

Specific secretory immune responses in the female genital tract following intranasal immunization with a recombinant adenovirus expressing glycoprotein B of herpes simplex virus

W. Scott Gallichan* and Kenneth L. Rosenthal*†

Previously, we demonstrated that intranasal (i.n.) but not intraperitoneal (i.p.) immunization with a recombinant adenovirus vector expressing glycoprotein B (gB) of herpes simplex virus type 1 (HSV-1) induced mucosal immune responses and conveyed long-term protection to mice against an i.n. challenge with heterologous HSV-2. We now show that i.n. immunization of female mice with this same vector, AdgB8, provides secretory and serum-derived humoral immune responses in the genital tract. Intranasal immunization induced anti-HSVgB IgA and IgG in vaginal washes of mice, whereas i.p. immunization only induced IgG, which appeared to be serum-derived. Interestingly, intravaginal (ivag) immunization with AdgB8 resulted in little or no anti-HSVgB IgA and only low levels of specific IgG in vaginal washes. All three routes of inoculation induced gB-specific serum IgG and IgA; however, i.n. immunized mice demonstrated the highest level of serum anti-HSVgB IgA. Additionally, ivag boosting with AdgB8 did not significantly alter the serum or vaginal wash antibody responses in i.n. or i.p. immunized mice. The IgG to IgA ratios of gB-specific and total antibody titres in the serum and vaginal washes of i.n. immunized mice indicated that the IgA in the vaginal washes was likely to be secretory. Furthermore, the titres of anti-HSVgB IgA relative to total IgA were higher in vaginal washes than sera, suggesting that the gB-specific vaginal wash IgA present in i.n. immunized mice was locally produced.

Keywords: Mucosal immunity; herpes simplex virus; genital tract sIgA; recombinant adenovirus; immunity to STDs; intranasal immunization

Protection of mucosal surfaces against re-infection by pathogens is largely dependent on secretory IgA, specifically polymeric IgA (pIgA), originating primarily from plasma cells located within the lamina propria¹. In rodents there may also be a contribution of pIgA from the circulation; circulatory pIgA is transported across the epithelium by secretory component (SC) in much the same manner as that produced locally by plasma cells². Mice given an intravenous injection of anti-influenza virus antibodies demonstrate specific transfer of pIgA into nasal secretions, but lack transfer of monomeric IgA². Furthermore, the titre of specific mucosal antibody is influenced by the route of immunization, with

application of antigen to the mucosa being the most effective in stimulating specific IgA secretion at the site of immunization³⁻⁸. Despite this, the induction of significant secretory immunity in the female genital tract by the use of local or systemic vaccinations has proven difficult and requires the use of adjuvants, high doses of vaccine and/or numerous applications⁸⁻¹⁴. Also, it is well documented that the induction of specific immune responses at one mucosal site may result in these responses appearing at distant mucosal surfaces, including the genital tract¹⁵⁻²⁰. We have utilized this knowledge in our approach to providing herpes simplex virus specific humoral immune responses in the female genital tract.

Herpes simplex virus type-2 (HSV-2) is a sexually transmitted agent that attaches, penetrates and undergoes infectious cycles of replication in the epithelium of the genital tract²¹. Previous studies have demonstrated that humoral immunity plays an important role in limiting or blocking HSV infection of the murine genital tract epithelium. The passive transfer of antibodies

*Molecular Virology and Immunology Programme, Departments of Pathology and Biology, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada. †To whom correspondence should be addressed. (Received 18 January 1995; revised 24 April 1995; accepted 24 April 1995)

directed against glycoproteins of HSV either prevented vaginal HSV infection or rapidly cleared the virus thereby limiting inflammation and lethality^{22,23}.

