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Mucosal Vaccine Approaches for Prevention of HIV and SIV Transmission

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Abstract

Optimal protective immunity to HIV will likely require that plasma cells, memory B cells and memory T cells be stationed in mucosal tissues at portals of viral entry. Mucosal vaccine administration is more effective than parenteral vaccine delivery for this purpose. The challenge has been to achieve efficient vaccine uptake at mucosal surfaces, and to identify safe and effective adjuvants, especially for mucosally administered HIV envelope protein immunogens. Here, we discuss strategies used to deliver potential HIV vaccine candidates in the intestine, respiratory tract, and male and female genital tract of humans and nonhuman primates. We also review mucosal adjuvants, including Toll-like receptor agonists, which may adjuvant both mucosal humoral and cellular immune responses to HIV protein immunogens.

Keywords

HIV; SIV; nonhuman primates; mucosal adjuvants; rectal; vaginal; oral; nasal immunization TLR agonists; vaccine vectors; delivery vehicles

1. INTRODUCTION

The vast majority of human immunodeficiency type 1 (HIV) transmissions occur across mucosal barriers, whether we consider mother-to-child transmission or adult sexual transmission, and significant HIV replication occurs in the gastrointestinal mucosa [1–5]. Blood transmission is now mostly restricted to intravenous drug use, as transmission through blood products has been virtually eliminated thanks to the careful selection of donors and screening of blood products before their use. In the setting of mucosal transmission, different surfaces can be breached by the virus and these surfaces are very different in histological structure and immunologically competent cells that are available for their

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CONFLICT OF INTEREST

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protection. This translates in different efficiencies of infection at different sites for the same virus amount, with rectal infection being significantly more efficient that vaginal, which is more efficient than oral [6, 7]. Because HIV can spread in a few days to other compartments, immunological containment cannot rely on anamnestic systemic responses, as these responses may become effective too late [8, 9]. An effective vaccine needs to be able to establish resident mucosal memory cells that can relatively quickly block viral replication and peripheral spread after infection has occurred. Once persistent systemic infection is established, it seems unlikely that immunological control can eradicate the infection, even in the setting of an attenuated virus that can control an incoming wild type virus [10]. Mucosal routes of vaccination, which provide more significant antigen-specific mucosal responses than systemic vaccination, may be better suited to achieve these goals [11]. However, for protection against HIV, induction of antiviral responses in both mucosal and systemic compartments may be necessary to achieve virus control, and it is unlikely that only humoral or only cell-mediated immunity will be sufficient for this task. This review covers different HIV vaccine candidates administered via mucosal routes that have been or are currently being explored in humans and Nonhuman Primates (NHP) in the attempt to control HIV or Simian Immunodeficiency Virus (SIV) infection and disease progression.

2. RECOMBINANT VECTORS FOR HIV ANTIGEN DELIVERY

Thanks to the advent of recombinant DNA technology, in addition to the traditional approach of using an inactivated pathogen mixed with an adjuvant, there are now numerous ways to expose the immune system to a specific antigen or antigens. Immunization can deliver antigens as expressed from naked DNA molecules containing the sequences for the antigens, as purified proteins (not discussed here), or as part of a recombinant microbe that expresses the antigen we intend to target as part of its own makeup [12–15]. This last possibility permits the use of live vectors as vehicles for selected proteins of a specific pathogen, an approach that results in a more prolonged stimulation of the immune system than non-replicating antigens can accomplish. This approach also provides the option of choosing recombinant bacteria or viruses known to enter the body *via* a specific route and known to stimulate preferentially certain immune responses, therefore tailoring the stimulated quality and quantity of immunity at a particular site. Understanding how microbial components, also referred to as microbe-associated molecular patterns (MAMPs), can interact with specific pattern recognition receptors (PRR) in the host and stimulate distinct innate immunological pathways with subsequent diverse adaptive immune responses permits the selection of vectors suited to stimulate the response with protective characteristics, once the correlates of protection are defined [16–18]. The affected PRR, specific for the vector, are strictly linked to what can be described as the adjuvant signature of a certain vector, which influences both innate and adaptive responses more than the recombinant antigen inserted in the vector. Toll-like receptors (TLR), which can be differentially engaged by lipids, carbohydrates, nucleic acids or other molecules unique to microbes, are a subcategory of PRR. For instance, Gram-negative bacteria are likely to activate the TLR4 pathway due to their lipopolysaccharide (LPS) content [16, 19]. Flagellin in some bacteria permits stimulation of TLR5, while single-stranded RNA viruses can activate TLR7 and TLR8 receptors, which in turn activate the Type I interferon (IFN)

response [20, 21]. Unmethylated CpG motifs present in viral DNA are recognized by TLR9 [21]. In the paragraphs below we will review the different delivery systems that have been investigated as candidate HIV vaccines.

2.1. Recombinant Bacterial and Viral Vectors

Use of bacteria with attenuated pathogenic potential as vectors to deliver pathogen-associated antigens is a promising approach to vaccination [13, 14]. A variety of well-studied bacteria are available, especially for the enteric route [22]. The biology of expression systems in different bacteria is also well characterized. Furthermore, continual expression of recombinant proteins appears to be a more efficient way to expose the immune system to the selected antigen resulting in more potent immune responses [23, 24]. Bacteria with tropism for a specific body compartment can be selected, depending on where the immunity is desired. Among the bacteria that are considered for engineering HIV vaccines to administer orally are attenuated strains of *Salmonella typhimurium* and *Shigella flexneri* [25–30]. The vaginal use of Gram-positive *Lactobacillus jensenii*, has been investigated for the prevention of HIV infection, not as a vaccine vector but as a delivery vehicle for the expression of the entry inhibitor cyanovirin-N [31, 32]. This approach reduced by ~ 60% the transmission rate of CCR5-tropic $SHIV_{SF162P3}$, suggesting that engineered bacterial commensals might be used similarly to achieve protection against pathogens. Lastly, Bacillus Calmette and Guerin (BCG), which is an attenuated version of *Mycobacterium bovis*, has been explored as a vector for oral or intradermal antigen delivery, as it is one of the few vaccines that are given at birth and, if successful as a vector, could accomplish dual immunizations early in life [33–36]. However, it should be noted that BCG as a vaccine vector is contra-indicated in healthy breast-feeding infants with HIV-infected mothers because BCG immunization by either systemic or oral routes induces persistent immune activation, including enhanced frequencies of activated $CCR5^+$ $CD4^+$ T cells (preferred HIV target cells) in the circulation, and oral immunization of neonatal macaques with a BCG-SIV vector increased susceptibility to oral SIV infection [37, 38].

When considering viruses as vectors, a few options are available, some being DNA viruses, others being RNA viruses [15, 39]. A few poxviruses have been explored as HIV candidate vaccines, including canarypox, fowlpox and Modified Vaccinia Ankara (MVA) virus [40–44]. These viruses work well when administered *via* systemic routes, especially in prime-boost heterologous immunizations [42, 44–48]. Their use *via* mucosal routes is less established but fowlpox has been given to NHP *via* the nasal route and MVA has been utilized nasally, orally, rectally, and vaginally with some level of protection obtained [44, 46, 47, 49–52]. Adenoviruses have also been extensively explored as vectors, and given their tropism for the respiratory tract and intestine, they seem particularly suited for mucosal immunization [53–62]. Among viruses of the Hepersviridae family, the rhesus Cytomegalovirus (CMV) is the most advanced in preclinical trials with evidence of stimulation of immunity that can clear infection in at least 50% of NHP that received parenteral vaccination and were subsequently infected rectally with SIV [63–65]. However, due to its biology, it does not lend itself well to mucosal immunization. Within Herpesviridae, Varicella Zoster virus (VZV) has also been considered as a possible vector and administered subcutaneously and intra-tracheally with significant results [66]. The

human live attenuated oral Poliovirus, a virus in the family of Picornaviridae, is one of the most successful mucosal vaccines, and both Poliovirus and Vesicular Stomatitis virus (VSV), a member of the Rhabdoviridae family, have been explored as mucosal viral vectors in macaques with partial efficacy [67–70].

A confounding factor that may reduce immune responses to HIV transgene products delivered *via* bacterial or viral vectors is pre-existing anti-vector immunity, either as a result of natural exposure or vaccination [71–73]. Pre-existing immunity is believed to be responsible for the poor results obtained in humans orally immunized with live-attenuated *Salmonella enterica* vectors [22, 74, 75]. To overcome immunity to vaccine vectors, the most common strategy has been to administer higher doses, although this may compromise safety and preclude commercialization. High doses may also induce greater levels of anti-vector antibodies that obviate vector boosting by the same route. Prime-boosting with heterologous vectors may therefore be more effective [76–78]. Another approach found effective for priming HIV-specific B and T cells without inducing anti-vector immunity in vaccination regimens has been to use DNA as the priming vehicle [79, 80].

2.2. Recombinant DNA

DNA vaccines have a number of demonstrated and potential advantages over more conventional vaccine formulations. DNA is a relatively stable molecule, facilitating storage and handling, and the ease of safe manufacture, scale-up and distribution of DNA vaccines makes this technology attractive. Stimulation of both humoral and cellular responses is usually observed, as antigens are expressed in their native form and correctly presented to the immune system [12, 81]. DNA vaccines expressing HIV genes have been investigated in humans for their safety and ability to prime or boost virus-specific immune responses [82–84]. Several Simian Immunodeficiency Virus (SIV) or Simian-Human Immunodeficiency Virus (SHIV) DNA vaccine constructs have been evaluated for their ability to induce protection against challenge with SIV or SHIV [85–88]. Macaques have been inoculated with various plasmids expressing a partial viral genome and various SIV or HIV Envelope (Env) genes. In some cases, DNA vaccination was followed by administration of recombinant bacterial or viral vectors or Env protein in regimes defined as heterologous prime-boost [45, 89–102].

Although the results obtained thus far with DNA vaccination in animal models are promising, there is clearly a need to increase the potency of this technology, especially for induction of antibodies. A number of studies indicate that the magnitude of the antigen-specific response can be modulated by using codon-optimized sequences for the antigen production, by the addition of genes for certain cytokines and by administering the DNA *via* electroporation, an approach that has significantly reduced the amount of DNA required for systemic immunization while increasing efficacy in expression of the antigen encoded by the DNA [89, 92, 94, 103, 104]. Interestingly, it has proved possible to use electroporation for buccal DNA delivery in the oral cavity of guinea pigs and mice, which generated more antigen-specific IgA in vaginal secretions, IgG in serum and $CD4^+$ and $CD8^+$ T cells in blood than did topical DNA application [105]. However, there are no devices that could be used similarly to deliver DNA vaccine directly into the mucosa of the small intestine, rectum

or vagina. For mucosal administration at these sites, DNA must be shielded from nucleases in the lumen, for instance through encapsulation in a protective delivery vehicle. For this purpose, cationic liposomes (discussed below) have most often been utilized to administer DNA vaccines *via* mucosal routes in NHP.

3. MUCOSAL ADJUVANTS

To avoid induction of T regulatory cells and tolerance [106], mucosal vaccines, like parenteral vaccines, must contain immune stimulatory motifs that bind to host PRR to elicit pro-inflammatory mediators and up-regulation of costimulatory molecules required for activation of naive lymphocytes by antigen-presenting cells (APC) in regional lymph nodes [107]. The cytokines and chemokines produced by local cells play a critical role in modulating the adaptive response by influencing the development of specific T-helper cell subsets which differentially promote cellular and humoral responses [108, 109]. For example, Th17 cells promote IgA responses [110–113] whereas Th1 cells preferentially induce IgG. In the presence of TGFp, which is enriched in mucosal tissues [114], pro-inflammatory cytokines typically induce Th17-type responses [108, 115]. Thus, vaccines or infectious agents that generate strong inflammatory responses and Th1-biased IgG responses in the systemic compartment often elicit Th17 cells and IgA responses when administered at mucosal surfaces [116–118].

Administration of the same antigen and adjuvant by mucosal or parenteral routes may also produce qualitatively different immune responses because the composition of innate cells, which express unique combinations of PRR, varies in mucosal and systemic tissues [119–122]. The responsiveness of innate cells to PRR stimulation also differs between mucosal and systemic compartments. For instance, intestinal macrophages, but not peripheral macrophages, are refractory to TLR stimulation [114, 123]. The majority of cells first contacted by vaccines applied to mucosal surfaces are epithelial cells, and apical expression of PRR and responsiveness to MAMPs varies considerably among these cells in mucosal compartments [121, 124, 125]. For these reasons, results obtained after administration of vaccine and adjuvant in one mucosal tissue cannot necessarily be extrapolated to another mucosal tissue.

3.1. TLR Agonists

Protein and peptide immunogens typically lack sufficient innate stimulatory properties and must be co-administered with adjuvants to trigger an adequate inflammatory response by innate cells. Molecules that bind to PRR are a rational choice for adjuvants, and a variety of MAMPs, especially TLR ligands [109], have been explored as adjuvants for parenterally administered HIV or SIV subunit immunogens in humans and NHP [126–131]. However, only a few have been evaluated in vaccine efficacy trials [127] or tested for the ability to adjuvant immune responses to mucosally administered antigens in humans or NHP.

It should be noted that B and T lymphocytes express TLR [132, 133], and TLR agonists can directly modulate functions of these cells [134]. Humans and macaques have similar cellular TLR distributions and responsiveness to TLR ligands [135–138]. However, there are some

striking differences between mice and primates [137–139], especially for TLR4, TLR7 and TLR8. Results of some mouse vaccination studies must, therefore, be interpreted cautiously.

TLR2 can bind more MAMPs than other TLRs due to its propensity to form heterodimers with TLR1 or TLR6 [140]. Among its ligands are bacterial lipoproteins/peptides (TLR2/1) and fungal zymosan and β-glucan (TLR2/6). In NHP, TLR2/1 agonists have not shown much promise as mucosal adjuvants for enhancing antibody responses to soluble proteins. Rectal or sublingual co-administration of the TLR2/1-binding lipoprotein, PAM_3CSK_4 with β-galactosidase or ovalbumin (OVA), respectively, in macaques failed to generate antigen-specific antibodies in serum or secretions [141]. PAM_3CSK_4 also did not induce antibody responses to nasally co-administered keyhole limpet hemocyanin (KLH) in macaques, although a TLR7/8 agonist did [141]. However, TLR2/6 agonists might be more effective as mucosal adjuvants. Vaginal immunization of mice with the TLR2/6 agonist, FSL-1, improved vaginal IgA responses to co-delivered tetanus toxoid, whereas the TLR2/1 agonist, peptidoglycan, did not [142]. Others have reported that FSL-1 was more effective than PAM_3CSK_4 for generating Th17 and Th1 cells in the intestine of mice [143], and TLR6 stimulation promotes Th17 responses in the lung [144]. Mucosal administration of TLR2 ligands conjugated to antigens could also be more effective than admixed agonist mixtures for generating immune responses. Vaginal immunization of mice with a TLR2 ligand conjugated to a Herpes Simplex Virus 2 (HSV-2) peptide was shown to generate local HSV-2-specific memory CD8 T cells [145]. Intragastric immunization with antigen incorporated in β-glucan particles [146] also improved specific antibody responses, possibly by enhancing M cell uptake and induction of local immune responses [147]. Stimulation of M cells with a variety of TLR2 agonists has been shown to enhance apical-to-basolateral transcytosis of microparticles and recruitment of dendritic cells (DC) in follicles below [148, 149]. There is also an intriguing possibility that parenteral immunization with TLR2 agonists might generate antigen-specific lymphocytes that home to the intestine. Human peripheral blood B cells cultured with TLR2 ligands have been shown to produce J chain and IgA, up-regulate expression of CCR9 and CCR10 chemokine receptors and migrate toward CCL25 and CCL28 [150], chemoattractants respectively produced by epithelial cells in the small and large intestine [151–153]. Splenic DC of mice have also been reported to acquire the ability to imprint CD8 T cells with the α4β7 and CCR9 small intestinal-homing phenotype after culture with PAM_3CSK_4 [154]. On the other hand, TLR2 agonists may not be desirable components of HIV vaccines. Human T cells express TLR2 [133], and direct stimulation of activated naive and memory CD4 T cells from blood with TLR2 ligands has been found to result in proliferation, up-regulation of CCR5 expression, and enhanced HIV replication *in vitro* [155–159]. Mucosal or parenteral immunization with TLR2 agonists could therefore transiently increase both inflammation and numbers of HIV target cells in vaccine recipients, rendering them more susceptible to HIV infection during this time.

TLR3 binds double-stranded RNA [160, 161], and TLR3 ligands based on polyinosinic-polycytidylic acid (pIC) have demonstrated great potential for use as adjuvants in mucosal or systemic HIV vaccines. Type I IFNs produced in response to TLR3, TLR7 or TLR9 ligation promote cross-presentation of antigens to CD8 T lymphocytes by DC [162], but pIC has been found optimal to TLR7 and TLR9 agonists for generating gag-specific CD8 T cells in blood and lung of NHP immunized with gag protein by the subcutaneous (s.c.) route [131].

In NHP, TLR3 agonists have also augmented systemic IgG responses to parenteral VLP vaccines [163]. In mice, pIC has acted as a nasal, sublingual and vaginal adjuvant to enhance mucosal and systemic antibody responses to HIV gp140 or other protein immunogens [142]. TLR3 agonists have not yet been tested as mucosal adjuvants for preventative HIV vaccines in NHP or humans. However, studies of inactivated nasal influenza A vaccines formulated with the TLR3 agonist, rintatolimod (aka Ampligen®), indicate that TLR3 agonists can function as nasal adjuvants in primates [164, 165]. In humans, nasal administration of rintatolimod three days after nasal immunization with the FluMist influenza vaccine augmented nasal IgA responses [166]. Importantly, vaccination with TLR3 agonists at sites of mucosal HIV transmission may also be safe. In nonvaccinated Rhesus macaques, rectal administration of the pIC analogue, PICLC, just before or at the time of rectal SIV challenge tended to decrease susceptibility of macaques to infection, despite the presence of more activated $CD4^+$ T cells and $\alpha4\beta7^+$ memory $CD4^+$ T cells in the rectal mucosa and blood [167]. In SIV-infected macaques on antiretroviral therapy (ART), therapeutic immunization of the palatine and lingual tonsils with PICLC and inactivated SIV particles resulted in a 2-log reduction in rebound viremia after cessation of ART [168], although a TLR9 agonist with SIV particles failed to do so. Primary human endocervical and ectocervical epithelial cells cultured with pIC have also been reported to up-regulate IFNβ [169], a cytokine recently shown to protect macaques against vaginal SHIV transmission [170]. Taken together, these studies suggest that innate responses generated by TLR3 agonists administered with vaccines in the nasal, oral, rectal, and vaginal mucosae may augment humoral and cellular immune responses without increasing susceptibility to HIV infection. Additional studies evaluating the ability of TLR3 agonists to enhance immunogenicity and efficacy of mucosal SIV or SHIV vaccines in NHP are warranted by these encouraging results.

TLR4 agonists [171] include LPS, detoxified monophosphoryl lipid A (MPL), and the synthetic analogue, glucopyranosyl lipid A (GLA). Both MPL and GLA have been found to enhance humoral and $CD4^+$ T cell responses to parenterally administered HIV Env or SIV gag proteins in humans and macaques [126, 128, 131], albeit to a lesser degree than TLR3, TLR7/8 or TLR9 agonists. Whether TLR4 ligands can act as mucosal adjuvants in humans or NHP is unknown. In mice, there is conflicting data regarding the ability of TLR4 agonists to function as nasal adjuvants [142, 172, 173]. In minipigs [174], which have a respiratory tract more similar to humans [175], GLA did not enhance antibody responses to nasal protein immunogens. However, it is unclear how translatable these results are to humans. Determining whether TLR4 agonists can function as mucosal adjuvants for HIV vaccines will require more definitive studies in humans or NHP.

The TLR5 agonist, bacterial flagellin [176], can directly modulate B cell responses [20] and has enhanced humoral responses to i.m. influenza vaccines in humans [177]. There is some evidence that flagellin expressed as a fusion protein with antigen might function as an adjuvant in the nasal cavity of primates [178]. In studies with guinea pigs, nasal immunization with HIV virus-like particles (VLPs) constructed to express a membrane-anchored bacterial flagellin generated greater levels of Env-specific serum IgG and vaginal IgA than VLPs lacking or admixed with flagellin [179]. Flagellin has also augmented local T-cell responses to peptides administered in the mouse vagina, but efficient delivery at this

site required epithelial thinning and disruption by medroxyprogesterone and Nononoxyl 9 [180]. In the intestine, flagellin may enhance transport of particles by M cells [181] but it has not yet been tested for ability -as a surface coating - to enhance uptake of delivery vehicles containing vaccine.

