Published in final edited form as:
Am J Reprod Immunol. 2011 March ; 65(3): 361–367. doi:10.1111/j.1600-0897.2010.00923.x.

Methods for Evaluation of Humoral Immune Responses in Human Genital Tract Secretions

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Abstract

The compilation of epidemiological, virological, and immunological data clearly indicates that HIV-1 infection must be considered primarily as a disease of the mucosal immune system. The earliest and most dramatic alternations of the immune system occur in the mucosal compartment. However, the mucosal immune systems of the genital and intestinal tracts display remarkable immunological differences that must be considered in the evaluation of humoral immune responses in HIV-1 infected individuals or in volunteers immunized with experimental HIV vaccines. In this regard, marked differences in the dominant Ig isotypes, molecular forms of HIV-1-specific antibodies, and their distinct effector functions in the genital versus intestinal tracts must be carefully evaluated and considered in the measurement and interpretation of humoral immune responses. Appropriate controls and alternative 5immunochemical assays should be used to complement and confirm results generated by ELISA, which are prone to false positivity. Special precautions and rigorous controls must be used in the evaluation of antibody-mediated virus neutralization in external secretions of the genital and intestinal tracts.

Keywords

Antibodies; External secretions; HIV; Mucosal immunity

INTRODUCTION

The correct collection and processing of individual external secretions, as well as the use of appropriate immunochemical assays, are of paramount importance for the reliable evaluation of humoral immune responses to microbial infections or vaccinations. In a sharp contrast to serum or plasma, external secretions display several characteristic features that must be considered in the collection, processing, storage, and measurement of antibody responses[1-7]. With the exception of human colostrum collected at the very onset of lactation, all other external secretions contain much lower and enormously variable levels of immunoglobulins (Igs)[2] (Table I). This marked variability is due to the method of collection, dilution of the specimen (e.g., cervicovaginal secretion) with lavage fluid, variations in flow rates upon stimulation (e.g., parotid saliva or tears), the presence of endogenous and exogenous proteolytic enzymes which degrade Igs, binding of Igs to other components such as mucus, and the humoral status of the individual[2]. Furthermore, repeated freezing and thawing or

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lyophilization of external secretions greatly enhances the high propensity of IgA towards irreversible aggregation and denaturation and results in the measurable loss of total, as well as antigen-specific antibodies. It is therefore imperative to express the level of specific antibodies in the context of total Ig levels of individual isotypes to compensate for the great variabilites in Ig levels and potential losses due to the processing and storage of secretions. Alternatively, Ig levels have been correlated with the levels of other proteins/glycoproteins, such as human serum albumin (HSA) or transferrin, that are not produced locally in mucosal tissues, but are derived exclusively from the circulation and are present in external secretions due to passive transudation[8]. Consequently, the comparison of the ratios of Igs to HSA in sera or plasma and external secretions may provide insight into local versus circulation-derived Igs. To correct for the dilution of Igs by a mucosal lavage fluid, a tracer such as lithium chloride can be added to the fluid and its level can be measured in the original and collected fluid. This approach has been used for the measurement of Igs in cervicovaginal secretions obtained by vaginal lavage9.

COLLECTION AND PROCESSING OF FEMALE AND MALE GENITAL TRACT SECRETIONS

These procedures have been described in great detail, including the purchase of supplies, buffers and protease inhibitors, as well as precautions and exclusion criteria for collection in our previous publication[2,5,7,10]. For the purpose of this brief review, we selected the most pertinent points that relate to the aims of this conference.

Most importantly, Igs in the female cervicovaginal lavages (CVL) are produced locally in the uterus, particularly in the endocervix, or are derived from the circulation. Consequently, hysterectomy greatly reduces the total level of Igs in vaginal lavages11. Furthermore, the total levels as well as the molecular properties of Igs in CVL are highly variable, depending on the day of collection during the menstrual cycle. The lowest levels are measured at the time of or shortly after ovulation, and the highest shortly before ovulation and during menstruation12. In addition, pregnancy or the use of contraceptive drugs also influences Ig levels. Finally, increased levels of Igs in CVL collected shortly after sexual intercourse may be derived from semen. Due to the frequently unreliable information obtained by interviews with subjects, it is recommended for critical experiments to perform tests which disclose the presence of semen-derived proteins in CVL (SEMA or Humagen tests). Blood contamination can be easily assessed with the Hemastix test.