We previously demonstrated that intranasal (i.n.) immunization with an adenovirus vector containing the glycoprotein B (gB) gene of HSV-1 (AdgB8) induced systemic as well as local immune responses in the respiratory tract²⁴. The responses were both humoral and cellular, as evidenced by secretory anti-HSVgB IgA in the respiratory tract and HSV-specific CTL in the mediastinal lymph nodes draining the respiratory tract. These responses were also sufficient to provide long-term protection against subsequent i.n. challenge with heterologous HSV-2. In contrast, mice immunized i.p. did not generate significant levels of mucosal IgA and protection from i.n. challenge was short-lived²⁴.

Since HSV-2 is a sexually transmitted virus, we evaluated adenovirus vectors as potential mucosal vaccines by examining the ability of AdgB8 to induce IgA in the genital tracts of mice following local (ivag), distant (i.n.), or systemic (i.p.) immunization.

MATERIALS AND METHODS

Animals and cell cultures

Inbred female C57BL/6 mice (purchased from Charles River Canada, St. Constant, Quebec, Canada) were used for these studies. Two hundred and ninety-three cells were grown in *a*-MEM (Gibco Laboratories, Burlington, Canada), supplemented with 10% fetal calf serum (FCS; Gibco), and 1% penicillin-streptomycin and L-glutamine (Gibco).

Virus strains and immunization

The construction of the replication competent recombinant adenovirus type 5 vectors, AdgB8 and AdE3⁻, are reported elsewhere²⁵. In short, the vector AdgB8 contains the gB gene from HSV-1. The E3 deletion virus, AdE3⁻, does not contain any HSV genes and was used as a control. The recombinant adenoviruses were grown in 293 cells, purified twice on CsCl gradients, and titered on 293 cells. Mice in each group were immunized with 10⁸ p.f.u. of AdgB8 or AdE3⁻ in the indicated volumes of buffer. Mice immunized intranasally (i.n.) were ether anesthetized and virus in 10–20 μ l of phosphate-buffered saline pH 7.4 (PBS) was introduced into the nares by means of a micropipette²⁴. Intraperitoneal (i.p.) immunization was performed by injection of virus in 0.2 ml PBS. Mice immunized by intravaginal (ivag) inoculation were anesthetized and maintained using halothane for the period of inoculation. While under halothane anesthesia, mice were ivag washed with PBS and swabbed with a cotton applicator before virus in 10–20 μ l of PBS was instilled ivag for 1 h. Mice in six groups of 9–10 mice per group were immunized with AdgB8 or AdE3⁻. Mice immunized i.n. or i.p. received two doses of vaccine with a two-week interval. Mice immunized i.n./ivag or i.p./ivag received two doses of vaccine as before (i.e. i.n. or i.p.); however, they were then immunized twice ivag at two-week intervals. Mice immunized ivag received four doses of vaccine at two-week intervals. Control mice were immunized twice i.n. with 10⁸ p.f.u. of AdE3⁻ with a two-week interval.

Collection of fluids

Blood samples were obtained from the mice by retro-orbital bleeding. Vaginal fluid was collected by pipetting 30 μ l of PBS into and out of the vagina several times. The staging of the estrous cycle for each mouse was based on a smear from these washings²⁶. Diff-Quik (Baxter Scientific Products, Miami, FL) was used to stain the smears. The vaginal washings were then centrifuged to remove particulate matter and the supernatants were stored at -20°C.

ELISA

HSV-1 gB-specific ELISAs were done in flat-bottomed microtiter plates (Costar, Cambridge, MA). Plates were precoated with 2.5 μ g ml⁻¹ of recombinant HSV-2 gB (provided by R.L. Burke, Chiron, Emeryville, CA) in borate-buffered saline (BBS), pH 8.5, and kept overnight at 4°C. A Tris-buffered saline (TBS) solution containing 10 mg ml⁻¹ bovine serum albumin, pH 7.4, was used to block any plastic not precoated with HSV-gB. Serially diluted samples of either hyperimmune control, test sera, or sample supernatants were added, followed by either biotin-labelled goat anti-mouse IgG or IgA antibody (Southern Biotechnology Associates, Birmingham, AL). The labelling reagent was alkaline phosphate (ExtrAvidin; Sigma, St. Louis); *p*-nitrophenyl phosphate (npp) (Sigma) was used as a substrate in the serum IgG ELISA, and the ELISA Amplification System (EAS) (Gibco, Burlington, Ont.) was used as a substrate for the serum IgA and lavage IgA and IgG ELISAs. The ELISAs were read at 405 and 492 nm for the substrates npp and EAS, respectively. Antibody titres represent the inverse dilution of the sample at which twice the background absorbance of serum or lavage fluid from uninfected mice was reached.

