

## Mucosal Immune System of the Human Genital Tract

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In contrast to the pronounced dominance of secretory IgA over other immunoglobulin isotypes in human saliva, tears, milk, and gastrointestinal fluids, secretions of both female and male genital tracts contain more IgG than secretory IgA. Both IgG and IgA are derived, to a variable degree, from the systemic immunoglobulin pool as well as from local synthesis. The origin of IgG- and IgA-plasma cell precursors destined for the genital tract is unknown, but indirect evidence suggests that mucosal inductive sites localized in the rectum, small intestine, and especially in the nasal cavity contribute such precursors to the female genital tract. Several studies indicated that intranasal immunization of various species, including humans, was efficient at inducing antigen-specific antibody responses in the female genital tract; however, whether this route is also effective in males has not been explored.

Mucosae of the female and male genital tracts are the portals of entry for sexually transmitted diseases (STDs) of viral, bacterial, fungal, and parasitic origin; ~120 million cases of STDs are reported annually. Infection with human immunodeficiency virus (HIV) is no exception: epidemiologic data indicate that worldwide, 70%–90% of all HIV infections are acquired by heterosexual transmission (review in [1]). This route has the most rapidly rising incidence of new infections, especially among women, who are infected at higher rates than men. Thus, induction of immune responses at the major portals of entry of HIV may be important for protection against HIV infection.

Although innate immune factors, such as secretory leukocyte protease inhibitor, and cells, such as natural killer cells, can help contain HIV early after infection, antigen-specific humoral and cell-mediated immune responses will be required [2]. Furthermore, because HIV is transmitted primarily by heterosexual routes, any successful HIV vaccine should induce immune responses in both mucosal and systemic compartments. By definition, cytotoxic T lymphocytes (CTL) do not prevent primary infection because they do not lyse or attack free virus or virus-infected cells from histoincompatible (allogeneic) individuals [3]. Experience with other viral and bacterial infections [4, 5] has shown that both antibodies and CTL will be important in preventing infection or, if infection occurs, progression to HIV disease [6]. This conclusion is supported by evidence showing

that both antibodies and CTL are important in controlling primary HIV infections [7–11].

### Independence of Mucosal and Systemic Compartments of the Immune System

The immune system can be divided into two compartments that display considerable functional independence: the systemic compartment, represented by the bone marrow, spleen, and lymph nodes, and the mucosal compartment, represented by lymphoid tissues in mucosae and external secretory glands (reviewed in [12]). Numbers and types of cells involved in immune responses and their soluble products, primarily antibodies, are remarkably different in the mucosal and systemic compartments of the immune system.

The specific humoral defense of mucosal surfaces is provided by antibodies, predominantly of the IgA isotype, that are selectively transported by a receptor-mediated mechanism into external secretions [13]. In humans, IgA-producing cells and their ultimate product—secretory IgA (S-IgA)—dominate and are characteristic of the mucosal immune system. Comparison of the molecular properties of IgA in serum and secretions, such as proportions of subclasses, polymeric and monomeric forms, specific antibody activities, and of cells in the secretory tissues and bone marrow, in both healthy and disease states, suggest that in humans, serum and S-IgA systems display a high degree of independence [12].

Experiments that addressed the origin of mucosal antibodies led to the conclusion that an overwhelming proportion of such antibodies is produced locally in mucosal tissues and that in most species, including humans, antibodies derived from the circulation represent only a minor fraction [13]. Consequently, the level of protection against diseases of the respiratory and intestinal tract, such as influenza, cholera, and salmonellosis,

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correlate better with the levels of antibodies in corresponding external secretions than in serum [14].

### Immunoglobulins in Secretions of the Human Genital Tract

Studies of the mucosal immune system of the human genital tract have focused on female tissues and secretions, primarily due to the technically and ethically acceptable collection of secretions (vaginal washes and cervical mucus) and tissues (obtained during frequently performed hysterectomies and tubal ligations). Consequently, information concerning the levels of immunoglobulins during the menstrual cycle, their isotype distribution, physicochemical properties, and transport mechanisms and distribution of immunoglobulin-containing cells, antigen-presenting cells, and CD4<sup>+</sup> and CD8<sup>+</sup> cells in Fallopian tubes, uterus, and vagina have been reported in considerable detail (reviewed in [15]). The immunoglobulin level and isotype display strong hormone-dependent variations that are not as pronounced in other external secretions [16, 17]. In human cervical mucus, there were higher levels of IgG than IgA; this contrasts with other typical external secretions, such as saliva, tears, milk, and intestinal fluids, in which S-IgA is the dominant isotype.