The origin of Igs in semen and pre-ejaculate has not been clearly determined. However, based on the molecular properties of Igs in these fluids, it appears that both the local synthesis, mainly in the penile urethra, and the circulation contribute to the Ig pool in these fluids10,[13]. Importantly, for the measurement of humoral immune responses, it must be kept in mind that the seminal fluid contains high levels of proteolytic enzymes, which effectively and selectively digest monomeric (m) IgA and IgM[14]. Thus, the addition of suitable protease inhibitors to collected samples of seminal fluid is imperative2.

LEVELS, ISOTYPE DISTRIBUTION, AND MOLECULAR PROPERTIES OF IgS IN FEMALE AND MALE GENITAL TRACT SECRETIONS AS RELATED TO THE EVALUATION OF HUMORAL IMMUNE RESPONSES

In comparison to other external secretions (e.g., rectal wash) female and male genital tract secretions contain relatively high levels of Igs[2] (Table I). However, and in sharp contrast to intestinal washes, tears, saliva and milk, genital tract secretions do not display a marked dominance of secretory IgA (S-IgA); instead, IgG represents the dominant isotype[2].

Immunochemical and immunohistochemical studies of the genital tract fluids and tissues, and various vaccination protocols suggest that most of the IgG is derived from the circulation, particularly in semen10. However, the endocervix contains high numbers of IgG-producing cells15,16. Thus, systemic immunization, which stimulates vigorous IgG responses in plasma, induces parallel robust responses in semen and CVL[10,17-20]. Consequently, systemic immunization represents an effective route of immunization to induce protective responses in the human genital tract. Furthermore, ample studies indicate that systemic immunization protects mucosal surfaces against a selected spectrum of mucosal pathogens21,22, and also against infectious agents that enter the body through the mucosal membranes, but display a prompt systemic dissemination (e.g., HIV-1 or poliovirus). Moreover, initial systemic immunization prevents, in both humans and animals, the induction of so-called mucosal tolerance, which is defined by the unresponsiveness of T cells in the systemic compartment, when induced by the initial mucosal antigen encounter[23].

Molecular properties of IgA in genital tract secretions are distinct from those of IgA in saliva, intestinal tears, fluid, and milk. IgA in the latter fluids is represented by the dominant S-IgA form composed of polymeric (p), dimeric and tetrameric IgA, with J chain and secretory component (SC) acquired during the selective transepithelial transport of pIgA; mIgA is present in low quantities[24]. In contrast, IgA in male and female genital secretions is represented by approximately similar properties of typical S-IgA, pIgA devoid of SC, and mIgA10,25. Furthermore, the two IgA subclasses -- IgA1 and IgA2 -- are present in genital secretions in proportions that differ from other body fluids: in CVL there is a slight preponderance of IgA2 reflecting the higher proportion of IgA2 – producing cells in the endocervix15,[26], while in semen IgA1 dominates, and the percentage of this subclass detected in semen is similar to the levels found in serum[10]. (Table II). Thus, this diversity of molecular forms of IgA in genital secretions reflects their origin and suggests potential immunization approaches to achieve optimal responses[27].

