Published in final edited form as:
J Reprod Immunol. 2009 December ; 83(1-2): 196–200. doi:10.1016/j.jri.2009.07.005.

Mucosal Immunology of the genital and gastrointestinal tracts and HIV-1 infection

Jiri Mestecky[1,2,4], **Zina Moldoveanu**[1], **Phillip D. Smith**[1,2], **Zdenek Hel**[3], and **Rashada C. Alexander**[1]

[1] Department of Microbiology, University of Alabama at Birmingham, U.S.A

[2] Department of Medicine, University of Alabama at Birmingham, U.S.A

[3] Department of Pathology, University of Alabama at Birmingham, U.S.A

[4] Department of Microbiology and Immunology, Charles University, Prague, Czech Republic

Abstract

The male and female genital tracts are served by a local immune system that displays features distinguishing them from other mucosal sites. In contrast to the intestinal tract, where locally produced IgA is the dominant Ig, secretions of the male and female genital tract contain predominantly IgG of both local and systemic origin. Genital tract tissues also lack mucosal lymphoepithelial inductive sites analogous to intestinal Peyer's patches; consequently, local immunization or infections with sexually transmitted pathogens induce low immune responses. Human immunodeficiency virus 1 (HIV-1) infection must be primarily considered as a mucosal disease with extensive involvement of the systemic immune compartment. Although the majority of infections are acquired through the genital mucosa, a high rate of virus replication and profound $CD4^+$ T cell depletion occurs in the intestinal mucosa and other mucosal tissues shortly after infection. Evaluation of HIV-specific antibodies in sera and external secretions, including vaginal washes and semen, unexpectedly revealed a selective lack of IgA responses. Moreover, specific antibody-secreting cells in peripheral blood were of the IgG isotype, even in mucosally infected individuals. Whether humoral responses to previously or newly encountered antigens are compromised in HIV-1-infected persons is under current investigation.

1. Introduction

Mucosal tissues of the genital and intestinal tract are the most common sites of human immunodeficiency virus 1 (HIV-1) infection, with women infected at a higher frequency than men (Simon et al., 2006). Penetrating virus promptly infects subepithelial target cells, resulting in extensive depletion of the resident memory $CD4^+$ T cells in the intestine, as well as in other mucosal tissues (Brenchley et al., 2004; Hel et al., 2006; Mehandru et al., 2007). This profound depletion of mucosal $CD4^+$ T cells occurs irrespective of the route of initial entry. Although the mucosal tissues of the female genital tract are also severely affected, the biological parameters of HIV-1 replication in the genital mucosa and the implications for disease progression and transmission have not been well characterized. As a consequence of mucosal

Corresponding Author: Dr. Jiri Mestecky, Department of Microbiology, University of Alabama at Birmingham - Box 1, 845 19th Street South, Birmingham, AL 35294, USA, Tel: (205) 934-2225, Fax: (205) 934-3894, mestecky@uab.edu.

depletion of $CD4^+$T cells and the ensuing breakdown of essential immunoregulatory mechanisms, mucosal defenses are severely impaired, resulting in a high rate of secondary opportunistic infections that contribute significantly to the morbidity and mortality of HIV-1-infected patients. It appears that as a result of the damaged mucosal barrier, there is an increased absorption of microbial products into the systemic compartment which, in turn, contributes to chronic T cell activation, and eventual loss of $CD4^+$ T cell-mediated immunoregulation (Brenchley et al., 2006; Canani et al., 2003). Importantly, recent evidence suggests that the risk of HIV-1 infection and the rate of disease progression in HIV-1-infected women are significantly influenced by the application of certain types of hormonal contraceptives (Stringer et al., 2007).

Epidemiological, virological, and immunological evidence leads to the obvious conclusion that HIV-1 infection must be considered first and foremost as a mucosal disease - a conclusion of great importance and impact for the development of an HIV-1 vaccine.

