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Review

Antibody-mediated protection and the mucosal immune system of the genital tract: relevance to vaccine design

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ABSTRACT

Mucosal tissues of the genital tracts and the distal intestinal tract are portals of entry for infectious agents of sexually transmitted diseases, including HIV-1. Although the genital and intestinal tracts share a common embryologic origin and remain in anatomical proximity, these two sites display remarkably different immunologic features, including the levels, isotypes and molecular forms of immunoglobulins, and magnitudes and qualities of humoral and cellular immune responses. Thus, viral and bacterial infections of the genital tract or intravaginal immunizations induce, in the absence of mucosal adjuvants, minimal immune responses. Consequently, to induce relevant immune responses in the genital tract, alternative immunization routes have been explored, including systemic, intranasal, oral, or rectal immunization and their combinations. In limited studies performed in animals, systemic immunization with a subsequent mucosal (intranasal) immunization proved to be effective in the induction of humoral immune responses in genital tract secretions. The approaches have been explored to a limited extent in humans.

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1. Introduction

The mucosal tissues of the female and male genital and distal intestinal tracts are portals of entry for infectious agents of sexually transmitted diseases (STD) (for review see Russell et al., 2005). Although the genital and intestinal tracts are both considered components of the common mucosal immune system, these locales display remarkably distinct immunological differences that must be considered in the outcome of both humoral and cellular immune

responses induced by either infections or immunization with relevant vaccines (Russell and Mestecky, 2002). Both female and male genital tract tissues lack lymphoepithelial inductive mucosal sites analogous to such structures abundantly distributed in the intestinal tract, including the rectum (for review see Boyaka et al., 2005). In contrast to typical external secretions, such as intestinal fluid that contain mostly locally produced secretory IgA (S-IgA) as the dominant isotype, semen as well as cervico-vaginal fluid, contain more IgG than IgA and the highly variable levels of immunoglobulins in the female genital tract are under pronounced hormonal control (for review see Kutteh et al., 2005), due, in part, to the expression of immunoglobulin receptors involved in their transepithelial transport (Kaetzel and Mostov, 2005). Furthermore, both cervico-vaginal secretions and semen contain plasma-derived as

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well as locally produced immunoglobulins, mainly of the IgG isotype (Russell and Mestecky, 2002; Moldoveanu et al., 2005).

These marked immunological differences must be considered in the design of immunization strategies to protect genital tract mucosae against infections with *Neisseria gonorrhoeae*, human papilloma viruses (HPV), herpes simplex virus (HSV) type 2, *Chlamydia trachomatis*, HIV-1, and other infectious agents of STD. Although the genital and intestinal tracts share a common embryologic origin and remain in anatomical proximity, these two mucosal compartments display vastly different physiological functions with distinct immunological requirements. Because of the presence of Peyer's patches and rectal tonsils, oral or intra-rectal immunization induces both local and generalized immune responses through the common mucosal immune system, manifested in the parallel appearance of S-IgA antibodies at the site of exposure and in the secretions of anatomically remote mucosal tissues (Boyaka et al., 2005). In contrast, infections restricted to the human vaginal mucosa or intravaginal immunization in the absence of potent mucosal adjuvants, such as cholera toxin (CT), generate, at best, unimpressive humoral responses confined to the site of immunization (Russell and Mestecky, 2002). However, ascendant infections or intrauterine immunization in animals may result in vigorous humoral responses enhanced by adjuvants, because of the presence of cells engaged in the uptake, processing, and presentation of antigens in the uterine mucosa (Kutteh et al., 2005). Vaginal immunizations of women with soluble antigens or inactivated poliovirus, or infections with *N. gonorrhoeae*, *C. trachomatis*, group B streptococci, HSV type 2, or HPV induce weak to modest local and rarely systemic humoral immune responses (Russell and Mestecky, 2002). Attempts to induce humoral immune responses by various immunization routes in semen have been performed in comparison to females, less frequently (Russell and Mestecky, 2002; Anderson and Pudney, 2005; Moldoveanu et al., 2005). Systemic or mucosal (oral) immunization of young adult males with diphtheria or tetanus toxoids, pneumococcal polysaccharide or live attenuated *Salmonella typhi* Ty21a vaccines induced dominantly IgG antibodies in serum and semen (Moldoveanu et al., 2005). The effectiveness of intranasal route of immunization on the induction of specific antibodies in semen has not been evaluated.

