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Special Article

Reassessment of the Impact of Mucosal Immunity in Infection with the Human Immunodeficiency Virus (HIV) and Design of Relevant Vaccines

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WHY IS MUCOSAL IMMUNITY IMPORTANT IN HIV INFECTION?

The absolute majority of all infectious diseases is contracted through the large surface area of mucosal membranes which serve as portals of entry for the most important viral and bacterial pathogens (1). Infection by the human immunodeficiency virus (HIV) is no exception: Excluding drug users and recipients of blood products or tissues from HIV-infected donors, epidemiological data concerning the route of acquisition of HIV on a worldwide basis convincingly indicate that 70–80% of all AIDS cases are acquired by heterosexual transmission (2–10). In the United States, this category has the most rapidly rising incidence of new infections, with women becoming infected at a higher frequency than men (11, 12). HIV, either associated with cells in semen or present as free virions, infects susceptible cells (Langerhans cells, macrophages, T cells, and probably epithelial cells) in the genital tract (2, 7, 8). Because mucosal and systemic immune responses are elicited and regulated with a considerable degree of independence (13–15), induction of protective immunity at the most frequent portals of entry of infectious agents—mucosal membranes—is being considered with increasing emphasis in the design of novel vaccines including those against HIV (1, 4, 11, 16–21).

Studies performed in animal models and in humans have convincingly demonstrated that the levels of protection against diseases of the respiratory tract (e.g., influenza, respiratory syncytial virus) or of the intestinal tract (e.g., rotavirus, cholera, salmonellosis, shigellosis) correlate better with the levels of antibodies in corresponding external secretions than in serum (7, 22, 23). Therefore, several injectable vaccines that preferentially stimulate systemic immunity have proved to be of limited value, and alternative routes of immunization are currently being developed to stimulate mucosal immune responses (1, 24).

The importance of mucosal immunity in protection of rhesus macaques against infection with simian immunodeficiency virus (SIV) has been clearly demonstrated. Systemically immunized animals, resistant to a systemic challenge with SIV, were not protected when the virus was introduced by the genital route (25). In contrast, mucosal immunization with inactivated SIV in biodegradable microparticles resulted in the induction of protective immunity (26). These encouraging initial results underscore the importance of mucosal immunity in the development of vaccines that will protect against mucosal as well as systemic routes of acqui-

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sition of HIV infection (7, 8, 11). However, advances in these efforts are compromised by insufficient basic knowledge of HIV transmission and types of infectable cells in the genital tract. Mechanisms involved in the induction and regulation of the mucosal immune response in the genital tract, including the origin and differentiation pathways of B and T cells and the origin and hormonal regulation of selectively transported antibodies, require further extensive investigation. Routes of immunization and antigen-delivery systems that would lead to protective immunity in both female and male genital tracts are currently being pursued.

HOW CAN HIV CROSS THE MUCOSAL BARRIER?

The mechanism of mucosal transmission of HIV is poorly understood at present. Clearly, mucosal acquisition, particularly by heterosexual contact, is grossly inefficient compared to infection via blood or blood products. Several reports have indicated that the rate of male to female transmission is less than 0.2% per incidence of unprotected sexual contact (27–29). Female-to-male transmission rates are believed to be considerably lower (12). Nevertheless, some individuals have become infected after only one or two sexual contacts with an infected partner (29, 30). Increased rates of HIV transmission have been shown to be associated with cofactors such as genital ulcers or coincidental sexually transmitted diseases (31–34). However, it is apparent that loss of integrity of mucosal tissues is not necessary for transmission to occur, although trauma to rectal mucosae during homosexual intercourse most certainly would be expected to lead to enhanced rates of infection. Most infections arise from heterosexual intercourse, which must be assumed to be nontraumatic in nature, and transmission by artificial insemination has even been reported (35, 36). Similarly, studies using the rhesus macaque SIV model have shown that these animals can be infected via the cervicovaginal, rectal, and urethral routes (25, 37–39); in addition, vaginal transmission of HIV infection in the chimpanzee has been demonstrated (40). In all of these animal studies careful attention was paid to avoid trauma when virus was applied to the mucosal site.