Statistical analysis

Data were analyzed using the GraphPAD InStat program (Graph PAD Software, San Diego, CA). The paired and unpaired Student's *t*-test were used to determine *P* values and significance between groups. Comparison among the means of the groups was carried out using analysis of variance (ANOVA).

RESULTS

Comparison of different routes of immunization for the generation of HSVgB specific vaginal wash IgA and IgG

Groups of mice were immunized intranasally (i.n.), intraperitoneally (i.p.) or intravaginally (ivag) with two doses of recombinant adenovirus capable of expressing HSV-1 glycoprotein B (AdgB8). Subsequently, subgroups of mice immunized by these routes were further boosted twice with AdgB8 intravaginally. A control group consisted of mice immunized twice i.n. with recombinant adenovirus containing a deletion of the E3 region (AdE3⁻). Significant levels of HSVgB-specific IgA were observed in vaginal washes of mice immunized i.n. with AdgB8 when compared to control or i.p. immunized mice (Figure 1). In contrast, mice immunized i.p. showed low or undetectable levels of specific vaginal wash IgA (Figure 1). Intravaginal boosting of i.n. or i.p.

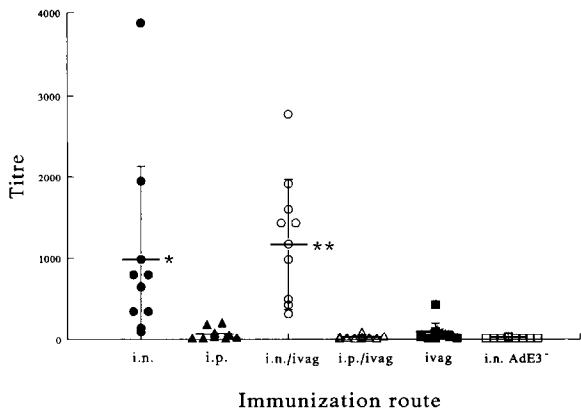


Figure 1 Anti-HSVgB IgA titres from the vaginal washes of mice immunized with AdgB8. Mice in each group were immunized at two-week intervals as follows: twice intranasally (i.n.); twice intraperitoneally (i.p.); twice intranasally followed by twice intravaginally (i.n./ivag); twice intraperitoneally followed by twice intravaginally (i.p./ivag); four times intravaginally (ivag); and twice intranasally with AdE3⁻ (i.n. AdE3⁻). Fifty-seven days post primary immunization vaginal washes were collected from each mouse and the titre of anti-HSVgB IgA determined by ELISA. Error bars indicate standard deviation. Differences between i.n. or i.n./ivag immunized groups and all other groups was determined by ANOVA: * $P \leq 0.05$; ** $P \leq 0.01$

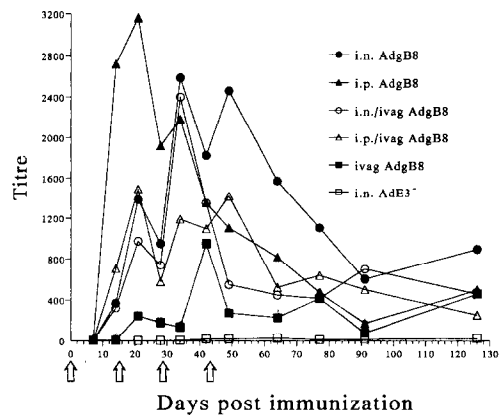


Figure 3 Time course of anti-HSVgB IgG titres in pooled vaginal washes of mice immunized with AdgB8. Mice in each group were immunized at two-week intervals as follows: twice intranasally (i.n.); twice intraperitoneally (i.p.); twice intranasally followed by twice intravaginally (i.n./ivag); twice intraperitoneally followed by twice intravaginally (i.p./ivag); four times intravaginally (ivag); and twice intranasally with AdE3⁻ (i.n. AdE3⁻). The arrows indicate the days on which mice were immunized. Vaginal washes from individual mice within each group were pooled and the IgG titres determined by ELISA

