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Mucosal immunity and protection against HIV/SIV infection: strategies and challenges for vaccine design

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Abstract

To date most HIV vaccine strategies have focused on parenteral immunization and systemic immunity. These approaches have not yielded the efficacious HIV vaccine urgently needed to control the AIDS pandemic. As HIV is primarily mucosally transmitted, efforts are being refocused on mucosal vaccine strategies, in spite of complexities of immune response induction and evaluation. Here we outline issues in mucosal vaccine design and illustrate strategies with examples from the recent literature. Development of a successful HIV vaccine will require in depth understanding of the mucosal immune system, knowledge that ultimately will benefit vaccine design for all mucosally transmitted infectious agents.

Keywords

HIV vaccine; mucosal immunity; mucosal immunization; cellular and humoral responses

The need for an HIV vaccine

Currently more than 33 million people are infected with human immunodeficiency virus type 1 (HIV), the majority living in Sub-Saharan Africa (www.unaids.org/en/KnowledgeCentre/HIVData/EpiUpdate/EpiUpdArchive/2007/default.asp). Astonishingly, over 7400 new infections occur every day, despite efforts to educate and promote safe sex practices. New infections among high risk groups are even increasing in the western world and former eastern block countries after years of decline [1]. This comes in part from the perception that AIDS is no longer a deadly disease, following the advent of highly active antiretroviral therapy (HAART) [2–4]. A vaccine remains the best hope to stop the epidemic.

HIV vaccine candidates have been recently reviewed, including those that have advanced to clinical trials [5,6]. Here we will focus on mucosal HIV vaccines, addressing current approaches and problems to be overcome. A comprehensive 2004 review summarized HIV/SIV mucosal vaccines, including protein-based, bacterial, DNA and viral vectored, and particulate [7]. Therefore, we have restricted the scope of this chapter primarily to studies conducted over the past 5 years. Citations are not all-inclusive, but selected as illustrative of points discussed.

Modes of mucosal HIV transmission

Worldwide more than 90% of all HIV infections occur via a mucosal route. In the USA, men having sex with men (MSM) exhibit the highest incidence, outnumbering all other risk

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groups combined [8]. Yet, the highest rate of new HIV infections occurs in sub-Saharan Africa, where the main mode of transmission is heterosexual contact [1]. Mother to child transmission during birth or breastfeeding also leads to a high infant infection rate in Africa and other developing countries. This route will not be addressed here, since HIV transmission can usually be avoided by drug therapy, if it is made available.

An effective vaccine will also need to protect against parenteral transmission. In the former Soviet Union, for example, a large proportion of new infections occurs among drug users [1]. But access of HIV to blood and tissues beneath the mucosal barrier can occur in multiple ways other than direct intravenous injection, and numerous factors can facilitate mucosal transmission. Common sexual practices may damage mucosal surfaces, giving HIV access to the underlying lamina propria [9,10]. The use of lubricants containing spermicides such as Nonoxynol-9, cellulose sulfate, and dextrin sulfate [11–13] can enhance infection by causing irritation and inflammation. Infections with organisms that cause sexually transmitted diseases (STDs) such as *Herpes simplex virus* type 2 (HSV-2), *Neisseria gonorrhoeae* candidiasis, syphilis (*Treponema pallidum*), and human papilloma virus [14–19] also can enhance HIV acquisition by causing breaks in the mucosal barrier, recruitment of target cells to the site of infection, and/or increased inflammatory responses. *Neisseria gonorrhoeae* enhances HIV susceptibility by inducing production and release of human defensins 5 and 6, part of the innate mucosal immune defense system, resulting in increased viral entry [18]. Other factors associated with increased risk of HIV infection include cervical ectopy, in which an increased proportion of the ectocervix is covered by simple columnar epithelium [20], chronic inflammatory bowel diseases in which lymphocytes can be highly activated, and conditions including hemorrhoids and pre-cancerous rectal polyps in which bleeding can occur. The plethora of factors which impact mucosal transmission render development of an effective vaccine all the more complex.

Mucosal barriers

The mucosal surfaces of the rectum and the male and female reproductive tracts differ, influencing susceptibility to HIV. The vagina is lined by a thick layer of stratified squamous non-keratinized epithelium. This non-mucus-secreting surface changes to mucussecreting, single layer columnar epithelium at the beginning of the cervical canal. The rectal surface is also covered for a relatively short distance with a single layer of stratified squamous epithelium which changes to the single layered columnar epithelium of the colon/large intestine. Rectal transmission of HIV is more likely, however, since the single layer stratified epithelium is easily damaged and bleeding often occurs, facilitating viral transmission to local tissue or the bloodstream. Overall, transmission frequency is greater rectally than by heterosexual intercourse [10], and higher from male to female compared to female to male [21,22]. In uncircumcised males the foreskin is a prime site of infection. The foreskin has weakly keratinized squamous epithelium on the outside, but the inner surface contains a high proportion of CCR5⁺/CD4⁺ T cells, macrophages and Langerhans cells [23,24]. Based on SIV infection experiments conducted in male rhesus monkeys [25], the urethra, lined by stratified columnar epithelium, may also be a site for viral transmission but this remains unproven in humans. The penis shaft and glands are more keratinized than the inner foreskin and potential target cells are less exposed and abundant [23]. Therefore infection here results predominantly from abrasions, lesions, and micro trauma, rather than by transcytosis, transmigration of infected cells, or dendritic cell (DC) sampling through the epithelium.