The target cells for HIV infection in mucosal sites have not been unequivocally identified, although there are several candidates. In the female genital tract, CD4+ T lymphocytes, cells of the monocyte/macrophage lineage and Langerhans cells are present in the vaginal epithelial submucosa (41) and have been shown to contain HIV which is infectious (42). Much less is known regarding the immunology of the male genital tract (43).

The gastrointestinal tract is clearly a key target of HIV infection. The mechanisms by which the gut may be infected are unclear at present but may include tissue injury, with resulting entry of virus which comes directly into contact with CD4+ cells, direct binding and internalization by intestinal epithelium, and uptake and transport by intestinal M cells to underlying lymphocytes and macrophages. Although it is known that CD4-bearing lymphoid cells are abundant in this tissue and can be shown to be infected with HIV (44), the question of whether mucosal epithelia can be *directly* infected has remained somewhat controversial. It has been reported that viral RNA can be detected in intestinal epithelium of biopsies obtained from infected patients (45, 46). Several other studies have shown that cells of intestinal epithelial origin can be infected *in vitro*, including cell lines (47–49) as well as freshly isolated cells (50, 51). Such infection can be achieved at least some of the time via a CD4-independent mechanism (48, 49, 52). In this regard, the glycosphingolipid galactosylceramide has been implicated as the alternate HIV receptor on intestinal epithelial cells and perhaps those of the genital tract as well (52).

HIV-1 has also been shown to be taken up and transported by intestinal M cells (53), which constitute a specialized epithelium above the lymphoid follicles (54). They are important in sampling of gut antigens and transporting such antigens to underlying lymphoid tissues. It is possible that M cells might function in delivery of the virus to intraepithelial lymphocytes and macrophages, without themselves becoming infected. However, these types of cells have not as yet been detected in the genital tract.

Mucosal immunity and physiology of the intestinal tract are profoundly influenced directly as well as indirectly in HIV infection. A variety of intestinal disorders, such as malabsorption and diarrhea, is known to occur commonly as complications of HIV-1 infection (44, 55). These disorders are frequently not associated with opportunistic infections, and it has been speculated that they result from primary HIV-1 disease (56). Ultrastructural studies have shown that HIV-1 induces hypertrophy of the Golgi complex and alters differentiation of the apical membrane of HT29 cells (57, 58).

Thus, although the basis for HIV-induced intestinal disease has not been established, the possibility that enteropathy is a direct result of virus infection remains real and provokes further concern with respect to prevention of infection of the gastrointestinal tract.

WHAT IS THE RELATIONSHIP BETWEEN MUCOSAL AND SYSTEMIC IMMUNITY?

Extensive studies have clearly shown that the immune system can be divided into two functionally independent compartments: systemic, represented by the bone marrow, spleen, thymus, and lymph nodes; and mucosal, represented by lymphoid tissues in mucosae and external secretory glands (13, 15, 59). For the development of vaccines, this compartmentalization is essential; induction of an immune response in one of these two systems may not necessarily be reflected in the other (1, 16, 60). In general, systemic immunization induces poor mucosal immunity; however, mucosal immunization offers the advantage in that some antigen delivery systems induce both mucosal and systemic immunity. Furthermore, these two systems do not display a parallel maturation pattern, and the products of immunocytes (i.e., antibodies and cytokines) differ in their quality and quantity (1, 13, 61).

Comparison of the molecular properties of antibodies in human serum and external secretions, on one hand, and of antibody-producing cells, on the other, clearly illustrates the remarkable independence of the two systems (13–15). It has been known for almost 30 years that polymeric IgA (pIgA) locally produced by the large number of plasma cells distributed in mucosal tissues and secretory glands is selectively transported by a receptor-mediated pathway through epithelial cells into external secretions (62, 63). In humans, plasma-derived immunoglobulins (Ig) of the IgG and IgA classes constitute only a minute fraction of secretory antibodies due to their inability to interact with the polymeric Ig receptor, also called secretory component (SC) expressed on epithelial cells. Numerous studies performed in humans demonstrated that usually less than 1% of antibodies in saliva, nasopharyngeal secretions, intestinal fluid, and bile are of plasma origin (62, 64). Thus, neither systemic active immunization with antigen nor passive administration of preformed antibodies is of value for protection of mucosal surfaces to prevent the initial entry of pathogens. Consequently, mucosal surfaces can be colonized by bacteria, and viruses can infect epithelial cells in spite of the presence of corresponding antibodies in the circulation. For example, systemic immunization with inactivated poliovirus may prevent the development of poliomyelitis but does not prevent infection in the gastrointestinal tract or in tonsils (65). Thus, these findings should be considered with increased awareness in the development of all vaccines destined to combat infections encountered through mucosae, including HIV. Furthermore, the ontogenies of the serum and secretory IgA systems display characteristic and independent patterns of maturation. Adult levels of mucosal IgA in external secretions are reached considerably earlier (1 month–2 years) than those of serum IgA (adolescence) (13, 61).