In humans, IgA occurs in two subclasses, IgA1 and IgA2, which differ in their protein and carbohydrate structures, distribution in various body fluids, and effector functions (reviewed in [18]). Specific antibodies to viral antigens, including HIV, are found in the IgA1 subclass, while anti-lipopolysaccharide or -polysaccharide antibodies are present mostly in the IgA2 subclass. Of most importance, while IgA antibodies in serum and respiratory and upper intestinal tract secretions belong mostly to the IgA1 isotype, both subclasses are found in equal proportions in rectum and large intestine [18]. When subclass-specific monoclonal antibodies were used to determine IgA1 and IgA2 levels in the female genital tract secretions, approximately equal proportions of IgA1 and IgA2 were detected [19, 20]. In this respect, female genital tract secretions resemble secretions of the lower intestinal tract rather than the upper intestinal or respiratory tract. The ratios of IgA1 and IgA2 and the predominance of polymeric IgA (pIgA) in cervical secretions indicate that much of the IgA originates from local production, not from plasma [20].

Immunohistochemical examination of tissue secretions or dispersed cells for the presence of antibody-secreting cells indicated that the uterine endocervix contained higher numbers of immunoglobulin-containing and immunoglobulin-secreting cells than did ectocervix, fallopian tubes, and vagina [19, 21]. IgA- and IgG-secreting cells were dominant. Almost all IgA-producing cells contain J chain, a marker of synthesis of pIgA. Furthermore, single-layered epithelial cells of fallopian tubes, uterus, endocervix, and ectocervical glands express the poly-

meric immunoglobulin receptor (pIgR; the extracellular part of which is called the secretory component [SC]), which is essential for selective transport of locally produced pIgA [13]. Thus, all structural and cellular components characteristic of an active transepithelial transport of pIgA are present. The mechanisms involved in the appearance of IgG in cervicovaginal secretions have not been elucidated. Immunoglobulins produced locally and transported from blood by uterine tissues provide humoral immunity in the vaginal canal; hysterectomy greatly reduces immunoglobulin levels in the vagina.

Although subepithelial connective tissue of human vagina contains dispersed IgA- and J chain-positive plasma cells, the multilayered epithelial cells do not stain for pIgR [19, 22]. Nevertheless, both IgA- and IgG-positive epithelial cells were frequently found on the luminal surface and dispersed among the multilayered epithelium. Such immunoglobulin-positive cells contained both  $\kappa$  and  $\lambda$  immunoglobulin light chains and human serum albumin but not C3 complement component, transferrin, or IgM. These data suggest that certain populations of vaginal epithelial cells can acquire, with some degree of selectivity, locally produced and plasma-derived proteins. However, mechanisms of immunoglobulin uptake are unknown, and the functional significance of intraepithelial immunoglobulin for the defense of the vaginal mucosa remains to be determined.

### Immunoglobulin in Male Genital Tract Secretions

Seminal plasma contains ~40 mg protein/mL (about half of protein concentration in serum). The bulk of these proteins is produced locally in the male genital tract tissues (reviewed in [23]). Although IgG, IgA, and IgM have been reported in both pre-ejaculate and seminal plasma, their relative levels have varied [23–30], perhaps due to differences in collection procedures, methods and standards used in immunoglobulin measurements, and the presence of proteolytic enzymes that are essential in liquefaction of semen but also degrade immunoglobulins with a selective degradation of IgM [31]. Even more striking are reported differences in the level of IgA (and S-IgA) and IgG in seminal fluid: One investigator [26] reported ~25 times higher levels of S-IgA than IgG (~1000 vs. ~40 mg/mL), which is similar to S-IgA/IgG ratios in other external secretions, while others [29] detected higher levels of IgG than IgA in this fluid. However, the pre-ejaculate contains more IgA than IgG (unpublished data mentioned in [32]). On the basis of parallel measurements of levels of plasma-derived proteins (e.g., albumin, transferrin) and immunoglobulin in split ejaculate, it appears that most IgG is derived from the circulation, while IgA, which is mainly represented by S-IgA, is of local origin [25, 29, 30, 32].

Although Brandtzaeg et al. [33] were unable to find immunoglobulin and SC expression in normal epididymis, seminal vesicles, or prostate by histochemical means and Northern blot

analyses for SC mRNA, Anderson and Pudney [34] reported prominent SC staining in all of these tissues collected from HIV-infected men with genital tract inflammation. Furthermore, epithelial cells in Littre's glands in penile urethra displayed prominent SC staining in most of the specimens collected from apparently normal tissues [32, 35]; plasma cells found in the vicinity of SC-positive epithelial cells stained prominently for IgA, J chain, IgG, and IgM. Thus, structural requisites for an operational S-IgA assembly are present in penile urethra but not in other segments of the male genital tract. Therefore, immunochemical and immunohistochemical data suggest that both plasma-derived and locally produced immunoglobulins are in seminal fluid.