2. Differences between the mucosal immune systems of the intestinal, female and male genital tracts and their relevance to HIV-1 infection

In sharp contrast to gastrointestinal secretions, saliva, tears and milk (in which secretory IgA (S-IgA) represents the dominant Ig isotype) human semen, cervicovaginal secretions and urine contain higher levels of IgG than IgA (Mestecky, 2007; Mestecky et al., 2005) (Table 1). Furthermore, the levels of Ig in female genital secretions are highly dependent on hormonal regulation and vary greatly at different stages of the menstrual cycle. Studies of the origin of Igs in genital secretions revealed that approximately half of the Igs are produced locally by plasma cells present in genital tract mucosa; the remaining Igs are derived from the circulation. This contrasts sharply with the origin of Igs in the intestine; under normal conditions, more than 90% of gut Igs are produced by plasma cells abundant in the subepithelial lamina propria. Thus, systemic immunization with HIV-1 antigens may induce substantial levels of HIV-1-specific antibodies in the secretions of the genital tract, but not in the intestinal tract, which would remain unprotected. Such considerations need to be taken into account in the design of strategies to induce humoral immune responses that block HIV-1 entry in both the genital and intestinal tract.

Less is known about the origin and molecular properties of Igs in male genital tract secretions. As in the female genital tract, IgG is the dominant Ig isotype, and limited studies of naturally-occurring and immunization-induced specific antibodies suggest that systemic immunization stimulates detectable responses in seminal plasma (Moldoveanu et al., 2005). Consequently, a sequential systemic and mucosal immunization will likely be required to achieve protection against HIV-1 entry at both mucosal sites.

3. HIV-1 entry into female genital and rectal mucosa

HIV-1 infection acquired through the female genital tract mucosa involves the translocation of virus across the epithelium, infection and replication in subepithelial mononuclear cells, and subsequent dissemination to initiate systemic infection. Studies aimed at the identification of entry, as well as target, cells in genital tissues have often yielded conflicting results (Broliden et al., 2009; Pope and Haase, 2003). The single layer of polarized, columnar epithelial cells in the endocervix may be more efficiently traversed by the virus than the multilayer, non-polarized squamous epithelium of the ectocervix and vagina. However, compounding factors, including microulcerations, mechanical breaks in the epithelium, co-infections with agents of sexually transmitted diseases, and the administration of hormonal contraceptives (see below) appear to enhance HIV-1 entry through the mucosal barrier. In addition to the epithelial cells, $CD4^+$ T cells, dendritic cells (DC) and Langerhans cells, which extend their processes between

epithelial cells and reach luminal surfaces, also may be involved in viral entry, although the results generated in various laboratories are somewhat discordant. Comparative immunohistochemical examination of human intestinal tract and vagina revealed major differences with respect to the density, distribution, and phenotypes of DC and Langerhans cells (Jameson et al., 2002) (Table 1). In the rectum, DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN) was copiously expressed throughout the lamina propria and $CCR5^+$ CD4 DCs were present just beneath the luminal epithelium. In sharp contrast, in the vagina, DC-$SIGN^+$ cells were absent within the vaginal epithelium, despite the abundance of Langerhans cells (Jameson et al., 2002). DC-$SIGN^+$ cells were detected in lamina propria and only a small proportion of these cells co-expressed CCR5. Thus, quantitative and qualitative differences in DCs and epithelial cell structures in the human intestinal and female genital tract tissues may contribute to the higher risk of HIV-1 transmission through rectal than vaginal intercourse.