The cells engaged in the production of antibodies destined for systemic and mucosal compartments display different tissue distribution and origin of precursors (13, 66, 67). Approximately 70% of all Ig-producing cells in the human body are found in mucosal tissues; the remaining 30% are found mainly in the bone marrow (the most important source of plasma IgG and IgA) and much less in the spleen and lymph nodes. In mucosae, some 80% of total Ig-producing plasma cells secrete IgA (13, 14, 59, 62). Precursors of these cells originate in the IgA-inductive sites such as gut-associated lymphoid tissues (GALT) including Peyer's patches (PP), solitary lymphoid nodules, and probably rectal tonsils (see below), bronchus-associated lymphoid tissues (BALT), and perhaps other potential inductive sites, such as palatine tonsils.

Less is known about the role of cell-mediated immunity (CMI), cytotoxic T lymphocytes (CTL), γδ T cells (68), and natural killer (NK) cells in the defense of mucosal membranes including the genital tract (8). The unavailability of mucosal tissues in sufficient quantities, usually low yields of isolated lymphocytes, and difficulties in generating virus-infected targets (for CTL) from outbred species (macaques, chimpanzees and human) present considerable obstacles in performing such studies.

THE COMMON MUCOSAL IMMUNE SYSTEM (CMIS) AND IgA-INDUCTIVE SITES: IS THE GENITAL TRACT A COMPONENT OF CMIS?

Extensive studies concerning the origin of precursors of mucosal IgA plasma cells revealed that the organized lymphoepithelial structures found

along the gastrointestinal and respiratory tracts are the main source of such cells (66, 67, 69). 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, such as interleukin-6 (IL-6), transforming growth factor β (TGFβ), and IL-10 derived from T cells and mucosal epithelial cells (70, 71). In animals, the evidence for this IgA cycle is based primarily on the adoptive transfer of cells from the GALT and BALT into recipients whose mucosal tissues and glands were populated by IgA plasma cells of donor origin. Furthermore, the oral administration of antigen led to the appearance of specific secretory IgA (S-IgA) in milk of immunized animals (72). These results were further validated and extended in many subsequent studies performed in a large number of animal species using diverse microbial antigens (16, 24, 60). Such specific S-IgA antibodies also appeared in secretions of the intestinal and respiratory tracts, as well as in tears, saliva, and milk.

Evidence for the existence of the CMIS in humans has been strengthened in recent years by several studies. In addition to the detection of specific S-IgA antibodies in remote secretions induced by natural exposure to antigens or oral immunization, analyses of IgA-secreting cells from peripheral blood and mucosal tissues provided strong evidence for this concept (16, 60, 73–76). These results provide a sound physiological basis for rational immunization protocols that exploit the potential of the CMIS—the design of vaccines that induce protective immunity at the portals of entry of most pathogens.

Although GALT, represented by ileojejunal PP, and BALT have been considered as primary sources of precursors of mucosal IgA plasma cells, additional lymphoid structures elsewhere in the body may serve a similar purpose. Accumulations of lymphoid tissues, such as palatine, lingual, and nasopharyngeal tonsils which constitute Waldeyer's ring, are strategically positioned at the beginning of the digestive and respiratory tracts (77–80). Several observations, summarized by Brandtzaeg and Halstensen (77) have suggested that these lymphoid tissues may serve as a source of precursors of IgA plasma cells found in the upper respiratory and digestive tracts.

