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Genital Delivery of Virus-like Particle and Pseudovirus Based Vaccines

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Abstract

Sexually transmitted diseases represent a significant cause of mortality and morbidity worldwide. The genital mucosa is the first line of defense against sexually transmitted pathogens and, like other mucosal tissues, it is colonized by resident immune cells that initiate an immune responses that can prevent the establishment and dissemination of infection. While it is clear that systemic vaccination is sufficient to provide protection against certain pathogens that infect the genital tract, such as human papillomavirus (HPV) and hepatitis B virus (HBV), it has not worked for other. Induction of local mucosal immune responses in the genital tract might increase the efficacy of vaccines targeting HSV, HIV and other sexually transmitted infections. Here, we describe recent promising efforts to induce adaptive immune responses in the genital tract using vaccines based on virus-like particles (VLPs) and pseudoviruses (PsVs).

Keywords

Virus-like Particle; mucosal immunity; HPV; STI

Virus-like Particle and Pseudovirus-based Vaccines

VLPs are comprised of viral structural proteins, which, when overexpressed, spontaneously self-assemble into particles that are superficially indistinguishable from the virus from which they were derived. VLPs typically indiscriminately package cellular nucleic acid, but they can also be made to encapsidate plasmid DNA to form PsVs. VLPs make superb vaccines because they are highly immunogenic, have excellent safety profiles, and are often easy to manufacture. VLPs evoke strong antibody responses because their dense, multivalent structures are highly stimulatory to B cells and elicit much stronger immune responses than monomeric or poorly organized antigens [1]. Their particulate nature also enhances cross-priming of CD8 T cell responses. VLPs can be used as stand-alone vaccines that target the viruses from which they are derived as well as scaffolds for enhancing the immunogenicity of associated antigenic components. Because they are hollow spheres, VLPs can also be loaded with a variety of cargos. In vaccine applications, these cargos can include adjuvants, to enhance immune responses, or, in the case of PsVs, the cargo can be DNA, for gene delivery. PsVs also induce strong antibody responses to the capsid, but they are typically used to deliver antigen genes to target cells for the induction of cellular immune responses. VLPs and PsVs can be derived from many different virus types, but VLPs and PsVs derived

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from viruses that normally infect the genital mucosa, such as HPV, may be particularly effective agents for gene delivery to this tissue.

Architecture of the genital tract

The genital tract is generally considered a poor inductive site of immune responses and induction of potent responses often requires the use of live infectious agents or strong adjuvants. The female genital tract mucosa is composed of two major types of epithelium; the uterus and the endo-cervix mucosa are composed of a single columnar epithelium and the vagina and the ecto-cervix mucosa have a squamous pluri-stratified epithelium. Whereas mucosa-associated lymphoid tissues are found in the respiratory tract and in the digestive tract mucosa, the genital tract mucosa is devoid of organized lymphoid tissues and notably of microfold (M) cells which are specialized in sampling the external milieu. In some inflammatory conditions, such as viral infection, lymphoid aggregates can be observed transiently in the submucosa of the vaginal epithelium. Despite the absence of organized lymphoid structures, the genital tract mucosa is drained by the iliac and inguinal lymph nodes and possesses an array of antigen presenting cells such as dendritic cells, located in both the pluri-stratified epithelium and in the submucosa, that can traffic to the draining lymph nodes to prime immune responses. For instance after intravaginal immunization, submucosal dendritic and Langerhans cells have been shown to prime Th1 and Th17 responses, respectively [2,3]. In this regard, the overall architecture of the immune system of the female genital tract is similar to other pluri-stratified epithelia such as the skin and the sublingual mucosa, keeping in mind that sexual hormones influence the genital tract epithelium.

Strategies for vaccination of the genital tract

The architecture of the genital tract and relative lack of immune sampling of the microbiota in this environment are barriers to the development of successful vaccination protocols. Simple instillation of VLPs with or without mucosal adjuvants is ineffective at inducing local antibody responses, presumably because of lack of access to immune cells and high rates of clearance from this site [4]. However, it has been shown that pre-treatment with Depo-Provera, followed by mechanical or chemical disruption of the vaginal epithelia allows for uptake of particles, access to the basal layers of the cervico-vaginal epithelium, and exposure to resident immune cells in the genital mucosa [5]. Mechanical disruption can be accomplished by the use of a cytobrush, and chemical disruption by application of nonoxydyl-9, a non-ionic detergent widely used in commercial spermicides. Following disruption, VLPs or PsV can be applied to the genital tract either in a gel or deposited as an aerosol using a high-pressure microsprayer syringe. These techniques can result in strong local adaptive immune responses in the genital tract [4,6].