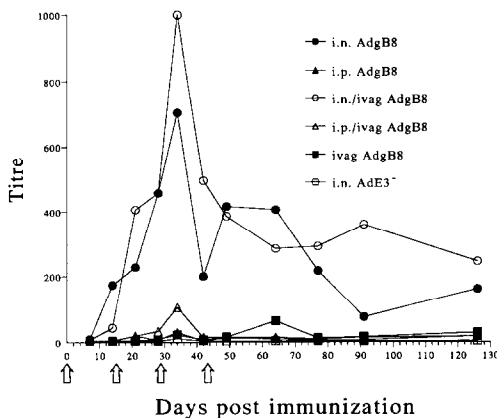


Figure 2 Time course of anti-HSVgB IgA titres in pooled vaginal washes of mice immunized with AdgB8. Mice in each group were immunized at two-week intervals as follows: twice intranasally (i.n.); twice intraperitoneally (i.p.); twice intranasally followed by twice intravaginally (i.n./ivag); twice intraperitoneally followed by twice intravaginally (i.p./ivag); four times intravaginally (ivag); and twice intranasally with AdE3⁻ (i.n. AdE3⁻). The arrows indicate the days on which mice were immunized. Vaginal washes from individual mice within each group were pooled and the IgA titres determined by ELISA

immunized mice with AdgB8 did not significantly increase these levels (Figure 1). Mice that were immunized four times ivag displayed little detectable vaginal wash anti-HSVgB IgA (Figure 1). Finally, mice immunized with AdE3⁻ displayed no significant vaginal wash anti-HSVgB IgA.

Vaginal wash samples from within groups of mice immunized by various routes were pooled and analyzed at various time points for specific anti-HSVgB IgA (Figure 2). Only mice immunized i.n. with AdgB8 demonstrated titres of anti-HSVgB IgA which persisted over the 126 days evaluated. Mice immunized i.n. with and without ivag AdgB8 boosting demonstrated titres of vaginal anti-HSVgB IgA that peaked 34 days post primary immunization. Intravaginal boosting with AdgB8 did not appear to affect these antibody levels

since titres generally decreased over the ivag boosting period. By two months following the second i.n. immunization, levels of anti-HSVgB IgA stabilized, especially in the ivag boosted group. Mice immunized i.p. or ivag with AdgB8 failed to demonstrate consistent levels of anti-HSVgB IgA in vaginal washes (Figure 2).

Mice immunized i.n. or i.p. with AdgB8 generated vaginal wash anti-HSVgB IgG within two weeks, peaking by 3–5 weeks (Figure 3). The levels of specific IgG varied considerably over time; however, mice immunized i.n. generated titres of anti-HSVgB IgG similar to that of i.p. immunized mice. When individual vaginal washes were analyzed, there was a broad range of responses in all groups (data not shown), similar to that observed for anti-HSVgB IgA. Intravaginal boosting on days 28 and 42 did not seem to significantly influence these levels. However, immunization of mice ivag four times did generate anti-HSVgB IgG in the vaginal wash which reached a peak titer at day 42. By 90 days the levels of specific IgG in the vaginal washes had decreased, but thereafter remained stable beyond 126 days (Figure 3). However, by 450 days post immunization, vaginal wash anti-HSVgB IgG and IgA levels had further decreased in all groups, and in a few cases was undetectable in individual mice (data not shown).