Follicular structures analogous to PP are also found in the large intestine, with especially pronounced accumulations in the rectum (81–83). The potential importance of the rectal lymphoid tissues as an IgA-inductive site and as a source of IgA plasma cell precursors is suggested by several studies. In humans, the distribution of plasma cells producing IgA1 and IgA2 antibodies in the lamina propria of the large intestine differs from that of other mucosal tissues by the pronounced predominance of IgA2 plasma cells (84–86). The fact that this is also the case in the female genital mucosal tissues (uterus, cervix, fallopian tubes, and vagina) (87) suggests that the rectal lymphoid tissues may be an important source of IgA precursors destined for the genital tract. However, it has not been conclusively established if in humans the genital tract is an integral component of the CMIS. It is unknown at present whether antibody-producing cells and T cells found in the genital tract originate, by analogy with other mucosal effector sites, mostly from PP or rectal lymphoid tissues. Furthermore, the effectiveness of oral, intravaginal, and rectal immunization routes (or their combinations) and systemic immunization in the induction of immune responses in human genital tract will require further evaluation.

Considered in the context of the CMIS, these studies suggest that further subcompartmentalization may exist and be controlled by a pronounced preference of homing of IgA plasma cell precursors. Thus, certain IgA-inductive sites provide precursor lymphocytes for a particular effector site. By extension, the intranasal route of immunization may be less effective in the induction of S-IgA in the genital tract than the introduction of antigens into the rectum. Thus, further comparative studies of the distribution of specific S-IgA antibodies in various external secretions induced by diverse mucosal immunization routes should be performed to address this point with implications important in the design of effective vaccines.

IMMUNOGLOBULINS (Ig) IN THE GENITAL TRACT: WHAT IS THEIR ORIGIN AND FUNCTION?

Early studies of the secretions of the female genital tract suggested that antibodies found in genital tract secretions originated from two compartments: the upper genital tract (fallopian tubes and uterus)

and the lower genital tract, which is chronically colonized by mucosal microorganisms (for review see Refs. 88 and 89). The upper genital tract secretions are the products of the peritoneal cavity, fallopian tube effluent, and hormonally dependent endometrial epithelium and stroma. These secretions are not readily accessible without invasive measures and appear to have lower rates of secretion than those of the lower tract. While vaginal fluid can be collected and quantitated separately, the cervicovaginal junction is a physiologic unit and the secretions there are usually collected and evaluated together.

Relative Ig concentrations in the genital tract are dependent on hormonal and local factors such as the presence of inflammation and probably the use of vaginal douches and contraceptives. The albumin/IgG ratio in cervical mucus, which is constant throughout the menstrual cycle, approximates that of serum. This suggests that both components originate from the systemic circulation. The IgA of cervical mucus is represented by S-IgA with a small component of monomeric IgA and IgM (88, 90, 91). This finding implies that the IgA in cervical mucus is predominantly of local origin. In the upper vaginal secretions of postmenopausal women the IgG level was reduced by ~50% after hysterectomy, but the IgA level was reduced 15-fold (92). Hysterectomy reduced IgA in vaginal fluid to 5% of controls but did not significantly alter IgG in mouse vaginal fluid (93). This demonstrates again the importance of the cervical contribution to the local IgA pool.

In comparison to external secretions of human salivary, lacrimal, and mammary glands and gastrointestinal fluids, the physicochemical properties of Ig found in the female genital tract have received less attention. Consequently, information is not available concerning the levels of mIgA and pIgA, presence of SC and J chain in pIgA molecules (indicating their local selective transport through epithelial cells rather than transudation), and proportion of total as well as antigen-specific IgA1 and IgA2 antibodies in fluids collected from fallopian tubes, uterus, and vagina, and the distribution and molecular properties of Ig isotypes in the genital tract secretions have not been well-defined in health or disease. Yet this information will be essential for the design of vaccines that should induce protective humoral immunity throughout the menstrual cycle and especially at ovulation when S-IgA becomes the dominant isotype in women's genital secretions (W. H. Kutteh, J. Mestecky, submitted for publication).

The presence and frequency of Ig-producing cells of IgA (including subclasses), IgG, or IgM isotypes and the presence of J chain in these cells and of SC in epithelial cells of female genital mucosa were determined by the immunofluorescence technique using antibodies that recognize various epitopes of the component polypeptide chains (87, 94). The endocervix and ectocervix displayed the highest accumulation of Ig-forming cells and such cells produced antibodies predominantly of the IgA isotype.