The TLR7/8 agonist [182], resiquimod (R848), has enhanced systemic immune responses to HIV proteins co-administered parenterally in macaques [128–131]. In mucosal immunization studies with macaques, R848 given with β-galactosidase by the rectal route or with ovalbumin by the sublingual route did not elicit antibody responses [141]. However, R848 did function as a nasal adjuvant for KLH in these animals. Importantly, nasal immunization with R848 enhanced KLH-specific serum IgG and vaginal IgA responses without generating adverse inflammatory responses in the nasal cavity [141]. R848 may therefore be safe as a nasal adjuvant in humans. R848 is also one of the few TLR agonists identified as a vaginal adjuvant [183]. Vaginal ring delivery of HIV gp140 protein with R848 in sheep significantly increased levels of anti-gp140 IgG in serum and IgA in vaginal secretions [183]. More studies of R848 as a mucosal adjuvant for HIV vaccines are warranted by these encouraging results.

TLR9 agonists [184], unmethylated synthetic CpG oligodeoxynucleotides (CpG ODNs), have also augmented humoral and cellular immune responses to parenterally administered HIV and SIV proteins or virus particles in macaques [128, 131, 185]. In mice, CpG ODNs have also been reported to function as nasal, oral, intestinal and vaginal adjuvants [142, 172, 186], but these results may be misleading as different innate cells in mice and primates express TLR9 [138, 187]. The type of CpG can also influence results. There are 4 classes of CPG ODNs which differ in their ability to activate B cells and to induce production of TNFα or IFNα by plasmacytoid DC (pDC) [187]. Type B CpG induces TNFα production by primate pDC whereas Type C induces more IFNα [188–190]. CpG-C may not be ideal for mucosal HIV vaccines. Vaginal administration of CpG-C in nonvaccinated macaques enhanced the risk of vaginal SIV transmission [191], albeit in larger doses than those typically used for immunization. Supplementation of inactivated SIV particles with CpG-C did not enhance SIV-specific humoral or cellular responses or improve the ability to control rectal SIV infection in macaques given tonsillar vaccinations [192]. Therapeutic parenteral or tonsillar vaccination of SIV-infected macaques on ART with CpG-C and inactivated SIV particles also failed to generate immune responses that reduced rebound viremia after cessation of ART, although a TLR3 agonist did so [168, 185]. Whether mucosal immunization with CpG-B ODNs may generate more beneficial anti-HIV innate and adaptive responses in humans or NHP is unknown. It is noteworthy that CpG-B has been administered in the human nasal cavity without producing adverse side effects [193].

3.2. Cytokines

Cytokines have been tested in many vaccine studies for their ability to enhance immune responses and protective efficacy of SIV and SHIV DNA vaccines. The first cytokine shown to improve the efficacy of a DNA vaccine in NHP was IL-2, expressed as a fusion protein with the Fc of human IgG1 to increase its half-life [194]. Both soluble IL-2/Ig and DNA encoding IL-2/Ig were able to adjuvant humoral and cellular responses induced by an i.m.

SHIV DNA vaccine, and each reduced viral replication and disease progression after intravenous $SHIV_{89.6P}$ infection [194, 195]. We found that supplementing a nasal SHIV DNA vaccine with IL-2/Ig DNA induced greater frequencies and more durable virus-specific IFNγ-and TNF α-producing $CD8^+$ T cells in the rectum and blood of NHP after nasal boosting with SHIV MVA [49]. Better protection against disease development was observed in these animals after rectal $SHIV_{89.6P}$ infection, and control was associated with frequencies of vaccine-induced gag-specific $CD8^+$ T cells.

Toward the goal of generating even greater HIV-specific $CD8^+$ T-cell responses with DNA vaccines, the Th1-promoting IL-12 cytokine has also been evaluated as an adjuvant in NHP, but mostly by the i.m. route, and with conflicting results regarding its ability to improve $CD8^+$ T-cell responses and vaccine efficacy [196–199]. We tested DNA encoding IL-12 as a nasal adjuvant for the DNA prime in a DNA/MVA vaccination strategy in NHP. When compared to animals nasally-primed with SHIV DNA and nasally-boosted with MVA, co-priming with IL-12 and SHIV DNA did not induce detectable differences in virus-specific cellular or humoral responses before or after rectal infection with $SHIV_{89.6P}$, nor did it alter viremia or the course of disease progression [49]. The lack of IL-12 effects in this study could not be attributed to degradation of DNA in the lumen of the nasal cavity because the DNA was protected by formulation in cationic liposomes, and identical co-administration of IL-2/Ig DNA and SHIV DNA in another group of animals did enhance T-cell responses and control of infection [49].

IL-15 has been explored as an adjuvant for augmenting $CD8^+$ T-cell responses to DNA vaccines in NHP. However, in macaques primed by the i.m. route with IL-15-adjuvanted SHIV or SIV DNA, little difference has been noted in the virus-specific $CD8^+$ T-cell responses [196, 200, 201], and vaccine efficacy for preventing vaginal or rectal SIV infection was not significantly improved [196, 201]. We found that nasal immunization of macaques with IL-15-adjuvanted DNA followed by nasal MVA boosting generated greater control of rectal SIV_{mac251} infection than the same regimen given by the i.m. route [47]. While this could be attributed to the use of a mucosal route for vaccine delivery, it is also possible that IL-15 may enhance responses to DNA vaccines given at mucosal surfaces. On the other hand, nasal priming with IL-2-and IL-15-adjuvanted SIV DNA did not elicit different cellular (or humoral) responses compared to macaques primed with GM-CSF, IL-12 and TNFα. After a nasal boost with MVA-SIV and vaginal challenge with SIV_{mac251}, both regimens were found equally effective for control of infection [51]. The inclusion of soluble IL-15 in both a colorectal SIV peptide prime and colorectal SIV-MVA boost also failed to induce higher quality $CD8^+$ T-cell responses or to enhance control of rectal SIV infection when compared to animals immunized with non-adjuvanted peptide and MVA [202]. However, the IL-15 rectally applied in this study was not protected by formulation in a protective delivery vehicle. Therefore, the bioactivity of IL-15 may have been reduced by proteolysis in the rectal lumen. Alternatively, the 300μg dose of IL-15 repeatedly administered may have been too large. IL-15 has been shown to be immunosuppressive when given in high doses [203].

To augment humoral responses to HIV DNA or subunit vaccines, several cytokines which directly stimulate B cells have been tested as mucosal adjuvants in animals. Of these, B-cell

Activating Factor (BAFF) and A Proliferation-Inducing Ligand (APRIL) have not proven effective as nasal adjuvants in mice for enhancing HIV-specific antibodies in serum or secretions when given in soluble form with HIV Env protein [204]. Thymic Stromal Lymphopoietin (TSLP) co-administered by the nasal route with HIV gp140 in mice did induce impressive increases in Env-specific systemic IgG and vaginal IgA antibodies [204]. However, in NHP, nasal immunization with TSLP and KLH infrequently generated KLH-specific IgG or IgA antibodies in serum or secretions [141]. Nasal immunization of macaques with human IL-1α, GM-CSF and an HIV peptide also did not increase IgA antibodies in the rectum or vagina [205], despite promising results in mice [206]. The use of human cytokines in macaques or administration of potentially non-optimal cytokine doses may be partly responsible for this disappointing outcome. Like IL-15, high doses of GM-CSF can also suppress immune responses, including IgA responses [207, 208].

A noteworthy molecular adjuvant used to enhance Env-specific systemic IgG and vaginal IgA antibodies generated by systemically administered DNA vaccines or HIV VLPs in mice is the CCL28 chemokine [209, 210], which attracts CCR10-expressing lymphocytes to the large intestine, and to the mouse uterus [211]. Recently, in macaques, co-delivery of CCL28 DNA with SIV DNA by electroporation was shown to enhance SIV-specific serum IgG and vaginal IgA responses, and after vaginal challenge with SIV_{smE660}, these animals had lower peak viremia and rapidly controlled viremia to undetectable levels [212]. Hopefully, this chemokine will be evaluated in future studies to determine if it can augment immune responses and improve efficacy of mucosal vaccines.

3.3. Attenuated Bacterial Enterotoxins

Promising adjuvants for mucosal HIV vaccines include attenuated bacterial enterotoxins such as the *Escherichia coli* heat-labile double mutant toxin, dmLT [213] and cholera toxin (CT) derivatives [214, 215], which function as adjuvants in the murine nasal cavity, sublingual mucosa, intestine and female genital tract [215–217], and typically generate mixed Th1/Th2/Th17 responses. The precise mechanisms through which these enterotoxin adjuvants exert their immune stimulatory activity is still under investigation [213, 214, 218]. Most cannot be administered in the human nasal cavity as local inflammatory responses can result in Bell's palsy [219, 220]. However, like dmLT, they should be safe in the intestine, oral cavity and probably the vagina [221]. There is evidence that the innate and adaptive responses generated by LT-based adjuvants might be favorable for control of HIV infection. Rectal immunization of macaques with SHIV peptides and the first iteration of dmLT, the single mutant mLT (aka LTR192G), was shown to generate local CTL which controlled rectal $SHIV_{Ku}$ infection [222]. Therapeutic transcutaneous vaccination of ART-treated SIV-infected macaques with DNA-encoding SIV proteins and the LT holotoxin has also been found to dramatically reduce viremia following cessation of ART [223]. It is not widely appreciated that these enterotoxins can be used as parenteral adjuvants [213]. Indeed, there is a possibility that dmLT-adjuvanted parenteral HIV vaccines might generate HIV-specific intestinal immune responses. Intradermal immunization of mice with peptide and dmLT (but not CpG) was recently shown to generate α4β7-expressing peptide-specific $CD4^+$ T cells in the circulation, followed by increases of these cells in the small and large intestine [224]. Intradermal immunization of mice with dmLT-adjuvanted inactivated poliovaccine also

generated polio-specific intestinal IgA responses [225]. However, it remains unclear whether immunity induced by parenteral vaccines supplemented with dmLT would be as protective as mucosal vaccines containing this adjuvant.

3.4. Chitosan, Endocine, and ISCOMs

Some bioadhesives that reduce mucociliary clearance and improve the retention time of vaccines at mucosal surfaces have also been reported to function as adjuvants in mice [226]. The mucoadhesive, chitosan [227], is a chitin-derived cationic polymer that can enhance both vaccine retention and uptake at columnar epithelial cell surfaces by transiently opening tight junctions [228]. In humans, nasal insufflation of a lyophilized diptheria toxoid formulated with chitosan modestly increased titers of neutralizing antibody in serum and IL-5 producing T cells in blood of humans [229]. However, chitosan is weakly inflammatory in the human nasal cavity [230], and nasal administration of chitosan with an HIV gp140 protein in humans did not elicit anti-Env antibodies [231]. Generating strong humoral responses to mucosally administered HIV proteins formulated in chitosan will likely require supplementation with more potent adjuvants.

Endocine (aka Eurocrine L3) is an emulsion of the anionic lipids, mono-olein and oleic acid, that forms liposomes less than 100nm in diameter. In addition to enhancing uptake of antigens at mucosal surface, Endocine has some immune stimulatory activity, which has been linked to activation of danger-associated molecular pattern molecules by RNA released from damaged cells at the site of administration [232]. Therapeutic nasal vaccination of HIV-infected humans with HIV gag peptides in Endocine has been reported to increase lymphoproliferative responses in blood, and gag-specific serum IgG, rectal IgA and nasal IgA antibodies, although very modestly [233]. In mice, Endocine has impressively adjuvanted antibody responses to HIV VLPs given in the nasal cavity [234]. However, in macaques, four nasal immunizations with Endocine and the same VLPs did not elicit systemic or vaginal antibodies [235]. It is therefore unclear whether Endocine has sufficient stimulatory activity to elicit strong distal mucosal antibodies to nasally administered HIV proteins in humans.

Saponin derivatives in the ISCOM delivery vehicle (see below) have proinflammatory activity, and have functioned as adjuvant for ISCOM-formulated antigens given to mice by systemic or mucosal routes [236, 237]. However, ISCOMs also have not yet been tested for adjuvant activity at mucosal surfaces of primates. In addition, better results have been obtained in mice when mucosally-administered ISCOM vaccines were supplemented with an additional adjuvant [217, 238].

4. FORMULATIONS FOR MUCOSAL DELIVERY OF VACCINES

Although mucosal vaccination should provide more optimal mucosal immunity than systemic immunization, the same innate physical and chemical barriers that inhibit pathogen entry at mucosal surfaces pose obstacles in the efficient uptake of mucosal vaccines (reviewed in [239]). Physical impediments include commensals, mucus, mucociliary clearance and epithelial cell barriers which may consist of a single layer of columnar epithelium with impermeable tight junctions, as in the intestine, or multiple layers of

permeable squamous epithelium that lack tight junctions, which can be up to 50 cells deep in the vagina [240]. Chemical constraints include degradative enzymes (proteases, glycolases and nucleases) produced by both the local microbiota and epithelial cells, the host defense antimicrobial peptides and proteins produced by epithelial cells [241–243], and, for peroral (ingested) vaccines, the low pH and bile acids in the stomach [239]. A variety of protective vehicles have been utilized with success to deliver intact HIV or SIV antigens across these barriers, as discussed below.

4.1. Enteric Capsules

One solution to protect peroral vaccines against hydrolysis in the stomach is to administer them in a neutralizing bicarbonate solution, which has worked well for bacterial vaccines, such as the Dukoral® whole-inactivated cholera vaccine and the live-attenuated Ty21a *Samonella typhi* vaccine [244, 245]. Peroral administration of freeze-dried vaccine in enteric-coated capsules that are resistant to hydrolysis in low pH solutions has also been used to preserve vaccine integrity during transit through the stomach, and is another method commonly used to orally deliver the live-attenuated Ty21a vaccine [245]. Enteric capsules typically consist of gelatin or Hydroxy-Propyl-Methylcellulose (HPMC) capsules containing lyophilized antigen and stabilizing agents. Surfaces of the capsules are coated with various mixtures of Eudragit® methacrylic acid esters that are insoluble at low pH [246]. Capsules coated with Eudragit® formulations that dissolve at a specific pH and after a certain period of time can be used to deliver vaccines to specific segments of the small intestine and even the distal colon [247, 248]. In macaques, intragastric administration of freeze-dried replication-defective adenovirus type 5 (Ad5) expressing HIV Gag or Env in capsules coated with Eudragit® solutions designed to dissolve in the jejunum has elicited antigen-specific lymphoproliferative responses in blood and specific IgA in salivary and vaginal secretions despite significant loss of vector activity, possibly due to the absence of stabilizing excipients [247]. Recombinant adeno-associated viruses have also been delivered successfully in the intestine of macaques using enteric capsules [249]. An obvious disadvantage of this approach, though, is that the contents of capsules are released in the lumen where they may be degraded before being internalized.

4.2. Poly(lactide-co-Glycolic Acid) Particles

A delivery vehicle that does not release its contents until after internalization at mucosal surfaces would be advantageous for induction of immune responses. Apical surfaces of intestinal M cells are largely devoid of mucus, and these cells readily transcytose bacteria- and virus-sized particles into underlying lymphoid follicles for phagocytosis by DC [11, 250]. This includes antigens encapsulated in poly(lactide-co-glycolic acid) (PLG) biodegradable particles (reviewed in [251]), which slowly release their contents following internalization by Antigen-Presenting Cells (APC). M cells have also been identified in the nasopharyngeal tonsil (adenoids) of humans [252] and may similarly mediate uptake of PLG particles applied in the nasal cavity. For vaginal immunization, available data suggest that hydrophobic surfaces of particles should be modified with polyethylene glycol or other substances for optimal diffusion through mucus [253, 254], and smaller (20–40nm) particles may better penetrate vaginal epithelium and reach draining lymph nodes [255]. However, the utility of PLG particles as protective delivery vehicles for vaccines is limited because

organic solvents are required for antigen incorporation, and the lactic and glycolic acids released during particle dissolution can further degrade antigen [251]. Hence, while the immunogenicity of some HIV peptides and the adjuvant activity of some TLR agonists is retained [248, 256], encapsulated HIV Env proteins or DNA vaccines are typically denatured [239, 257]. This may also explain why oral immunization of humans with PLG particles containing an HIV V3 peptide dose of 1mg failed to elicit detectable immune responses, although the lack of an adjuvant was more likely responsible [258] In mice, a converse strategy of adsorbing DNA onto the PLG particle surface has been reported to be superior to naked DNA for nasal immunization [259]. It is unlikely, though, that proteins or DNA vaccines could be effectively administered in this way in the harsher luminal environments of the oral cavity, intestine and vagina.

4.3. Cationic Liposomes

Phopholipid bilayer-based liposomes with an aqueous core (reviewed in [260]), and often supplemented with cholesterol to increase stability, have been used with success in NHP to deliver SIV peptides by the rectal route [217] and DNA vaccines by numerous mucosal routes [50, 88, 202]. In humans, freeze-dried liposomes containing protein immunogen have also been administered perorally in enteric capsules for induction of salivary IgA antibodies [261]. However, mucosal delivery of vaccines incorporated within liposomes may primarily induce T cell responses as liposomes fuse with cell membranes resulting in cytosolic delivery of antigen [260]. Positively charged (cationic) liposomes, rather than negatively charged (anionic) liposomes, are most often used for vaccine delivery because DNA and most protein or peptide antigens are negatively charged and readily associate with cationic liposomes through electrostatic interactions. Cationic liposomes have also been reported to be more efficiently internalized by DC due to their ability to bind to heparan sulfate proteoglycans on the surface of these cells [251]. Epithelial cells also express heparan sulfate proteoglycans. Mucosal immunization with liposomal DNA may therefore result in transfection of epithelial cells, which can function as APC [262–264], but may be sloughed in a matter of days. Nonetheless, we and others have found that SIV DNA encapsulated in cationic liposomes can successfully generate SIV-specific T cells when delivered in the rectum, small intestine, oral cavity, vagina or nasal cavity of macaques [50, 88]. However, we should note that administration of naked DNA in the primate nasal cavity is also effective for induction of antigen-specific T cells [47, 49, 51, 52].

4.4. Immune Stimulatory Complexes (ISCOMs)

ISCOMs [265] are 40–50nm nanoparticles consisting of cholesterol, phosphatidylcholine and *Quillaja* saponin derivatives [238], which form cage-like structures through hydrophobic interactions. The saponin component has proinflammatory activity and bestows this delivery vehicle with some immune stimulatory activity [236]. Any protein or peptide with a hydrophobic region can be incorporated into ISCOMs [266], and both humoral and cellular responses have been induced by ISCOM-formulated antigens [265]. ISCOMs enhance uptake of proteins in the murine intestine [267] and they have been used to deliver SHIV peptides in the rectum of macaques, which elicited CTL and low levels of neutralizing antibodies in blood, and reduced peak viremia after rectal $SHIV_{162P4}$ infection [268]. ISCOMs have also been used for protein immunization in the murine female reproductive

tract [217] and in the upper or lower respiratory tract of mice or sheep [269]. However, with the exception of the rectal route, ISCOMs have not yet been tested as delivery vehicles at other mucosal surfaces in humans or NHP.

