

Immunization for Protection of the Reproductive Tract: A Review

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PROBLEM: Local application of non-replicating antigens to the female reproductive tract is ineffective in stimulating the common mucosal immune system, and induces only weak genital antibody responses. Studies of immune responses to genital infections such as gonorrhea also support the concept that, lacking mucosal immune inductive sites, the reproductive tract is ill-equipped to mount effective immune responses.

METHOD OF STUDY: Intranasal (i.n.) and intravaginal (i.vag.) routes of immunization of mice with a protein antigen coupled to cholera toxin (CT) B subunit, or genetically engineered as chimeric proteins with the A2/B subunits of CT or type II heat-labile enterotoxin, were compared for their ability to induce specific antibody responses in vaginal fluids, saliva, and serum.

RESULTS: Mice immunized i.n. developed substantially stronger vaginal immunoglobulin A (IgA) and immunoglobulin G (IgG) and serum IgG and IgA antibodies, than those immunized i.vag. which also failed to develop salivary antibodies. Vaginal antibody responses induced i.n. persisted for at least 1 year, and were recallable by booster immunization after a prolonged period.

CONCLUSIONS: Such alternative strategies for inducing potent genital antibody responses offer the prospect of prophylactic immunization against genital infections. Further studies are required to evaluate their applicability to humans, and to comprehend the cellular and molecular mechanisms involved in delivering effective immune responses to the reproductive tracts.

Key words:
IgA antibodies, intranasal immunization, mucosal immune system, protective immunity

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INTRODUCTION – IS THE GENITAL TRACT PART OF THE COMMON MUCOSAL IMMUNE SYSTEM?

Although the concept of the common mucosal immune system (CMIS) has been the dominant paradigm in mucosal immunology for more than two decades, it has become increasingly clear that the CMIS is not uniform, and that the human reproductive tracts represent components of this system with unique features.^{1,2} For example, whereas locally produced secretory immunoglobulin A (IgA) is present in the genital secretions, the dominant Ig isotype is immunoglobulin G (IgG), much of which is derived from the circulation. Furthermore, both female and male genital tracts lack organized lymphoepithelial structures resembling intestinal Peyer's

patches where mucosal immune responses are induced and disseminated to remote effector sites. The hormone-dependent lymphoid aggregates consisting of CD8⁺ T cells, B cells, and macrophages in the uterine stratum basalis³ (see also this volume) are of a different composition and probably have a different function.

Numerous experiments have shown that the local instillation of non-replicating antigens into the vagina of experimental animals or of human female volunteers can result in the development of specific antibodies in the local secretions^{4–8} Generally, however, the levels of these responses are quite modest and moreover the responses are not disseminated either to remote mucosal sites or to the systemic compartment represented by serum antibodies. Furthermore, local immunization of the male tract is unlikely to be practicable. When mice were immunized intravaginally

(i.vag.) with a bacterial protein antigen coupled to cholera toxin (CT) B subunit plus CT as adjuvant, weak specific antibody responses in both IgA and IgG isotypes were detected in vaginal wash fluids 7 days after the last of three immunizations (Table I). However, no antibodies were detected in saliva (Table I) and only low levels of IgG antibodies were found in the serum.⁹ In contrast, when the same immunogen was administered without adjuvant intranasally (i.n.), mice developed substantially greater levels of IgA and IgG antibodies in vaginal fluids, and also IgA antibodies in saliva (Table I) as well as IgG and IgA antibodies in serum.⁹ Analysis of the molecular forms of IgA antibodies in murine vaginal washes indicated that these were predominantly polymeric and similar to those found in saliva, consistent with S-IgA, although smaller amounts of possibly monomeric IgA were also present.⁹

The presence of polymeric IgA-secreting plasma cells in subepithelial tissue of the female genital tract, particularly in the endocervix and to a lesser extent in the fallopian tubes and uterus, has been well documented^{10,11} and polymeric Ig receptor (pIgR), the membrane precursor form of secretory component (SC) has been demonstrated in the overlying epithelium.¹⁰ Thus in these locations, and in the penile urethral glands of Littre in the male tract,¹² S-IgA is assembled and transported into the lumen. The mechanisms whereby IgG from the circulation, or produced by resident IgG-secreting plasma cells, is transferred to the lumen remain unclear.