Comparison of different routes of immunization for the generation of specific serum IgA and IgG

Serum from within groups of mice was pooled at each time point investigated. Pooled serum samples from mice immunized i.p. with AdgB8 demonstrated high titres of anti-HSVgB IgG as seen in Figure 4, and indeed, antibody was still present over a year later (data not shown). Mice immunized i.n. developed similar levels of serum anti-HSVgB IgG (Figure 4) which were also present one year post immunization. Boosting of the i.n. and i.p. immunized groups by ivag inoculation did not increase the levels of specific serum IgG (Figure 4). Mice immunized i.p. displayed a peak titre of serum anti-HSVgB IgG on day 21 which corresponded to a peak

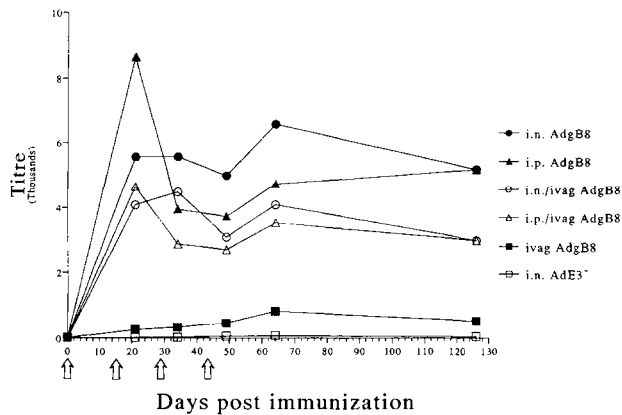


Figure 4 Time course of anti-HSVgB IgG titres in pooled sera of mice immunized with AdgB8. Mice in each group were immunized at two-week intervals as follows: twice intranasally (i.n.); twice intraperitoneally (i.p.); twice intranasally followed by twice intravaginally (i.n./ivag); twice intraperitoneally followed by twice intravaginally (i.p./ivag); four times intravaginally (ivag); and twice intranasally with AdE3⁻ (i.n. AdE3⁻). The arrows indicate the days on which mice were immunized. Serum from individual mice within each group was pooled and the IgG titres determined by ELISA

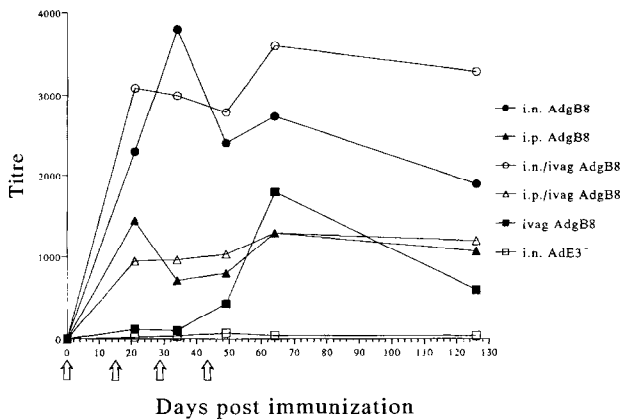


Figure 5 Time course of anti-HSVgB IgA titres in pooled sera of mice immunized with AdgB8. Mice in each group were immunized at two-week intervals as follows: twice intranasally (i.n.); twice intraperitoneally (i.p.); twice intranasally followed by twice intravaginally (i.n./ivag); twice intraperitoneally followed by twice intravaginally (i.p./ivag); four times intravaginally (ivag); and twice intranasally with AdE3⁻ (i.n. AdE3⁻). The arrows indicate the days on which mice were immunized. Serum from individual mice within each group was pooled and the IgA titres determined by ELISA

titre of anti-HSVgB IgG in the vaginal wash on day 21. Mice immunized ivag with AdgB8 demonstrated only a modest serum anti-HSVgB IgG response following four inoculations (Figure 4). When mice were examined individually, all mice immunized i.n. or i.p. demonstrated serum anti-HSVgB IgG, whereas not all mice immunized ivag exhibited specific antibody (data not shown). Control mice immunized with AdE3⁻ had no specific anti-HSVgB IgG (Figure 4).