4.5. Influenza Virosomes

Influenza virosomes are 150–200nm phosphatidyl-choline-enriched liposomes supplemented with the influenza A Neuraminidase (NA) and Hemagglutinin (HA) viral proteins [270, 271]. The HA assists in targeting of virosomes to APC due to its recognition of sialic acid residues abundantly expressed on DC and macrophages, and it likely enhances mucosal uptake of virosomes through binding to sialic acid-containing molecules ubiquitously expressed on apical surfaces of epithelial cells [272]. In mice, virosomes containing incorporated DNA, proteins or peptides have induced humoral and cellular immune responses after delivery by the nasal, pulmonary, sublingual or vaginal routes [273–276]. To generate stronger antibody responses, antigens can also be expressed on the surface of the virosome through integration into the lipid bilayer [270], although they would be unprotected. In one study, HIV peptides were covalently attached to the influenza proteins on the outer surface of gp120 and saponin containing virosomes. However, nasal immunization of macaques with these surface modified virosomes did not produce good antibody responses [277]. In contrast, i.m. priming and nasal boosting of macaques with gp41 membrane proximal external region (MPER) peptide and an immunodominant domain-deleted gp41 integrated into the virosome lipid bilayer was successful for generating protective neutralizing and transcytosis-inhibiting antibodies [278] in rectal and vaginal secretions [279]. In humans, a similar i.m./nasal vaccination regimen with virosomes containing only the MPER peptide elicited more modest mucosal antibody responses [280]. Whether this may have been due to the presence of pre-existing anti-influenza antibodies in serum or the upper respiratory tract is unclear. In humans, pre-existing serum antibodies have been correlated with reduced systemic IgG responses to influenza following i.m. immunization with the Inflexal V® influenza virosome vaccine [281]. However, in other clinical studies with adults who presumably had pre-existing anti-influenza HA antibodies, excellent serum antibody responses have been generated to non-influenza antigens after i.m. administration of virosome-based hepatitis A virus and malaria vaccines [282, 283]. Additional studies will be required to determine the extent to which pre-existing mucosal and serum IgG antibodies to influenza proteins may limit the utility of the virosome as a mucosal delivery vehicle in humans.

Disadvantages of delivering mucosal vaccines in the above liposome-based vehicles are the increased costs associated with vaccine manufacture and short shelf-life [284]. While new lipid-based nanovesicles are in development to circumvent these issues [284], the problem still remains that encapsulation of proteins inside liposomes and PLG particles that are primarily internalized by DC not only shields them from degradative enzymes but also from B cells. New methods for delivery of HIV Env proteins to mucosal B cells are urgently needed to more effectively generate anti-Env antibodies in the intestine and genital tract.

5. ORAL AND RECTAL ROUTES OF SIV VACCINATION

Immunization in the oral cavity and gastrointestinal mucosa using peroral administration has significant appeal because of the simplicity of administration and therefore its feasibility even in settings with limited health care resources [285]. Rectal immunization, important because the rectum is a site of HIV transmission, primarily stimulates responses in the intestine, and it is not excessively complicated in terms of administration, as the vaccine could be formulated in suppositories, a common delivery tool for therapy [286]. However, it would not be as well received as oral immunization. Immunization in the large intestine through peroral administration is feasible, though, as the vaccine can be formulated in enteric-coated capsules that specifically dissolve in the colon or rectum [248], as discussed above.

Gastrointestinal immunization in the context of the NHP model for AIDS has been explored with a variety of platforms, including DNA, peptide, proteins, viruses, and bacteria. These candidate vaccines were delivered exclusively to the oral cavity, tonsils, small intestine, rectum or the entire gastrointestinal (GI) tract. It is unclear to what extent oral vaccination, whether targeted to the oral cavity or the GI tract can protect in the context of HIV/SIV infection. The highest antibody titers are usually achieved at the mucosal site of antigen exposure and decrease at distant sites [287–292]. Although HIV replicates at significant levels in the intestinal mucosa, it enters the body predominantly *via* the genital tract or the rectum, requiring a more diversified immunity than pathogens that infect exclusively by the oral route [5]. The rationale for considering an oral vaccine capable of stimulating both mucosal and systemic immunity comes from humans and NHP vaccine studies [293–295] and from data involving highly exposed but persistently seronegative (HEPS) uninfected individuals [296–298], which suggest that it is unlikely that vaccine approaches that stimulate a single arm of the immune system will provide effective prevention of chronic systemic infection [293–295, 299–308]. Achieving humoral and cellular mucosal responses at different mucosal sites in addition to systemic responses, which is possible with oral immunization, may result in protection from chronic infection, as this combination has been suggested key to HIV resistance in highly exposed sex workers and discordant heterosexual couples [296, 297]. In the next paragraphs, we will review what we have learned so far when oral and intestinal immunization approaches were investigated in preclinical trials.

We used SIV and SHIV recombinant DNA+MVA *via* the oral cavity, the small intestine and rectal routes, using a SHIV challenge rectally and an SIV challenge either rectally or vaginally [46, 50, 88, 309]. We determined that mucosal and systemic responses to multiple viral antigens produced by SHIV or SIV DNA vaccine boosted by a matched recombinant MVA can be induced to different degrees after rectal or mixed systemic/mucosal vaccination in male and female animals. However, mucosal antibody responses were sporadic and short-lived. After challenge, we observed control of viremia and delay of $CD4^+$ T-cell loss and AIDS. When the vaccinated animals were compared to controls, on average a significant reduction in viremia was observed. Post-challenge immunological correlates of protection were systemic anti-SIV Gag + Env $CD4^+$/IL-2^+, $CD4^+$/IFNγ^+, and $CD8^+$/TNFα^+ T cells and vaginal anti-SIV Gag + Env $CD8^+$ T-cell total responses. When a similar SIV vaccination, further boosted by mLT-adjuvanted inactivated SIV particles, and given *via* the

oral route was directly compared to the small intestinal route in female animals challenged vaginally, we found that immunizations generated mucosal SIV-specific IgA at different sites and limited levels of SIV-specific IgG antibodies in plasma and mainly against Gag and Pol proteins after particle immunization [50]. No SIV-specific IgG antibodies were detected in secretions. Oral immunization was effective in inducing SIV-specific IgA in vaginal secretions and generated greater IgA responses in rectal secretions and saliva when compared to the small intestinal immunization route or vaginal and nasal secretions evaluated in the same study. These immunizations stimulated systemic T-cell responses against Gag and Env, albeit to a different extent, with oral immunization inducing a higher magnitude response. SIV-specific T cells producing IFNγ dominated these responses. Vaccination also induced $CD4^+$ and $CD8^+$ T-cell responses in the rectal and vaginal mucosa with greater functional heterogeneity than in blood samples. Rectal T-cell responses were significantly greater in the orally vaccinated animals than in the other animals. The most balanced, higher-magnitude vaginal T-cell responses were observed after intestinal vaccination. Of the routes tested, oral vaccination provided the most overall diverse and significant response to the vaccine. After vaginal challenge with SIV_{mac251}, 50% of the orally vaccinated animals suppressed viremia to undetectable levels and viremia did not rebound after $CD8^+$ T-cell depletion, while suppression of viremia occurred to a significantly lower degree in intestinally vaccinated animals and in controls [46]. Regardless of the route of vaccination, mucosal vaccinations prevented loss of $CD4^+$ T central memory cells and $CD4^+$ α4β7+ T cells and reduced immune activation. None of the orally vaccinated animals had developed AIDS after 72–84 weeks of infection, when the trial was closed.

When considering viral vectors given *via* the oral route, an interesting approach was provided by recombinant live attenuated oral polioviruses, each expressing a small SIV peptide and covering, when combined in a single vaccination, the entire Gag, Pol and Env protein sequences [67]. Oral immunization of 7 Cynomolgus macaques with this vaccine stimulated both humoral and cell-mediated systemic and mucosal responses. These responses provided protection from vaginal challenge in some animals, which required a significantly higher number of challenges to become infected, and 2 were completely protected from infection, while 2 other animals showed significant reduction of viremia after infection. All remained disease free by week 48, when 50% of the 12 controls had developed AIDS [68].

Vaccination of the palatine and lingual tonsils with aldrithiol 2 (AT-2)-inactivated SIVmac particles mixed with CpG adjuvants was tested in Chinese Rhesus macaques [192]. Anti-SIV rectal IgA were of limited magnitude and the titer did not increase after challenge. Fifty percent of the animals were fully protected from homologous rectal challenge. In the infected animals, viremia control was limited and transient [192]. A SIV-based, Single Cycle Immunodeficiency Virus (SCIV) was also used as an oral spray administered at the tonsils and followed by oral challenge [310]. Although reduction of peak viremia was observed, this immunization provided very limited benefits compared to others. This route of vaccination provided slightly better results when a recombinant MVA vaccination was used in the SHIV model, where better viremia control and protection from $CD4^+$ T-cell loss was observed. In both cases, systemic and mucosal responses were stimulated. The easier ability to protect

against CXCR4-tropic SHIVs compared to SIV needs to be kept in mind when these different outcomes are considered [311].

Rectal immunization of macaques using SIV and HIV peptides mixed with mLT induced responses at multiple mucosal sites and although it did not prevent infection, viremia after challenge was significantly reduced to undetectable levels in the intestinal mucosa and in blood [222]. These investigators observed equally significant results when they tested a combination of multiple SIV Gag and HIV Env and Tat peptides and the attenuated vaccinia virus strain NYVAC expressing SIV gag/pol and HIV env genes. Excellent T-cell responses in blood, colon and mesenteric lymph nodes were observed and delayed dissemination of SHIV occurred after rectal challenge [312]. When focusing on rectal vaccination with viral vectors, significant results were also observed in macaques rectally immunized with SIV *gag, env* and *rev*-expressing replication-competent adenovirus type 5 (Ad5), which stimulated secretory IgA at multiple mucosal sites and memory cell-mediated responses both systemically and mucosally [61]. Importantly, when combined with parenteral SIV gp120 immunization, this study showed that although the antibody responses were non-neutralizing, vaccination induced antibodies that mediated Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cellular Viral Inhibition (ADCVI) and transcytosis inhibition [61].

Among the bacterial vectors investigated for oral immunization, recombinant live attenuated *Salmonella typhimurium* strains expressing SIV Gag and Env fragments have been evaluated for their capability to stimulate anti-SIV immunity when given alone or combined with boosting by recombinant MVA [25–29, 313]. Cell-mediated responses were induced in blood and colonic mucosa but these responses did not provide control of viremia after rectal or intravenous challenge. *Shigella flexneri* strains have been investigated to deliver DNA vaccine plasmids in a mouse model and succeeded in stimulating both cell-mediated and humoral responses [30]. However, data in NHP are not yet available.

An interesting approach to HIV vaccination is the use of recombinant mycobacterial vectors, which could simultaneously achieve vaccination for TB and HIV. A single oral dose of an attenuated Mtb-SIV vaccine given to infant macaques during the first week of life stimulated immune responses to TB and SIV antigens, and anti-SIV responses increased after MVA-SIV boosting. T-cell responses reached the highest magnitude in the intestine and in oral lymph nodes. Mucosal SIV-specific IgA could be detected in saliva and intestinal secretion, while IgG was present in blood. Significantly lower peak viremia after challenge correlated with pre-challenge SIV Env-specific salivary and intestinal IgA responses and higher-avidity SIV Env-specific IgG in plasma, and in these animals $CD4^+$ T-cell populations were better preserved over time compared to non-controlling animals [35, 36].

6. VAGINAL SIV OR SHIV VACCINATION

In studies with NHP, vaccine-mediated protection against vaginal SIV or SHIV infection has been associated with the induction of CD8 T-resident memory (Trm) cells and local anti-Env IgA-or IgG-antibody responses, especially to gp41, in the female genital tract [10, 279, 314–316]. Transudated serum IgG antibodies may also contribute to protection in genital tract

tissues if they can neutralize virus, mediate phagocytosis or kill infected cells through ADCC [127, 317, 318]. These immune effectors could be generated by systemically administered replication-competent viral vectors that have some propensity to replicate in the female genital tract. Even replication-incompetent viral vectors, such as the SIV-expressing NYVAC poxvirus vector, can induce specific CD8 T cells in the genital tract of primates following parenteral immunization [319]. However, a replication-competent Ad5-SIV generated more SIV-specific CD8 T cells in vaginal tissues of macaques when immunization was performed by the vaginal route [61], consistent with reports that more virus-specific CD8 T cells are generated at sites of immunization [248, 320, 321]. Parenteral immunization with non-replicating vectors and protein immunogens is also ineffective for generating IgA or IgG plasma cells in the female genital tract [127, 287]. The vaginal route has been found optimal for induction of local IgA and IgG antibody responses in the female genital tract of NHP and women [287, 289–291]. In several animal studies, vaginal immunization with DNA or other non-replicating vaccines has also proved more effective than parenteral immunization for preventing infections by vaginally transmitted pathogens [322–324].

Vaginal immunization can, however, be challenging. The phase of the menstrual cycle may need to be taken into account for some vaccine antigens. Vaginal immunization of women with a whole inactivated cholera vaccine containing CTB has been found equally effective for generating local CTB-specific antibodies when immunization was performed during either the mid-follicular or mid-luteal phase of the menstrual cycle. However, no antibodies were generated to cholera LPS in women immunized during the mid-luteal phase [290]. The reason for this is unclear. It may be related to reduced uptake of free LPS or contact of cholera vibrios with the epithelium during the luteal phase, a time when mucus is more viscous [325]. Mucus interferes with the ability of many antigens, including HIV particles [326], to penetrate the epithelium and contact DC. In studies tracking the fate of tracer proteins or nanoparticles applied in the vagina of mice or macaques, very little tracer has been detected in the stroma of the vagina or ectocervix. Most of it remains sequestered in mucus at apical epithelial surfaces or within the superficial layers of sloughing epithelium [255, 326–328]. For this reason, induction of antibody responses in the female genital tract will require large doses of antigen, in addition to adjuvant. Even large doses of HIV Env protein administered by the vaginal route in the absence of adjuvant have proved relatively ineffective for generating local IgA-or IgG-antibody responses in humans and NHP [231, 329–331].

Generating strong local antibody responses with the vaginal immunization route has also proved difficult using viral-vectored vaccines, including HIV-expressing canarypox in humans [332] and replication-competent SIV Ad5 [61]. Vaginal administration of SIV DNA in cationic liposomes, MVA and SIV particles formulated with mLT adjuvant in macaques did elicit SIV Env-specific vaginal IgA responses in some animals, but they were of low magnitude and did not persist [50]. Vaginal immunization of macaques with HPV pseudovirions expressing SIV gp120 env, gag and pol generated negligible anti-Env IgA in vaginal secretions [333]. Recently, however, sheep immunized with vaginal rings that release gp140 and R848 adjuvant within 24 hours were shown to develop impressive levels of HIV gp140-specific IgA and IgG in vaginal secretions [183]. In serum, high concentrations of

anti-gp140 IgG but not IgA were also found in these animals. It would be interesting to know if this immunization strategy could confer protection against SHIV infection in macaques.

Since low doses of antigen can stimulate T-cell responses, vaginally administered SIV vaccines have, not surprisingly, proved more effective for generating antigen-specific $CD4^+$ and $CD8^+$ T cells in the genital tract of NHP [46, 61, 333]. Evaluating cells in genital tract tissues of humans is difficult in clinical studies. However, vaginal immunization of women with HIV gp140 conjugated to the *Mycobacterium tuberculosis* heat shock protein 70 has been shown to generated gp140-specific CD4 and CD8 T cells in blood [334]. Interestingly, the innate antiviral restrictive enzyme, APOBEC3G, and chemokines (CCL3 and CCL5) which down-regulate CCR5 expression by $CD4^+$ T cells were up-regulated in these women. In addition, $CD4^+$ T cells from the blood of these women were found to be more resistant to HIV infection *in vitro* when compared to their pre-immunization T cells [334].

Vaginal immunization using viral vectored vaccines can also generate T cells in the large intestine [50, 61, 335]. In a small study with macaques, vaginal administration of replication-incompetent helper-dependent adenoviral vectors expressing HIV gp140 induced Env-specific $CD4^+$ T cells in the rectum, and this vaginal immunization regimen was as effective as i.m. vaccination for reducing acute viremia after rectal challenge with $SHIV_{162P3}$ [335].

Despite the induction of local antiviral CD8 T cells, nonreplicating vaginal SIV vaccines have not prevented vaginal SIV transmission or significantly reduced viremia in NHP. Only vaginal immunization with the nonpathogenic $SHIV_{89.6}$ has proved effective for this [336]. Interestingly, $SHIV_{8.96}$ does not generate Th1-biased responses, in contrast to most SIV vaccines. $CD4^+$ T cells recruited to the genital tract of $SHIV_{89.6}$-immunized macaques include T regulatory cells, and following SIV_{mac251} infection these cells suppress local $CD4^+$ T-cell proliferation and inflammation, allowing local $CD8^+$ T cells to eliminate infected cells in the genital tract without increasing the pool of available activated $CCR5^+$ $CD4^+$ target cells [337]. Replication-incompetent vaccines designed to mimic the cellular responses generated by SHIV89.6 and induce Env-specific antibodies to CCR5-tropic viruses may be ideal for vaginal immunization, and achieving protection against HIV in the female genital tract.

7. PENILE VACCINATION

In the last few years, there has been considerable progress identifying modes of HIV transmission in the male genital tract [338–341] and the local immune effectors that could potentially contribute to protection at this site [340, 342–344]. In the urethral mucosa, IgA plasma cells dominate over IgG plasma cells [345], but male genital tract secretions, like those in the female genital tract, typically contain more IgG due to transudation (or possibly FcRn-mediated transport) of large amounts of serum IgG [346]. Thus, i.m. vaccination can generate IgG antibodies in these fluids [347]. However, HIV resistance in men has been linked to HIV-neutralizing IgA in foreskin [298, 348], and induction of local HIV-specific IgA responses will likely require that vaccine be administered locally or by another mucosal

route. Unfortunately, there is very little information on this topic. The male genital tract is the most understudied site for vaccine administration. In the single penile immunization study that we are aware of, a pediatric nasogastric feeding tube was used to topically apply chimeric SIV p27-Ty yeast transposon virus-like particles conjugated to cholera toxin B subunit (CTB) [349] in the urethra of male macaques [350]. Two urethral immunizations induced p27-specific IgA and IgG antibodies in urethral fluids and serum. Interestingly, p27-specific lymphoproliferative responses were also detected in blood, and these were comparable to those in animals vaccinated by the i.m. route with p27-Ty particles and alum [350]. This study clearly demonstrates the feasibility of generating virus-specific humoral and cellular responses by immunization in the male genital tract. However, more practical methods are needed for vaccine delivery at this site.

Additional studies evaluating the immune responses generated in the male genital tract after delivery of vaccines by more practical immunization routes would also inform HIV vaccine development. It is known that i.m. immunization with non-replicating Ad26- and Ad35-SIV vectors can generate gag-specific $CD4^+$ and $CD8^+$ T cells in foreskin of macaques [351], and i.m. vaccination would provide serum IgG antibodies at this site. To generate both antigen-specific T cells and IgA plasma cells in the male genital tract, nasal immunization may be optimal to oral immunization. In men orally immunized with the live attenuated *Salmonella* typhi vaccine, only low levels of *Salmonella*-specific IgA antibodies were generated in semen [347]. In studies using the highly immunogenic CTB as an antigen, nasal immunization of men has been found more effective than oral immunization for generating IgA antibodies in urogenital fluid [292]. More studies will be needed to determine which vaccination route or combination of routes would be most effective for generating protective immunity to HIV in the male genital tract.

8. RESPIRATORY TRACT VACCINATION

Antibody responses generated by mucosal immunization of humans or NHP with protein immunogens can be remarkably confined to the site of immunization [288, 352, 353]. For instance, immunization of one palatine tonsil in humans generates IgG and IgA antibody-secreting cells in the immunized tonsil but not in the non-immunized tonsil [352]. Administration of vaccine in a single nostril of humans similarly induces greater levels of nasal IgA antibodies in the immunized than in the non-immunized nostril [354]. Considering this, and that unique combination of receptors are utilized for homing of lymphocytes to different mucosal compartments [355, 356], it should not be surprising that there is even less cross-talk between the intestine and the genital tract. Indeed, in women and female macaques, oral or rectal immunization with live-attenuated Salmonella Typhi [357–359], non-adjuvanted inactivated influenza vaccine [360], CTB [289–291] or SIV p55 gag protein with CT adjuvant [361] has not consistently elicited specific antibodies in cervical and vaginal secretions. Local vaginal immunization has been found optimal to oral and rectal routes for induction of genital tract antibody responses, but vaginal immunization has not generated antibodies in the rectum [289–291]. This is problematic as a vaccine intended to prevent sexual transmission of HIV should ideally induce antibodies in both the rectum and genital tract of men and women. The nasal route began to attract attention as a possible means to accomplish this when it was reported that nasal vaccination of mice could generate

IgA plasma cells in the intestine [362], IgA antibodies in vaginal scretions [362], CTL in mesenteric and iliac lymph nodes [363] and protection against vaginal HSV-2 infection [364]. It was subsequently confirmed that nasal immunization in humans and NHP could also induce specific antibodies in the rectum and both the male and female genital tract [290, 292, 365, 366].