A large number of IgA-positive cells costained with reagents that recognize J chain; this finding strongly suggests that they were engaged in the synthesis of pIgA (62). SC was occasionally detected in the endometrial glands and at a high frequency in the epithelial cells of the endocervix; squamous epithelial cells were SC negative. Furthermore, examination of tissues positive for IgA-producing cells with monoclonal antibodies that distinguish human IgA1 and IgA2 subclasses (84, 85) revealed approximately equal proportions of cells positive for IgA1 or IgA2. Comparison of the distribution of IgA1- or IgA2-producing cells in various human lymphoid and mucosal tissues (84–86) indicated that, in this respect, the female genital tract strongly resembles the lower intestinal tract.

If we accept the premise that specific antibodies in plasma and in external secretions are important in protection against viral infections (22), including HIV and SIV (95), their induction in infected and immunized individuals should be of benefit to the host. Although mucosal antibodies can neutralize free viruses, little is known about their ability to interfere with intracellular viral assembly. This is especially important in HIV infection because the virus is present in the free form as well as in cells in the form of incompletely assembled virus, viral RNA, or unintegrated and integrated DNA provirus (96, 97). However, recent *in vitro* studies have suggested that epithelial cells which express specific receptors for pIgA and IgM (SC) internalize such antibodies and, by an unknown mechanism, interfere with viral infection, presumably by intracellular neutralization (98). These findings are relevant to HIV infection because epithelial cells that express SC are infectable by the virus. Furthermore, CD4+ monocytes/macrophages also express receptors for Fc of IgA (99), which is internalized. The effect of HIV-specific IgA on virus infectivity and intracellular neutralization has not been evaluated (100).

HIV as well as HIV-specific antibodies have been detected in virtually all external secretions (7). However, some standard markers for mucosal immunity appear depressed in HIV-infected patients: Salivary IgA levels are actually significantly decreased (101, 102), and it has been suggested that this is a consequence of selective depletion of IgA2 (102). Further, the numbers of IgA plasma cells in the gastrointestinal tract are decreased (103, 104) and the IgA subclass ratio which normally characterizes these cells (see below) is altered such that the numbers of IgA2 cells are often markedly depleted (103).

Although levels of IgA in serum are second only to those of IgG (105), the function of serum IgA has essentially remained enigmatic. However, many diverse functions of S-IgA have been shown, including virus neutralization (1, 106). Intracellular neutralization of virus by IgA antibodies is a recently described function of IgA (98) which has been suggested but not yet investigated with respect to protection against HIV disease (100).

We (107) and others (108) have recently studied whether IgA antibodies isolated from the sera of HIV-infected individuals can neutralize HIV *in vitro* and have found that serum IgA anti-HIV has a limited capacity to perform this function, although neutralization was clearly accomplished by IgA isolated from some donors. However, the ability of mucosally derived IgA antibodies to neutralize HIV has not been studied, and elucidation of the potential function of S-IgA anti-HIV isolated from external secretions is important.

IgA antibodies have also been shown to mediate antibody-dependent cell mediated cytotoxicity (ADCC) (106). Our own findings in this regard indicate that IgA isolated from the sera of HIV-infected donors is equally competent to IgG isolated from the same individuals in mediation of ADCC of HIV-infected cells (109). Again, the role of S-IgA anti-HIV antibodies in ADCC remains to be determined.

A potential function of IgA antibodies that has not been adequately addressed is IgA-mediated enhancement of HIV infection. It is well-known that anti-HIV antibodies can be shown under defined conditions to enhance infection of susceptible cells, provided that the cells have receptors for the Fc portion of anti-HIV antibodies or for complement components which bind to HIV-anti-HIV immune complexes. This phenomenon has been widely described for a variety of cell types and has raised questions regarding the potential dangers of vaccination against HIV (110, 111). Virtually all of these studies have been done using whole serum or purified IgG, and little attention has been paid to the potential for IgA anti-HIV antibodies to mediate enhancement of HIV infection. Since many cells (monocytes/macrophages, polymorphonuclear neutrophils, epithelial cells, and eosinophils) bear receptors for the Fc portion of IgA (15, 99, 112), this possibility must be considered, especially in light of the high levels of IgA1-containing circulating immune complexes known to exist in AIDS patients (113, 114). Although the presence of IgG receptors on rectal epithelium has prompted concerns about the potential for IgG antibodies to mediate enhancement of infection of the gastrointestinal tract (115), similar concerns regarding IgA mediated-enhancement of intestinal epithelium, possibly via the polymeric immunoglobulin receptor (SC) on epithelial cells, should be addressed. Interestingly, in an analogous study, pIgA antibodies to Epstein–Barr virus (EBV) have been shown to carry EBV into HT29 cells not normally susceptible to this virus (116).

