stage it is not known how other factors

such as STIs, helminths, compliance, and the diversity of HIV-1 subtypes in Africa will affect their efficacy.

## CONCLUSION

The control of the HIV-1 epidemic will need a multi-pronged approach and additional tools are urgently needed. Better understanding of the immunology of the mucosal surface provides an important opportunity for such discoveries. This research cannot be confined to developed nations, given the unique characteristics of the epidemic in Africa. We have reviewed some of the current literature on the mucosal immunology of co-infections commonly occurring in Africa, immunological aspects of current interventions, and discussed some challenges of developing a mucosal vaccine against HIV-1. A number of gaps in our knowledge of HIV-1 transmission need to be addressed and some have already been identified in the Global HIV-1 Vaccine Enterprise Strategic plan. Some of the issues discussed in this review are also a priority in our research programs as we continue to build capacity in HIV-1 vaccine research and trials in Africa.

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## DISCLOSURE

The authors declare no conflict of interest.

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