Crossing of mucosal membranes by HIV

HIV infection can occur following exposure to both cell-free and cell-associated virus. The first barrier the virus encounters at mucosal surfaces is mucus which protects the epithelium of the female reproductive tract and the male and female rectum and colon. In general, mucus contains secreted antibodies (mostly IgA), defense related proteins such as beta-defensins, lactoferrin, lysozyme, serine and cysteine proteases and other enzymes, as well as protease inhibitors [26–28]. To date few investigators have explored the effect of mucus characteristics on HIV transmission. In addition to providing a protective barrier [29], specific IgA in mucus can bind and trap virus, thus preventing direct contact with the underlying epithelium. This “immune exclusion” could be exploited in a vaccine context [30].

If the virus clears this barrier, then cells within the epithelium, lamina propria, submucosa or dermis, are the main targets for HIV infection. The cells susceptible to infection and/or responsible for the uptake, transport and presentation to susceptible cells include Langerhans cells (LC), DC, tissue macrophages, and resting or activated T-cells [10,31–35]. Gut membranous or microfold cells (M-cells) specialize in transcytosing antigen from the lumen [36], and are also able to transcytose virus particles from the apical luminal side to the lamina propria on the basal side where potential HIV target cells are abundant. Additionally, antigen transport involving the neonatal Fc receptor and/or the direct sampling of the lumen by intraepithelial DC may also play a role in HIV transmission [37–41]. Finally, virus may cross the mucosal barrier by transmigration of infected cells through spaces between epithelial cells as demonstrated with infected monocytes *in vitro* [42]. It is unclear if this occurs *in vivo*.

In SIV infection most naturally occurring strains exclusively use the CCR5 co-receptor, and in humans, the initially transmitted HIV seed virus is CCR5 tropic [43,44]. This initial selection is mediated, at least in part, by mucosal epithelial cells [45,46]. Primary intestinal epithelial cells (IEC) lack CD4 but express galactosylceramide, which serves as an alternate primary receptor, and CCR5 but not CXCR4. Thus, CCR5-tropic strains are taken up by the IEC and rapidly transcytosed to underlying target cells. For reviews of other co-receptors used by HIV and SIV, see Cilliers and Morris [47] and Clapham and McKnight [48]. A further potential co-receptor for HIV in gut mucosal tissue is the homing receptor, alpha-4 beta-7 ($\alpha 4\beta 7$), expressed on activated memory T-cells which home to gut mucosal sites [49]. HIV gp120 can bind $\alpha 4\beta 7$, activating LFA-1, an integrin involved in virological synapse formation. This interaction may facilitate cell-to-cell spread of HIV in the gut mucosa, and explain the rapid depletion of CD4⁺ memory T-cells at this site.

HIV can also access the lamina propria via DC transport facilitated by expression of CCR5 or CXCR4 and galactosylceramide, resulting in presentation *in trans* to T-cells and productive infection. [37,50,51]. DC in the lamina propria also express DC-SIGN, which can bind and present HIV *in trans* to T-cells [52–54]. In this case, the DC sample the lumen through the epithelial cell layer. In both humans and rhesus macaques, DC-SIGN and CCR5 double positive cells in the rectal mucosa lie just below the luminal single cell epithelial layer. DC-SIGN can also present infectious virus *in trans* in a CCR5 independent fashion [52,55], and can enhance CXCR4 virus transmission to T-cells [56]. For a recent review of DC-SIGN-mediated HIV transmission, see de Witte et al. [57]. Finally, the DC immunoreceptor (DCIR), a type II transmembrane molecule of the C-type lectin receptor family, acts as a ligand for HIV-1 and can present the virus *in trans* to T-cells, mediating DC-SIGN-independent infection [58].

Overall, the window of opportunity to prevent mucosal HIV infection is very narrow. Once the virus has traversed the mucosal barrier, it establishes a small local infection which rapidly spreads within 3 or 4 days to the local lymphoid tissue [59,60]. From there it can disseminate systemically, leading to the rapid depletion of CD4⁺ T-cells in the gut associated lymphoid tissue (GALT) [59,61,62].

The ideal vaccine

To prevent both systemic and mucosal infection, a preventive HIV vaccine needs to stimulate both arms of the adaptive immune system, eliciting strong cellular immunity, memory cells and antibodies at mucosal surfaces and throughout the body [30]. The antibodies, both IgG and IgA, should ideally be able to neutralize primary viruses [33,63] since HIV disseminates faster than a recall CTL response can be initiated [60,64]. In fact, neutralizing mucosal IgA antibodies have been isolated from HIV exposed uninfected individuals [21,60,65]. Vaccine-elicited antibodies may also possess additional protective functions, including inhibition of transcytosis [66], antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell mediated viral inhibition (ADCVI). The latter functions have been associated with protection [67–69]. CTL responses exhibited by non-infected but exposed sex workers illustrate the participation of cellular immunity in protective efficacy [70]. Further, CD8 depletion studies in the rhesus macaque system have illustrated the contribution of cellular immunity to control of chronic viremia [71]. Overall, the ideal HIV vaccine will need to engage all components of the immune system.