After translocation across the epithelium, mononuclear cells in the lamina propria of the genital or intestinal tracts become primary targets of HIV-1 infection. Macrophages isolated from the intestinal lamina propria do not support HIV-1 replication, whereas vaginal macrophages do (Shen et al., 2009). This is likely due to the marked phenotypic differences between macrophages isolated from these two compartments, particularly the expression of CD4, CCR5 and low levels of CXCR4 receptors on vaginal, but not intestinal cells. Furthermore, unlike intestinal macrophages, cells from the vagina and ectocervix express receptors for bacterial lipopolysaccharides (CD14), IgA (CD89) and IgG (CD16, CD32, and CD64). Although both macrophages and T cells from the vagina support HIV-1 replication, only T cells from the intestine do so. Interestingly, rectal macrophages appear to be permissive to HIV-1 infection and replication.

4. Hormonal contraception and altered immunity to HIV-1 infection

Safe and effective methods of pregnancy prevention represent a critical component of health care with respect to reducing infant mortality, especially in women infected with HIV-1. Consequently, the use of highly efficient contraceptives, such as depot medroxy-progesterone acetate (DMPA), has gained world-wide acceptance. Unfortunately, multiple studies in humans and rhesus macaques suggest an association between the use of progesterone-based contraceptives and an increased risk of HIV-1 or simian immunodeficiency virus (SIV) infection, higher viral burden, increased viral shedding, and broadened viral diversity (Baeten et al., 2005; Marx et al., 1996; Stringer and Antonsen, 2008). Furthermore, recent studies indicate that women who use progesterone-based contraceptives display accelerated disease progression and mortality compared to women who do not use hormonal contraception (Stringer et al., 2007). Although the molecular and cellular pathways involved in these undesirable outcomes are not clear, progesterone and its derivatives regulate a number of immunological mechanisms, including the inhibition of the cytotoxic activity of T and NK cells, decreased production and altered glycosylation of IgG and IgA antibodies (thereby altering effector functions of both Igs), modulation of cytokine production, changes in lymphocyte migration and proliferation, and upregulation of HIV-1 receptor expression on $CD4^+$ T cells. These data suggest that hormonally-based contraceptives may perturb the mucosal defense mechanisms operational in the female genital tract resulting in an increased risk of HIV-1 infection and accelerated disease progression.

5. The role of mucosal antibodies in the prevention of HIV-1 or SIV infection

The primary aim of all vaccines is to induce humoral and cellular immune responses to prevent infection and disease. The correlates of protection against HIV-1 infection and prevention of disease progression to AIDS have not been unambiguously defined. HIV-1-specific antibodies, particularly those capable of virus neutralization, and cytotoxic T cells have been considered

in prevention and local limitation of viral infection at mucosal sites of HIV-1 or SIV entry (Shacklett et al., 2009).

The protective role of antibodies in the prevention of mucosal infection has been demonstrated in the macaque SIV model. Systemically administered monoclonal virus-neutralizing antibodies of the IgG isotype protected female monkeys from vaginal viral challenge (Baba et al., 2000; Mascola et al., 2000). Because IgG from the circulation contributes significantly to the secretions of the female genital tract, it is likely that these antibodies exhibited their protective effect by interaction with the virus in the vagina and prevented its entry. The local protective effect of antibodies was demonstrated by direct vaginal application before the viral challenge (Veazey et al., 2003). It should be recognized, however, that the neutralizing monoclonal antibodies were of the IgG isotype. Furthermore, systemic (intramuscular) and/or mucosal (intranasal) immunization of macaques with HIV-1_{SF162} envelope protein induced protective immune responses upon intravaginal challenge with homologous $SHIV_{SF162P4}$ (Barnett et al., 2008). Serum neutralizing antibodies against the challenge virus correlated with protection. The presence and demonstrated protective effect of antibodies, irrespective of the route of immunization, is likely due to the fact that both locally produced and circulating systemic antibodies contribute to a significant degree to the pool of Ig in genital tract secretions (Mestecky et al., 2005). Whether HIV-1- or SIV-neutralizing (or even non-neutralizing) antibodies of the IgA and IgM isotypes also display protective activities has not been evaluated in this model. However, IgA antibodies induced in mice by mucosal immunization with gp41 envelope protein derived from HIV-1 were capable of blocking *in vitro* transcytosis of HIV-1 through tumor colonic carcinoma or endometrial epithelial cell lines grown in a confluent monolayer (Matoba et al., 2004).