### **Common Mucosal Immune System and Its Compartments: Relevance to the Induction of Immune Responses in the Genital Tract**

Extensive studies concerning the origin of precursors of mucosal IgA plasma cells performed in animal models revealed that the organized lymphoepithelial structures found along the gastrointestinal and respiratory tracts are the main source of such cells [12, 36, 37]. These precursors, which are committed to IgA synthesis, mature in mesenteric lymph nodes and enter the circulation through the thoracic duct. Subsequently, they lodge in the lamina propria of the intestinal, respiratory, and genital tracts and in the mammary, salivary, and lacrimal glands, where terminal differentiation into IgA plasma cells occurs under the influence of locally produced cytokines [36–38]. Evidence for the existence of the common mucosal system in humans include the parallel detection of specific S-IgA antibodies in remote secretions induced by natural exposure to antigens or oral immunization and analyses of IgA-secreting cells from peripheral blood and mucosal tissues [39].

Lymphoid tissues, such as palatine, lingual, and nasopharyngeal tonsils are strategically positioned at the beginning of the digestive and respiratory tracts. They are continuously exposed to ingested and inhaled antigens and possess structural features similar to both lymph nodes and mucosa-associated lymphoid tissues [40]. A lymphoepithelium is present that contains M cells for selective antigen uptake. Thus, B and T cells, plasma cells and M cells, and antigen-processing and -presenting cells have been shown to occur in abundance.

Several recent studies have emphasized the importance of inductive sites in the nasal cavity for the generation of mucosal and systemic immune responses that may exceed in magnitude those induced by oral immunization [41–47]. Experiments done in rodents, rhesus monkeys, chimpanzees, and humans convincingly demonstrated that viral or bacterial antigens instilled into the nasal cavity induce superior immune responses in local secretions, such as saliva and, surprisingly, also in female genital tract secretions. Because neither IgG nor IgA antibodies in vaginal washes correlated with serum antibody responses, it is

assumed that antibodies of both isotypes are predominantly of local origin. This finding may have important implications in the design of vaccines effective in the induction of immune responses in the genital tract.

Although most investigations of IgA inductive sites have primarily centered on Peyer's patches and the appendix, analogous follicular structures are also found in the large intestine, especially in the rectum [48, 49]. Indeed, recent results on the effectiveness of various immunization routes for inducing simian immunodeficiency virus (SIV)-specific antibodies in secretions of the female genital tract have demonstrated the superiority of intrarectal immunization [50, 51]. Vaginal IgA and IgG antibodies also were induced in women rectally immunized with viral or bacterial vaccines [52–56].

Studies done in animals and in humans have shown that induction of mucosal immunity, as measured by specific IgA and IgG antibody responses in the female reproductive tract, required intensive local immunization with repeated large doses of antigen (reviewed in [18]). Whereas systemic immunization resulted in lower local responses, booster immunizations given in the vagina or uterine horns often resulted in an enhanced response in the reproductive tract. Recent comparative studies [57] of the effectiveness of vaginal, pelvic, and parenteral immunizations in the induction of IgA and IgG antibodies in murine vaginal fluid indicated that local (vaginal) immunization induced a weak response. In contrast, subserous and intraperitoneal immunizations with the same antigen resulted in IgA and IgG antibodies in vaginal washings, while subcutaneous injection induced only IgG antibodies. Mucosal inductive sites, such as Peyer's patches, are characterized by the presence of absorptive epithelial M cells, antigen-processing and -presenting cells, and B and T cell-containing areas [12]. Such structures are absent from the female genital tract. This is a probable reason for limited immune responses induced by local intravaginal immunization, particularly with nonreplicating antigens (reviewed in [15]).

In macaques, sequential vaginal, rectal, and oral immunizations with a recombinant particulate SIV antigen elicited both mucosal and systemic immune responses manifested by S-IgA and IgG in the vaginal fluid, IgA and IgG antibodies in serum, and T cell proliferative and helper functions in the genital lymph nodes and peripheral blood [50, 51]. Using SIV incorporated into microspheres, Marx et al. [58] demonstrated that oral or intratracheal immunization following systemic priming induces a protective immune response against vaginal challenge with SIV.

Induction of genital tract immune responses by various immunization routes would have profound implications for the prevention of STDs, including AIDS. Further studies using innovative approaches should be initiated to evaluate critically the role of such inductive sites in human mucosal immunity of the genital tract.

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