Compartmentalization of mucosal inductive sites

Mucosal immune inductive sites are collectively termed the mucosa-associated lymphoid tissue (MALT). The MALT is further subdivided into the nasal- or nasopharynx-associated lymphoid tissue (NALT), the bronchus-associated lymphoid tissue (BALT), and the gut associated lymphoid tissue (GALT) [38,65,72–74]. These sites are anatomically separated but functionally connected by the common mucosal immune system, so that induction of an immune response at one site leads to an effector response at a different mucosal site, mediated by homing receptors on induced memory T- and B-cells [75,76]. Table 1 lists homing receptors expressed on cells in the blood and their ligands expressed at various tissue locations. Strangely enough, the MALT is not all-inclusive, and the female reproductive tract is not associated with the MALT [75]. However, DC which take up antigens from the lumen, can home to an associated draining lymph node, in the case of the female genital tract, the iliac lymph node [75]. For vaccine design, the choice of mucosal inductive site is critical in determining the distal effector site to which induced memory cells will home. Unfortunately not all sites are equal in inducing mucosal immune responses. The magnitude of the response is also dependent to some extent on the type of immunogen, adjuvant, and delivery method. Recent reviews have summarized inducer sites and the distal mucosal sites at which they elicit immunity and memory [76,77]. These linked inducer-effector sites are briefly considered in the discussion that follows.

Nasal vaccination

Intranasal (IN) vaccination is a preferred immunization route due to ease of administration and the lesser amount of antigen needed to induce an immune response. Thus, many HIV/SIV vaccines have been evaluated by this route [78]. Depending on the mode of delivery and the nature of the vaccine, potential side effects resulting from vaccine reaching the olfactory bulb and brain must be ruled out [65]. However, an IN approach is feasible, as shown by the licensing of the nasally administered FluMist [74]. Nasal vaccines target the NALT leading to responses locally and in the lung and female reproductive tract [77]. Nasal immunizations have consistently elicited IgA and IgG responses and antigen specific

cytotoxic T-lymphocytes (CTL) in the cervicovaginal mucosa but low to no responses in the intestine and rectal mucosa [30,64,65,77,79]. Therefore to elicit comprehensive protection, a combination approach with additional oral or rectal immunization might prove useful [30].

The efficacy of nasal vaccines is often impaired due to delivery problems and the rapid clearance of free antigen [74]. For a nasal vaccine to be effective, adsorption to the epithelium and uptake by resident DC or transcytosis by M-cells is essential. Natural receptors of live vectored vaccines facilitate infection of the epithelium and promote uptake and transcytosis. With other immunogens, adjuvants can be used. The most thoroughly investigated mucosal adjuvants are *E.coli* heat labile enterotoxin (LT) and cholera toxin (CT) [77]. Both are extremely potent, but can have severe side effects in humans when given orally or intranasally. For example, nasal administration of an inactivated flu vaccine enhanced with LT has been associated with development of Bell's palsy in some vaccinated individuals [80], although the same vaccine administered parenterally has not [81]. Efforts to render these adjuvants nontoxic have led to CTB following removal of the toxic A subunit and to mutant LT adjuvants such as LTK-63. The latter was well-tolerated in a phase 1 human trial of an intranasally administered influenza vaccine [82], although a larger trial will be needed to fully evaluate safety. The LTK-63 adjuvant has subsequently been successfully used in pre-clinical HIV vaccine studies in mice [83] and rhesus macaques [84].

It is not the intent of this chapter to review mucosal adjuvants in depth. Novel adjuvants used recently to augment IN vaccines in the HIV field include NE, an oil-in-water nanoemulsion [85]. IN administration of HIV gp120 mixed with NE to mice and guinea pigs elicited potent anti-envelope IgG with broad neutralizing activity against multiple clade B isolates, and bronchial, vaginal, and serum anti-IgA antibodies. Systemic Th1 cellular immune responses were also induced suggesting that NE may have broad applicability. Similarly, in mice, the cationic lipid adjuvant, Eurocine N3, has enhanced vaginal and rectal IgA responses and systemic humoral and cellular responses following IN administration of plasmid DNA HIV vaccines [86].

In contrast to adjuvants, cytokines have not been very effective in enhancing immune responses or protective efficacy of mucosally administered DNA vaccines. Three IN immunizations of rhesus macaques with SHIV plasmid DNA, with or without DNA encoding IL-2/Ig or IL-12, followed by a single IN boost with MVA expressing SIV Gag, Pol, and Env elicited humoral and cellular systemic and mucosal immune responses [87]. But upon intrarectal challenge with SHIV_{89.6P}, only the group co-immunized with the IL-2/Ig plasmid showed some protection against disease development. In a follow-up rhesus macaque study, 3 IN coimmunizations with DNA encoding IL-12 and SHIV plasmid DNA followed by IM or IN boosting with MVA encoding SIV/HIV antigens was similarly ineffective in consistently eliciting mucosal IgA antibodies [88].