The protective activities of IgA antibodies specific for HIV-1 have been inferred from studies of semen or vaginal washes collected from HIV-1-exposed, but seronegative sex-workers (for reviews, see (Alexander and Mestecky, 2007; Hirbod and Broliden, 2007; Mestecky, 2007; Miyazawa et al., 2009). The authors of these studies suggested that local IgA antibodies induced by viral exposure protect by interaction with HIV-1 in mucosal secretions or within epithelial cells, which internalize the virus as well as IgA (Broliden et al., 2001; Devito et al., 2002; Kaul et al., 2001). In contrast, several subsequent studies have not confirmed the presence of HIV-1-specific IgA antibodies in such cohorts (Dorrell et al., 2000; Skurnick et al., 2002). To resolve this apparent controversy, comparative blind studies need to be performed to exclude highly probable difficulties with reliable analyses of IgA immune responses to HIV (Wright et al., 2002). It is highly unlikely that low IgA responses are related to the differences in HIV clades in North America, Europe or Africa. IgG antibodies are reliably detected with remarkable concordance among six different laboratories in sera and external secretions of HIV-infected individuals, irrespective of HIV clades or geographical location (Wright et al., 2002). However, it is possible that HIV inherently stimulates low IgA, but not IgG, responses due to genetically-dependent variances in the association of specific antibodies with selected Ig isotypes among humans, as well as a variety of vertebrate species. In this regard, HIV-1 in infected humans or chimpanzees, and SIV in infected macaques stimulate excellent IgG responses in sera and external secretions, while IgA responses are weak or absent in the majority of subjects (Belec et al., 1995; Israel and Marx, 1995; Mestecky et al., 2004; Raux et al., 1999; Schafer et al., 2002; Wright et al., 2002). Interestingly, HIV-1-specific IgG antibodies may exhibit their protective effect as mediators of antibody-dependent cell-mediated cytotoxicity (ADCC); cervical fluids collected from women with high titers of specific IgG antibodies in sera and secretions displayed lower genital viral loads than women with only serum antibodies (Nag et al., 2004).

The puzzling paucity of IgA responses in HIV-1-infected humans and chimpanzees, and SIV-infected macaques, or humans immunized mucosally with experimental HIV vaccines deserves

further investigation. It has been amply documented that mucosal infections or immunizations with viral or bacterial vaccines commonly induce preferential IgA responses in external secretions (Boyaka et al., 2005). Obviously, this is not the case with HIV or SIV. Whether the absence or low level of IgA responses to HIV-1 and SIV in infected subjects extends to previously or newly encountered antigens and probable alterations in underlying immunoregulatory mechanisms remains to be addressed. Furthermore, the inescapable realization that HIV-1-infection is predominantly a mucosal disease should re-focus future studies with due consideration to the evaluation of both humoral and cellular mucosal immune responses. It is disheartening to accept that in hundreds of world-wide HIV vaccine trials, mucosal responses are totally disregarded (Lehner et al., 2005) and the functionally distinct and immunologically unique features of the genital and intestinal mucosal immune systems are mostly not accounted for in the design and delivery of experimental HIV-1 vaccines and analysis of ensuing immune responses. This is especially applicable to the analyses of the highly desirable induction of HIV-1-neutralizing antibodies; sera are routinely evaluated, but external secretions of the genital and intestinal tract, as the most common sites of infection, are inexplicably ignored. Thus, concerted efforts of research teams and funding agencies will be required to rectify this obvious deficiency.