Nasal vaccination would also be more practical and acceptable worldwide than rectal or vaginal vaccination. Unfortunately, after Bell's palsy was reported in study subjects receiving nasal vaccines with the LTK63 enterotoxin adjuvant [220], some have viewed nasal HIV vaccine development as being futile. However, numerous nasal vaccines are in clinical development, and they have been confirmed safe in the human nasal cavity. These include recombinant chimpanzee Ad and MVA vectors expressing respiratory syncytial virus (RSV) antigens [367], recombinant Sendai virus expressing human parainfluenza virus type 1 antigens [368], inactivated influenza vaccines adjuvanted with *Neisseria meningitidis* outer membrane vesicles [369] or squalene [370], a *Shigella* LPS-containing vaccine [371], and live attenuated RSV, Bordetella pertussis and influenza vaccines [372–374]. Developing an HIV nasal vaccine should therefore be quite feasible.

As one would expect, nasally-administered replication-competent vaccines, such as attenuated SHIVs, have proved most effective in NHP for preventing or controlling mucosal infections by $SHIV_{89.6P}$ or SIV_{mac239} [336, 375]. Cynomolgus macaques nasally immunized with replicating poliovirus vectors expressing SIV genes also developed both T-cell responses and mucosal antibodies, that together were likely responsible for the sterile protection or significant control of infection observed following vaginal SIV_{mac251} challenge [68]. Non-replicating vaccines can also provide protection against rectal or vaginal immunodeficiency virus infections when applied simply as liquid drops in the nasal cavity, and perhaps as an aerosol in the lungs [376]. We have found that nasal priming of Rhesus macaques with DNA expressing SHIV or SIV VLP followed by nasal boosting with MVA-SHIV or -SIV resulted in significant control of rectal $SHIV_{89.6P}$ or vaginal SIV_{mac251} infection [49, 51, 52]. Nasal administration of these vaccines seeded both the rectal and genital tract mucosa with virus-specific T cells, and these were likely responsible for control because only very low levels of systemic and mucosal antibodies were generated by these DNA/single MVA boost vaccines. Importantly, nasal DNA/MVA immunization generated more T cells in the colorectal mucosa and significantly better control of infection when compared to i.m. immunization with the same vaccine components [47].

Interestingly, some non-replicating vaccines that failed to protect macaques when given solely by the i.m. or nasal route have demonstrated greater efficacy when given by both routes. For example, i.m. priming with SIV gag and IL-12 encoding DNA followed by nasal boosting with a vesicular stomatitis virus (VSV) vector expressing SHIV genes generated greater protection against $SHIV_{89.6P}$ than did nasal immunization with the VSV construct alone [377, 378]. A SHIV DNA/fowlpox vaccine that did not result in control of $SHIV_{162P3}$ vaginal infection when the components were given only by the i.m. or nasal routes did significantly reduce viremia in pig-tailed macaques immunized with the DNA by the i.m. route and the fowlpox by the nasal route [44]. Excellent antibody-mediated protection against vaginal $SHIV_{162P3}$ transmission was obtained in Chinese Rhesus macaques

vaccinated by the i.m. route with influenza virosomes containing a gp41 MPER peptide and recombinant gp41 lacking the immunodominant domain, but better protection was generated by i.m. priming followed by nasal boosting [279]. In studies focused on induction of antibodies for preventing mother-to-child breast milk transmission, i.m. DNA/MVA vaccination of lactating female macaques followed by nasal boosting with gp120 and R848 adjuvant has been found to generate more gp120-specific IgA antibodies in breast milk when compared to i.m. boosting [379]. However, serum IgG antibodies generated by nasal immunization were less effective at mediating ADCC. Simultaneous parenteral and nasal vaccination may be one approach for generating immune effectors in both the systemic and mucosal compartments. We have found that simultaneous i.m. and nasal DNA/MVA administration resulted in more dramatic control of viremia when compared to immunization by the nasal route alone [52].

CONCLUSION AND FUTURE PERSPECTIVES

The studies reviewed above clearly support the fact that mucosal administration of replication-incompetent T cell-inducing SIV/SHIV vaccines can be just as efficacious, if not better [46, 222], than parenteral vaccination for control of mucosal immunodeficiency virus infections. Given the efficacy of SIV DNA/MVA delivered by the intragastric or buccal routes for controlling vaginal SIV infection [46], peroral HIV vaccine development may deserve more attention because an ingested vaccine would contact tissues in both oral cavity and intestine. In the absence of safe, replication-competent vaccines that would induce strong Env-specific mucosal antibody responses in addition to T-cell responses, there is a need to identify Env immunogens and adjuvants that can stimulate production of these antibodies in the intestine and genital tract, which should improve efficacy of HIV vaccines [279, 380]. Vaccines delivered by the nasal route can induce T cells that control rectal or vaginal SIV or SHIV infections [46, 47, 49, 52], and because the nasal route can also generate antibodies in the rectum and genital tract, the identification of safe adjuvants for nasally-administered Env proteins could have a large impact on vaccine development. However, without a clearly suitable small animal model, NHP will likely be required for adjuvant studies. Vaccines that additionally generate multifunctional serum IgG antibodies that transudate into mucosal tissues would further augment protective immunity to HIV [381]. Whether simultaneous mucosal and parenteral vaccination strategies will be required for this is unclear, but should be explored. The ability to shorten vaccination regimens by performing simultaneous immunizations rather than prime-boost immunizations with antibody-inducing Env immunogens and T cell-inducing DNA plasmids or viral vectors should also be investigated because vaccine regimens that require numerous boosts are less likely to result in full compliance. There has been considerable progress in the design of recombinant Env immunogens that can induce HIV-neutralizing antibodies [382], and with improved mucosal delivery systems for these, the goal of an HIV-free generation might be achieved.

REFERENCES

[1]. Brenchley JM, Schacker TW, Ruff LE, et al. CD4$^+$ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004; 200(6): 749–59. [PubMed: 15365096]

[2]. Li Q, Duan L, Estes JD, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 2005; 434(7037): 1148–52. [PubMed: 15793562]

[3]. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med 2004; 200(6): 761–70. [PubMed: 15365095]

[4]. Santangelo PJ, Rogers KA, Zurla C, et al. Whole-body immunoPET reveals active SIV dynamics in viremic and antiretroviral therapy-treated macaques. Nat Methods 2015; 12(5): 427–32. [PubMed: 25751144]

[5]. Xu H, Wang X, Veazey RS. Mucosal immunology of HIV infection. Immunol Rev 2013; 254(1): 10–33. [PubMed: 23772612]

[6]. Chenine AL, Siddappa NB, Kramer VG, et al. Relative transmissibility of an R5 clade C simian-human immunodeficiency virus across different mucosae in macaques parallels the relative risks of sexual HIV-1 transmission in humans via different routes. J Infect Dis 2010; 201(8): 1155–63. [PubMed: 20214475]

[7]. Keele BF, Estes JD. Barriers to mucosal transmission of immunodeficiency viruses. Blood 2011; 118(4): 839–46. [PubMed: 21555745]

[8]. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu Rev Med 2011; 62: 127–39. [PubMed: 21054171]

[9]. Miller CJ, Li Q, Abel K, et al. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. J Virol 2005; 79(14): 9217–27. [PubMed: 15994816]

[10]. Adnan S, Reeves RK, Gillis J, et al. Persistent low-level replication of SIV Delta nef drives maturation of antibody and CD8 T cell responses to induce protective immunity against vaginal SIV infection. PLoS Pathog 2016; 12(12): e1006104.

[11]. Neutra MR, Kozlowski PA. Mucosal vaccines: The promise and the challenge. Nat Rev Immunol 2006; 6(2): 148–58. [PubMed: 16491139]

[12]. Kutzler MA, Weiner DB. DNA vaccines: Ready for prime time? Nat Rev Genet 2008; 9(10): 776–88. [PubMed: 18781156]

[13]. Lin IY, Van TT, Smooker PM. Live-attenuated bacterial vectors: Tools for vaccine and therapeutic agent delivery. Vaccines (Basel) 2015; 3(4): 940–72. [PubMed: 26569321]

[14]. Unnikrishnan M, Rappuoli R, Serruto D. Recombinant bacterial vaccines. Curr Opin Immunol 2012; 24(3): 337–42. [PubMed: 22541723]

[15]. Parks CL, Picker LJ, King CR. Development of replication-competent viral vectors for HIV vaccine delivery. Curr Opin HIV AIDS 2013; 8(5): 402–11. [PubMed: 23925000]

[16]. Cao X. Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease. Nat Rev Immunol 2016; 16(1): 35–50. [PubMed: 26711677]

[17]. O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors-redefining innate immunity. Nat Rev Immunol 2013; 13(6): 453–60. [PubMed: 23681101]

[18]. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol 2011; 30(1): 16–34. [PubMed: 21235323]

[19]. Fukata M, Abreu MT. TLR4 signalling in the intestine in health and disease. Biochem Soc Trans 2007; 35(Pt 6): 1473–8. [PubMed: 18031248]

[20]. Oh JZ, Ravindran R, Chassaing B, et al. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. Immunity 2014; 41(3): 478–92. [PubMed: 25220212]

[21]. Bauer S, Pigisch S, Hangel D, Kaufmann A, Hamm S. Recognition of nucleic acid and nucleic acid analogs by Toll-like receptors 7, 8 and 9. Immunobiology 2008; 213(3–4): 315–28. [PubMed: 18406377]

[22]. Kotton CN, Hohmann EL. Enteric pathogens as vaccine vectors for foreign antigen delivery. Infect Immun 2004; 72(10): 5535–47. [PubMed: 15385450]

[23]. Binet R, Letoffe S, Ghigo JM, Delepelaire P, Wandersman C. Protein secretion by Gram-negative bacterial ABC exporters--a review. Gene 1997; 192(1): 7–11. [PubMed: 9224868]

[24]. Hahn HP, von Specht BU. Secretory delivery of recombinant proteins in attenuated Salmonella strains: Potential and limitations of Type I protein transporters. FEMS Immunol Med Microbiol 2003; 37(2–3): 87–98. [PubMed: 12832111]

[25]. Chin'ombe N, Bourn WR, Williamson AL, Shephard EG. Oral vaccination with a recombinant Salmonella vaccine vector provokes systemic HIV-1 subtype C Gag-specific CD4+ Th1 and Th2 cell immune responses in mice. Virol J 2009; 6: 87–96. [PubMed: 19555490]

[26]. Evans DT, Chen LM, Gillis J, et al. Mucosal priming of simian immunodeficiency virus-specific cytotoxic T-lymphocyte responses in rhesus macaques by the Salmonella type III secretion antigen delivery system. J Virol 2003; 77(4): 2400–9. [PubMed: 12551977]

[27]. Fouts TR, Tuskan RG, Chada S, Hone DM, Lewis GK. Construction and immunogenicity of Salmonella typhimurium vaccine vectors that express HIV-1 gp120. Vaccine 1995; 13(17): 1697–705. [PubMed: 8719522]

[28]. Franchini G, Robert-Guroff M, Tartaglia J, et al. Highly attenuated HIV type 2 recombinant poxviruses, but not HIV-2 recombinant Salmonella vaccines, induce long-lasting protection in rhesus macaques. AIDS Res Hum Retroviruses 1995; 11(8): 909–20. [PubMed: 7492438]

[29]. Shata MT, Reitz MS Jr., DeVico AL, Lewis GK, Hone DM. Mucosal and systemic HIV-1 Env-specific CD8(+) T-cells develop after intragastric vaccination with a Salmonella Env DNA vaccine vector. Vaccine 2001; 20(3–4): 623–9. [PubMed: 11672930]

[30]. Vecino WH, Morin PM, Agha R, Jacobs WR Jr., Fennelly GJ. Mucosal DNA vaccination with highly attenuated Shigella is superior to attenuated Salmonella and comparable to intramuscular DNA vaccination for T cells against HIV. Immunol Lett 2002; 82(3): 197–204. [PubMed: 12036602]

[31]. Brichacek B, Lagenaur LA, Lee PP, Venzon D, Hamer DH. In vivo evaluation of safety and toxicity of a Lactobacillus jensenii producing modified cyanovirin-V in a rhesus macaque vaginal challenge model. PLoS One 2013; 8(11): e78817.

[32]. Lagenaur LA, Sanders-Beer BE, et al. Prevention of vaginal SHIV transmission in macaques by a live recombinant Lactobacillus. Mucosal Immunol 2011; 4(6): 648–57. [PubMed: 21734653]

[33]. Aldovini A, Young RA. Development of a BCG recombinant vehicle for candidate AIDS vaccines. Int Rev Immunol 1990; 7(1): 79–83. [PubMed: 2132881]

[34]. Aldovini A, Young RA. Humoral and cell-mediated immune responses to live recombinant BCG-HIV vaccines. Nature 1991; 351(6326): 479–82. [PubMed: 2046750]

[35]. Jensen K, Pena MG, Wilson RL, et al. A neonatal oral Mycobacterium tuberculosis-SlV prime/intramuscular MVA-SIV boost combination vaccine induces both SIV and Mtb-specific immune responses in infant macaques. Trials Vaccinol 2013; 2: 53–63. [PubMed: 24454591]

[36]. Jensen K, Nabi R, Van Rompay KK Jr., et al. Vaccine-elicited mucosal and systemic antibody responses are associated with reduced simian immunodeficiency viremia in infant rhesus macaques. J Virol 2016; 90(16): 7285–302. [PubMed: 27252535]

[37]. Gasper MA, Hesseling AC, Mohar I, et al. BCG vaccination induces HIV target cell activation in HIV-exposed infants in a randomized trial. J Clin Invest Insight 2017; 2(7): e91963.

[38]. Jensen K, Dela Pena-Ponce MG, Piatak M Jr., et al. Balancing trained immunity with persistent immune activation and the risk of simian immunodeficiency virus infection in infant macaques vaccinated with attenuated Mycobacterium tuberculosis or Mycobacterium bovis BCG vaccine. Clin Vaccine Immunol 2017; 24(1): pii: e00360.

[39]. Schnell MJ. Viral vectors as potential HIV-1 vaccines. FEMS Microbiol Lett 2001; 200(2): 123–9. [PubMed: 11425463]

[40]. Pegu P, Vaccari M, Gordon S, et al. Antibodies with high avidity to the gp120 envelope protein in protection from simian immunodeficiency virus SIV(mac251) acquisition in an immunization regimen that mimics the RV-144 Thai trial. J Virol 2013; 87(3): 1708–19. [PubMed: 23175374]

[41]. Price PJ, Torres-Dominguez LE, Brandmuller C, Sutter G, Lehmann MH. Modified vaccinia virus Ankara: innate immune activation and induction of cellular signalling. Vaccine 2013; 31(39): 4231–4. [PubMed: 23523404]

[42]. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009; 361(23): 2209–20. [PubMed: 19843557]

[43]. Teigler JE, Phogat S, Franchini G, Hirsch VM, Michael NL, Barouch DH. The canarypox virus vector ALVAC induces distinct cytokine responses compared to the vaccinia virus-based vectors MVA and NYVAC in rhesus monkeys. J Virol 2014; 88(3): 1809–14. [PubMed: 24257612]

[44]. Kent SJ, Dale CJ, Ranasinghe C, et al. Mucosally-administered human-simian immunodeficiency virus DNA and fowlpoxvirus-based recombinant vaccines reduce acute phase viral replication in macaques following vaginal challenge with CCR5-tropic SHIVSF162P3. Vaccine 2005; 23(42): 5009–21. [PubMed: 15985317]

[45]. Lai L, Kwa SF, Kozlowski PA, et al. SIVmac239 MVA vaccine with and without a DNA prime, similar prevention of infection by a repeated dose SIVsmE660 challenge despite different immune responses. Vaccine 2012; 30(9): 1737–45. [PubMed: 22178526]

[46]. Manrique M, Kozlowski PA, Cobo-Molinos A, et al. Resistance to infection, early and persistent suppression of simian immunodeficiency virus SIVmac251 viremia, and significant reduction of tissue viral burden after mucosal vaccination in female rhesus macaques. J Virol 2014; 88(1): 212–24. [PubMed: 24155376]

[47]. Manrique M, Kozlowski PA, Wang SW, et al. Nasal DNA-MVA SIV vaccination provides more significant protection from progression to AIDS than a similar intramuscular vaccination. Mucosal Immunol 2009; 2(6): 536–50. [PubMed: 19741603]

[48]. Munseri PJ, Kroidl A, Nilsson C, et al. Priming with a simplified intradermal HIV-1 DNA vaccine regimen followed by boosting with recombinant HIV-1 MVA vaccine is safe and immunogenic: A phase IIa randomized clinical trial. PLoS One 2015; 10(4): e0119629.

[49]. Bertley FM, Kozlowski PA, Wang SW, et al. Control of simian/human immunodeficiency virus viremia and disease progression after IL-2-augmented DNA-modified vaccinia virus Ankara nasal vaccination in nonhuman primates. J Immunol 2004; 172(6): 3745–57. [PubMed: 15004179]

[50]. Manrique M, Kozlowski PA, Cobo-Molinos A, et al. Immunogenicity of a vaccine regimen composed of simian immunodeficiency virus DNA, rMVA, and viral particles administered to female rhesus macaques via four different mucosal routes. J Virol 2013; 87(8): 4738–50. [PubMed: 23408627]

[51]. Manrique M, Kozlowski PA, Cobo-Molinos A, et al. Long-term control of simian immunodeficiency virus mac251 viremia to undetectable levels in half of infected female rhesus macaques nasally vaccinated with simian immunodeficiency virus DNA/recombinant modified vaccinia virus Ankara. J Immunol 2011; 186(6): 3581–93. [PubMed: 21317390]

[52]. Manrique M, Micewicz E, Kozlowski PA, et al. DNA-MVA vaccine protection after X4 SHIV challenge in macaques correlates with day-of-challenge antiviral $CD4^+$ cell-mediated immunity levels and postchallenge preservation of $CD4^+$ T cell memory. AIDS Res Hum Retroviruses 2008; 24(3): 505–19. [PubMed: 18373436]

[53]. Emmer KL, Wieczorek L, Tuyishime S, Molnar S, Polonis VR, Ertl HC. Antibody responses to prime-boost vaccination with an HIV-1 gp145 envelope protein and chimpanzee adenovirus vectors expressing HIV-1 gp140. AIDS 2016; 30(16): 2405–14. [PubMed: 27525550]

[54]. Cheng C, Wang L, Ko SY, et al. Combination recombinant simian or chimpanzee adenoviral vectors for vaccine development. Vaccine 2015; 33(51): 7344–51. [PubMed: 26514419]

[55]. Brocca-Cofano E, McKinnon K, Demberg T, et al. Vaccine-elicited SIV and HIV envelope-specific IgA and IgG memory B cells in rhesus macaque peripheral blood correlate with functional antibody responses and reduced viremia. Vaccine 2011; 29(17): 3310–9. [PubMed: 21382487]

[56]. Demberg T, Florese RH, Heath MJ, et al. A replication-competent adenovirus-human immunodeficiency virus (Ad-HIV) tat and Ad-HIV env priming/Tat and envelope protein boosting regimen elicits enhanced protective efficacy against simian/human immunodeficiency

virus SHIV89.6P challenge in rhesus macaques. J Virol 2007; 81(7): 3414–27. [PubMed: 17229693]

[57]. Hidajat R, Xiao P, Zhou Q, et al. Correlation of vaccine-elicited systemic and mucosal nonneutralizing antibody activities with reduced acute viremia following intrarectal simian immunodeficiency virus SIVmac251 challenge of rhesus macaques. J Virol 2009; 83(2): 791–801. [PubMed: 18971271]

[58]. Lakhashe SK, Velu V, Sciaranghella G, et al. Prime-boost vaccination with heterologous live vectors encoding SIV gag and multimeric HIV-1 gp160 protein: Efficacy against repeated mucosal R5 clade C SHIV challenges. Vaccine 2011; 29(34): 5611–22. [PubMed: 21693155]

[59]. Patterson LJ, Robert-Guroff M. Replicating adenovirus vector prime/protein boost strategies for HIV vaccine development. Expert Opin Biol Ther 2008; 8(9): 1347–63. [PubMed: 18694354]

[60]. Xiao P, Zhao J, Patterson LJ, et al. Multiple vaccine-elicited nonneutralizing antienvelope antibody activities contribute to protective efficacy by reducing both acute and chronic viremia following simian/human immunodeficiency virus SHIV89.6P challenge in rhesus macaques. J Virol 2011; 84(14): 7161–73.