Mice immunized i.n. with AdgB8 demonstrated serum anti-HSVgB IgA titres that were 2–4 times higher than mice immunized i.p. (Figure 5). Intravaginal boosting did not appear to have a marked effect on anti-HSVgB IgA serum levels in i.n. or i.p. immunized mice (Figure 5). Although four ivag immunizations with recombinant AdgB8 induced relatively low levels of serum anti-HSVgB IgG, the titres of serum anti-HSVgB IgA rose to levels comparable to that in i.p. immunized mice

(Figure 5). These results indicate that i.n. AdgB8 immunization induced higher levels of specific serum IgA, but comparable levels of specific serum IgG, relative to i.p. immunization.

Comparison of anti-HSVgB IgG to IgA ratios in the serum and vaginal washes

In order to begin to address the source of the vaginal wash anti-HSVgB IgA, we determined the IgG to IgA ratios of specific and total antibodies in the serum and vaginal wash fluids of i.n. AdgB8 immunized mice. Four weeks post i.n. immunization, serum and vaginal wash anti-HSVgB and total IgG and IgA titres were determined for each mouse at estrus or on a daily basis over two estrus cycles. The ratio of IgG to IgA in the vaginal washes was found to be significantly lower than in the serum ($P < 0.05$; paired Student's *t*-test) in all instances (Table 1). This was true for specific anti-HSVgB and total IgG to IgA ratios for all three groups, and in fact, in every mouse we have examined (data not shown). A significantly lower IgG to IgA ratio in the vaginal washes relative to the sera indicates an increase in the titre of IgA relative to IgG in the vaginal wash. Therefore, the IgA in the vaginal fluids is higher than would be expected if it was only present due to serum transudation (i.e. IgG in the vaginal fluids originates from serum transudation through the vaginal epithelium)²⁷. Furthermore, the ratio of specific anti-HSVgB IgA to total IgA was, in all cases, higher in vaginal washes as compared to serum (Table 1). This demonstrates that the specific IgA present in the vaginal fluids of i.n. immunized mice was higher than in the serum, when examined as a ratio of the total IgA. Although these results suggest local production, the presence of specific anti-HSVgB IgA producing lymphocytes in the genital tract remains to be determined.

DISCUSSION

Previously, we demonstrated that intranasal (i.n.) AdgB8 immunization induced secretory IgA specific for gB of HSV-1 in lung and nasal washes, whereas intraperitoneal (i.p.) immunization did not induce local IgA²⁴. Here we extend these results by demonstrating that i.n. AdgB8 immunization induced anti-HSVgB IgG and IgA antibodies in the genital tracts of mice. In contrast, no anti-HSVgB IgA appeared in vaginal washes following i.p. or ivag AdgB8 immunization. These results indicate that i.n. immunization of mice with a recombinant adenovirus is an effective method for inducing specific immune responses at local and distant mucosal surfaces. The genital tract may therefore serve as an effector site for immune responses generated within other tissues that make up the common mucosal immune system^{1,16,17}. In support of this, Natuk *et al.* demonstrated anti-HIV antibody responses in the vaginal fluids of chimpanzees following oral or i.n. immunization with adenovirus type 4-, 5-, and 7-vectored vaccines expressing either the HIV *env* or *gag*-protease genes²⁸. In particular, intranasal immunization appeared to induce the highest antibody responses. Others have also observed that following i.n. immunization with bacteria-CTB conjugates²⁹ or HSV-1 subunits³⁰, specific

Table 1 Comparison between the ratios of vaginal wash and serum antibodies in mice immunized intranasally with AdgB8