Vectored vaccines are less dependent on adjuvants. Both bacterial and viral vectors have proven useful for IN administration of HIV/SIV vaccines, alone, or in combination with other immunogens. A relatively new approach is IN vaccination with recombinant bacteria. Oral immunization can be problematic due to inefficient uptake of the vaccine vector due to the presence of food and other microorganisms, low pH and abundant proteolytic enzymes. However, following two IN immunizations of mice with recombinant *Salmonella enterica* serovar Typhi expressing HIV Gag or Env or both the animals developed systemic antibody and cellular immune responses, and mucosal antibodies in feces [89]. However IgA antibodies to HIV gp120 were undetectable in saliva and vaginal secretions. We speculate that the absence of vaginal antibodies, generally elicited following IN vaccination, might have resulted from the live bacterial vector preferentially traveling to the gut *in vivo* rather than colonizing the upper respiratory tract.

Most vectored HIV/SIV vaccines designed for nasal administration have been used in combination approaches as will be discussed below. Since nasal vaccination induces cellular and humoral responses in the female genital tract, it is an obvious choice as a vaccine strategy, given that the main route of HIV transmission worldwide is heterosexual intercourse [64,77]. Overall a nasal vaccine is believed to be culturally more acceptable than an intravaginal (IVag) vaccine. Moreover, the IN route has been more effective compared to IVag immunization [65,79].

Intratracheal/aerosol vaccination

Vaccines applied either intratracheally (IT) or by aerosol through the nose target mainly the BALT, a diverse inductive site with large follicles similar to Peyer's patches at the bronchial bifurcations [73,90]. Aerosol vaccination with a nebulizer or inhaler can potentially elicit potent immune responses, due to the high surface area of the lung. Antibody responses induced in the BALT are mostly of the IgA type, and effector and memory T- and B-cells induced there can home to distant mucosal sites [90]. That aerosol vaccination via the BALT might be feasible for an HIV vaccine was demonstrated in non-human primates vaccinated with the molecularly attenuated vaccinia vector (NYVAC) encoding an HIV clade C envelope [91]. The approach was both safe, causing no lung or brain pathology, and immunogenic. Our own approach, based on replication-competent Ad type 5 host range mutant (Ad5hr) recombinants, has used simultaneous oral plus IN priming, followed by an IT immunization. The IT route would not be suitable for administration of replicating Adrecombinants to people, however. In rhesus macaques that are not as permissive as humans for replication of the Ad vector, it has been safe and effective. This strategy elicits strong cellular immunity and when followed by IM boosting with envelope protein, the vaccine approach has elicited potent, durable protection against a mucosal SIV_{mac251} challenge [92,93] and a 4-log reduction in chronic viremia after an intravenous (IV) SHIV_{89,6P} challenge [94].

Oral vaccination

Oral vaccination elicits mucosal immune responses mainly in the gut, and mammary and salivary glands. Low to no responses are induced in the genital tract [64,77,95]. Major obstacles for oral vaccination include the dose and stability of the vaccine during passage through the GI tract [76]. The vaccine becomes diluted by saliva and gastric fluids, and inactivated by the acid pH of the stomach and digestive enzymes. Formulating vaccines in enteric coated capsules which are resistant to stomach acid but dissolve in the neutral pH of the intestine can overcome these problems. Both replication-defective [96] and replicationcompetent Ad-recombinant vaccines [97] have been administered using this approach. Bacterial vectors are also well-suited to oral delivery, especially lactic acid bacteria (see Wells and Mercenier [98] for a recent review). Bacterial recombinants are eventually overgrown by normal gut flora, however prior to this time, expression levels and induction of immune responses are dependent on the type of antigen, the bacterial strain, and the nature of the antigen (secreted, anchored or intracellular). Oral vaccination of mice with a recombinant *Lactococcus lactis* expressing surface bound HIV env together with cholera toxin led to systemic cellular immunity and systemic and mucosal humoral immune responses. Reduced viremia resulted when the mice were challenged intraperitoneally with vaccinia virus expressing HIV env [99]. Cynomolgous macaques primed intradermally with recombinant live attenuated *mycobacterium bovis* BCG expressing SIV *nef*, *gag* or *env* followed by oral or rectal boosting with rBCG developed good antibody and cellular immune responses [100].

Oral vaccination may target the tonsils rather than the GI tract. Non-replicating Ad-SIV recombinants are immunogenic in rhesus macaques when administered as an oral aerosol spray [101]. This approach resulted in diminished viral loads following oral challenge with SIV_{mac239}. Additionally, an HIV *gag* recombinant based in a non-pathogenic protozoan parasite vector, *Leishmania tarentolae* was shown to infect human tonsils *in vitro* [102], suggesting use as an oral vaccine. Mice vaccinated with the recombinant intraperitoneally developed strong cellular responses to HIV Gag, although antibody responses were low.

Finally, a novel oral strategy, not yet applied in the HIV field, is sublingual vaccination [103]. Administration of antigen by this route can elicit systemic and mucosal cellular and humoral immune responses. Sublingual immunization with an influenza virus vaccine was safe, and protected mice against a lethal influenza challenge [104].