Analyses of purified serum IgA from HIV-infected individuals have indicated that specific IgA antibodies can be regularly demonstrated only in the IgA1 subclass (117, 118); dominant IgA1 anti-HIV antibodies were also observed in two S-IgA samples isolated from colostrum of healthy HIV-seropositive mothers (118). Our laboratory has observed that IgA1 antibodies seem to be directed almost exclusively against *env* glycoproteins. In many subjects, a total lack of IgA1 reactivity to *gag* and *pol* proteins was accompanied by intact IgG responses to these same antigens (118). It is important to emphasize that this striking restriction of the IgA anti-HIV response to the IgA1 subclass is of potential functional importance, since this isotype is exquisitely sensitive to IgA1 proteases produced by many pathogenic organisms responsible for secondary infections in AIDS patients (22).

INDUCTION OF IMMUNE RESPONSES IN THE GENITAL TRACT: IMPLICATIONS FOR HIV VACCINE DEVELOPMENT, ROUTES OF IMMUNIZATION, AND EFFECTIVE ANTIGEN-DELIVERY SYSTEMS

The ultimate goal of a potential HIV vaccine is to induce protective immunity systemically as well as at mucosal surfaces of the genital tract. The importance of the mucosal compartment in the protection

of the genital tract has been convincingly and dramatically demonstrated by Miller *et al.* (25, 119): Rhesus monkeys systemically vaccinated with inactived SIV were not protected against SIV applied to the genital mucosa. However, 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 (120, 121). Using SIV incorporated into microspheres, Marx *et al.* (26) have demonstrated that oral or intratracheal immunization following systemic priming induces a protective immune response against vaginal challenge with SIV.

Other studies performed in animal models have generally shown that the induction of mucosal immunity as measured by specific IgA and IgG antibody responses in the female reproductive tract requires intensive local immunization with repeated challenges of large doses of antigen (11, 88, 89, 122, 123). The intravaginal route of antigen administration is relatively ineffective (93, 124) in the induction of local immune responses unless a combination of mucosal (oral and rectal) and systemic immunization is used. The low efficiency of intravaginal immunization may be due to poor absorption of antigens and the apparent absence of an IgA-inductive site, analogous to GALT or BALT, in the genital tract. Furthermore, if the female genital tract were an efficient inductive site, repeated deposition of sperm during sexual intercourse should result in prompt induction of immune responses with reduced fertility.

Whereas systemic immunization resulted in lower local responses, booster immunization given in the vagina or uterine horns often resulted in an increased response in the reproductive tract. Recent comparative studies (125) of the effectiveness of vaginal, pelvic, and parenteral immunization in the induction of IgA and IgG antibodies in murine vaginal washings indicated that local (vaginal) immunization induced an unimpressive response. In contrast, subserous and intraperitoneal immunizations with the same antigen resulted in both IgA and IgG responses in this fluid, while subcutaneous injection induced only IgG antibodies. The authors speculated that the effectiveness of intraperitoneal immunization was due to vigorous stimulation of regional lymph nodes. However, an alternative explanation may be offered based on recent studies of the peritoneal origin of the precursors of mucosal IgA plasma cells (126). Thus, it is probable that this route of immunization stimulates peritoneal IgA precursors that subsequently populate the genital tract. Oral immunization has been attempted in laboratory experiments in mice and rats with limited success. Female mice immunized with homologous sperm by oral intubation show partial reduction in fertility. Allardyce and Rademaker (127) combined oral with vaginal booster immunization and were successful in demonstrating vaginal IgA anti-sperm antibodies in association with reduced fertility. In addition, in rats oral administration of sperm cells resulted in anti-sperm antibodies of the IgA class in vaginal fluids with antibody titers being associated with length of infertility (127).