BIOLOGICAL IMPORTANCE OF EVALUATION OF MOLECULAR FORMS OF IgA IN GENITAL TRACT SECRETIONS

Molecular heterogeneity of IgA in human body fluids with respect to the pIgA, mIgA and IgA subclass distribution of specific antibodies reflects the relative effectiveness and sites of immunization, duration of the immune response, and type of antigen[27]. Interestingly, irrespective of the site of mucosal or systemic immunization or infections, initial IgA responses are manifested by the presence of pIgA in both sera and secretions[27]. In prolonged IgA responses to certain antigens, a conversion from pIgA to mIgA has been observed. The biological significance of this phenomenon becomes obvious when the functions of these two forms are compared[24,27-29]. Due to the presence of 4 to 8 antigen-binding sites in dimeric and tetrameric IgA molecules, respectively, the avidity of specific antibodies in pIgA as compared to mIgA is remarkably increased and results, for example, in enhanced virus neutralization activity of pIgA27,28,30. Furthermore, pIgA displays increased reactivity with Fcα receptors expressed on various cell populations including the phagocytic and epithelial cells[31]. Most importantly, only J chain-containing pIgA interacts with the epithelial polymeric Ig receptor (pIgR) essential in the selective transepithelial transport of pIgA into external secretions[32]. Intraepithelial pIgA can also effectively participate in the neutralization of virus-infected cells33. The extracellular form of pIgR remains associated with pIgA in the form of SC. The acquisition of SC endows S-IgA with an increased resistance to proteolytic enzymes34, and due to its high content of *N*-linked carbohydrate side-chains, SC participates in glycan-mediated inhibition of the adherence of mucosal bacteria to corresponding receptors expressed on the surfaces of epithelial cells[35].

Evaluation of naturally occurring or immunization-induced IgA1 and IgA2 antibodies specific for a variety of antigens revealed several important findings[27]. Antibodies specific for protein and glycoprotein antigens were found predominantly in the IgA1 subclass, while antibodies to polysaccharides, lipopolysaccharides, and teichoic acid antigens were dominantly of the IgA2 subclass. These differences were further accentuated in response to systemic or mucosal immunization[27]. The influenza virus vaccine induced almost exclusively IgA1 responses, while the polyvalent pneumococcal polysaccharide vaccine induced IgA2 responses[24,27,29] . Thus, these results indicate that the character of an antigen influences the outcome of the immune response with respect to the IgA subclass. Association of the IgA immune response with the IgA1 subclass may be detrimental due to the sensitivity of IgA1 to uniquely specific bacterial IgA proteases, which in some experiments impaired antibody-dependent protective activity[36].

Collectively, detailed evaluation of IgA antibodies with respect to their pIgA or mIgA form and IgA subclass association provides invaluable information concerning their potential protective function.

HUMORAL IMMUNE RESPONSES IN GENITAL TRACT SECRETIONS IN HIV-1-INFECTED, EXPOSED BUT SERONEGATIVE, OR VACCINATED INDIVIDUALS

HIV-1-specific antibodies are easily and reliably detectable in external secretions, including those of the female and male genital tract, by a variety of immunochemical assays[5]. Further studies concerning the isotypes of such antibodies revealed surprising discrepancies[37,38]. HIV-1-specific antibodies of the IgG isotype have been detected with a remarkable concordance in all laboratories involved. In contrast, the presences of such antibodies in the IgA isotype were met with a surprising lack of unity. In most studies, HIV-1-specific antibodies of the IgA isotype are either absent or present at very low levels in sera and all external secretions of the HIV-1-infected individuals examined[37,38]. This unexpected conclusion is based on blindly performed evaluations of the same samples distributed to several laboratories using a variety of assays37. This obvious discordance with the results indicating the dominance in external secretions of HIV-1-specific antibodies of the IgA isotype calls for a conclusive resolution of the existing controversy. In addition to conventional assays such as ELISA, which has proven to generate sometimes unreliable, false-positive results39-41, other tests such as chemiluminescence-enhanced western blot (ECL-WB) appear to be exquisitely sensitive and more reliable because the reactivities of antibodies with individual HIV-1 antigens can be easily discerned[5,37,38]. Using this assay, HIV-1-specific antibodies, dominantly of the IgG isotype, were easily detected and found at high frequencies in all plasma and serum samples and also in external secretions, even those in which total IgA is dominant[37,38]. Therefore, it appears that HIV-1 results in a selective, and in most individuals, a profound suppression of IgA responses in the mucosal and systemic compartments[38].

Consequently, attempts to induce possibly more biologically effective (see above) pIgA HIV-1-specific responses in external secretions by immunization approaches, which direct humoral responses toward dominant IgA (e.g., use of certain mucosal adjuvants, co-administration of selected cytokines, conjugation of HIV-1 antigens to polysaccharides etc.), should be vigorously pursued in HIV-1 vaccinology. It must be stressed, however, that low IgA responses specific for HIV-1 antigens do not reflect the diminished production of total IgA: levels of total IgA in sera and external secretions of HIV-1-infected individuals are either within normal limit or may be even elevated[37,38].