Because of the marked differences in the mucosal immune systems of the genitourinary and intestinal tracts, vaccination strategies should be designed to target both compartments in order to induce protective immunity at these common sites of entry for numerous microbial agents (e.g., HIV-1, HPV, and the gonococcus).

Although the importance of cell-mediated immune responses in the clearance of infected cells (including those in the genital tract of animal models) has been amply documented (for review see Parr and Parr, 2005), the dominant role of antibodies in the prevention of infection is undisputable: "Most, if not all, effective vaccines protect via pre-existing antibodies..." (Zinkernagel and Hengartner, 1997). The validity of this statement can be extended to two new vaccines against human papilloma virus, whose protective activity is antibody-dependent (Schiller and Lowy,

Table 1

Functional differences of IgA and IgG antibodies in secretions of the genital tract.

	IgA	IgG
Dominant levels	++	+++
Antigen-binding sites	4 (dimers)	2
	8 (tetramers)	
Effectiveness in virus neutralization	+++	+
Glycan-mediated inhibition of microbial adherence	++	–
Resistance to proteolytic enzymes of bacterial or endogenous origin	±	++
Interactions with humoral factors of innate immunity	+	–
Complement activation (inflammatory potential)	–	+++
Receptors on		
Epithelial cells	++	(+?)
Phagocytic cells	++	++
Intracellular (intraepithelial) virus neutralization	++	(–?)

From: Baba et al. (2000), Mascola et al. (2000), Veazay et al. (2003), Kutteh et al. (2005), Russell and Kilian (2005), Woof and Mestecky (2005) Lamm (2007), and Mestecky and Russell (2009).

Some of the above-described protective functions have been demonstrated *in vitro* using IgA or IgG isolated from serum or other external secretions (Russell and Kilian, 2005).

2006). Consequently, in this short review paper, we focus on our studies concerning the functional uniqueness of antibodies of the IgA, as compared to IgG, isotypes and strategies effective in the induction of humoral immune responses.

2. Functional differences in mucosal antibodies of the IgG and IgA isotypes

Marked dominance of S-IgA in the intestinal fluid, saliva, milk and tears as opposed to the dominance of IgG in genital tract secretions and urine (Jackson et al., 2005) prompts the question of the functional consequences of S-IgA and IgG and their potential in the protection of mucosal tissues. Although specific IgG and S-IgA antibodies, or IgA in general, interact with corresponding antigens, the biological consequences are remarkably different (Table 1). S-IgA in its dimeric or tetrameric forms contains 4–8 antigen-binding sites (in contrast to 2 for IgG) and due to the "bonus effect of multi-valency" displays, for example, virus neutralization activity which may be several orders of magnitude greater than that of Igs in their monomeric form (Renegar et al., 1998; Russell and Kilian, 2005). Furthermore, S-IgA, which functions in the environment containing endogenous as well as exogenous (bacterial) proteases, is remarkably resistant to proteolysis due to an intrinsic low susceptibility to proteases, potentiated by association with secretory component (SC) acquired during the transepithelial transport (Corthesy, 2007; Kaetzel and Mostov, 2005). In addition to the specific antibody activity, IgA- and SC-associated glycans are likely to play an important role as inhibitors in the receptor-mediated interactions of microorganisms with epithelial cells (Russell and Kilian, 2005; Mestecky and Russell, 2009).

Due to the expression of corresponding receptor on functionally and histologically diverse cell populations

(e.g., epithelial cell, polymorphonuclear leukocytes, monocytes and macrophages), IgA and IgG is internalized and selectively transported through the epithelial cells, using the polymeric Ig receptor (pIgR), into external secretions (Kaetzel and Mostov, 2005). Thus, polymeric IgA produced locally by subepithelial plasma cells in the intestinal tract and uterine endocervix is taken up and transported as S-IgA into corresponding fluids. Interestingly, virus-specific IgA internalized by epithelial cells can effectively interfere with the intracellular assembly of viruses (Lamm, 2007; Russell and Kilian, 2005). In contrast to the intestinal and endocervical epithelial cells, vaginal epithelial cells in women do not express pIgR (Kutteh et al., 2005), but internalize, by an unknown mechanism, both IgA and IgG. Functional consequences remain, however, unknown.