These considerations and findings sustain the notion that the genital tracts represent *effector* sites of the CMIS, but as inductive sites they serve only for the generation of *local* responses.

TABLE I. Antibody responses to *Streptococcus mutans* AgI/II in vaginal wash and saliva collected 7 days after the third immunization with AgI/II-CTB conjugate given at 10-day intervals

Response ^a	Route of immunization	
	i.n.	i.vag.
Vaginal IgA	38.2 ×/±2.4	2.7 ×/±2.0
Vaginal IgG	30.5 ×/±1.3	4.7 ×/±3.4
Salivary IgA	48.2 ×/±1.3	0

^aAntibodies assayed by ELISA and expressed as percent of total corresponding Ig isotype concentrations (geometric mean ×/±S.D., n = 5) (data from ref. 9).

MUCOSAL IMMUNE RESPONSES IN HUMAN GONORRHEA

We have previously presented data on the local mucosal IgA and IgG as well as circulating immunoglobulin M (IgM), IgG, or IgA antibody responses against *Neisseria gonorrhoeae* in females diagnosed with gonococcal cervicitis^{13–15} Although variable, these responses were low, whether measured in cervical mucus, vaginal washes, serum, or saliva, and showed little or no significant difference from antibodies measured in individuals from the same population who were not currently infected with *N. gonorrhoeae*. Furthermore, subjects having a previous history of gonococcal infection, and those with rectal as well as genital infection, did not display significantly elevated responses. Likewise in males with gonococcal urethritis, we found only low levels of antigenococcal IgG and IgA antibodies in urethral swab samples, and these were not significantly higher in infected compared with currently uninfected subjects (Table II).¹⁵ Male subjects returning for a second visit approximately 2 weeks after diagnosis, however, tended to show significantly higher serum IgG antibodies than at their first visit.¹⁵ In a further study of male subjects from Malawi, blood plasma IgA1 antibodies were significantly elevated in those with gonococcal (or non-gonococcal) urethritis compared with those without current urethritis, but seminal fluid antibodies were not significantly different between the groups (S.R. Hedges *et al.*, personal communication).

Overall therefore our findings with respect to antibody responses in cases of uncomplicated genital tract gonococcal infections are (i) that the local and systemic antibody response to infection is low; (ii) the cytokine response to infection is also low; (iii) subjects with prior history of infection show no higher responses; (iv) rectal infection does not appear to enhance the response; and (v) gonococcal IgA1 protease activity does not appear to account for the lack of detectable

TABLE II. Antibodies to *Neisseria gonorrhoeae* cells in urethral swab samples from males infected with gonococcal urethritis or currently uninfected

Antibody isotype	Median antibody level (range) ng/mL ^a	
	Uninfected n = 14	GC-infected n = 36
IgA1	0 (0–99)	0 (0–170)
IgA2	77 (0–354)	213 (0–432)
IgG	0 (0–442)	119 (0–685)

^aAntibodies assayed by ELISA (data from ref. 15).

IgA1 antibodies. These findings are consistent both with the limited ability of the reproductive tracts to mount immune responses to epithelial infections, and with the well-known clinical observation that gonorrhea can be contracted repeatedly with no effective immunity arising from previous infections. They also suggest that gonococci may be able to interfere with the normal course of an immune response.¹⁵ An important corollary, however, of these findings is that if antibodies to conserved gonococcal antigens can be induced by other routes and methods of immunization, and delivered to the genital tract, then vaccination against gonorrhea may become feasible. Results from our laboratory and elsewhere indicate that the i.n. route of immunization may be particularly suitable for generating strong genital tract antibody responses.^{16–19}