References

Alexander R, Mestecky J. Neutralizing antibodies in mucosal secretions: IgG or IgA? Curr HIV Res 2007;5:588–93. [PubMed: 18045115]

Baba TW, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med 2000;6:200–6. [PubMed: 10655110]

Baeten JM, et al. Effect of contraceptive methods on natural history of HIV: studies from the Mombasa cohort. J Acquir Immune Defic Syndr 2005;38(Suppl 1):S18–21. [PubMed: 15867603]

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

Belec L, et al. Cervicovaginal overproduction of specific IgG to human immunodeficiency virus (HIV) contrasts with normal or impaired IgA local response in HIV infection. J Infect Dis 1995;172:691–7. [PubMed: 7658060]

Boyaka, PN., et al. Mucosal vaccines: an overview. In: Mestecky, J., et al., editors. Mucosal Immunology. Vol. 3. Elsevier/Academic Press; Amsterdam: 2005. p. 855-873.

Brenchley JM, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12:1365–71. [PubMed: 17115046]

Brenchley JM, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004;200:749–59. [PubMed: 15365096]

Broliden K, et al. Introduction: Back to basics: mucosal immunity and novel HIV vaccine concepts. J Intern Med 2009;265:5–17. [PubMed: 19093956]

Broliden K, et al. Functional HIV-1 specific IgA antibodies in HIV-1 exposed, persistently IgG seronegative female sex workers. Immunol Lett 2001;79:29–36. [PubMed: 11595287]

Canani RB, et al. Effects of HIV-1 Tat protein on ion secretion and on cell proliferation in human intestinal epithelial cells. Gastroenterology 2003;124:368–76. [PubMed: 12557143]

Devito C, et al. Cross-clade HIV-1-specific neutralizing IgA in mucosal and systemic compartments of HIV-1-exposed, persistently seronegative subjects. J Acquir Immune Defic Syndr 2002;30:413–20. [PubMed: 12138348]

Dorrell L, et al. Absence of specific mucosal antibody responses in HIV-exposed uninfected sex workers from the Gambia. AIDS 2000;14:1117–22. [PubMed: 10894275]

Hel Z, et al. HIV infection: first battle decides the war. Trends Immunol 2006;27:274–81. [PubMed: 16679064]

Hirbod T, Broliden K. Mucosal immune responses in the genital tract of HIV-1-exposed uninfected women. J Intern Med 2007;262:44–58. [PubMed: 17598814]

Israel ZR, Marx PA. Nonclassical mucosal antibodies predominate in genital secretions of HIV-1 infected chimpanzees. J Med Primatol 1995;24:53–60. [PubMed: 8613973]

Jameson B, et al. Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. J Virol 2002;76:1866–75. [PubMed: 11799181]

Kaul R, et al. Mucosal IgA in exposed, uninfected subjects: evidence for a role in protection against HIV infection. AIDS 2001;15:431–2. [PubMed: 11273233]

Lehner T, et al. European Union and EDCTP strategy in the global context: recommendations for preventive HIV/AIDS vaccines research. Vaccine 2005;23:5551–6. [PubMed: 16153752]

Marx PA, et al. Progesterone implants enhance SIV vaginal transmission and early virus load. Nat Med 1996;2:1084–9. [PubMed: 8837605]

Mascola JR, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000;6:207–10. [PubMed: 10655111]

Matoba N, et al. A mucosally targeted subunit vaccine candidate eliciting HIV-1 transcytosis-blocking Abs. Proc Natl Acad Sci U S A 2004;101:13584–9. [PubMed: 15347807]

Mehandru S, et al. Mechanisms of gastrointestinal CD4+ T-cell depletion during acute and early human immunodeficiency virus type 1 infection. J Virol 2007;81:599–612. [PubMed: 17065209]

Mestecky J. Humoral immune responses to the human immunodeficiency virus type-1 (HIV-1) in the genital tract compared to other mucosal sites. J Reprod Immunol 2007;73:86–97. [PubMed: 17354294]

Mestecky J, et al. Paucity of antigen-specific IgA responses in sera and external secretions of HIV-type 1-infected individuals. AIDS Res Hum Retroviruses 2004;20:972–88. [PubMed: 15585085]