Rectal/colonic vaccination

Whereas earlier studies indicated that intrarectal (IR) or colonic vaccination elicited only local immunity [77], recent studies have shown more distal responses. IR vaccination with a rotavirus vaccine induced systemic IgG and IgA antibodies in mice [105]. Moreover, in mice, colonic antigen administration induced higher IgA antibody levels in the colon and vagina and higher serum IgG levels compared to orally administered antigen [106]. However, protective efficacy elicited by IR vaccination has been modest. Multiple IR immunizations with peptide vaccines mixed with mutant *E. coli* labile toxin plus GM-CSF, IL-12 and CpG oligodeoxynucleotide followed by NYVAC-recombinant boosts led only to a delayed and blunted peak viremia in a rhesus macaque SHIV_{ku2} challenge model [107]. Similarly, animals primed three times IR with plasmid DNA in a liposome solution followed by recombinant MVA exhibited reduced peak viremia upon IR challenge with SHIV_{89,6P} [108]. Antibody responses following IR vaccination were highly variable, and only the MVA boosted group developed detectable serum antibodies to SHIV.

Intravaginal vaccination

Local humoral responses to vaginally-applied antigens or infections can be elicited [75,79] but generally are mostly unimpressive [109], since the female genital tract lacks secondary lymphoid tissue and has no direct association with the MALT. Timing of immunizations is critical due to hormonal variations, making large scale application of intravaginal (IVag) vaccines highly complex. A comparison of IN and IVag administration of cholera toxin B in female volunteers showed that both elicited serum IgG and IgA responses, but only IVag immunization, administered on days 10 and 24 of the menstrual cycle elicited potent cervical antibodies. In contrast, IN immunization was optimal for induction of IgA antibodies in vaginal secretions, suggesting that a combined IN/IVag regimen might be optimal. The hormonal influence on the transfection efficiency of plasmid DNA by vaginal electroporation was also recently illustrated in murine studies [110]. However, while transfection was achieved by this method, it is not likely that it would be acceptable to women. IVag immunization with HIV gp160 was recently evaluated in a phase I trial. Following 3 low dose immunizations, with or without adjuvant, no gp160-specific antibodies were elicited in serum or secretory fluids. However, IN immunization with the same dose of gp160 was similarly ineffective [111].

Combination approaches

A variety of parenteral and mucosal combination strategies have been explored in attempts to elicit mucosal immunity at multiple sites, as well as the systemic immunity needed to prevent transmission by blood and control systemic spread of HIV should initial sterilizing immunity not be achieved following vaccination. These experiments are complex, with

many variables in addition to immunization route and the nature of the immunogen influencing the outcome. For example, in mice, studies of IN, IM, and combination strategies for HIV gp140 plus adjuvant immunizations showed the interval between immunizations as well as immunization route greatly influenced the balance between systemic and mucosal antibody responses [112].

HIV DNA and protein immunogens administered to rhesus macaques IN or in combined IN/IM regimens have elicited IgG and IgA responses in sera and vaginal secretions as well as cellular immunity. Following a rest period, IM immunization significantly boosted cellular and humoral immune responses, the latter able to neutralize several HIV clade B strains [113]. The importance of the IM component was confirmed in a later study in which rhesus macaques immunized with HIV Env protein IM, IN/IM or IM/IN developed neutralizing antibody and apparent sterilizing immunity and completely resisted IVag challenge with SHIV_{SF162P4} [84]. In contrast the IN only immunized macaques were not protected. Serum neutralizing antibody was associated with the protection, and was not present in the IN group prior to challenge. The mechanism by which serum antibody protects against IVag challenge remains to be determined. But previous passive transfer studies have shown that neutralizing antibody administered intravenously can protect against vaginal challenge [114].

A similar result was reported by Kent et al. [115]. Pigtail macaques immunized IN with multigenic HIV and SHIV DNA vaccines were not protected against a SHIV_{SF162P3} challenge, whereas IM priming followed by mucosal boosting, either IN or IR, with multigenic recombinant fowlpox virus led to modest, transient reductions in acute phase viremia. Here, neutralizing antibodies were absent prior to challenge, and mucosal antibodies were not evaluated.

Combination Oral/IM immunization using attenuated *Listeria monocytogenes* recombinants has also been evaluated in rhesus macaques [116]. Sequential oral immunizations elicited primarily cellular immune responses in peripheral blood, while Oral/IM immunization elicited predominantly serum and mucosal antibodies. Unfortunately, cellular immune responses at mucosal sites were not evaluated. We recently compared IN/Oral versus Oral/Oral administrations of replication-competent Ad5hr-SIV recombinants followed by IM protein boosts [97]. Following challenge with SIV_{mac251}, both immunization groups showed equivalent, significant reduction in chronic viremia compared to controls. Surprisingly, however, the IN/Oral group exhibited stronger cellular immune responses in PBMC, generally associated with control of chronic infection. Further studies revealed equivalent T-cell memory responses in BAL and the presence of viral-specific T-cells expressing gut homing receptors in both groups. Thus, the peripheral blood did not appropriately illustrate the sum total of cellular immunity elicited. Initial priming by the IN route, however, elicited better systemic and mucosal antibody responses, including significantly higher serum binding titers, stronger ADCC responses and higher ADCVI activity. Anti SIVgp120 IgA antibodies in lung lavage, and better transcytosis inhibition were exhibited by rectal secretions. The enhanced antibody responses in the IN/Oral group were significantly correlated with reduced acute phase viremia (Hidajat et al., submitted for publication).