STRATEGIES FOR INDUCING GENITAL TRACT ANTIBODIES

A wide variety of novel approaches to developing mucosal immune responses by the application of antigens in different delivery systems has been investigated, many with considerable success. These include the coadministration of immunogens with adjuvants active at mucosal surfaces, coupling immunogens to carrier molecules that promote their uptake at mucosal inductive sites, expression of antigens in live attenuated bacterial or viral vectors that can colonize mucosal tissues, and the incorporation of antigens into a variety of microparticulate or adhesive vehicles that are taken up in mucosal inductive sites.²⁰ Among these we have focused particularly on the use of enterotoxins such as the type II heat-labile enterotoxins of *E. coli* which have advantageous mucosal adjuvant properties²¹ and on the coupling of vaccine antigens to the non-toxic binding B subunits of these enterotoxins by chemical

TABLE III. Antibody responses^a to i.n. immunization with SBR^b alone or in the form of chimeric immunogens constructed from CT or LT(IIa)

Immunogen	Serum IgG ($\mu\text{g/mL}$)	Vag. wash IgA (%Ab/Ig) ^c	Saliva IgA (%Ab/Ig) ^c
SBR	8.8 \pm 2.1	0.06 \pm 0.04	0.78 \pm 0.07
SBR-CTA2/B	77.0 \pm 6.4	3.5 \pm 1.0	3.4 \pm 0.5
SBR-LT (IIa)A2/B	46.0 \pm 4.1	2.5 \pm 0.9	1.1 \pm 0.6

^aAntibodies (mean \pm S.D.) were measured by ELISA in serum, vaginal wash, and saliva collected 7 days after the third immunization given at 10-day intervals (data from ref. 24).

^bSBR: saliva-binding region of *Streptococcus mutans* AgI/II.

^cAntibody as percent of total corresponding Ig isotype concentration.

conjugation or genetic engineering.^{22–24} When mice were immunized i.n. with a recombinant chimeric protein constructed from a bacterial antigen coupled to the A2 and B subunits of CT or type IIa heat-labile enterotoxin, strong serum IgG and IgA, as well as salivary and vaginal IgA antibody responses were induced (Table III).²⁴ We have further found that responses induced by mucosal immunization of mice with similar materials can persist for 1–2 years or be recalled by booster immunization, suggesting the maintenance of immunological memory within the mucosal immune system over a prolonged time.^{9,25}

CONCLUDING REMARKS

Numerous questions remain to be solved in the quest to comprehend the development of specific immune responses in the genital tract, and how these may be manipulated for desirable purposes such as prophylaxis against sexually transmitted infections, or immune contraception. Among these are the ‘homing’ mechanisms whereby mucosally induced lymphocytes preferentially relocate in genital tract tissues especially, as it appears, after i.n. immunization. The currently best understood mucosal addressin-receptor system, consisting of MAdCAM-1 expressed on intestinal endothelium and $\alpha\beta 7$ integrin expressed on lymphocytes induced in Peyer’s patches²⁶ probably does not apply to the genital tract which appears to lack MAdCAM-1.²⁷ Studies in humans have shown that tonsil-induced lymphocytes express both $\alpha\beta 7$ integrin and L-selectin, the homing receptor for peripheral lymph nodes.²⁸ Whether VCAM-1 and ICAM-1, which have been described in genital tract tissues²⁷ and their ligands $\alpha\beta 1$ and $\alpha\beta 2$ integrins, respectively, can satisfactorily account for genital homing of nasally induced lymphocytes remains to be determined.

Studies need to be initiated in humans to evaluate innovative strategies for eliciting mucosal immunity in the genital tract, to determine whether the promising approaches developed in animal models are applicable to humans. Challenges include generating appropriate antibody responses that can act locally against pathogens invading the genital tract as well as systemically against those that succeed in penetrating beyond the genital tissues. In addition, the induction of cell-mediated immunity and cytotoxic T cells in the mucosa, and the cellular and molecular mechanisms governing the generation and recall of memory in the mucosal immune system need to be comprehended.

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