Mestecky J, et al. Immunologic uniqueness of the genital tract: challenge for vaccine development. Am J Reprod Immunol 2005;53:208–14. [PubMed: 15833098]

Miyazawa M, et al. The ‘immunologic advantage’ of HIV-exposed seronegative individuals. AIDS 2009;23:161–75. [PubMed: 19098485]

Moldoveanu Z, et al. Human male genital tract secretions: both mucosal and systemic immune compartments contribute to the humoral immunity. J Immunol 2005;175:4127–36. [PubMed: 16148163]

Nag P, et al. Women with cervicovaginal antibody-dependent cell-mediated cytotoxicity have lower genital HIV-1 RNA loads. J Infect Dis 2004;190:1970–8. [PubMed: 15529262]

Pope M, Haase AT. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. Nat Med 2003;9:847–52. [PubMed: 12835704]

Raux M, et al. Comparison of the distribution of IgG and IgA antibodies in serum and various mucosal fluids of HIV type 1-infected subjects. AIDS Res Hum Retroviruses 1999;15:1365–76. [PubMed: 10515152]

Schafer F, et al. Lack of simian immunodeficiency virus (SIV) specific IgA response in the intestine of SIV infected rhesus macaques. Gut 2002;50:608–14. [PubMed: 11950804]

Shacklett BL, et al. Mucosal T-cell responses to HIV: responding at the front lines. J Intern Med 2009;265:58–66. [PubMed: 19093960]

Shen R, et al. Macrophages in vaginal but not intestinal mucosa are monocyte-like and permissive to HIV-1. J Virol. 2009 (In press).

Simon V, et al. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. Lancet 2006;368:489–504. [PubMed: 16890836]

Skurnick JH, et al. Correlates of nontransmission in US women at high risk of human immunodeficiency virus type 1 infection through sexual exposure. J Infect Dis 2002;185:428–38. [PubMed: 11865394]

Stringer E, Antonsen E. Hormonal contraception and HIV disease progression. Clin Infect Dis 2008;47:945–51. [PubMed: 18715161]

Stringer EM, et al. A randomized trial of the intrauterine contraceptive device vs hormonal contraception in women who are infected with the human immunodeficiency virus. Am J Obstet Gynecol 2007;197:144–8. [PubMed: 17689627]

Veazey RS, et al. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat Med 2003;9:343–6. [PubMed: 12579198]

Wright PF, et al. Detection of mucosal antibodies in HIV type 1-infected individuals. AIDS Res Hum Retroviruses 2002;18:1291–300. [PubMed: 12487817]

Table 1

Comparative immunological features of the human intestinal and genital tracts

	Intestinal	Genital
Ig isotypes	IgA ⋙ IgG	IgG > IgA In male and female secretions
Molecular forms of IgA	S-IgA ⋙ pIgA > mIgA	S-IgA = pIgA = mIgA
	IgA1 > < IgA2 (IgA2 dominant in the large intestine)	IgA1 > IgA2 (IgA2 dominant in the female secretions)
Origin of Ig	Local ⋙ systemic	Local = systemic
Hormonal regulation	–	+++
Epithelium	Single layer of polarized enterocytes	Stratified multilayered cells in vagina and ectocervix
DC/Langerhans' cells		
DC-SIGN	+++	–
CCR 5	+++	+
Macrophages		
CD 14	–	+
CD 89 (FcαR)	–	+
CD16 (FγR)	–	+
CD4	-	++
HIV-1 infectable	–	+
Plasma cells	IgA ⋙ IgG	IgG > IgA Endocervix
	IgA1 > IgA2 (small intestine)	IgA2 > IgA1
	IgA2 > IgA1 (large intestine)	
Lymphoepithelial inductive sites	++	–
Humoral responses to:		
Local infections or immunizations	+++	±
Systemic immunization	±	++
Intranasal immunization	±	++