[61]. Xiao P, Patterson LJ, Kuate S, et al. Replicating adenovirus-simian immunodeficiency virus (SIV) recombinant priming and envelope protein boosting elicits localized, mucosal IgA immunity in rhesus macaques correlated with delayed acquisition following a repeated low-dose rectal SIV(mac251) challenge. J Virol 2012; 86(8): 4644–57. [PubMed: 22345466]

[62]. Valentin A, McKinnon K, Li J, et al. Comparative analysis of SIV-specific cellular immune responses induced by different vaccine platforms in rhesus macaques. Clin Immunol 2014; 155(1): 91–107. [PubMed: 25229164]

[63]. Hansen SG, Ford JC, Lewis MS, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature 2011; 473(7348): 523–7. [PubMed: 21562493]

[64]. Hansen SG, Jr MP, Ventura AB, Hughes CM, et al. Immune clearance of highly pathogenic SIV infection. Nature 2013; 502(7469): 100–4. [PubMed: 24025770]

[65]. Hansen SG, Sacha JB, Hughes CM, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. Science 2013; 340(6135): e1237874.

[66]. Traina-Dorge V, Pahar B, Marx P, et al. Recombinant varicella vaccines induce neutralizing antibodies and cellular immune responses to SIV and reduce viral loads in immunized rhesus macaques. Vaccine 2010; 28(39): 6483–90. [PubMed: 20654666]

[67]. Crotty S, Lohman BL, Lu FX, Tang S, Miller CJ, Andino R. Mucosal immunization of cynomolgus macaques with two serotypes of live poliovirus vectors expressing simian immunodeficiency virus antigens: stimulation of humoral, mucosal, and cellular immunity. J Virol 1999; 73(11): 9485–95. [PubMed: 10516057]

[68]. Crotty S, Miller CJ, Lohman BL, et al. Protection against simian immunodeficiency virus vaginal challenge by using Sabin poliovirus vectors. J Virol 2001; 75(16): 7435–52. [PubMed: 11462016]

[69]. Marthas ML, Van Rompay KK, Abbott Z, et al. Partial efficacy of a VSV-SIV/MVA-SIV vaccine regimen against oral SIV challenge in infant macaques. Vaccine 2011; 29(17): 3124–37. [PubMed: 21377510]

[70]. Schell JB, Bahl K, Folta-Stogniew E, et al. Antigenic requirement for Gag in a vaccine that protects against high-dose mucosal challenge with simian immunodeficiency virus. Virology 2015; 476: 405–12. [PubMed: 25591175]

[71]. Sharpe S, Polyanskaya N, Dennis M, et al. Induction of Simian Immunodeficiency Virus (SIV)-specific CTL in rhesus macaques by vaccination with modified vaccinia virus Ankara expressing SIV transgenes: Influence of pre-existing anti-vector immunity. J Gen Virol 2001; 82(Pt 9): 2215–23. [PubMed: 11514732]

[72]. Kannanganat S, Nigam P, Velu V, et al. Preexisting vaccinia virus immunity decreases SIV-specific cellular immunity but does not diminish humoral immunity and efficacy of a DNA/MVA vaccine. J Immunol 2010; 185(12): 7262–73. [PubMed: 21076059]

[73]. Priddy FH, Brown D, Kublin J, et al. Safety and immunogenicity of a replication-incompetent adenovirus type 5 HIV-1 clade B gag/pol/nef vaccine in healthy adults. Clin Infect Dis 2008; 46(11): 1769–81. [PubMed: 18433307]

[74]. Frey SE, Lottenbach KR, Hill H, et al. A Phase I, dose-escalation trial in adults of three recombinant attenuated Salmonella Typhi vaccine vectors producing Streptococcus pneumoniae surface protein antigen PspA. Vaccine 2013; 31(42): 4874–80. [PubMed: 23916987]

[75]. Kantele A, Kantele JM, Arvilommi H, Makela PH. Active immunity is seen as a reduction in the cell response to oral live vaccine. Vaccine 1991; 9(6): 428–31. [PubMed: 1887674]

[76]. Bolton DL, Santra S, Swett-Tapia C, et al. Priming T-cell responses with recombinant measles vaccine vector in a heterologous prime-boost setting in non-human primates. Vaccine 2012; 30(41): 5991–8. [PubMed: 22732429]

[77]. Tatsis N, Lasaro MO, Lin SW, et al. Adenovirus vector-induced immune responses in nonhuman primates: responses to prime boost regimens. J Immunol 2009; 182(10): 6587–99. [PubMed: 19414814]

[78]. Sauermann U, Radaelli A, Stolte-Leeb N, et al. Vector order determines protection against pathogenic simian immunodeficiency virus infection in a triple component vaccine by balancing CD4(+) and CD8(+) T-cell responses. J Virol 2017; pii: JVI.01120–17.

[79]. Koup RA, Roederer M, Lamoreaux L, et al. Priming immunization with DNA augments immunogenicity of recombinant adenoviral vectors for both HIV-1 specific antibody and T-cell responses. PLoS One 2010; 5(2): e9015. [PubMed: 20126394]

[80]. Clarke DK, Hendry RM, Singh V, et al. Live virus vaccines based on a Vesicular Stomatitis Virus (VSV) backbone: Standardized template with key considerations for a risk/benefit assessment. Vaccine 2016; 34(51): 6597–609. [PubMed: 27395563]

[81]. Liu MA. DNA vaccines: A review. J Intern Med 2003; 253(4): 402–10. [PubMed: 12653868]

[82]. Boyer JD, Chattergoon MA, Ugen KE, et al. Enhancement of cellular immune response in HIV-1 seropositive individuals: A DNA-based trial. Clin Immunol 1999; 90(1): 100–7. [PubMed: 9884357]

[83]. Calarota S, Bratt G, Nordlund S, et al. Cellular cytotoxic response induced by DNA vaccination in HIV-1-infected patients. Lancet 1998; 351(9112): 1320–5. [PubMed: 9643795]

[84]. MacGregor RR, Boyer JD, Ugen KE, et al. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: Safety and host response. J Infect Dis 1998; 178(1): 92–100. [PubMed: 9652427]

[85]. Haigwood NL, Pierce CC, Robertson MN, et al. Protection from pathogenic SIV challenge using multigenic DNA vaccines. Immunol Lett 1999; 66(1–3): 183–8. [PubMed: 10203053]

[86]. Lu S, Arthos J, Montefiori DC, et al. Simian immunodeficiency virus DNA vaccine trial in macaques. J Virol 1996; 70(6): 3978–91. [PubMed: 8648735]

[87]. Robinson HL, Montefiori DC, Johnson RP, et al. Neutralizing antibody-independent containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations. Nat Med 1999; 5(5): 526–34. [PubMed: 10229229]

[88]. Wang SW, Kozlowski PA, Schmelz G, et al. Effective induction of simian immunodeficiency virus-specific systemic and mucosal immune responses in primates by vaccination with proviral DNA producing intact but noninfectious virions. J Virol 2000; 74(22): 10514–22. [PubMed: 11044096]

[89]. Felber BK, Valentin A, Rosati M, Bergamaschi C, Pavlakis GN. HIV DNA vaccine: stepwise improvements make a difference. Vaccines 2014; 2(2): 354–79. [PubMed: 26344623]

[90]. Iyer SS, Amara RR. DNA/MVA Vaccines for HIV/AIDS. Vaccines 2014; 2(1): 160–78. [PubMed: 26344473]

[91]. Kulkami V, Rosati M, Bear J, et al. Comparison of intradermal and intramuscular delivery followed by in vivo electroporation of SIV Env DNA in macaques. Hum Vaccin Immunother 2013; 9(10): 2081–94. [PubMed: 23811579]

[92]. Kulkarni V, Rosati M, Jalah R, et al. DNA vaccination by intradermal electroporation induces long-lasting immune responses in Rhesus macaques. J Med Primatol 2014; 43(5): 329–40. [PubMed: 24810337]

[93]. Li J, Valentin A, Kulkarni V, et al. HIV/SIV DNA vaccine combined with protein in a co-immunization protocol elicits highest humoral responses to envelope in mice and macaques. Vaccine 2013; 31(36): 3747–55. [PubMed: 23624057]

[94]. Lindsay RW, Ouellette I, Arendt HE, et al. SIV antigen-specific effects on immune responses induced by vaccination with DNA electroporation and plasmid IL-12. Vaccine 2013; 31(42): 4749–58. [PubMed: 23954384]

[95]. Muthumani K, Bagarazzi M, Conway D, et al. A Gag-Pol/Env-Rev SIV239 DNA vaccine improves CD4 counts, and reduce viral loads after pathogenic intrarectal SIV(mac)251 challenge in Rhesus macaques. Vaccine 2003; 21(7–8): 629–37. [PubMed: 12531331]

[96]. Muthumani K, Kudchodkar S, Zhang D, et al. Issues for improving multiplasmid DNA vaccines for HIV-1. Vaccine 2002; 20(15): 1999–2003. [PubMed: 11983262]

[97]. Patel V, Jalah R, Kulkarni V, et al. DNA and virus particle vaccination protects against acquisition and confers control of viremia upon heterologous simian immunodeficiency virus challenge. Proc Natl Acad Sci USA 2013; 110(8): 2975–80. [PubMed: 23359688]

[98]. Rosati M, Bergamaschi C, et al. DNA vaccination in rhesus macaques induces potent immune responses and decreases acute and chronic viremia after SIVmac251 challenge. Proc Natl Acad Sci U S A 2009; 106(37): 15831–6. [PubMed: 19717425]

[99]. Boyer JD, Robinson TM, Maciag PC, et al. DNA prime Listeria boost induces a cellular immune response to SIV antigens in the rhesus macaque model that is capable of limited suppression of SIV239 viral replication. Virology 2005; 333(1): 88–101. [PubMed: 15708595]

[100]. Kwissa M, Amara RR, Robinson HL, et al. Adjuvanting a DNA vaccine with a TLR9 ligand plus FLT3 ligand results in enhanced cellular immunity against the simian immunodeficiency virus. J Exp Med 2007; 204(11): 2733–46. [PubMed: 17954572]

[101]. Lai L, Kwa S, Kozlowski PA, et al. Prevention of infection by a granulocyte-macrophage colony-stimulating factor co-expressing DNA/modified vaccinia Ankara simian immunodeficiency virus vaccine. J Infect Dis 2011; 204(1): 164–73. [PubMed: 21628671]

[102]. Lai L, Vodros D, Kozlowski PA, et al. GM-CSF DNA: An adjuvant for higher avidity IgG, rectal IgA, and increased protection against the acute phase of a SHIV-89.6P challenge by a DNA/MVA immunodeficiency virus vaccine. Virology 2007; 369(1): 153–67. [PubMed: 17698160]

[103]. Liu J, Kjeken R, Mathiesen I, Barouch DH. Recruitment of antigen-presenting cells to the site of inoculation and augmentation of human immunodeficiency virus type 1 DNA vaccine immunogenicity by in vivo electroporation. J Virol 2008; 82(11): 5643–9. [PubMed: 18353952]

[104]. Rosati M, Valentin A, Jalah R, et al. Increased immune responses in rhesus macaques by DNA vaccination combined with electroporation. Vaccine 2008; 26(40): 5223–9. [PubMed: 18468743]

[105]. Kichaev G, Mendoza JM, Amante D, et al. Electroporation mediated DNA vaccination directly to a mucosal surface results in improved immune responses. Hum Vaccin Immunother 2013; 9(10): 2041–8. [PubMed: 23954979]

[106]. Mestecky J, Russell MW, Elson CO. Perspectives on mucosal vaccines: is mucosal tolerance a barrier? J Immunol 2007; 179(9): 5633–8. [PubMed: 17947632]

[107]. Coquet JM, Rausch L, Borst J. The importance of co-stimulation in the orchestration of T helper cell differentiation. Immunol Cell Biol 2015; 93(9): 780–8. [PubMed: 25801480]

[108]. Schmitt N, Ueno H. Regulation of human helper T cell subset differentiation by cytokines. Curr Opin Immunol 2015; 34: 130–6. [PubMed: 25879814]

[109]. Coffman RL, Sher A, Seder RA. Vaccine adjuvants: Putting innate immunity to work. Immunity 2010; 33(4): 492–503. [PubMed: 21029960]

[110]. Hirota K, Turner JE, Villa M, et al. Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. Nat Immunol 2013; 14(4): 372–9. [PubMed: 23475182]

[111]. Cao AT, Yao S, Gong B, Nurieva RI, Elson CO, Cong Y. Interleukin (IL)-21 promotes intestinal IgA response to microbiota. Mucosal Immunol 2015; 8(5): 1072–82. [PubMed: 25586558]

[112]. Dann SM, Manthey CF, Le C, et al. IL-17A promotes protective IgA responses and expression of other potential effectors against the lumen-dwelling enteric parasite Giardia. Exp Parasitol 2015; 156: 68–78. [PubMed: 26071205]

[113]. Christensen D, Mortensen R, Rosenkrands I, Dietrich J, Andersen P. Vaccine-induced Th17 cells are established as resident memory cells in the lung and promote local IgA responses. Mucosal Immunol 2017; 10(1): 260–70. [PubMed: 27049058]

[114]. Smith PD, Smythies LE, Shen R, Greenwell-Wild T, Gliozzi M, Wahl SM. Intestinal macrophages and response to microbial encroachment. Mucosal Immunol 2011; 4(1): 31–42. [PubMed: 20962772]

[115]. Moens L, Tangye SG. Cytokine-mediated regulation of plasma cell generation: IL-21 takes center stage. Front Immunol 2014; 5(article 65): 1–13. [PubMed: 24474949]

[116]. DePaolo RW, Kamdar K, Khakpour S, Sugiura Y, Wang W, Jabri B. A specific role for TLR1 in protective T(H)17 immunity during mucosal infection. J Exp Med 2012; 209(8): 1437–44. [PubMed: 22778390]

[117]. Gallorini S, Taccone M, Bonci A, et al. Sublingual immunization with a subunit influenza vaccine elicits comparable systemic immune response as intramuscular immunization, but also induces local IgA and TH17 responses. Vaccine 2014; 32(20): 2382–8. [PubMed: 24434044]

[118]. Orr MT, Beebe EA, Hudson TE, et al. Mucosal delivery switches the response to an adjuvanted tuberculosis vaccine from systemic TH1 to tissue-resident TH17 responses without impacting the protective efficacy. Vaccine 2015; 33(48): 6570–8. [PubMed: 26541135]

[119]. Douagi I, Gujer C, Sundling C, et al. Human B cell responses to TLR ligands are differentially modulated by myeloid and plasmacytoid dendritic cells. J Immunol 2009; 182(4): 1991–2001. [PubMed: 19201852]

[120]. Soloff AC, Barratt-Boyes SM. Enemy at the gates: Dendritic cells and immunity to mucosal pathogens. Cell Res 2010; 20(8): 872–85. [PubMed: 20603644]

[121]. McClure R, Massari P. TLR-dependent human mucosal epithelial cell responses to microbial pathogens. Front Immunol 2014; 5: 386–99. [PubMed: 25161655]

[122]. Sips M, Krykbaeva M, Diefenbach TJ, et al. Fc receptor-mediated phagocytosis in tissues as a potent mechanism for preventive and therapeutic HIV vaccine strategies. Mucosal Immunol 2016; 9(6): 1584–95. [PubMed: 26883728]

[123]. Dennis EA, Robinson TO, Smythies LE, Smith PD. Characterization of human blood monocytes and intestinal macrophages. Curr Protoc Immunol 2017; 118(14.3): 1–14. [PubMed: 28762487]

[124]. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: Cellular responses and interactions. Immunol Rev 2005; 206: 306–35. [PubMed: 16048557]

[125]. Herbst-Kralovetz MM, Quayle AJ, Ficarra M, et al. Quantification and comparison of toll-like receptor expression and responsiveness in primary and immortalized human female lower genital tract epithelia. Am J Reprod Immunol 2008; 59(3): 212–24. [PubMed: 18201283]

[126]. Joachim A, Bauer A, Joseph S, et al. Boosting with subtype C CN54rgp140 protein adjuvanted with glucopyranosyl lipid adjuvant after priming with HIV-DNA and HIV-MVA Is safe and enhances immune responses: a phase I trial. PLoS One 2016; 11(5): e0155702.

[127]. Kasturi SP, Kozlowski PA, Nakaya HI, et al. Adjuvanting a simian immunodeficiency virus vaccine with Toll-like receptor ligands encapsulated in nanoparticles induces persistent antibody responses and enhanced protection in TRIM5alpha restrictive macaques. J Virol 2017; 91(4): e01844.

[128]. Moody MA, Santra S, Vandergrift NA, et al. Toll-like receptor 7/8 (TLR7/8) and TLR9 agonists cooperate to enhance HIV-1 envelope antibody responses in rhesus macaques. J Virol 2014; 88(6): 3329–39. [PubMed: 24390332]

[129]. Wille-Reece U, Flynn BJ, Lore K, et al. HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in nonhuman primates. Proc Natl Acad Sci U S A 2005; 102(42): 15190–4. [PubMed: 16219698]

[130]. Wille-Reece U, Flynn BJ, Lore K, et al. Toll-like receptor agonists influence the magnitude and quality of memory T cell responses after prime-boost immunization in nonhuman primates. J Exp Med 2006; 203(5): 1249–58. [PubMed: 16636134]

[131]. Park H, Adamson L, Ha T, et al. Polyinosinic-polycytidylic acid is the most effective TLR adjuvant for SIV Gag protein-induced T cell responses in nonhuman primates. J Immunol 2013; 190(8): 4103–15. [PubMed: 23509365]

[132]. Bekeredjian-Ding I, Jego G. Toll-like receptors: Sentries in the B-cell response. Immunology 2009; 128(3): 311–23. [PubMed: 20067531]

[133]. Expression Kabelitz D. and function of Toll-like receptors in T lymphocytes. Curr Opin Immunol 2007; 19(1): 39–45. [PubMed: 17129718]

[134]. Manicassamy S, Pulendran B. Modulation of adaptive immunity with Toll-like receptors. Semin Immunol 2009; 21(4): 185–93. [PubMed: 19502082]

[135]. Gujer C, Sundling C, Seder RA, Karlsson Hedestam GB, Lore K. Human and rhesus plasmacytoid dendritic cell and B-cell responses to Toll-like receptor stimulation. Immunology 2011; 134(3): 257–69. [PubMed: 21977996]

[136]. Jesudason S, Collins MG, Rogers NM, Kireta S, Coates PT. Non human primate dendritic cells. J Leukoc Biol 2012; 91(2): 217–28. [PubMed: 22124138]

[137]. Ketloy C, Engering A, Srichairatanakul U, et al. Expression and function of Toll-like receptors on dendritic cells and other antigen presenting cells from non-human primates. Vet Immunol Immunopathol 2008; 125(1–2): 18–30. [PubMed: 18571243]

[138]. Thompson EA, Lore K. Non-human primates as a model for understanding the mechanism of action of toll-like receptor-based vaccine adjuvants. Curr Opin Immunol 2017; 47: 1–7. [PubMed: 28715767]

[139]. Kwissa M, Nakaya HI, Oluoch H, Pulendran B. Distinct TLR adjuvants differentially stimulate systemic and local innate immune responses in nonhuman primates. Blood 2012; 119(9): 2044–55. [PubMed: 22246032]

[140]. Basto AP, Leitao A. Targeting TLR2 for vaccine development. J Immunol Res 2014; 2014(article 619410): 1–22.

[141]. Veazey RS, Siddiqui A, Klein K, et al. Evaluation of mucosal adjuvants and immunization routes for the induction of systemic and mucosal humoral immune responses in macaques. Hum Vaccin Immunother 2015; 11(12): 2913–22. [PubMed: 26697975]

[142]. Buffa V, Klein K, Fischetti L, Shattock RJ. Evaluation of TLR agonists as potential mucosal adjuvants for HIV gp140 and tetanus toxoid in mice. PLoS One 2012; 7(12): e50529.