Pronounced and presumably protective local IgA responses in the CVL of highly exposed but persistently seronegative women observed in some studies have not been confirmed by others[42]. Obviously, a blindly performed comparative evaluation in several independent laboratories experienced in the evaluation of HIV-1-specific antibodies in external secretions is called for to resolve this glaring controversy.

In contrast to extensive studies of the induction of mucosal IgA responses in animals immunized by a variety of routes with a broad spectrum of SIV-derived antigens[43], limited experiments have been performed in humans vaccinated with experimental HIV vaccines by systemic and much less frequently utilized mucosal immunization routes[38,44]. So far, no immunization protocols and approaches have been reported that would generate vigorous IgA responses in sera and external secretions of volunteers immunized with experimental HIV vaccines[42].

DETECTION OF HIV-NEUTRALIZING ANTIBODIES

Because of their demonstrable protective effect in an animal model, induction of virus-neutralizing antibodies in genital and intestinal secretions is a desired goal in HIV vaccinology44›45. Although the evaluation of antibody-dependent HIV-neutralization in the sera of HIV-1-infected or immunized individuals has been extensively pursued and neutralization assays against a broad spectrum of HIV-1 viruses are described in detail46, analogous studies are not commonly performed on external secretions for a variety of reasons. First of all, in contrast to other viruses (e. g., influenza virus), HIV-1 induces, in most individuals, low levels of HIV-neutralizing antibodies that reach titers of several hundred or thousand-fold dilution of serum, depending on the target virus type used for the neutralization assays[47]. To avoid virus-induced cytopathic effects that interfere with accurate measurements of neutralization, molecular cloned full-length HIV-1 X4 tropic (NL4.3) and pseudotype R5 tropic viruses (SF162 and YU2) are first titered in TZM-bl indicator cells. HIV-1-neutralizing antibodies are then measured with a standard inoculum of 200 TCID50 (average 200,000 RLU) for NL4.3 and SF162 and 280 TCID50 (average 280,000 RLU) for YU2 in neutralization assays with serum samples. As described above, external secretions (including those of the genital tract) contain, in comparison to serum or plasma, much lower and highly variable levels of total Igs (see Table I) which preclude the detection of low levels of HIV-specific antibodies. Concentration of the collected fluids does not remedy this problem due to the aggregation and “stickiness” of Igs to other components (e.g., mucin) and membranes. Moreover, these secretions are usually collected in low volumes to avoid the undesirable dilution of the sample (for example, CVL by the lavage fluid) and the limited volume does not allow extensive evaluations of neutralization against different tiers of HIV-1 viruses. The detection of HIV-1-specific antibodies is further compromised by the above-described dominant association of HIV-1-specific antibodies with the IgG isotype, which in some secretions (e.g., intestinal fluid, milk, and saliva) constitute an extremely low percentage of the total Igs. Most importantly, external secretions contain, in addition to antibodies, other humoral factors of innate immunity (e.g., SLPI, lactoferrin, lysozyme and others48), which display antibody-independent HIV “neutralizing” activity. To ascertain that the observed neutralization activity is indeed mediated by HIV-1-specific antibodies, virus neutralization should be performed and compared before and after a selective removal of Ig of various isotypes by, for example, immunosorbent beads coupled to suitable lectins or antibodies specific for individual Ig isotypes. If possible, bound Igs can be selectively desorbed from the beads and re-evaluated for their neutralizing activity. This is, however, a difficult task due to the extremely low yields of desorbed Igs. As pointed out above, low volumes of external secretions and low levels of Igs and HIV-specific antibodies, and possible interference with innate humoral factors limit the number of assays, particularly evaluations of neutralizing activities against

viruses representing various tiers of sensitivities, and the ability to perform detailed characterization of molecular properties of HIV-1-neutralizing antibodies.