Group ^a	Anti-gB IgG/IgA ratios		Total IgG/IgA ratios		Anti-gB IgA/total IgA	
	Wash ^b	Serum ^c	Wash	Serum	Wash	Serum
1	0.23 ± 0.14	16.6 ± 15.3*	0.35 ± 0.43	16.1 ± 8.79***	0.091 ± 0.110	0.019 ± 0.013
2	7.46 ± 8.58	40.8 ± 7.59***	1.18 ± 1.79	6.68 ± 5.30*	0.047 ± 0.024	0.030 ± 0.013*
3	1.60 ± 1.39	45.5 ± 21.9***	0.57 ± 0.51	3.42 ± 1.78***	0.035 ± 0.019	0.011 ± 0.002**

^aSamples taken 4 weeks post intranasal immunization with 10⁸ p.f.u. of AdgB8. Group 1 represents nine mice sampled during estrus, and groups 2 and 3 represent the means of samples from individual mice sampled over two estrous cycles, i.e. 8 days. ^bExpressed as a ratio of titres ± standard deviation. ^cSignificant difference between vaginal washes and serums by the paired Student's *t*-test: **P*≤0.05; ***P*≤0.01; ****P*≤0.005

vaginal wash antibodies were present; however, in the latter study no specific-IgA was detected. Furthermore, vaginal wash antibodies appeared following oral administration of sperm³¹, bacteria³², viruses³³, or conjugates of antigen with cholera toxin B subunit (CTB)^{8,14}. These studies demonstrate that the murine genital tract can serve as an effector site for mucosal immune responses, and in particular, intranasal immunization with recombinant adenoviruses is an effective vaccination strategy for inducing secretory IgA in the genital tract.

Although humoral immune responses were evident in the genital tract following i.n. immunization with AdgB8, ivag immunization did not induce secretory anti-HSVgB IgA in vaginal washes. Indeed, little to no anti-HSVgB IgA was detected even after four ivag immunizations. In addition, while others have shown increased immune responses in the genital tract following mucosal boosting^{8,14,34-36}, we did not observe an increase in secretory IgA following ivag boosting of i.n. or i.p. immunized mice. These findings suggest that the murine genital tract is not a good inductive site for the generation of mucosal immune responses following recombinant adenovirus immunization. This may reflect the lack of functional mucosal-associated lymphoid tissue²⁷ (MALT) able to initiate mucosal immune responses in the vagina, or the inability of recombinant adenoviruses to infect and express in the murine genital tract. Although virus titres were not determined, ivag immunization did induce serum anti-HSVgB IgA and IgG, suggesting that recombinant adenoviruses were able to infect the genital tract and induce systemic immune responses in the absence of secretory IgA. Interestingly, immune responses in the genital tract are present following local administration of various antigens; however, adjuvants or the use of sutures to tie off the uterus were generally involved in these animal models^{7,8,27,37}. In contrast to protein antigens, invasive microorganisms may penetrate the epithelium and induce local immune responses³⁸. McDermott and colleagues observed IgG in the genital tract following ivag inoculation of mice with an attenuated strain of HSV-2; however, no specific IgA was detected¹². These studies and our results imply that direct vaccination of the vagina may not be as effective at generating secretory IgA in the genital tract as immunization of other mucosal tissues.

The presence of IgG antibodies can also be important for protection of mucosal surfaces and, in fact, IgG antibodies specific for HSV or HSVgB can protect the genital tract from HSV challenge even in the absence of T-cells^{22,23}. Our results demonstrate the presence of vaginal wash anti-HSVgB IgG following all three routes

of AdgB8 immunization that persist for at least 128 days. Furthermore, in agreement with our previous results and those of others^{24,39} serum IgG antibodies specific for HSVgB were also detected following i.n. and i.p. immunization. Additionally, serum anti-HSVgB IgG was observed following four ivag immunizations and while low compared to either of the other routes, seemed to remain constant. Interestingly, the peak pooled serum levels of specific IgG in i.p. immunized mice occurred on day 21 which corresponded with the peak vaginal wash anti-HSVgB IgG suggesting that the vaginal wash IgG was derived from serum transudation. This is consistent with other studies demonstrating that IgG transudates from the serum into the vaginal lumen²⁷.