Little is known concerning the induction of an effective immune response in the human female genital tract. The occurrence of specific antibodies in cervicovaginal secretions from women with sexually transmitted diseases and other local infections has supported the contention that a mucosal immune response can be induced in the genital tract (11, 88, 89, 122, 123). Active immunization of the cervical–vaginal mucosa by direct exposure to antigens has resulted in secretory antibody production (128). Local immunization of the vagina or uterus with inactivated polio virus resulted in specific IgA and IgG antiviral antibodies in vaginal secretions but only IgG antibodies were found in the uterus. The concept of an independent genital mucosal immune system is also supported by the identification of local IgA and IgG antibodies as well as systemic antibodies against sperm cells in some infertile men and women (129). Such antibodies may occur in sera or secretions concurrently or independently.

Systematic studies of the induction of humoral and cellular immune responses in the human genital tract by mucosal or systemic immunization routes (and their combination) have not been performed. In view of the independence of the mucosal and systemic immune compartments and predominant route of HIV acquisition, this information will be necessary for the design of potential vaccines protective against HIV as well as other sexually transmitted diseases.

Antigen-Delivery Systems for the Induction of Mucosal and Systemic Immune Responses

Empirical experience with mucosal immunization has resulted in a generally accepted conclusion that considerably high doses of antigens are required. This is due to the elimination of antigens by peristalsis and outflow of secretions, existence of effective mechanical (epithelial cells) and chemical (e.g., mucins) barriers, and degradation and denaturation of antigens by enzymes and acids. Thus, only minute quantities of fully potent antigens reach the mucosal lymphoid tissues. Consequently, several strategies recently reviewed (1, 18–21, 60, 130, 131) have been proposed to circumvent this problem.

The ability of some microorganisms to colonize and infect mucosa of the intestinal tract and the potential for inclusion of genes from unrelated microorganisms which code for relevant antigens represent an attractive possibility for design of novel vaccines effective in the protection of mucosal surfaces (132–134). Although several bacterial species, including genetically modified strains of *Salmonellae, Escherichia coli, Mycobacteria* (BCG), *Yersinia enterocolitica*, and *Lactobacilli* have been considered (24, 76), most of the experimental work has been performed with extensively attenuated by genetic modifications strains of *Salmonellae* and, more recently, BCG (135–138). In addition, polio- and adenoviruses (139, 140) have also been considered as vectors suitable for mucosal immunization. The development of recombinant vaccines based on the attenuated strains of poliovirus is attractive given the fact that the vaccine is routinely given to the population.

These approaches have several obvious advantages. Colonization and infection with live microorganisms are known to induce long-lasting, vigorous immune responses in both mucosal and systemic compartments (141). Furthermore, the possibility of introduction of genetic material coding for many different and unrelated antigens into a single microbial vector would reduce the need for multiple immunizations to a single dose and yet induce protection against several diseases. For example, oral immunization with BCG expressing genes encoding for HIV glycoproteins and fragments of tetanus toxin, or many other candidate antigens of medical importance, resulted in the induction of corresponding antibodies (137, 138). Furthermore, such vaccines would be easily administered and inexpensive to produce—factors that are of paramount importance for large-scale immunizations in the Third World countries. However, a number of related questions and problems associated with this approach must be considered. The most important aspect concerns the reusability of individual vectors. Immune responses are induced not only against the desired antigen expressed in a given microbial vector, but also to the vector itself (75). In point of fact, the response to the vector is dominant in some systems. Therefore, such immune responses could limit the effectiveness of subsequent secondary or tertiary immunizations with the same microorganism as recently demonstrated (75). However, multiple administrations of the Sabin poliovirus are routinely used for immunization.

Some of the antigens considered are poorly expressed in a given vector or are not released from the periplasmic space until after the death of a bacterium. Furthermore, the antigens expressed in a bacterial vector are likely to differ from the structure of such antigens derived from the original source. For example, the secondary and tertiary structures of native glycoproteins will be dissimilar to those expressed in *Salmonellae* or *E. coli*, due to the inability of these bacteria to glycosylate the protein, and thus the epitopes on a given glycoprotein may be altered. The absence of carbohydrates on gp120 of HIV expressed in bacterial vectors (50% of the total molecular mass of gp120 is contributed by N- and O-linked side chains) may pose serious problems concerning the relevance and specificity of antibodies induced by immunization with non-glycosylated proteins. Carbohydrate determinants apparently play an important role in the antigenicity of glycosylated HIV glycoproteins (142). The absence of carbohydrates would not be a limitation, though, for the generation of a cell-mediated immune response. Taken together, the limitations of using a single recombinant vaccine necessitates the development of several compatible approaches involving the use of different recombinant organisms in conjunction with non-recombinant-based methods (e.g., microencapsulated antigen).