[143]. Morgan ME, Koelink PJ, Zheng B, et al. Toll-like receptor 6 stimulation promotes T-helper 1 and 17 responses in gastrointestinal-associated lymphoid tissue and modulates murine experimental colitis. Mucosal Immunol 2014; 7(5): 1266–77. [PubMed: 24670426]

[144]. Moreira AP, Cavassani KA, Ismailoglu UB, et al. The protective role of TLR6 in a mouse model of asthma is mediated by IL-23 and IL-17A. J Clin Invest 2011; 121(11): 4420–32. [PubMed: 22005301]

[145]. Zhang X, Chentoufi AA, Dasgupta G, et al. A genital tract peptide epitope vaccine targeting TLR-2 efficiently induces local and systemic CD8+ T cells and protects against herpes simplex virus type 2 challenge. Mucosal Immunol 2009; 2(2): 129–43. [PubMed: 19129756]

[146]. De Smet R, Demoor T, Verschuere S, et al. Beta-Glucan microparticles are good candidates for mucosal antigen delivery in oral vaccination. J Control Release 2013; 172(3): 671–8. [PubMed: 24041710]

[147]. Kraehenbuhl JP, Neutra MR. Epithelial M cells: differentiation and function. Annu Rev Cell Dev Biol 2000; 16: 301–32. [PubMed: 11031239]

[148]. Chabot S, Wagner JS, Farrant S, Neutra MR. TLRs regulate the gatekeeping functions of the intestinal follicle-associated epithelium. J Immunol 2006; 176(7): 4275–83. [PubMed: 16547265]

[149]. Chabot SM, Chernin TS, Shawi M, et al. TLR2 activation by proteosomes promotes uptake of particulate vaccines at mucosal surfaces. Vaccine 2007; 25(29): 5348–58. [PubMed: 17582662]

[150]. Liang Y, Hasturk H, Elliot J, et al. Toll-like receptor 2 induces mucosal homing receptor expression and IgA production by human B cells. Clin Immunol 2011; 138(1): 33–40. [PubMed: 20947433]

[151]. Lazarus NH, Kunkel EJ, Johnston B, Wilson E, Youngman KR, Butcher EC. A common mucosal chemokine (mucosae-associated epithelial chemokine/CCL28) selectively attracts IgA plasmablasts. J Immunol 2003; 170(7): 3799–805. [PubMed: 12646646]

[152]. Kunkel EJ, Kim CH, Lazarus NH, et al. CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA Ab-secreting cells. J Clin Invest 2003; 111(7): 1001–10. [PubMed: 12671049]

[153]. Hieshima K, Kawasaki Y, Hanamoto H, et al. CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. J Immunol 2004; 173(6): 3668–75. [PubMed: 15356112]

[154]. Wang S, Villablanca EJ, De Calisto J, et al. MyD88-dependent TLR½ signals educate dendritic cells with gut-specific imprinting properties. J Immunol 2011; 187(1): 141–50. [PubMed: 21646294]

[155]. Caron G, Duluc D, Fremaux I, et al. Direct stimulation of human T cells via TLR5 and TLR7/8: flagellin and R-848 up-regulate proliferation and IFN-gamma production by memory $CD4^+$ T cells. J Immunol 2005; 175(3): 1551–7. [PubMed: 16034093]

[156]. Komai-Koma M, Jones L, Ogg GS, Xu D, Liew FY. TLR2 is expressed on activated T cells as a costimulatory receptor. Proc Natl Acad Sci U S A 2004; 101(9): 3029–34. [PubMed: 14981245]

[157]. Henrick BM, Yao XD, Rosenthal KL, team Is. HIV-1 structural proteins serve as PAMPs for TLR2 heterodimers significantly Increasing Infection and innate immune activation. Front Immunol 2015; 6(article 426): 1–15. [PubMed: 25657648]

[158]. Thibault S, Tardif MR, Barat C, Tremblay MJ. TLR2 signaling renders quiescent naive and memory $CD4^+$ T cells more susceptible to productive infection with X4 and R5 HIV-type 1. J Immunol 2007; 179(7): 4357–66. [PubMed: 17878330]

[159]. Bolduc JF, Ouellet M, Hany L, Tremblay MJ. Toll-like receptor 2 ligation enhances HIV-1 replication in activated $CCR6^+$ $CD4^+$ T cells by increasing virus entry and establishing a more permissive environment to infection. J Virol 2017; 91(4): e01402–16.

[160]. Zhang SY, Herman M, Ciancanelli MJ, et al. TLR3 immunity to infection in mice and humans. Curr Opin Immunol 2013; 25(1): 19–33. [PubMed: 23290562]

[161]. Toussi DN, Massari P. Immune adjuvant effect of molecularly-defined Toll-like receptor ligands. Vaccines 2014; 2(2): 323–53. [PubMed: 26344622]

[162]. Le Bon A, Etchart N, Rossmann C, et al. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. Nat Immunol 2003; 4(10): 1009–15. [PubMed: 14502286]

[163]. Stahl-Hennig C, Eisenblatter M, Jasny E, et al. Synthetic double-stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques. PLoS Pathog 2009; 5(4): e1000373.

[164]. Ichinohe T, Ainai A, Ami Y, et al. Intranasal administration of adjuvant-combined vaccine protects monkeys from challenge with the highly pathogenic influenza A H5N1 virus. J Med Virol 2010; 82(10): 1754–61. [PubMed: 20827774]

[165]. Saito S, Ainai A, Suzuki T, et al. The effect of mucoadhesive excipient on the nasal retention time of and the antibody responses induced by an intranasal influenza vaccine. Vaccine 2016; 34(9): 1201–7. [PubMed: 26802605]

[166]. Overton ET, Goepfert PA, Cunningham P, et al. Intranasal seasonal influenza vaccine and a TLR-3 agonist, rintatolimod, induced cross-reactive IgA antibody formation against avian H5N1 and H7N9 influenza HA in humans. Vaccine 2014; 32(42): 5490–5. [PubMed: 25128802]

[167]. Aravantinou M, Frank I, Hallor M, et al. PolyICLC exerts pro-and anti-HIV effects on the DC-T cell milieu in vitro and in vivo. PLoS One 2016; 11(9): e0161730.

[168]. Vagenas P, Aravantinou M, Williams VG, et al. A tonsillar PolyICLC/AT-2 SIV therapeutic vaccine maintains low viremia following antiretroviral therapy cessation. PLoS One 2010; 5(9): e12891.

[169]. Andersen JM, Al-Khairy D, Ingalls RR. Innate immunity at the mucosal surface: role of Toll-like receptor 3 and Toll-like receptor 9 in cervical epithelial cell responses to microbial pathogens. Biol Reprod 2006; 74(5): 824–31. [PubMed: 16421230]

[170]. Veazey RS, Pilch-Cooper HA, Hope TJ, et al. Prevention of SHIV transmission by topical IFN beta treatment. Mucosal Immunol 2016; 9(6): 1528–36. [PubMed: 26838048]

[171]. Reed SG, Hsu FC, Carter D, Orr MT. The science of vaccine adjuvants: Advances in TLR4 ligand adjuvants. Curr Opin Immunol 2016; 41: 85–90. [PubMed: 27392183]

[172]. Gwinn WM, Johnson BT, Kirwan SM, et al. A comparison of non-toxin vaccine adjuvants for their ability to enhance the immunogenicity of nasally-administered anthrax recombinant protective antigen. Vaccine 2013; 31(11): 1480–9. [PubMed: 23352329]

[173]. Arias MA, Van Roey GA, Tregoning JS, et al. Glucopyranosyl Lipid Adjuvant (GLA), a synthetic TLR4 agonist, promotes potent systemic and mucosal responses to intranasal immunization with HIVgp140. PLoS One 2012; 7(7): e41144.

[174]. McKay PF, King DF, Mann JF, Barinaga G, Carter D, Shattock RJ. TLR4 and TLR7/8 adjuvant combinations generate different vaccine antigen-specific immune outcomes in minipigs when administered via the ID or IN routes. PLoS One 2016; 11(2): e0148984.

[175]. Yang J, Dai L, Yu Q, Yang Q. Histological and anatomical structure of the nasal cavity of Bama minipigs. PLoS One 2017; 12(3): e0173902.

[176]. Vijay-Kumar M, Gewirtz AT. Flagellin: key target of mucosal innate immunity. Mucosal Immunol 2009; 2(3): 197–205. [PubMed: 19242410]

[177]. Taylor DN, Treanor JJ, Strout C, et al. Induction of a potent immune response in the elderly using the TLR-5 agonist, flagellin, with a recombinant hemagglutinin influenza-flagellin fusion vaccine (VAX125, STF2.HA1 SI). Vaccine 2011; 29(31): 4897–902. [PubMed: 21596084]

[178]. Honko AN, Sriranganathan N, Lees CJ, Mizel SB. Flagellin is an effective adjuvant for immunization against lethal respiratory challenge with Yersinia pestis. Infect Immun 2006; 74(2): 1113–20. [PubMed: 16428759]

[179]. Vassilieva EV, Wang BZ, Vzorov AN, et al. Enhanced mucosal immune responses to HIV virus-like particles containing a membrane-anchored adjuvant. MBio 2011; 2(1): e00328–10.

[180]. Lee SE, Hong SH, Verma V, et al. Flagellin is a strong vaginal adjuvant of a therapeutic vaccine for genital cancer. Oncoimmunology 2016; 5(2): e1081328.

[181]. Chabot SM, Shawi M, Eaves-Pyles T, Neutra MR. Effects of flagellin on the functions of follicle-associated epithelium. J Infect Dis 2008; 198(6): 907–10. [PubMed: 18721059]

[182]. Vasilakos JP, Tomai MA. The use of Toll-like receptor 7/8 agonists as vaccine adjuvants. Expert Rev Vaccines 2013; 12(7): 809–19. [PubMed: 23885825]

[183]. McKay PF, Mann JF, Pattani A, et al. Intravaginal immunisation using a novel antigen-releasing ring device elicits robust vaccine antigen-specific systemic and mucosal humoral immune responses. J Control Release 2017; 249: 74–83. [PubMed: 28115243]

[184]. Iho S, Maeyama J, Suzuki F. CpG oligodeoxynucleotides as mucosal adjuvants. Hum Vaccin Immunother 2015; 11(3): 755–60. [PubMed: 25751765]

[185]. Wang Y, Blozis SA, Lederman M, Krieg A, Landay A, Miller CJ. Enhanced antibody responses elicited by a CpG adjuvant do not improve the protective effect of an aldrithiol-2-inactivated simian immunodeficiency virus therapeutic AIDS vaccine. Clin Vaccine Immunol 2009; 16(4): 499–505. [PubMed: 19225080]

[186]. Newsted D, Fallahi F, Golshani A, Azizi A. Advances and challenges in mucosal adjuvant technology. Vaccine 2015; 33(21): 2399–405. [PubMed: 25865473]

[187]. Bode C, Zhao G, Steinhagen F, Kinjo T, Klinman DM. CpG DNA as a vaccine adjuvant. Expert Rev Vaccines 2011; 10(4): 499–511. [PubMed: 21506647]

[188]. Abel K, Wang Y, Fritts L, et al. Deoxycytidyl-deoxyguanosine oligonucleotide classes A, B, and C induce distinct cytokine gene expression patterns in rhesus monkey peripheral blood mononuclear cells and distinct alpha interferon responses in TLR9-expressing rhesus monkey plasmacytoid dendritic cells. Clin Diagn Lab Immunol 2005; 12(5): 606–21. [PubMed: 15879022]

[189]. Vollmer J, Weeratna R, Payette P, et al. Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. Eur J Immunol 2004; 34(1): 251–62. [PubMed: 14971051]

[190]. Teleshova N, Kenney J, Jones J, et al. CpG-C immunostimulatory oligodeoxyribonucleotide activation of plasmacytoid dendritic cells in rhesus macaques to augment the activation of IFN-gamma-secreting simian immunodeficiency virus-specific T cells. J Immunol 2004; 173(3): 1647–57. [PubMed: 15265893]

[191]. Wang Y, Abel K, Lantz K, Krieg AM, McChesney MB, Miller CJ. The Toll-like receptor 7 (TLR7) agonist, imiquimod, and the TLR9 agonist, CpG ODN, induce antiviral cytokines and chemokines but do not prevent vaginal transmission of simian immunodeficiency virus when applied intravaginally to rhesus macaques. J Virol 2005; 79(22): 14355–70. [PubMed: 16254370]

[192]. Vagenas P, Williams VG, Piatak M Jr., et al. Tonsillar application of AT-2 SIV affords partial protection against rectal challenge with SIVmac239. J Acquir Immune Defic Syndr 2009; 52(4): 433–42. [PubMed: 19779309]

[193]. Mansson A, Bachar O, Adner M, Cardell LO. Nasal CpG oligodeoxynucleotide administration induces a local inflammatory response in nonallergic individuals. Allergy 2009; 64(9): 1292–300. [PubMed: 19243360]

[194]. Barouch DH, Craiu A, Kuroda MJ, et al. Augmentation of immune responses to HIV-1 and simian immunodeficiency virus DNA vaccines by IL-2/Ig plasmid administration in rhesus monkeys. Proc Natl Acad Sci U S A 2000; 97(8): 4192–7. [PubMed: 10759543]

[195]. Barouch DH, Santra S, Schmitz JE, et al. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science 2000; 290(5491): 486–92. [PubMed: 11039923]

[196]. Demberg T, Boyer JD, Malkevich N, et al. Sequential priming with simian immunodeficiency virus (SIV) DNA vaccines, with or without encoded cytokines, and a replicating adenovirus-SIV recombinant followed by protein boosting does not control a pathogenic SIVmac251 mucosal challenge. J Virol 2008; 82(21): 10911–21. [PubMed: 18753198]

[197]. Hirao LA, Wu L, Khan AS, et al. Combined effects of IL-12 and electroporation enhances the potency of DNA vaccination in macaques. Vaccine 2008; 26(25): 3112–20. [PubMed: 18430495]

[198]. Jalah R, Patel V, Kulkarni V, et al. IL-12 DNA as molecular vaccine adjuvant increases the cytotoxic T cell responses and breadth of humoral immune responses in SIV DNA vaccinated macaques. Hum Vaccin Immunother 2012; 8(11): 1620–9. [PubMed: 22894956]

[199]. Winstone N, Wilson AJ, Morrow G, et al. Enhanced control of pathogenic Simian immunodeficiency virus SIVmac239 replication in macaques immunized with an interleukin-12 plasmid and a DNA prime-viral vector boost vaccine regimen. J Virol 2011; 85(18): 9578–87. [PubMed: 21734035]

[200]. Boyer JD, Robinson TM, Kutzler MA, et al. Protection against simian/human immunodeficiency virus (SHIV) 89.6P in macaques after coimmunization with SHIV antigen and IL-15 plasmid. Proc Natl Acad Sci U S A 2007; 104(47): 18648–53. [PubMed: 18000037]

[201]. Dubie RA, Maksaereekul S, Shacklett BL, et al. Co-immunization with IL-15 enhances cellular immune responses induced by a vif-deleted simian immunodeficiency virus proviral DNA vaccine and confers partial protection against vaginal challenge with SIVmac251. Virology 2009; 386(1): 109–21. [PubMed: 19193388]

[202]. Sui Y, Zhu Q, Gagnon S, et al. Innate and adaptive immune correlates of vaccine and adjuvant-induced control of mucosal transmission of SIV in macaques. Proc Natl Acad Sci U S A 2010; 107(21): 9843–8. [PubMed: 20457926]

[203]. Yin J, Dai A, Laddy DJ, et al. High dose of plasmid IL-15 inhibits immune responses in an influenza non-human primates immunogenicity model. Virology 2009; 393(1): 49–55. [PubMed: 19683780]

[204]. Van Roey GA, Arias MA, Tregoning JS, Rowe G, Shattock RJ. Thymic stromal lymphopoietin (TSLP) acts as a potent mucosal adjuvant for HIV-1 gp140 vaccination in mice. Eur J Immunol 2012; 42(2): 353–63. [PubMed: 22057556]

[205]. Egan MA, Chong SY, Hagen M, et al. A comparative evaluation of nasal and parenteral vaccine adjuvants to elicit systemic and mucosal HIV-1 peptide-specific humoral immune responses in cynomolgus macaques. Vaccine 2004; 22(27–28): 3774–88. [PubMed: 15315859]

[206]. Bradney CP, Sempowski GD, Liao HX, Haynes BF, Staats HF. Cytokines as adjuvants for the induction of anti-human immunodeficiency virus peptide immunoglobulin G (IgG) and IgA antibodies in serum and mucosal secretions after nasal immunization. J Virol 2002; 76(2): 517–24. [PubMed: 11752142]

[207]. Schell JB, Bahl K, Rose NF, et al. Viral vectored granulocyte-macrophage colony stimulating factor inhibits vaccine protection in an SIV challenge model: protection correlates with neutralizing antibody. Vaccine 2012; 30(28): 4233–9. [PubMed: 22537983]

[208]. Kannanganat S, Wyatt LS, Gangadhara S, et al. High doses of GM-CSF inhibit antibody responses in rectal secretions and diminish modified vaccinia Ankara/Simian immunodeficiency

virus vaccine protection in TRIM5alpha-restrictive macaques. J Immunol 2016; 197(9): 3586–96. [PubMed: 27683750]

[209]. Rainone V, Dubois G, Temchura V, et al. CCL28 induces mucosal homing of HIV-1-specific IgA-secreting plasma cells in mice immunized with HIV-1 virus-like particles. PLoS One 2011; 6(10): e26979.

[210]. Hu K, Luo S, Tong L, et al. CCL19 and CCL28 augment mucosal and systemic immune responses to HIV-1 gp140 by mobilizing responsive immunocytes into secondary lymph nodes and mucosal tissue. J Immunol 2013; 191(4): 1935–47. [PubMed: 23858028]

[211]. Cha HR, Ko HJ, Kim ED, et al. Mucosa-associated epithelial chemokine/CCL28 expression in the uterus attracts CCR10+ IgA plasma cells following mucosal vaccination via estrogen control. J Immunol 2011; 187(6): 3044–52. [PubMed: 21832166]

[212]. Kutzler MA, Wise MC, Hutnick NA, et al. Chemokine-adjuvanted electroporated DNA vaccine induces substantial protection from simian immunodeficiency virus vaginal challenge. Mucosal Immunol 2016; 9(1): 13–23. [PubMed: 25943275]

[213]. Clements JD, Freytag LC. Parenteral vaccination can be an effective means of inducing protective mucosal responses. Clin Vaccine Immunol 2016; 23(6): 438–41. [PubMed: 27122485]

[214]. Lycke N Recent progress in mucosal vaccine development: potential and limitations. Nat Rev Immunol 2012; 12(8): 592–605. [PubMed: 22828912]

[215]. Lebens M, Terrinoni M, Karlsson SL, et al. Construction and preclinical evaluation of mmCT, a novel mutant cholera toxin adjuvant that can be efficiently produced in genetically manipulated Vibrio cholerae. Vaccine 2016; 34(18): 2121–8. [PubMed: 26973069]

[216]. Norton EB, Lawson LB, Freytag LC, Clements JD. Characterization of a mutant Escherichia coli heat-labile toxin, LT(R192G/L211A), as a safe and effective oral adjuvant. Clin Vaccine Immunol 2011; 18(4): 546–51. [PubMed: 21288994]

[217]. Marks E, Helgeby A, Andersson JO, Schon K, Lycke NY. CD4(+) T-cell immunity in the female genital tract is critically dependent on local mucosal immunization. Eur J Immunol 2011; 41(9): 2642–53. [PubMed: 21681740]

[218]. Larena M, Holmgren J, Lebens M, Terrinoni M, Lundgren A. Cholera toxin, and the related nontoxic adjuvants mmCT and dmLT, promote human Th17 responses via cyclic AMP-protein kinase A and inflammasome-dependent IL-1 signaling. J Immunol 2015; 194(8): 3829–39. [PubMed: 25786687]

[219]. van Ginkel FW, Jackson RJ, Yoshino N, et al. Enterotoxin-based mucosal adjuvants alter antigen trafficking and induce inflammatory responses in the nasal tract. Infect Immun 2005; 73(10): 6892–902. [PubMed: 16177369]

[220]. Lewis DJ, Huo Z, Barnett S, et al. Transient facial nerve paralysis (Bell's palsy) following intranasal delivery of a genetically detoxified mutant of Escherichia coli heat labile toxin. PLoS One 2009; 4(9): e6999. [PubMed: 19756141]

[221]. Lundgren A, Bourgeois L, Carlin N, et al. Safety and immunogenicity of an improved oral inactivated multivalent enterotoxigenic Escherichia coli (ETEC) vaccine administered alone and together with dmLT adjuvant in a double-blind, randomized, placebo-controlled Phase I study. Vaccine 2014; 32(52): 7077–84. [PubMed: 25444830]

[222]. Belyakov IM, Hel Z, Kelsall B, et al. Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. Nat Med 2001; 7(12): 1320–6. [PubMed: 11726972]

[223]. Fuller DH, Rajakumar P, Che JW, et al. Therapeutic DNA vaccine induces broad T cell responses in the gut and sustained protection from viral rebound and AIDS in SIV-infected rhesus macaques. PLoS One 2012; 7(3): e33715.