Finally, under *normal* circumstances, IgA, unlike IgG, does not activate any of the three complement (C) pathways (Russell and Kilian, 2005; Russell et al., 1997). As a matter of fact, IgA antibodies display a strong anti-inflammatory effect. Thus, immune complexes formed in mucosal tissues between antigens and IgA do not activate C; in sharp contrast, IgG-containing immune complexes activate C, which attract polymorphonuclear leukocytes and cause damage of the mucosal barrier with enhanced absorption of by-stander antigens (for review see Russell and Kilian, 2005). Nevertheless, IgG found in a free form in external secretions of the genital, respiratory, and also in the intestinal tract appears to be functionally effective and prevents infections with respiratory intestinal pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, influenza virus, and probably *Salmonella*, *Shigella*, and other mucosal pathogens (Underdown, 2005).

3. Induction of humoral responses in external secretions of genital and intestinal tracts

Neutralizing antibodies of the IgG isotype specific for HIV-1 which were administered either systemically or

intravaginally protected rhesus macaque monkeys against intravaginal challenge with SHIV (Baba et al., 2000; Mascola et al., 2000; Veazay et al., 2003). These experiments clearly demonstrated that antibodies exhibit their protective effect in the genital tract. Therefore, their induction by active immunization is a highly desirable goal. However, in view of the marked immunological differences between the genital and intestinal tracts, alternative immunization strategies must be considered to achieve protection at both mucosal sites. Although systemic immunization generally induces weak humoral responses in intestinal secretions, the dominance of IgG in seminal plasma and cervico-vaginal secretions, as well as the significant contribution of IgG from the circulation to genital secretions and urine, provide a compelling explanation for the protective effect of systemically induced IgG antibodies in the genitourinary tract. The comparative studies concerning the effectiveness of various mucosal immunization routes on the parallel induction of antibodies in genital and intestinal secretions have been explored mainly in animal models, with only a few studies performed in humans (for review see Russell and Mestecky, 2002). Thus, oral, sublingual, rectal, intravaginal, and intranasal immunizations with a broad variety of antigens have been evaluated with several noteworthy observations (Table 2). Interestingly, the intranasal route of antigen administration, with or without suitable mucosal adjuvants, induced both systemic and mucosal humoral immune responses, including those in female genital tract secretions (Russell and Mestecky, 2002; Wu and Russell, 1997). Apparently, lymphoid cells originating from the nasal mucosal inductive sites display on their surfaces homing receptors with a certain degree of specificity for systemic (e.g., L-selectin) or mucosal effector sites (e.g., $\alpha 4\beta 7$, $\alpha 4\beta 1$ and others) which direct such cells to their target tissues (Quiding-Järbrink et al., 1997) where, under the influence of locally produced cytokines, their terminal differentiation to plasma cells occurs.

In spite of the demonstrable effectiveness of intranasal immunization for the induction of humoral responses in

Table 2
Mucosal site of antigen encounter determines the quality of immune responses.

Site of immunization	Antibody response			Cell-mediated immunity	Mucosal tolerance	Note
	Local	Other mucosal sites	Systemic			
Nasal	++	+	+	+	+	Humans/animals
		(Female genital tract) (Male genital tract?)				
Oral						
(Local, no Ag ingestion)	±	?	±	+	+	Humans/animals
Sublingual	+	+	+	+	+	Humans/animals
Ingestion	+	+	+	+	+	Humans/animals
		(Female and male genital tract)				
Rectal	+	+	+(?)	+(?)	?	Humans/animals
		(Female genital tract)				
Vaginal	±	–	–	+(?)	+ in mice ? in humans	Humans/animals (menstrual cycle-dependent)

From: Wu and Russell (1997), Russell and Mestecky (2002, 2010), Boyaka et al. (2005), Kutteh et al. (2005), Moldoveanu et al. (2005) and Mestecky et al. (2007).