Tolerance, immunogenicity and homing

All mucosal vaccines have to overcome tolerance [30,77,117], regardless of administration route. Tolerance can be induced at several mucosal sites but seems limited to the “local” inductive site [90,118]. It is highly dependent on the nature of the antigen, the dosage, the method of delivery and on whether or not adjuvant is used [117,119,120]. The antigen dosage influences induction of regulatory cell-driven or anergydriven tolerance [120]. To

overcome oral or nasal tolerance, the vaccine has to elicit a strong immune response. The best delivery vehicles may therefore be modified mucosal pathogens, including viruses and bacteria, or immunogenic parts of pathogens as adjuvant (e.g. *Cholera* toxin, *E.coli* toxin). Mimicking a pathogen by expression of pathogen associate molecular pattern (PAMP), will result in recognition by pathogen recognition receptors (PRR) such as Toll like receptors. The expression density of the PRRs on the surface of epithelial cells and DC differs among cells of the blood, lung, and gut [121], influencing the pathogen sensing sensitivity and therefore the dose needed to elicit an immune response. Recognition of the vaccine vehicle leads to release of immune mediators by the DCs or epithelial cells and initiation of a response. Infection by the vector will also cause an inflammatory reaction leading to the influx and activation of monocytes and neutrophils which secrete cytokines and chemokines [75,121,122]. For an overview of resident intestinal mucosal T-cells during inflammatory responses and vaccination see Montufar-Solis, Garza et al. [123].

The imprinting of the desired homing address for a particular mucosal site on T- and B-cells in the draining lymph nodes by DC is a key factor for the development of a successful mucosal HIV vaccine. A homing address for the gut is dependent on the availability of retinoic acid (RA) for the DC within Peyer's patches and the mesenteric lymph node [124,125]. For the induction of IgA secreting cells, RA plays a major role together with IL-6 and IL-5 [126]. The most important gut homing receptors are $\alpha 4\beta 7$ and CCR9. But lymphocytes don't necessarily have to express CCR9 to enter the colon, suggesting an additional mechanism [125,127]. Cells which home to the lung mucosa express $\alpha 4\beta$ [27]. Homing to the skin is dependent on P- and E-selectins [41,128]. Cells which home to other tissue sites, for example the genital tract, will also be imprinted with appropriate receptors (Table 1). This raises the question of whether vaccines administered parenterally, such as DNA vaccines that are generally administered intradermally or IM, will be effectively imprinted for trafficking to mucosal sites. Perhaps coadministration of DNA with cytokines, adjuvants such as microemulsion or PLGA-particles [129,130], or RA will lead to generation of strong mucosal as well as systemic responses. Further studies are needed to address cell homing and a potential connection between the MALT and skin associated lymphoid tissue.

Innate immunity

It has been estimated that innate immunity is up to 99% effective in preventing natural HIV infection at mucosal sites [131]. This protection is mediated by a variety of mechanisms including innate immune proteins, type I interferons, and β -chemokines. α -defensins, for example, are able to interfere with the binding of HIV gp120 to CD4 [132]. As highlighted by Haynes, it would be beneficial if functions of the innate immune system could be harnessed for vaccine development [22]. To date, the focus has been on bridging innate and adaptive immunity by vaccine elicited antibodies and effector cells including NK-cells, monocyte/macrophages and $\gamma\delta$ T-cells. Whether neutrophils, which possess phagocytotic and degranulating properties, and bear Fc receptors able to bind antibody also mediate protective functions that bridge innate and adaptive immunity has not been investigated.

Conclusion and Future strategies

Human clinical trials of HIV vaccines have focused on parenteral immunization and systemic immunity. Yet the candidates to date, including envelope protein, and replication-defective poxvirus and Ad vectors, with or without DNA, have not elicited sustained or strong immunity [133–136] or protective efficacy [137]. Overall, mucosal immunity has not been addressed in these trials, and mucosal immune responses have not even been evaluated. While only a few clinical trials have directly addressed mucosal immunization strategies

(Table 2), the HIV field is now shifting to a greater consideration of mucosal vaccine development. We have advocated the use of mucosal replicating vectors [138] and have selected replication-competent Ad recombinants for our own vaccine strategy. The record of safety and efficacy of oral Ad4 and Ad7 vaccines in the US military [139], together with their ability to elicit potent immunity and natural tropism for mucosal epithelia make Ad ideal vehicles for mucosal vaccination either by the oral, IN, or IR route [76,140,141]. Using replicating Ad recombinants in pre-clinical studies in non human primates, we have shown strong systemic and mucosal immune responses and potent protection against SIV and SHIV challenges as recently reviewed [142]. But as illustrated in this chapter, a number of other promising mucosal strategies are available, and are being actively pursued by other investigators. These studies will be facilitated by increasing knowledge of the mucosal immune system, gleaned in part from a greater understanding of immune responses to mucosal pathogens. As discussed above, these pathogens may prove useful following attenuation as vaccine vehicles to appropriately stimulate potent mucosal immunity.