[224]. Frederick DR, Goggins JA, Sabbagh LM, Freytag LC, Clements JD, McLachlan JB. Adjuvant selection regulates gut migration and phenotypic diversity of antigen-specific CD4+ T cells following parenteral immunization. Mucosal Immunol 2018; 11(2): 549–61. [PubMed: 28792004]

[225]. Norton EB, Bauer DL, Weldon WC, Oberste MS, Lawson LB, Clements JD. The novel adjuvant dmLT promotes dose sparing, mucosal immunity and longevity of antibody responses to the

inactivated polio vaccine in a murine model. Vaccine 2015; 33(16): 1909–15. [PubMed: 25765967]

[226]. Lawson LB, Norton EB, Clements JD. Defending the mucosa: Adjuvant and carrier formulations for mucosal immunity. Curr Opin Immunol 2011; 23(3): 414–20. [PubMed: 21511452]

[227]. Smith A, Perelman M, Hinchcliffe M. Chitosan: A promising safe and immune-enhancing adjuvant for intranasal vaccines. Hum Vaccin Immunother 2014; 10(3): 797–807. [PubMed: 24346613]

[228]. Deli MA. Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. Biochim Biophys Acta 2009; 1788(4): 892–910. [PubMed: 18983815]

[229]. McNeela EA, Jabbal-Gill I, Illum L, Pizza M, et al. Intranasal immunization with genetically detoxified diphtheria toxin induces T cell responses in humans: enhancement of Th2 responses and toxin-neutralizing antibodies by formulation with chitosan. Vaccine 2004; 22(8): 909–14. [PubMed: 15161067]

[230]. Sigsgaard T, Thorne PS, Schlunssen V, et al. The change in nasal inflammatory markers after intranasal challenges with particulate chitin and lipopolysaccharide: a randomized, double-blind, placebocontrolled, crossover study with a positive control. Int Forum Allergy Rhinol 2015; 5(8): 716–23. [PubMed: 25851155]

[231]. Cosgrove CA, Lacey CJ, Cope AV, et al. Comparative Immunogenicity of HIV-1 gp140 vaccine delivered by parenteral, and mucosal routes in female volunteers; MUCOVAC2, a randomized two centre study. PLoS One 2016; 11(5): e0152038.

[232]. Hayashi M, Aoshi T, Ozasa K, et al. RNA is an adjuvanticity mediator for the lipid-based mucosal adjuvant, Endocine. Sci Rep 2016; 6: e29165.

[233]. Brekke K, Lind A, Holm-Hansen C, et al. Intranasal administration of a therapeutic HIV vaccine (Vacc-4x) induces dose-dependent systemic and mucosal immune responses in a randomized controlled trial. PLoS One 2014; 9(11): e112556.

[234]. Buonaguro L, Devito C, Tornesello ML, et al. DNA-VLP prime-boost intra-nasal immunization induces cellular and humoral anti-HIV-1 systemic and mucosal immunity with cross-clade neutralizing activity. Vaccine 2007; 25(32): 5968–77. [PubMed: 17629365]

[235]. Buonaguro L, Tagliamonte M, Visciano ML, et al. Immunogenicity of HIV virus-like particles in rhesus macaques by intranasal administration. Clin Vaccine Immunol 2012; 19(6): 970–3. [PubMed: 22461530]

[236]. Quinn KM, Yamamoto A, Costa A, et al. Coadministration of polyinosinic: Polycytidylic acid and immunostimulatory complexes modifies antigen processing in dendritic cell subsets and enhances HIV gag-specific T cell immunity. J Immunol 2013; 191(10): 5085–96. [PubMed: 24089189]

[237]. Helgeby A, Robson NC, Donachie AM, et al. The combined CTA1-DD/ISCOM adjuvant vector promotes priming of mucosal and systemic immunity to incorporated antigens by specific targeting of B cells. J Immunol 2006; 176(6): 3697–706. [PubMed: 16517738]

[238]. Eliasson DG, Helgeby A, Schon K, et al. A novel non-toxic combined CTA1-DD and ISCOMS adjuvant vector for effective mucosal immunization against influenza virus. Vaccine 2011; 29(23): 3951–61. [PubMed: 21481325]

[239]. McEntee C, Lavelle EC, O'Hagan DT. Antigen delivery systems I: Nonliving microparticles, liposomes, and immune-stimulating complexes (ISCOMs) In: Mestecky J, Strober W, Russell MW, Kelsall BL, Cheroutre H, Lambrecht BN, editors. Mucosal Immunology. 4th ed Boston: Elsevier; 2015 p. 1211–31.

[240]. Rodriguez-Garcia M, Patel MV, Wira CR. Innate and adaptive anti-HIV immune responses in the female reproductive tract. J Reprod Immunol 2013; 97(1): 74–84. [PubMed: 23432874]

[241]. Mukherjee S, Hooper LV. Antimicrobial defense of the intestine. Immunity 2015; 42(1): 28–39. [PubMed: 25607457]

[242]. Ghosh M Secreted mucosal antimicrobials in the female reproductive tract that are important to consider for HIV prevention. Am J Reprod Immunol 2014; 71(6): 575–88. [PubMed: 24754244]

[243]. Zhang P, Summer WR, Bagby GJ, Nelson S. Innate immunity and pulmonary host defense. Immunol Rev 2000; 173: 39–51. [PubMed: 10719666]

[244]. Akhtar M, Qadri F, Bhuiyan TR, et al. Kinetics of antibody-secreting cell and fecal IgA responses after oral cholera vaccination in different age groups in a cholera endemic country. Vaccine 2017; 35(2): 321–8. [PubMed: 27916412]

[245]. Levine MM, Ferreccio C, Abrego P, Martin OS, Ortiz E, Cryz S. Duration of efficacy of Ty21a, attenuated Salmonella typhi live oral vaccine. Vaccine 1999; 17(Suppl 2): S22–7. [PubMed: 10506405]

[246]. Maroni A, Moutaharrik S, Zema L, Gazzaniga A. Enteric coatings for colonic drug delivery: State of the art. Expert Opin Drug Deliv 2017; 14(9): 1027–9. [PubMed: 28749188]

[247]. Mercier GT, Nehete PN, Passeri MF, et al. Oral immunization of rhesus macaques with adenoviral HIV vaccines using enteric-coated capsules. Vaccine 2007; 25(52): 8687–701. [PubMed: 18063450]

[248]. Zhu Q, Talton J, Zhang G, et al. Large intestine-targeted, nanoparticle-releasing oral vaccine to control genitorectal viral infection. Nat Med 2012; 18(8): 1291–6. [PubMed: 22797811]

[249]. Hara H, Ono F, Nakamura S, et al. An oral abeta vaccine using a recombinant adeno-ssociated virus vector in aged monkeys: Reduction in plaque amyloid and increase in abeta oligomers. J Alzheimers Dis 2016; 54(3): 1047–59. [PubMed: 27567868]

[250]. Mabbott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A. Microfold (M) cells: Important immunosurveillance posts in the intestinal epithelium. Mucosal Immunol 2013; 6(4): 666–77. [PubMed: 23695511]

[251]. McNeela EA, Lavelle EC. Recent advances in microparticle and nanoparticle delivery vehicles for mucosal vaccination. Curr Top Microbiol Immunol 2012; 354: 75–99. [PubMed: 21904984]

[252]. Ogasawara N, Kojima T, Go M, et al. Epithelial barrier and antigen uptake in lymphoepithelium of human adenoids. Acta Otolaryngol 2011; 131(2): 116–23. [PubMed: 21062118]

[253]. Ensign LM, Cone R, Hanes J. Nanoparticle-based drug delivery to the vagina: A review. J Control Release 2014; 190: 500–14. [PubMed: 24830303]

[254]. Mohideen M, Quijano E, Song E, et al. Degradable bioadhesive nanoparticles for prolonged intravaginal delivery and retention of elvitegravir. Biomaterials 2017; 144: 144–54. [PubMed: 28829952]

[255]. Howe SE, Konjufca VH. Protein-coated nanoparticles are internalized by the epithelial cells of the female reproductive tract and induce systemic and mucosal immune responses. PLoS One 2014; 9(12): e114601.

[256]. Kasturi SP, Skountzou I, Albrecht RA, et al. Programming the magnitude and persistence of antibody responses with innate immunity. Nature 2011; 470(7335): 543–7. [PubMed: 21350488]

[257]. Singh M, Chesko J, Kazzaz J, et al. Adsorption of a novel recombinant glycoprotein from HIV (Env gp120dV2 SF162) to anionic PLG microparticles retains the structural integrity of the protein, whereas encapsulation in PLG microparticles does not. Pharm Res 2004; 21(12): 2148–52. [PubMed: 15648244]

[258]. Lambert JS, Keefer M, Mulligan MJ, et al. A Phase I safety and immunogenicity trial of UBI microparticulate monovalent HIV-1 MN oral peptide immunogen with parenteral boost in HIV-1 seronegative human subjects. Vaccine 2001; 19(23–24): 3033–42. [PubMed: 11311997]

[259]. Singh M, Kazzaz J, Ugozzoli M, Malyala P, Chesko J, O'Hagan DT. Polylactide-co-glycolide microparticles with surface adsorbed antigens as vaccine delivery systems. Curr Drug Deliv 2006; 3(1): 115–20. [PubMed: 16472100]

[260]. Bernasconi V, Norling K, Bally M, Hook F, Lycke NY. Mucosal vaccine development based on liposome technology. J Immunol Res 2016; 2016: 5482087.

[261]. Childers NK, Zhang SS, Michalek SM. Oral immunization of humans with dehydrated liposomes containing Streptococcus mutans glucosyltransferase induces salivary immunoglobulin A2 antibody responses. Oral Microbiol Immunol 1994; 9(3): 146–53. [PubMed: 7936720]

[262]. Nguyen HH, Boyaka PN, Moldoveanu Z, et al. Influenza virus-infected epithelial cells present viral antigens to antigen-specific CD8+ cytotoxic T lymphocytes. J Virol 1998; 72(5): 4534–6. [PubMed: 9557755]

[263]. Hatano R, Yamada K, Iwamoto T, et al. Antigen presentation by small intestinal epithelial cells uniquely enhances IFN-gamma secretion from $CD4^+$ intestinal intraepithelial lymphocytes. Biochem Biophys Res Commun 2013; 435(4): 592–6. [PubMed: 23684621]

[264]. Ochiel DO, Rossoll RM, Schaefer TM, Wira CR. Effect of oestradiol and pathogen-associated molecular patterns on class II-mediated antigen presentation and immunomodulatory molecule expression in the mouse female reproductive tract. Immunology 2012; 135(1): 51–62. [PubMed: 22043860]

[265]. Morelli AB, Becher D, Koernig S, Silva A, Drane D, Maraskovsky E. ISCOMATRIX: A novel adjuvant for use in prophylactic and therapeutic vaccines against infectious diseases. J Med Microbiol 2012; 61(Pt 7): 935–43. [PubMed: 22442293]

[266]. Lycke N, Bemark M. Mucosal adjuvants and long-term memory development with special focus on CTA1-DD and other ADP-ribosylating toxins. Mucosal Immunol 2010; 3(6): 556–66. [PubMed: 20844480]

[267]. Furrie E, Smith RE, Turner MW, Strobel S, Mowat AM. Induction of local innate immune responses and modulation of antigen uptake as mechanisms underlying the mucosal adjuvant properties of immune stimulating complexes (ISCOMS). Vaccine 2002; 20(17–18): 2254–62. [PubMed: 12009281]

[268]. Pahar B, Cantu MA, Zhao W, et al. Single epitope mucosal vaccine delivered via immuno-stimulating complexes induces low level of immunity against simian-HIV. Vaccine 2006; 24(47–48): 6839–49. [PubMed: 17050045]

[269]. Wee JL, Scheerlinck JP, Snibson KJ, et al. Pulmonary delivery of ISCOMATRIX influenza vaccine induces both systemic and mucosal immunity with antigen dose sparing. Mucosal Immunol 2008; 1(6): 489–96. [PubMed: 19079216]

[270]. Cusi MG. Applications of influenza virosomes as a delivery system. Hum Vaccin 2006; 2(1): 1–7. [PubMed: 17012895]

[271]. Moser C, Muller M, Kaeser MD, Weydemann U, Amacker M. Influenza virosomes as vaccine adjuvant and carrier system. Expert Rev Vaccines 2013; 12(7): 779–91. [PubMed: 23885823]

[272]. Garcia-Sastre A Influenza virus receptor specificity: Disease and transmission. Am J Pathol 2010; 176(4): 1584–5. [PubMed: 20203283]

[273]. Gargett T, Grubor-Bauk B, Miller D, et al. Increase in DNA vaccine efficacy by virosome delivery and co-expression of a cytolytic protein. Clin Transl Immunology 2014; 3(6): e18. [PubMed: 25505966]

[274]. Blom RAM, Amacker M, van Dijk RM, et al. Pulmonary delivery of virosome-bound antigen enhances antigen-specific CD4(+) T cell proliferation compared to liposome-bound or soluble antigen. Front Immunol 2017; 8(article 359): 1–17. [PubMed: 28149297]

[275]. Pedersen GK, Ebensen T, Gjeraker IH, et al. Evaluation of the sublingual route for administration of influenza H5N1 virosomes in combination with the bacterial second messenger c-di-GMP. PLoS One 2011; 6(11): e26973.

[276]. De Bernardis F, Arancia S, Sandini S, Graziani S, Norelli S. Studies of Immune Responses in Candida vaginitis. Pathogens 2015; 4(4): 697–707. [PubMed: 26473934]

[277]. Koopman G, Bogers WM, van Gils M, et al. Comparison of intranasal with targeted lymph node immunization using PR8-Flu ISCOM adjuvanted HIV antigens in macaques. J Med Virol 2007; 79(5): 474–82. [PubMed: 17385685]

[278]. Zhou M, Ruprecht RM. Are anti-HIV IgAs good guys or bad guys? Retrovirology 2014; 11(article 109): 1–11. [PubMed: 24383984]

[279]. Bomsel M, Tudor D, Drillet AS, et al. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. Immunity 2011; 34(2): 269–80. [PubMed: 21315623]

[280]. Leroux-Roels G, Maes C, Clement F, et al. Randomized Phase I: Safety, Immunogenicity and Mucosal Antiviral Activity in Young Healthy Women Vaccinated with HIV-1 Gp41 P1 Peptide on Virosomes. PLoS One 2013; 8(2): e55438.

[281]. Berger CT, Greiff V, Mehling M, et al. Influenza vaccine response profiles are affected by vaccine preparation and preexisting immunity, but not HIV infection. Hum Vaccin Immunother 2015; 11(2): 391–6. [PubMed: 25692740]

[282]. Cech PG, Aebi T, Abdallah MS, et al. Virosome-formulated Plasmodium falciparum AMA-1 & CSP derived peptides as malaria vaccine: randomized phase 1b trial in semi-immune adults & children. PLoS One 2011; 6(7): e22273.

[283]. Chappuis F, Farinelli T, Deckx H, et al. Immunogenicity and estimation of antibody persistence following vaccination with an inactivated virosomal hepatitis A vaccine in adults: A 20-year follow-up study. Vaccine 2017; 35(10): 1448–54. [PubMed: 28190741]

[284]. Grimaldi N, Andrade F, Segovia N, et al. Lipid-based nanovesicles for nanomedicine. Chem Soc Rev 2016; 45(23): 6520–45. [PubMed: 27722570]

[285]. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Biol 2010; 8(article 129): 1–10. [PubMed: 20051105]

[286]. Loehr BI, Rankin R, Pontarollo R, et al. Suppository-mediated DNA immunization induces mucosal immunity against bovine herpesvirus-1 in cattle. Virology 2001; 289(2): 327–33. [PubMed: 11689054]

[287]. Eriksson K, Quiding-Jarbrink M, Osek J, et al. Specific-antibody-secreting cells in the rectums and genital tracts of nonhuman primates following vaccination. Infect Immun 1998; 66(12): 5889–96. [PubMed: 9826370]

[288]. Eriksson K, Quiding-Jarbrink M, Osek J, et al. Anatomic segmentation of the intestinal immune response in nonhuman primates: differential distribution of B cells after oral and rectal immunizations to sites defined by their source of vascularization. Infect Immun 1999; 67(11): 6210–2. [PubMed: 10531293]

[289]. Kozlowski PA, Cu-Uvin S, Neutra MR, Flanigan TP. Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. Infect Immun 1997; 65(4): 1387–94. [PubMed: 9119478]

[290]. Kozlowski PA, Williams SB, Lynch RM, et al. Differential induction of mucosal and systemic antibody responses in women after nasal, rectal, or vaginal immunization: influence of the menstrual cycle. J Immunol 2002; 169(1): 566–74. [PubMed: 12077289]

[291]. Wassen L, Schon K, Holmgren J, Jertborn M, Lycke N. Local intravaginal vaccination of the female genital tract. Scand J Immunol 1996; 44(4): 408–14. [PubMed: 8845036]

[292]. Rudin A, Riise GC, Holmgren J. Antibody responses in the lower respiratory tract and male urogenital tract in humans after nasal and oral vaccination with cholera toxin B subunit. Infect Immun 1999; 67(6): 2884–90. [PubMed: 10338495]

[293]. Demberg T, Robert-Guroff M. Mucosal immunity and protection against HIV/SIV infection: strategies and challenges for vaccine design. Int Rev Immunol 2009; 28(1): 20–48. [PubMed: 19241252]

[294]. McMichael AJ. HIV vaccines. Annu Rev Immunol 2006; 24: 227–55. [PubMed: 16551249]

[295]. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 2012; 366(14): 1275–86. [PubMed: 22475592]

[296]. Ruiz MJ, Salido J, Abusamra L, et al. Evaluation of Different Parameters of Humoral and Cellular Immune Responses in HIV Serodiscordant Heterosexual Couples: Humoral Response Potentially Implicated in Modulating Transmission Rates. EBioMedicine 2017; 26: 25–37. [PubMed: 29129698]

[297]. Fenizia C, Rossignol JF, Clerici M, Biasin M. Genetic and immune determinants of immune activation in HIV-exposed seronegative individuals and their role in protection against HIV infection. Infect Genet Evol 2017; pii: S1567–1348(17)30439–2.

[298]. Hirbod T, Kong X, Kigozi G, et al. HIV acquisition is associated with increased antimicrobial peptides and reduced HIV neutralizing IgA in the foreskin prepuce of uncircumcised men. PLoS Pathog 2014; 10(10): e1004416.

[299]. Pantophlet R, Burton DR. GP120: Target for neutralizing HIV-1 antibodies. Annu Rev Immunol 2006; 24: 739–69. [PubMed: 16551265]

[300]. Hendricks EE, Ludlage E, Bussell S, George K, Wegner FH, Mansfield KG. Wasting syndrome and disruption of the somatotropic axis in simian immunodeficiency virus-infected macaques with Mycobacterium avium complex infection. J Infect Dis 2004; 190(12): 2187–94. [PubMed: 15551219]

[301]. Letvin NL. Progress toward an HIV vaccine. Annu Rev Med 2005; 56: 213–23. [PubMed: 15660510]

[302]. Gottardo R, Bailer RT, Korber BT, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. PLoS ONE 2013; 8(9): e75665.

[303]. Zolla-Pazner S, deCamp AC, Cardozo T, et al. Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. PLoS ONE 2013; 8(1): e53629.