the genital tract secretions, there are concerns about the safety of nasal vaccines co-administered with enterotoxin-based adjuvants (Fujihashi et al., 2002). Sublingual immunization represents an alternative, non-invasive route, which may avoid problems with toxicity, but displays shared and important advantages with the intranasal route. These include an efficient uptake of antigens due to the high number of existing antigen-presenting cells at this site (Moingeon et al., 2006), the highly limited interference of uptake of vaccine antigens by abundant intestinal microbiota and food-derived substances, absence of proteolytic enzymes, and the exposure to low pH in the stomach. However, only few studies have been published in which the sublingual route of immunization was explored for the induction of immune responses in external secretions, but not for the genital tract (Cuburu et al., 2007; Song et al., 2008). The results of these studies suggest that, using the appropriate amount and form of vaccine antigens, the sublingual route should be explored for the induction of responses in the genital tract.

In addition to inactivated or attenuated microorganisms or their most relevant isolated antigens given with or without adjuvants by systemic or mucosal immunization routes, a novel type of vaccines based on the expression of microbial antigens in host cells through the introduction of DNA encoding microbial antigens has been explored in extensive studies (Liu, 2003). Advantages of DNA-based vaccines are numerous; they are safe, non-toxic and antigens are produced in hosts' cells exploiting biosynthetic machinery, including relevant glycosylation, which may be of critical importance.

However in spite of initial enthusiasm justified by experiments performed mostly in mice, subsequent studies, including those performed in primates, yielded disappointing results. One of the potential explanations for the low efficacy of intramuscularly injected DNA vaccines to adult animals and humans appears to be related to the low level of protein synthesis in skeletal muscles when compared to other tissues. For example, in the growing 3–6-week-old rats, the absolute synthetic rate (mg of protein/day/100 g body weight) is 15.9 ± 3.2 in the muscle, but 695 ± 209 in the liver (Combe et al., 2004). When DNA is targeted to the liver by hydrodynamic intravenous application, high and long-lasting humoral and cellular immune responses are induced, which exceed such responses induced by the intramuscular application of equal doses of DNA by several orders of magnitude (Raska et al., 2008). When systemic DNA immunization is combined with a subsequent mucosal (e.g., intranasal) route of protein application, high levels of specific IgA and IgG antibodies were induced in the genital tract secretions of mice immunized with DNA encoding HIV-1 envelope antigens and boosted with a recombinant gp120 protein (Raska et al., 2008). Consequently, alternate means of targeting DNA to cell types endowed with a high level of proteosynthetic activity are currently being explored in several laboratories. Another advantage of the systemic priming – mucosal boosting immunization protocol is the markedly reduced risk of the induction of mucosal tolerance defined by systemic T cell unresponsiveness to antigens encountered first by the mucosal route (Mowat et al., 2005). Diminished sys-

temic T cell responses may interfere with the defenses against infectious agents, such as HIV-1 or SIV, in which T cells play an essential protective role (Mestecky et al., 2007).

It is well recognized that the absolute majority of infectious agents enter the body through the large surface area of mucosal tissues, which contains more cells involved in the immune responses, including antigen-presenting cells, phagocytes, T and B lymphocytes and plasma cells than the systemic immune compartment (Mestecky et al., 2003). However, immune responses initiated by infections or vaccinations are rarely evaluated in external secretions and mucosal tissues, due to the difficulties encountered during the non-standard collection procedures, processing of samples and immunologic assays that are suitable to such specimens (Jackson et al., 2005).

Keeping in mind the unique immunological features of the genital tract as compared to other compartments of the mucosal immune system as well as marked but, unfortunately, frequently ignored differences between mucosal immune systems of humans and numerous species of commonly used laboratory animals, extreme caution should be applied in the interpretation and generalization of data generated in various, often irrelevant, models. This is particularly true in the area of vaccinology, as related to the genital tract. Anatomical, histological (e.g., the thickness of the vaginal wall and cellular compartments), physiological (e.g., frequency of sexual encounters and hormonal conception), and immunological differences including the expression of Ig receptors, densities of Ig-producing cells, phenotypes of B and T cells, macrophages, antigen-presenting cells, variabilities in the level or Ig during the menstrual cycle, difficulties in the evaluation of cell-mediated immune response in humans, inevitable ethical restrictions, and enormous variabilities among different laboratories in the collection and processing secretions and tissues from the human genital tract should be considered and adequately addressed in future studies.

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