CONCLUSIONS

Acceptance of the fact that HIV-1 infection is dominantly a mucosal disease should refocus future studies with due consideration to the mucosal immune system. Specific areas which will require additional studies include:

1. Evaluation of mucosal immune responses at the most frequent sites of HIV-1 entry, the female and male genital tracts and the ano-rectal mucosae is essential. It is disheartening to conclude that in almost all currently funded HIV vaccine trails, humoral responses at these two critical sites have not been systematically evaluated as clearly and justifiably recommended[49].
2. The functionally and immunologically distinct mucosal sites – genital versus intestinal mucosa–must be independently evaluated with respect to the immunization strategies and evaluations of immune responses to experimental HIV vaccines. Although systemic immunization is likely to induce a protective IgG-dependent immune response in the genital tract, the intestinal tract mucosa will remain largely unprotected. Immunization protocols that will provide concomitant protection at both of these sites, indeed the most important sites, of HIV-1 entry need to be developed.
3. Collection, processing, storage, and evaluations of humoral immune responses in external secretions require a unified approach. Although the detailed protocols for the collection and processing of human external secretions have been developed and described in detail[2,7], they have not been implemented in most of the current studies. This is also true of assays used for the evaluation of humoral immune responses in external secretions. Critical and rigorous evaluation of blindly distributed samples of sera and external secretions should be initiated in order to eliminate assays that may yield misleading results.
4. Due to the remarkable diversity of the molecular forms and functional differences of HIV-1-specific antibodies of various isotypes, detailed characterizations of these antibodies should be considered in the evaluation of humoral immune responses of HIV-1-infected individuals, and of volunteers immunized with experimental HIV vaccines.
5. Evaluation of HIV-1-neutralizing antibodies in external secretions will require extensive additional studies. Specifically, low levels of total as well as HIV-1-specific antibodies in all external secretions require the development of sensitive, reliable, and highly discriminatory assays to distinguish antibody versus humoral innate-mediated virus-neutralizing activity.
6. Molecular-cellular mechanisms responsible for normal or even increased levels of total IgA in sera and secretions but low immune responses to HIV-1 in the IgA isotype in HIV-infected individuals, volunteers immunized with HIV experimental vaccines as well as in HIV-infected chimpanzees and SIV-infected macaques need to be further explored to provide a rational basis for this unique phenomenon and exploit the generated data in the design of vaccines that would stimulate immune responses in the IgA isotype.

Acknowledgments

We thank Ms. Patricia V. Grayson for editorial assistance. Experimental results included in this review were supported by NIH grants P01AI083027, P01AI071739, T32AI07493, and DK64400.