As greater emphasis is placed on a mucosal HIV vaccine, the lack of methods to readily evaluate mucosal immunity will hinder vaccine development. Blood, a tissue easily sampled and assayed does not adequately reflect mucosal cellular and humoral immune responses. Tissue biopsies and mucosal secretions are required, and their collection requires invasive procedures. Development of highly sensitive and surrogate assays to monitor induced mucosal immunity on readily obtained blood samples will be needed in order to monitor large scale human trials for induction of mucosal immune responses. Standardization of procedures for evaluation of antibodies in mucosal secretions will also be required, due to variable antibody levels in these samples, and variability in quantitation of these antibodies between laboratories [143]. Such efforts will be rewarded when they culminate in an urgently needed and efficacious vaccine, able to protect against both systemic as well as mucosal HIV exposures.

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Table 1

Homing receptors expressed on blood cells and the ligands/chemokines recognized.

Receptor	Receptor expressed on	Ligand(s)	Ligand expressed/secreted by	Reference
$\alpha 2\beta 1$ (VLA-2) or $\alpha 1\beta 1$	T, B	Collagen	Venules (site of acute inflammation)	[125]
$\alpha 4\beta 7$ (LPAM-1)	B, T, Treg	MAdCAM-1	Small intestine and colon (GALT, Peyer's Patches, mesenteric LN), high endothelial venules (HEV)	[40–41,75,124–125,127,144–147]
$\alpha E\beta 7$	DC, T, Treg	E-cadherin	Acutely inflamed endothelial cells (most organs), skin microvessels	[75,125,127,145–146]
$\alpha 4\beta 1$ (VLA-4)	T, Treg	VCAM-1, Fibronectin	HEV (peripheral lymph nodes), venules (skin)	[124–125,144–146]
E-selectin ligand	Treg	E-selectin (CD62E)	Skin, vascular endothelial cells, HEV	[41,75,125,127,144,146,148]
P-selectin ligand (PSGL-1 (CD162))	Treg, T, B, monos	P-selectin (CD62P)	Skin, vascular endothelial cells	[41,75,125,127,144,146,148]
CXCR1 (CDw181)	DC	CXCL6 (CKA-3), CXCL8 (IL-8)	Inflamed tissue (gut)	[41]
CXCR3 (CD183)	T, NK, Treg	CXCL10 (IP-10), CXCL9 (MIG), CXCL11 (ITAC)	vascular endothelial cells (during mucosal inflammation), (CXCL9) high endothelia venules	[75,124–125,146–147,149–151]
CXCR4 (CD184)	T, B, monos, DC, Treg, stemcells	CXCL12 (SDF-1 α)	Lymph node, bone marrow (marrow stromal cells), skin (keratinocytes)	[41,124,125,127,145,147,149,152,153]
CXCR5 (CD185)	B, T	CXCL13 (BCA-1)	Peyer's Patches, secondary lymphoid organs	[125,154]
CXCR6 ((Bonzo) CDw186)	T, T _H -17	CXCL16	follicle associated epithelial cells, lung	[75,124,147,155]
CX ₃ CR1	DC, monos, T, NK	CX3CL1 (Fractalkine)	Lamina propria, terminal ileal epithelium, skin, mesenteric venules	[41,75,147,149,152]
CCR1 (CD191)	T, DC	CCL-3 (MIP-1 α), CCL-5 (RANTES), CCL-7 (MCP-3), CCL-9 (MIP-1 γ)	Inflamed tissue, Peyer's Patches (follicle associated epithelial cells)	[41,75]
CCR2 (CD192)	DC, T, Treg	CCL-2 (MCP-1), CCL-7 (MCP-3), CCL-13 (MCP-4)	(CCL-2) vascular endothelial cells (during mucosal inflammation) and HEV, inflamed tissue	[41,124,146–148]
CCR4 (CD194)	T, Treg, T _H -17	CCL-17 (TARC), CCL-22 (MDC)	Skin (normal/inflamed), asthmatic airways, inflammation	[41,125,144–149,152,156]
CCR5 (CD195)	T, DC, Treg	CCL-3 (MIP-1 α), CCL-4 (MIP-1 β), CCL-5 (RANTES)	Inflamed tissue	[41,75,124,146–149]
CCR6 (CD196)	T, B, DC, Treg, T _H -17	CCL-20 (MIP-3 α)	Skin (keratinocytes), Peyer's Patches (follicle associated epithelial cells), small intestine enterocytes, inflamed tonsils, inflamed tissue	[41,75,124,145–150,156]
CCR7 (CD197)	T, B, DC, Treg	CCL-19 (ELC), CCL-21 (SLC)	Lymph node, (CCL-19) stromal cells and mucosal DC, (CCL-21) HEV, stromal cells	[41,75,124–125,127,144–149,152]

Receptor	Receptor expressed on	Ligand(s)	Ligand expressed/secreted by	Reference
CCR8 (CDw198)	T, monos, DC, Treg	CCL-1	Skin (normal/ allergic reaction)	[145–147,149,152]
CCR9 (CD199)	T, B, DC	CCL-25 (TECK)	Small and large intestine epithelial cells (GALT), vaginal epithelia	[40–41,75,124–125,127,144–145,147,149,152]
CCR10	T, B	CCL-27 (CTACK), CCL-28 (MEC)	(CCL-27) Skin (normal or inflamed) and vaginal epithelium, (CCL-28) Small, large intestine and vaginal epithelia	[41,75,124,127,144–145,147,149,152]
L-selectin (CD62L)	pDC, T, monos, NK, Treg	L-selectin ligand (PNAd)	HEV (peripheral lymph nodes)	[124–125,127,144–146,148]