VLPs for Inducing Antibody Responses

Mucosal antibodies can provide sterilizing immunity by blocking the earliest stages of infection of mucosal epithelial cells, typically through direct neutralization or opsonization of pathogens. Antibodies in genital tract secretions largely consist of locally produced secretory IgA (sIgA) and locally or systemically produced IgG, which can transudate or exudate into cervical secretions [7]. Given the effectiveness of the parenteral HPV and HBV vaccines at preventing infection in the genital tract, an important question is whether genital vaccination and local production of antibodies will confer additional protective advantages for other vaccines. Although parenteral immunization elicits IgG, it does not induce sIgA production. sIgA has unique characteristics that enhance its anti-pathogen activity: it can be produced at high levels, it is actively transported into mucosal secretions, it is resistant to proteases in these secretions, it is anti-inflammatory, and it can eliminate antigens by

basolateral-to-apical transcytosis. In addition, the secretory component of sIgA specifically binds mucus and so can inhibit microbial movement through mucus [8], potentially decreasing the likelihood of transmission by enhancing this barrier function. These characteristics are compelling factors supporting the development of genital vaccines that induce strong local sIgA responses.

Although intranasal and sublingual vaccination elicit weak serum IgG antibody responses but relatively strong IgA antibody response in the genital secretions [9], it is notoriously difficult to induce local antibody responses by vaginal immunization using conventional recombinant antigens. Recently, we have shown that vaginal aerosol immunization with HPV and bacteriophage VLPs can induce high titer sIgA and IgG in vaginal secretions as well as systemic IgG responses [6]. A single vaginal immunization with HPV VLPs without adjuvant was sufficient to confer protection from genital challenge with HPV PsV. Our belief is that the combination of a highly immunogenic antigen (such as the VLP) and an effective vaginal immunization protocol is critical for the induction of strong cervico-vaginal antibody responses. In future studies, it will be important to assess the durability of the cervicovaginal sIgA responses.

PsVs for inducing T cell responses

Vaccine modalities able to induce genital cytotoxic T cell responses may present an advantage for preventing or controlling initial replication of sexually transmitted infection such as Herpes simplex viruses and HIV. The best route of immunization to achieve strong genital CD8+ T cell responses is still debated; whereas mucosal immunization induces preferential trafficking of T cells to mucosal sites by promoting the expression of a network of mucosal homing molecules by memory CD8+ T cells, systemic immunization has also been shown to induce genital CD8+ T cell responses. Nevertheless, local genital immunization may provide an advantage over other remote vaccination modalities by promoting preferential recruitment or *in situ* proliferation of CD8+ T cells. For instance, it was shown that antigen-specific CD4+ T cells present in the genital tract are involved in the recruitment of circulating memory CD8+ T cells to the genital tract during HSV-2 secondary infection [10]. In addition, resident memory CD8+ T cells can proliferate *in situ* upon *de novo* antigen expression during reactivation of neurotropic viruses [11,12]. Thus, in a context of intravaginal vaccination, these two mechanisms may help to induce long-lived resident memory CD8+ T cells and confer protection as reported in the skin during HSV infection [13].

Genetic vaccination using naked DNA, live attenuated viral vectors or replication-incompetent vectors have been shown to induce robust CD8+ T cell responses. However, for genital vaccination, naked DNA does not seem to be practical or may require new formulation in order increase gene transduction, and intravaginal delivery of live attenuated viruses or bacteria might raise safety concerns. Hence, replication-incompetent viral vectors may represent the most viable option, provided that tropism of the viral vectors allows efficient gene transduction in the mucosa. For these reasons, HPV PsV [14], which naturally infect basal keratinocytes, are attractive vehicles for genetic delivery of antigens to the genital tract. Expression of the transduced gene(s) after chemical disruption of the vaginal epithelium appears to be transient and restricted to wounded keratinocytes in mice and non human primates [5,15]. An initial study in mice demonstrated that HPV PsV intravaginal immunization induces CD8 and CD4 T cell responses with a broad cytokine secretion profile, in blood and in the lungs. Furthermore, HPV PsV intravaginal immunization was able to prime local and systemic IgA and IgG responses [16]. Studies to characterize the induction of cervicovaginal T cells by this strategy are currently underway. Because HPV PsVs mainly transduce basal keratinocytes, the induction of CD8+ T cell responses may

require cross-priming by dendritic cells which might be enhanced by the use of a molecular or genetic adjuvant. However, HPV16 VLPs induce acute activation of innate immune responses, including TLR4-mediated signaling and promotion of inflammatory cytokine production by myeloid and plasmacytoid dendritic cells, which may account for the high intrinsic immunogenicity of HPV PsV [17,18]. Pre-existing immunity is an issue for most viral vectors and the success of the current HPV vaccine may limit the use of certain types of HPV. Therefore use of rare human types or animal types may be needed to overcome vaccine-induced neutralization.

Conclusions

Although the practicality and feasibility of intravaginal immunization may be questioned, the experience of clinical trials with topical microbicides against sexually-transmitted infections should pave the way for the development of an intravaginal vaccine that would be widely accepted by women. Thus, we believe that VLPs and PsVs constitute promising platforms for delivery of antigens to the genital tract.

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