REFERENCES

1. Wright PF, Kozlowski PA, Rybczyk GK, Goepfert P, Staats HF, VanCott TC, Trabottoni D, Sannella E, Mestecky J. Detection of mucosal antibodies in HIV-1 type 1-infected individuals. AIDS Res Hum Retroviruses 2002;18:1291–1300. [PubMed: 12487817]
2. Jackson, S.; Mestecky, J.; Moldoveanu, Z.; Spearman, P. Appendix I: Collection and processing of human mucosal secretions. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 1647-1659.
3. Mestecky J, Moldoveanu Z, Russell MW. Immunological uniqueness of the genital tract: Challenge for vaccine development. Am J Reprod Immunol 2005;53:208–214. [PubMed: 15833098]
4. Mestecky J. Humoral immune responses to the human immunodeficiency virus type-1 (HIV-1) in the genital tract as compared to other mucosal sites. J Reprod Immunol 2007;73:86–97. [PubMed: 17354294]
5. Moldoveanu, Z.; Mestecky, J. Mucosal antibody responses to HIV. In: Prasad, VR.; Kalpana, GV., editors. HIV Protocols. Methods Molec Biol. 2nd edn. Vol. 48. Humana Press/Springer Science; New York: 2009. p. 333-345.
6. Mestecky J, Moldoveanu Z, Smith PD, Hel Z, Alexander RC. Mucosal immunology of the genital and gastrointestinal tracts and HIV-1 infection. J Reprod Immunol 2009;83:196–200. [PubMed: 19853927]
7. Mestecky, J.; Jackson, S.; Moldoveanu, Z.; Spearman, P.; Wright, P.; Zimmerman, E.; Wagner, L.; Crumbo, K.; Ashley, R.; McElrath, J.; Anderson, D.; Kutteh, WH.; Erb, S.; Mathieson, BJ.; Walker, MC. Manual for collection and processing of mucosal specimens. Laboratory for Assessment of Mucosal Immune Responses Induced by AIDS Vaccines in Clinical Trial Volunteers, National Institute of Health, Division of AIDS; Bethesda, MD: 1999.
8. Delacroix DA, Hodgson HJ-F, McPherson A, Dive C, Vaerman J-P. Selective transport of polymeric immunoglobulin A in bile. Quantitative relationships of monomeric and polymeric immunoglobulin A, immunoglobulin M and other proteins in serum, bile, and saliva. J Clin Invest 1982;70:230–241. [PubMed: 7096566]
9. Belec L, Meillet D, Levy M, Georges A, Tevi-Benissan C, Pillot J. Dilution assessment of cervicovaginal secretions obtained by vaginal washing for immunological assays. Clin Diagn Lab Immunol 1995;2:57–61. [PubMed: 7719914]
10. Moldoveanu Z, Huang W-Q, Kulhavy R, Pate MS, Mestecky J. Human male genital tract secretions: Both mucosal and systemic immune compartments contribute to the humoral immunity. J Immunol 2005;175:4127–4136. [PubMed: 16148163]
11. Jalanti R, Isliker H. Immunoglobulins in human cervico-vaginal secretions. Int Arch Allergy Appl Immunol 1977;53:402–408. [PubMed: 558170]
12. Kutteh WH, Prince SJ, Hammonds KR, Kutteh CC, Mestecky J. Variations in immunoglobulins and IgA subclasses of human uterine cervical secretions around the time of ovulation. Clin Exp Immunol 1996;104:538–542. [PubMed: 9099941]
13. Anderson, DJ.; Pudney, J. Human male genital tract immunity and experimental models. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 1647-1659.
14. Tjokronegoro A, Sirisinha S. Degradation of immunoglobulins by secretions of human reproductive tract. J Reprod Fertil 1974;38:221–225. [PubMed: 4210216]
15. Kutteh WH, Hatch KD, Blackwell RE, Mestecky J. Secretory immune system of the female reproductive tract: 1. Immunoglobulin and secretory component-containing cells. Obstet Gynecol 1988;71:56–60. [PubMed: 3336542]

16. Crowley-Nowick PA, Bell M, Edwards RP, McCallister D, Gore H, Kanbour-Shakir A, Mestecky J, Partridge EE. Normal uterine cervix: Characterization of isolated lymphocyte phenotypes and immunoglobulin secretion. Amer J Reprod Immunol 1995;34:214–247.

17. Kutteh, WH.; Mestecky, J.; Wira, CR. Mucosal immunity in the human female reproductive tract. In: Mestecky, J.; Lamm, ME.; Strober, W.; Bienenstock, J.; McGhee, JR.; Mayer, L.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 1631-1646.

18. Bouvet JP, Belec L, Pires R, Pillot J. Immunoglobulin G antibodies in human vaginal secretions after parenteral vaccination. Infect Immun 1994;62:3957–3961. [PubMed: 8063413]

19. Russell MW, Mestecky J. Humoral immune responses to microbial infections in the genital tract. Microbes Infect 2002;4:667–677. [PubMed: 12048036]

20. Mestecky J, Raska M, Novak J, Alexander RC, Moldoveanu Z. Antibody–mediated protection and the mucosal immune system of the genital tract: Relevance to vaccine design. J Reprod Immunol 2010;85:81–85. [PubMed: 20236708]

21. Mestecky J, Nguyen H, Czerkinsky C, Kiyono H. Oral immunization – An Update. Curr Opin Gastroenterol 2008;24:713–719. [PubMed: 19122521]

22. Underdown, BJ. Parenteral immunization induces mucosal protection: A challenge to the mucosal immunity paradigm. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 831-840.