Abbreviations:

T, T-cell (CD4⁺, CD8⁺, multiple subtypes); Treg, T-regulatory cell (CD4⁺FoxP3⁺); T_H-17, CD8⁺ or CD4⁺ T-cell secreting IL-17; B, B-cell (multiple subtypes); NK, NK-cell; monos, Monocytes; DC, Dendritic cell (myeloid or plasmacytoid DC, Langerhans Cell) VLA-2, VLA-4 (Very Late Appearing Antigen-2, -4); LPAM-1 (Lymphocyte Peyer's Patch Adhesion Molecule-1); MAdCAM-1 (mucosal vascular addressin cell adhesion molecule-1); VCAM-1 (vascular cell adhesion molecule-1); PSGL-1 (P-selectin glycoprotein ligand-1); CXCL6 (CKA-3, Chemokine alpha 3); CXCL10 (IP-10, Interferon- γ -inducible protein of 10 kDa); CXCL9 (MIG, monokine induced by interferon- γ); CXCL11 (ITACI, Interferon-inducible T Cell Alpha Chemoattractant); CXCL12 (SDF-1 α , Stromal-Derived Factor-1 α); CXCL13 (BCA-1, B Cell-Attracting Chemokine 1); CCL-3 (MIP-1 α , Macrophage Inflammatory Protein 1 α); CCL-5 (RANTES, Regulated Upon Activation, Normal T Cell Expressed and Secreted); CCL-7 (MCP-3, Monocyte Chemotactic Protein 3); CCL-9 (MIP-1 γ , Macrophage Inflammatory Protein 1 γ); CCL-2 (MCP-1, Monocyte Chemotactic Protein-1); CCL-13 (MCP-4, Monocyte Chemotactic Protein-4); CCL-17 (TARC, Thymus and Activation-Regulated Chemokine); CCL-22 (MDC, Macrophage-Derived Chemokine); CCL-4 (MIP-1 β , Macrophage Inflammatory Protein 1 β); CCL-20 (MIP-3 α , Macrophage Inflammatory Protein 3 α); CCL-19 (ELC, Epstein-Barr virus-Induced-Molecule-1-Ligand-Chemokine); CCL-21 (SLC, Secondary Lymphoid tissue Chemokine); CCL-25 (TECK, Thymus-Expressed Chemokine); CCL-27 (CTACK, Cutaneous T Cell- Attracting Chemokine); CCL-28 (MEC, Mucosae-Associated Epithelial Chemokine); L-selectin ligand (PNAd, Peripheral Node Addressin)

Table 2

Clinical trials of prophylactic mucosal HIV vaccine candidates.

Clinical Trial Identifier	Type/Start Year	Status	Delivery	Description
NCT00000798	Phase 1/1999	completed	oral	HIV-1 MN peptide in microparticles
NCT00000846	Phase 1/1999	completed	Intramuscular prime/oral boost	HIV-1 PND peptide prime, microparticulate monovalent branched peptide boost
NCT00000884	Phase 1/1999	completed	Intramuscular/oral/intranasal/intrarectal/intravaginal or combinations	ALVAC-HIV vCP205 (expresses gp120) or ALVAC-RG vCP2058 (rabies vaccine); prime by various routes ALVAC-HIV vCP205 or ALVAC-RG, IN boost or AIDSVAX B/B or Imovax (rabies vaccine), IM boost
NCT00001053	Phase 1/1999	completed	Intramuscular prime/oral or rectal boost	HIV p17/p24:Ty-VLP (Virus like particles) with or without Alum followed by oral or rectal boosting
NCT00062530	Phase 1/2003	not yet open	oral	weakened <i>Salmonella typhi</i> expressing HIV-1 BaL gp120
NCT00122564	Phase 1/2005	terminated	intranasal or intravaginal	HIV-1 gp160 (MN/LAI) with or without DC-Chol ^f
NCT00369031	Phase 1/2006	terminated	intranasal prime/intramuscular boost	HIV gp140ΔV2 with or without LTK63 prime; booster with MF59 adjuvant
NCT00637962	Phase 1/2008	recruiting	intravaginal	HIV gp140 (ZM96) plus Carbopol 974

Additional information can be found at <http://clinicaltrials.gov/ct2/search>

Abbreviations/definitions:

Microparticles: biodegradable polymers polylactide and poly-co-glycolide with entrapped antigen

PND: principal neutralizing determinant

ALVAC: live canary pox vector

AIDSVAX B/B: a bivalent vaccine consisting of MN rgp120/HIV-1 and GNE8 rgp120/HIV-1 antigens in alum adjuvant

DC-chol: cationic lipid (3 β -[N-(N', N'-dimethylaminoethane)carbamoyl]cholesterol)

LTK63: a nontoxic mutant of Escherichia coli heat labile enterotoxin, LT