[304]. Beyrer C, Artenstein AW, Rugpao S, et al. Epidemiologic and biologic characterization of a cohort of human immunodeficiency virus type 1 highly exposed, persistently seronegative female sex workers in northern Thailand. Chiang Mai HEPS Working Group. J Infect Dis 1999; 179(1): 59–67. [PubMed: 9841823]

[305]. Plummer FA, Ball TB, Kimani J, Fowke KR. Resistance to HIV-1 infection among highly exposed sex workers in Nairobi: what mediates protection and why does it develop? Immunol Lett 1999; 66(1–3): 27–34. [PubMed: 10203031]

[306]. Clerici M, Salvi A, Trabattoni D, et al. A role for mucosal immunity in resistance to HIV infection. Immunol Lett 1999; 66(1–3): 21–5. [PubMed: 10203030]

[307]. Clerici M, Barassi C, Devito C, et al. Serum IgA of HIV-exposed uninfected individuals inhibit HIV through recognition of a region within the alpha-helix of gp41. AIDS 2002; 16(13): 1731–41. [PubMed: 12218383]

[308]. Lo Caputo S, Trabattoni D, Vichi F, et al. Mucosal and systemic HIV-1-specific immunity in HIV-1-exposed but uninfected heterosexual men. Aids 2003; 17(4): 531–9. [PubMed: 12598773]

[309]. Wang SW, Bertley FM, Kozlowski PA, et al. An SHIV DNA/MVA rectal vaccination in macaques provides systemic and mucosal virus-specific responses and protection against AIDS. AIDS Res Hum Retroviruses 2004; 20(8): 846–59. [PubMed: 15320989]

[310]. Stahl-Hennig C, Kuate S, Franz M, et al. Atraumatic oral spray immunization with replication-deficient viral vector vaccines. J Virol 2007; 81(23): 13180–90. [PubMed: 17898066]

[311]. Feinberg MB, Moore JP. AIDS vaccine models: Challenging challenge viruses. Nat Med 2002; 8(3): 207–10. [PubMed: 11875482]

[312]. Belyakov IM, Kuznetsov VA, Kelsall B, et al. Impact of vaccine-induced mucosal high-avidity CD8+ CTLs in delay of AIDS viral dissemination from mucosa. Blood 2006; 107(8): 3258–64. [PubMed: 16373659]

[313]. Chin'ombe N, Ruhanya V. Recombinant Salmonella bacteria vectoring HIV/AIDS vaccines. Open Virol J 2013; 7: 121–6. [PubMed: 24478808]

[314]. Ambrose Z, Larsen K, Thompson J, et al. Evidence for early local viral replication and local production of antiviral immunity upon mucosal simian-human immunodeficiency virus SHIV (89.6) infection in Macaca nemestrina. J Virol 2001; 75(18): 8589–96. [PubMed: 11507204]

[315]. Genesca M, McChesney MB, Miller CJ. Antiviral CD8+ T cells in the genital tract control viral replication and delay progression to AIDS after vaginal SIV challenge in rhesus macaques immunized with virulence attenuated SHIV 89.6. J Intern Med 2009; 265(1): 67–77. [PubMed: 19093961]

[316]. Li Q, Zeng M, Duan L, et al. Live simian immunodeficiency virus vaccine correlate of protection: local antibody production and concentration on the path of virus entry. J Immunol 2014; 193(6): 3113–25. [PubMed: 25135832]

[317]. Barnett SW, Srivastava IK, Kan E, et al. Protection of macaques against vaginal SHIV challenge by systemic or mucosal and systemic vaccinations with HIV-envelope. AIDS 2008; 22(3): 339–48. [PubMed: 18195560]

[318]. Alpert MD, Harvey JD, Lauer WA, et al. ADCC develops over time during persistent infection with live-attenuated SIV and is associated with complete protection against SIV(mac)251 challenge. PLoS Pathogens 2012; 8(8): e1002890.

[319]. Stevceva L, Alvarez X, Lackner AA, et al. Both mucosal and systemic routes of immunization with the live, attenuated NYVAC/simian immunodeficiency virus SIV(gpe) recombinant vaccine result in gag-specific CD8(+) T-cell responses in mucosal tissues of macaques. J Virol 2002; 76(22): 11659–76. [PubMed: 12388726]

[320]. Cuburu N, Graham BS, Buck CB, et al. Intravaginal immunization with HPV vectors induces tissue-resident CD8+ T cell responses. J Clin Invest 2012; 122(12): 4606–20. [PubMed: 23143305]

[321]. Belyakov IM, Ahlers JD. Mucosal immunity and HIV-1 infection: Applications for mucosal AIDS vaccine development. Curr Top Microbiol Immunol 2012; 354: 157–79. [PubMed: 21203884]

[322]. Vajdy M, Gardner J, Neidleman J, et al. Human immunodeficiency virus type 1 Gag-specific vaginal immunity and protection after local immunizations with sindbis virus-based replicon particles. J Infect Dis 2001; 184(12): 1613–6. [PubMed: 11740739]

[323]. Gupta S, Janani R, Bin Q, et al. Characterization of human immunodeficiency virus Gag-specific gamma interferon-expressing cells following protective mucosal immunization with alphavirus replicon particles. J Virol 2005; 79(11): 7135–45. [PubMed: 15890953]

[324]. Schautteet K, De Clercq E, Jonsson Y, et al. Protection of pigs against genital Chlamydia trachomatis challenge by parenteral or mucosal DNA immunization. Vaccine 2012; 30(18): 2869–81. [PubMed: 22387629]

[325]. Mall AS, Habte H, Mthembu Y, Peacocke J, de Beer C. Mucus and Mucins: do they have a role in the inhibition of the human immunodeficiency virus? Virol J 2017; 14(1): e192.

[326]. Carias AM, McCoombe S, McRaven M, et al. Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. J Virol 2013; 87(21): 11388–400. [PubMed: 23966398]

[327]. King BF. The permeability of nonhuman primate vaginal epithelium: a freeze-fracture and tracer-perfusion study. J Ultrastruct Res 1983; 83(1): 99–110. [PubMed: 6304332]

[328]. Parr MB, Parr EL. Antigen recognition in the female reproductive tract: I. Uptake of intraluminal protein tracers in the mouse vagina. J Reprod Immunol 1990; 17(2): 101–14. [PubMed: 2338672]

[329]. Pialoux G, Hocini H, Perusat S, et al. Phase I study of a candidate vaccine based on recombinant HIV-1 gp160 (MN/LAI) administered by the mucosal route to HIV-seronegative volunteers: The ANRS VAC14 study. Vaccine 2008; 26(21): 2657–66. [PubMed: 18068876]

[330]. Lewis DJ, Fraser CA, Mahmoud AN, et al. Phase I randomised clinical trial of an HIV-1(CN54), clade C, trimeric envelope vaccine candidate delivered vaginally. PLoS One 2011; 6(9): e25165.

[331]. Cranage MP, Fraser CA, Cope A, et al. Antibody responses after intravaginal immunisation with trimeric HIV-1 CN54 clade C gp140 in Carbopol gel are augmented by systemic priming or boosting with an adjuvanted formulation. Vaccine 2011; 29(7): 1421–30. [PubMed: 21187177]

[332]. Wright PF, Mestecky J, McElrath MJ, et al. Comparison of systemic and mucosal delivery of 2 canarypox virus vaccines expressing either HIV-1 genes or the gene for rabies virus G protein. J Infect Dis 2004; 189(7): 1221–31. [PubMed: 15031791]

[333]. Gordon SN, Doster MN, Kines RC, et al. Antibody to the gp120 V1/V2 loops and $CD4^+$ and $CD8^+$ T cell responses in protection from SIVmac251 vaginal acquisition and persistent viremia. J Immunol 2014; 193(12): 6172–83. [PubMed: 25398324]

[334]. Lewis DJ, Wang Y, Huo Z, et al. Effect of vaginal immunization with HIVgp140 and HSP70 on HIV-1 replication and innate and T cell adaptive immunity in women. J Virol 2014; 88(20): 11648–57. [PubMed: 25008917]

[335]. Weaver EA, Nehete PN, Nehete BP, et al. Comparison of systemic and mucosal immunization with helper-dependent adenoviruses for vaccination against mucosal challenge with SHIV. PLoS One 2013; 8(7): e67574.

[336]. Abel K, Compton L, Rourke T, et al. Simian-human immunodeficiency virus SHIV89.6-induced protection against intravaginal challenge with pathogenic SIVmac239 is independent of the route of immunization and is associated with a combination of cytotoxic T-lymphocyte and alpha interferon responses. J Virol 2003; 77(5): 3099–118. [PubMed: 12584336]

[337]. Genesca M, Ma ZM, Wang Y, et al. Live-attenuated lentivirus immunization modulates innate immunity and inflammation while protecting rhesus macaques from vaginal simian immunodeficiency virus challenge. J Virol 2012; 86(17): 9188–200. [PubMed: 22696662]

[338]. Ganor Y, Zhou Z, Bodo J, et al. The adult penile urethra is a novel entry site for HIV-1 that preferentially targets resident urethral macrophages. Mucosal Immunol 2013; 6(4): 776–86. [PubMed: 23187317]

[339]. Zhou Z, Barry de Longchamps N, Schmitt A, et al. HIV-1 efficient entry in inner foreskin is mediated by elevated CCL5/RANTES that recruits T cells and fuels conjugate formation with Langerhans cells. PLoS Pathog 2011; 7(6): e1002100.

[340]. Prodger JL, Gray R, Kigozi G, et al. Foreskin T-cell subsets differ substantially from blood with respect to HIV co-receptor expression, inflammatory profile, and memory status. Mucosal Immunol 2012; 5(2) :121–8. [PubMed: 22089029]

[341]. Dinh MH, Anderson MR, McRaven MD, et al. Visualization of HIV-1 interactions with penile and foreskin epithelia: clues for female-to-male HIV transmission. PLoS Pathog 2015; 11(3): e1004729.

[342]. Anderson D, Politch JA, Pudney J. HIV infection and immune defense of the penis. Am J Reprod Immunol 2011; 65(3): 220–9. [PubMed: 21214659]

[343]. Sennepin A, Real F, Duvivier M, et al. The human penis is a genuine immunological effector site. Front Immunol 2017; 8(article 1732): 1–18. [PubMed: 28149297]

[344]. Rothaeusler K, Ma ZM, Qureshi H, et al. Antiviral antibodies and T cells are present in the foreskin of simian immunodeficiency virus-infected rhesus macaques. J Virol 2012; 86(13): 7098–106. [PubMed: 22532691]

[345]. Pudney J, Anderson DJ. Immunobiology of the human penile urethra. Am J Pathol 1995; 147(1): 155–65. [PubMed: 7604877]

[346]. Mestecky J, Alexander RC, Wei Q, Moldoveanu Z. Methods for evaluation of humoral immune responses in human genital tract secretions. Am J Reprod Immunol 2011; 65(3): 361–7. [PubMed: 21087333]

[347]. Moldoveanu Z, Huang WQ, Kulhavy R, Pate MS, Mestecky J. Human male genital tract secretions: both mucosal and systemic immune compartments contribute to the humoral immunity. J Immunol 2005; 175(6): 4127–36. [PubMed: 16148163]

[348]. Prodger JL, Hirbod T, Kigozi G, et al. Immune correlates of HIV exposure without infection in foreskins of men from Rakai, Uganda. Mucosal Immunol 2014; 7(3): 634–44. [PubMed: 24150258]

[349]. Adams SE, Dawson KM, Gull K, Kingsman SM, Kingsman AJ. The expression of hybrid HIV: Ty virus-like particles in yeast. Nature 1987; 329(6134): 68–70. [PubMed: 3041226]

[350]. Lehner T, Tao L, Panagiotidi C, et al. Mucosal model of genital immunization in male rhesus macaques with a recombinant simian immunodeficiency virus p27 antigen. J Virol 1994; 68(3): 1624–32. [PubMed: 8107223]

[351]. Balandya E, Miller AD, Beck M, et al. Adenovirus serotype 26 and 35 vectors induce simian immunodeficiency virus-specific T lymphocyte responses in foreskin in rhesus monkeys. J Virol 2014; 88(7): 3756–65. [PubMed: 24429370]

[352]. Quiding-Jarbrink M, Granstrom G, Nordstrom I, Holmgren J, Czerkinsky C. Induction of compartmentalized B-cell responses in human tonsils. Infect Immun 1995; 63(3): 853–7. [PubMed: 7868256]

[353]. Mills KH, Cosgrove C, McNeela EA, et al. Protective levels of diphtheria-neutralizing antibody induced in healthy volunteers by unilateral priming-boosting intranasal immunization associated with restricted ipsilateral mucosal secretory immunoglobulin A. Infect Immun 2003; 71(2): 726–32. [PubMed: 12540551]

[354]. Huo Z, Sinha R, McNeela EA, et al. Induction of protective serum meningococcal bactericidal and diphtheria-neutralizing antibodies and mucosal immunoglobulin A in volunteers by nasal insufflations of the Neisseria meningitidis serogroup C polysaccharide-CRM197 conjugate vaccine mixed with chitosan. Infect Immun 2005; 73(12): 8256–65. [PubMed: 16299322]

[355]. Anjuere F, Bekri S, Bihl F, et al. B cell and T cell immunity in the female genital tract: Potential of distinct mucosal routes of vaccination and role of tissue-associated dendritic cells and natural killer cells. Clin Microbiol Infect 2012; 18(Suppl 5): 117–22. [PubMed: 22882377]

[356]. Davila SJ, Olive AJ, Starnbach MN. Integrin alpha4beta1 is necessary for $CD4^+$ T cell-mediated protection against genital Chlamydia trachomatis infection. J Immunol 2014; 192(9): 4284–93. [PubMed: 24659687]

[357]. Nardelli-Haefliger D, Kraehenbuhl JP, Curtiss R 3rd, et al. Oral and rectal immunization of adult female volunteers with a recombinant attenuated Salmonella typhi vaccine strain. Infect Immun 1996; 64(12): 5219–24. [PubMed: 8945569]

[358]. Kantele A, Hakkinen M, Moldoveanu Z, et al. Differences in immune responses induced by oral and rectal immunizations with Salmonella typhi Ty21a: evidence for compartmentalization within the common mucosal immune system in humans. Infect Immun 1998; 66(12): 5630–5. [PubMed: 9826335]

[359]. Kutteh WH, Kantele A, Moldoveanu Z, Crowley-Nowick PA, Mestecky J. Induction of specific immune responses in the genital tract of women after oral or rectal immunization and rectal boosting with Salmonella typhi Ty 21a vaccine. J Reprod Immunol 2001; 52(1–2): 61–75. [PubMed: 11600178]

[360]. Crowley-Nowick PA, Bell MC, Brockwell R, et al. Rectal immunization for induction of specific antibody in the genital tract of women. J Clin Immunol 1997; 17(5): 370–9. [PubMed: 9327336]

[361]. Kubota M, Miller CJ, Imaoka K, et al. Oral immunization with simian immunodeficiency virus p55gag and cholera toxin elicits both mucosal IgA and systemic IgG immune responses in nonhuman primates. J Immunol 1997; 158(11): 5321–9. [PubMed: 9164952]

[362]. Wu HY, Russell MW. Induction of mucosal immunity by intranasal application of a streptococcal surface protein antigen with the cholera toxin B subunit. Infect Immun 1993; 61(1): 314–22. [PubMed: 8418053]

[363]. Gallichan WS, Rosenthal KL. Long-lived cytotoxic T lymphocyte memory in mucosal tissues after mucosal but not systemic immunization. J Exp Med 1996; 184(5): 1879–90. [PubMed: 8920875]

[364]. Gallichan WS, Rosenthal KL. Long-term immunity and protection against herpes simplex virus type 2 in the murine female genital tract after mucosal but not systemic immunization. J Infect Dis 1998; 177(5): 1155–61. [PubMed: 9592997]

[365]. Russell MW, Moldoveanu Z, White PL, Sibert GJ, Mestecky J, Michalek SM. Salivary, nasal, genital, and systemic antibody responses in monkeys immunized intranasally with a bacterial protein antigen and the cholera toxin B subunit. Infect Immun 1996; 64(4): 1272–83. [PubMed: 8606090]

[366]. Rudin A, Johansson EL, Bergquist C, Holmgren J. Differential kinetics and distribution of antibodies in serum and nasal and vaginal secretions after nasal and oral vaccination of humans. Infect Immun 1998; 66(7): 3390–6. [PubMed: 9632610]

[367]. Green CA, Scarselli E, Sande CJ, et al. Chimpanzee adenovirus-and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. Sci Transl Med 2015; 7(300): e126.

[368]. Adderson E, Branum K, Sealy RE, et al. Safety and immunogenicity of an intranasal Sendai virus-based human parainfluenza virus type 1 vaccine in 3-to 6-year-old children. Clin Vaccine Immunol 2015; 22(3): 298–303. [PubMed: 25552633]

[369]. Lambkin-Williams R, Gelder C, Broughton R, et al. An intranasal proteosome-adjuvanted trivalent influenza vaccine Is safe, immunogenic & efficacious in the human viral influenza challenge model. Serum IgG and Mucosal IgA are important correlates of protection against illness associated with infection. PLoS One 2016; 11(12): e0163089.

[370]. Madan A, Segall N, Ferguson M, et al. Immunogenicity and safety of an AS03-adjuvanted H7N9 pandemic influenza vaccine in a randomized trial in healthy adults. J Infect Dis 2016; 214(11): 1717–27. [PubMed: 27609809]

[371]. Riddle MS, Kaminski RW, Williams C, et al. Safety and immunogenicity of an intranasal Shigella flexneri 2a Invaplex 50 vaccine. Vaccine 2011; 29(40): 7009–19. [PubMed: 21787825]

[372]. Malkin E, Yogev R, Abughali N, et al. Safety and immunogenicity of a live attenuated RSV vaccine in healthy RSV-seronegative children 5 to 24 months of age. PLoS One 2013; 8(10): e77104.

[373]. Thorstensson R, Trollfors B, Al-Tawil N, et al. A phase I clinical study of a live attenuated Bordetella pertussis vaccine--BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. PLoS One 2014; 9(1): e83449.

[374]. Rudenko L, Kiseleva I, Stukova M, et al. Clinical testing of pre-pandemic live attenuated A/H5N2 influenza candidate vaccine in adult volunteers: results from a placebo-controlled, randomized double-blind phase I study. Vaccine 2015; 33(39): 5110–7. [PubMed: 26296497]

[375]. Enose Y, Ui M, Miyake A, et al. Protection by intranasal immunization of a nef-deleted, nonpathogenic SHIV against intravaginal challenge with a heterologous pathogenic SHIV. Virology 2002; 298(2): 306–16. [PubMed: 12127792]

[376]. Bolton DL, Song K, Wilson RL, et al. Comparison of systemic and mucosal vaccination: impact on intravenous and rectal SIV challenge. Mucosal Immunol 2012; 5(1): 41–52. [PubMed: 22031182]

[377]. Egan MA, Chong SY, Rose NF, et al. Immunogenicity of attenuated vesicular stomatitis virus vectors expressing HIV type 1 Env and SIV Gag proteins: comparison of intranasal and intramuscular vaccination routes. AIDS Res Hum Retroviruses 2004; 20(9) :989–1004. [PubMed: 15585086]

[378]. Egan MA, Chong SY, Megati S, et al. Priming with plasmid DNAs expressing interleukin-12 and simian immunodeficiency virus gag enhances the immunogenicity and efficacy of an experimental AIDS vaccine based on recombinant vesicular stomatitis virus. AIDS Res Hum Retroviruses 2005; 21(7): 629–43. [PubMed: 16060834]

[379]. Fouda GG, Amos JD, Wilks AB, et al. Mucosal immunization of lactating female rhesus monkeys with a transmitted/founder HIV-1 envelope induces strong Env-specific IgA antibody responses in breast milk. J Virol 2013; 87(12): 6986–99. [PubMed: 23596289]

[380]. Watkins JD, Sholukh AM, Mukhtar MM, et al. Anti-HIV IgA isotypes: differential virion capture and inhibition of transcytosis are linked to prevention of mucosal R5 SHIV transmission. AIDS 2013; 27(9): F13–F20. [PubMed: 23775002]

[381]. Sholukh AM, Watkins JD, Vyas HK, et al. Defense-in-depth by mucosally administered anti-HIV dimeric IgA2 and systemic IgG1 mAbs: complete protection of rhesus monkeys from mucosal SHIV challenge. Vaccine 2015; 33(17): 2086–95. [PubMed: 25769884]

[382]. Pauthner M, Havenar-Daughton C, Sok D, et al. Elicitation of robust tier 2 neutralizing antibody responses in nonhuman primates by HIV envelope trimer immunization using optimized approaches. Immunity 2017; 46(6): 1073–88. [PubMed: 28636956]