23. Mestecky J, Russell MW, Elson CO. Perspective on mucosal vaccines: Is mucosal tolerance a barrier? J Immunol 2007;179:5633–5638. [PubMed: 17947632]

24. Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev 2005;206:64–82. [PubMed: 16048542]

25. Kutteh WH, Hammond K, Prince S, Wester R, Mestecky J. Production of immunoglobulin A by the cervix of the human female genital tract. Serono Symp Reprod Immunol 1993;97:151–158.

26. Pakkanen S, Kantele A, Moldoveanu Z, Hedges S, Hakkinen M, Mestecky J, Kantele A. Expression of homing receptors on IgA1 and IgA2 plasmablasts in blood reflects differential distribution of IgA1 and IgA2 in various body fluids. Clin Vaccine Immunol 2010;17:393–401. [PubMed: 20089794]

27. Russell MW, Lue C, van den Wall Bake AWL, Moldoveanu Z, Mestecky J. Molecular heterogeneity of human IgA antibodies during an immune response. Clin Exp Immunol 1992;87:1–6. [PubMed: 1733625]

28. Russell, MW.; Kilian, M. Biological activities of IgA. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 267-289.

29. Mestecky J, Lue C, Tarkowski A, Ladjeva I, Peterman JH, Moldoveanu Z, Russell MW, Brown TA, Radl J, Haaijman JJ, Kiyono H, McGhee JR. Comparative studies of the biological properties of human IgA subclasses. Protides Biol Fluids 1989;36:173–182.

30. Renegar KB, Jackson GDF, Mestecy J. In vitro comparison of the biologic activities of monoclonal monomeric IgA, polymeric IgA and Secretory IgA. J Immunol 1998;160:1219–1223. [PubMed: 9570537]

31. Woof, JM.; van Egmond, M.; Kerr, MA. Fc receptors. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 251-265.

32. Kaetzel CS. The polymeric immunogloubin receptor: bridging innate and adaptive immune responses at mucosal surfaces. Immunol Rev 2005;206:83–99. [PubMed: 16048543]

33. Lamm, ME. Protection of mucosal epithelia by IgA: Intracellular neutralization and excretion of antigens. In: Kaetzel, CS., editor. Mucosal Immune Defense: Immunoglobuin A. Springer Science +Business Media, LLC; New York: 2007. p. 173-182.

34. Crottet P, Corthesy B. Secretory component delays the conversion of secretory IgA into antigen-binding competent F(ab')2: a possible implication for mucosal defense. J Immunol 1998;161:5445–5453. [PubMed: 9820520]