A recent report has shown that plants such as tobacco, lettuce, tomato, or banana can be used for the expression of viral (hepatitis B) antigens (143). This novel approach has stimulated considerable interest in potential utilization of this "edible antigen delivery system" for an inexpensive mass immunization by the oral route in human and veterinary medicine.

Cholera toxin (CT) and its B subunit bind to all nucleated cells, especially to intestinal epithelial cells, through a specific GM 1 ganglioside receptor. CT is the most potent oral immunogen, and in addition, CTB promotes significant mucosal and serum antibodies to proteins when coadministered by the mucosal route (144, 145). Furthermore, coadministration of viral antigens with CT significantly enhances S-IgA antiviral responses in external secretions as well as in serum (144, 146).

To avoid their degradation and denaturation by pepsin and hydrochloric acid in the stomach, vaccine antigens have been incorporated into gelatin capsules, liposomes, and biodegradable microspheres, subsequently coated with substances that became soluble in the alkaline pH of the small intestine. Biodegradable microspheres have been used for systemic and oral immunizations in several recent studies (21, 26, 130, 131, 147). The microspheres are usually composed of biodegradable and biocompatible materials, such as poly DL-lactide-co-glycolide (DL-PLG) copolymers with antigens incorporated within such particles during their preparation. Controlled biodegradation, whose rates may range from several days to months depending on the lactide–glycolide proportion, proceeds by hydrolysis of ester bonds to yield the catabolizable products lactic and glycolic acids. Microspheres are absorbed from the gastrointestinal tract through PP, where they are retained and subsequently release antigen. The incorporation of antigens into biodegradable microspheres has several advantages including the protection of antigen from proteolysis and possible coincorporation of immunological adjuvants or cytokines that may further enhance the immune response. Furthermore, a single injection or ingestion of microspheres with programmed short and long biodegradative times may induce overlapping primary and long-lasting secondary immune responses, thus eliminating the need for booster immunization. Small microspheres (1–5 μm) were also absorbed but not retained in PP (131) and were found in the spleen and lymph nodes; thus, a concurrent systemic and secretory immune response may be induced by ingestion of microspheres of appropriate sizes. As a dry powder, microspheres containing antigens are stable, and with the small number of antigens tested thus far, the results indicate that their immunogenicity can be preserved for many months. Promising results have been obtained with more complex antigens, such as influenza virus (147) and SIV (26).

Finally, gene vaccines or the use of DNA encoding for relevant antigens are gaining deserved attention (148). Such vaccines given by the intramuscular as well as the mucosal (intranasal) routes have induced protective immune responses against influenza in animal models and the application of this technology to HIV and SIV vaccines is being pursued. In addition to the effectiveness of several immunization routes and minute amounts of DNA required, the antigens produced by the immunized individual are likely to resemble native antigens and contain relevant carbohydrate moieties.

CONCLUSIONS

The development of vaccines that would induce specific immune responses in the genital tract secretions would have far-reaching implications for not only the prevention of AIDS and sexually transmitted diseases but also the immunological control of fertility (4, 76, 88, 89, 123, 124, 149). Most of the currently studied vaccines utilize systemic routes of immunization which are of limited value for the prevention of mucosa-contracted diseases. The relative contribution of antigen-sensitized cells from PP or other inductive sites (e.g., rectal tonsils) to remote or adjacent effector sites (e.g., genital tract) as manifested by the appearance of corresponding S-IgA antibodies has not been studied extensively in humans despite its unquestionable practical importance. Exploration of immunization routes that are effective for induction of mucosal immune responses and that are based primarily on current knowledge of the origin of antibodies and of specific antibody-forming cells in mucosal tissues, together with novel antigen delivery systems (1, 76, 89, 130), is likely to reduce the incidence of many infectious diseases including AIDS and also reduce the cost of administration of such vaccines.

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