35. Mestecky J, Russell MW. Specific antibody activity, glycan heterogeneity and polyreactivity contribute to the protective activity of S-IgA at mucosal surfaces. Immunol Lett 2009;124:57–62. [PubMed: 19524784]
36. Kilian, M.; Russell, MW. Microbial evasion of IgA functions. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 291-303.
37. Wright PF, Kozlowski PA, Rybczyk GK, Goepfert P, Staats HF, VanCott TC, Trabattoni D, Sannella E, Mestecky J. Detection of mucosal antibodies in HIV-1 type1-infected individuals. AIDS Res Hum Retroviruses 2002;18:1291–1300. [PubMed: 12487817]
38. Mestecky J, Jackson S, Moldoveanu Z, Nesbit LR, Kulhavy R, Prince S, Sabbaj S, Mulligan MJ, Goepfert PA. Paucity of antigen-specific IgA responses in sera and external secretions of HIV-1-infected individuals. AIDS Res Hum Retroviruses 2004;20:972–988. [PubMed: 15585085]
39. Jackson S, Prince S, Kulhavy R, Mestecky J. False positivity of enzyme-linked immunosorbent assay for measurement of secretory IgA antibodies directed at HIV-1 antigens. AIDS Res Hum Retroviruses 2000;16:595–602. [PubMed: 10777150]
40. Raux M, Finkielsztejn L, Salmon-Ceron D, Bouchez H, Excler JL, Dulioust E, Grouin JM, Sicard D, Blondeau C. Development and standardization of methods to evaluate the antibody response to an HIV-1 candidate vaccine in secretions and sera of seronegative vaccine recipients. J Immunol Methods 1999;222:111–124. [PubMed: 10022378]
41. Raux M, Finkielsztejn L, Salmon-Ceron D, Bouchez H, Excler JL, Dulioust E, Grouin JM, Sicard D, Bloudeau C. Comparison of antibodies in serum and various mucosal fluids of HIV type 1-infected subjects. AIDS Res Hum Retroviruses 1999;15:1365–1376. [PubMed: 10515152]
42. Mestecky J. Humoral immune responses to the human immunodeficiency virus type-1 (HIV-1) in the genital tract as compared to other mucosal sites. J Reprod Immunol 2007;73:86–97. [PubMed: 17354294]
43. Lehner, T.; Bergmeier, LA. Mucosal infection and immune responses to simian immunodeficiency virus. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal Immunology. 3rd edn. Elsevier Academic Press; Amsterdam, The Netherlands: 2005. p. 1179-1197.
44. Azizi A, Ghunaim H, Diaz-Mitoma F, Mestecky J. Mucosal HIV vaccines: A holy grail or a dud? Vaccine 2010;28:4015–4026. [PubMed: 20412879]
45. Alexander RC, Mestecky J. Neutralizing antibodies in mucosal secretions: IgG or IgA? Current HIV Research 2007;5:588–593. [PubMed: 18045115]
46. Montefiori, DC. Measuring HIV neutralization in a luciferase reporter gene assay. In: Prasad, VR.; Kaplana, GV., editors. HIV Protocols. Methods Mol Biol. 2nd edn. Vol. 485. Humana Press/Springer Science; New York: 2009. p. 395-405.
47. Burton DR, Desrosiers RC, Doms RW, Koff WC, Kwong PD, Moore JP, Nabel GJ, Sodroski J, Wilson IA, Wyatt RT. HIV vaccine design and the neutralizaing antibody problem. Nat Immunol 2004;5:233–236. [PubMed: 14985706]
48. Dembert T, Robert-Guroff M. Mucosal immunity and protection against HIV/SIV infection: strategies and challenges for vaccine design. Int rev Immunol 2009;28:20–48. [PubMed: 19241252]
49. Lehner T, Hoelscher M, Clerici M, Gotch F, Pedneault L, Tartaglia J, Gray C, Mestecky J, Sattentau Q, van de Wijgert J, Toure C, Osmanova S, Schmitt RE, Debre P, Romaria M, Hoeveler A, Di Fabio S. European Union and EDCTP strategy in the global context: Recommendation for preventive HIV/AIDS vaccine research. Vaccine 2005;23:5551–5556. [PubMed: 16153752]

TABLE I

External Secretions Display Marked Variabilities in the Level and Isotype Distribution of Immunoglobulins

	(in µg/ml)		
Fluid	**IgA**	**IgG**	**IgM**
Cervico-vaginal lavage	21 – 260	10 – 467	~16
Uterine cervix	3 – 330	1 – 1,200	5 – 328
Pre-ejaculate	0.3 – 17.3	0.0 – 6.4	
Ejaculate (Seminal plasma)	11 – 23	16 – 33	0 – 8
Rectal fluid	6 – 800	0.1 – 6.5	30
Colostrum and milk	470 – 53,800	40 – 168	50 – 340
Serum	500 – 3,500	7,000 – 12,000	500 – 1,500

TABLE II

External Secretions Contain Highly Variable Molecular Forms of Immunoglobulins with (A) Distinct Functional Differences (B)

A

Fluid	Polymer	Monomer	IgA1	IgA2
	(%)		(%)	
Cervico-vaginal lavage uterine cervix	55 – 80	20 – 45	40 – 50	50 – 60
Ejaculate	60	40	83	17
Rectal/intestinal fluid	5	5	30	70
Serum	1 – 5	95 – 99	85	15

B

IgA subclasses		Polymers (p)/monomers (m)
IgA1:	susceptible to bacterial proteases	pIg-receptor-mediated transport
	antibodies to proteins, viruses (e.g., HIV, influenza)	4-8 antigen-binding sites or dimeric and tetrameric IgA (bonus effect of multivalency)
	dominant in the oral cavity respiratory tract and small intestine	virus neutralization S-IgA>pIgA>>mIgA
IgA2:	resistant to proteolysis	higher affinity of polymeric IgA for Fcα – receptors