Previous studies in mice have identified that specific IgA in vaginal fluids likely originates from the uterus since hysterectomized mice have only 5% of the vaginal wash IgA observed in controls⁹. Furthermore, secretory component (SC) is present in the uterine epithelium²⁷ and polymeric but not monomeric IgA appears on the surface of murine mucosal tissues⁵. In addition, IgA plasma cells are found in the body and horns of the uterus^{40,41} and can migrate from other mucosal tissues¹⁶, demonstrating that the genital tract is part of common mucosal immune system¹. These studies suggest that the vaginal wash IgA observed in i.n. AdgB8 immunized mice may be secretory in nature, originating from the uterus due to serum transudation or local antibody producing B cells. In order to examine these issues we compared the antibody ratios in the serum and vaginal washes of i.n. immunized mice. Our results demonstrate that the specific and total IgG to IgA antibody ratios in the vaginal washes were significantly lower than in the serum, indicating that there was more IgA relative to IgG in the lavages than in the serum. This would imply that the majority of both total and specific IgA present in the vaginal washes is secretory and derived from active transport and not serum transudation as in IgG. In order to begin to determine the origin of the specific IgA, we examined the ratio of specific anti-HSVgB IgA to total IgA titres between the serum and vaginal washes. If there was local production of specific IgA due to B cells in the uterine mucosa, one would expect the ratio to be higher in the lavages than in the serum. In our studies we found several times more specific to total IgA in the vaginal washes, indicating that perhaps there was a local B-cell component to the production of the HSVgB-specific IgA in i.n. immunized mice. Interestingly i.p. and ivag immunizations did result in serum but not vaginal wash IgA. This may be due to the induction of monomeric IgA or

alternatively to the lack of HSVgB-specific B cells in the genital tract.

Our work demonstrates that intranasal administration of AdgB8 induces not only antigen-specific systemic antibodies but pronounced mucosal IgA responses in the genital tract. This indicates that mucosal or more specifically i.n. administration of adenovirus vectors expressing immunogenic antigens should serve as excellent vaccine candidates for STDs such as HSV or HIV. Indeed, scientists and policy makers have recently generated criteria for an "ideal" AIDS vaccine⁴². One key property includes a vaccine candidate that will induce local immunity in the genital tract. Additionally, several studies have recently demonstrated that systemic vaccination with glycoprotein D (gD) of HSV results in a reduction in the recurrence of genital HSV lesions⁴³⁻⁴⁵. These results suggest that mucosal administration of recombinant adenovirus vectors which induce both systemic and mucosal responses may also serve as immunotherapeutic agents for recurrent HSV lesions.

ACKNOWLEDGEMENTS

We thank Rae Lyn Burke (Chiron, Emeryville, CA) for generously providing the recombinant gB used in the ELISAs; and Drs Frank L. Graham and David C. Johnson for provision of the recombinant adenovirus vector, AdgB8; and Karen Copeland and Carolyn Gallichan for critical evaluation of the manuscript. We acknowledge the Medical Research Council (MRC) of Canada for grant support.

REFERENCES

- Mestecky, J. The common mucosal immune system and current strategies for induction of immune response in external secretions. *J. Clin. Immunol.* 1987, **7**, 265-276
- Renegar, K. and Small, P. Passive transfer of local immunity to influenza virus infection by IgA antibody. *J. Immunol.* 1991, **146**, 1972-1978
- Waldman, R.H. and Ganguly, R. Techniques for eliciting mucosal immune response. *Acta Endocr., Copenh.* 1974, **194 (suppl.)**, 262-277
- Bienenstock, J. IgA synthesis and stimulation. In: *Progress Towards Better Vaccines* (Eds Bell, R. and Torrigiani, G.). Oxford University Press, Oxford, 1986, pp. 39-47
- Ogra, P.L. and Ogra, S.S. Local antibody response to polio-vaccine in the human female genital tract. *J. Immunol.* 1973, **110**, 1307-1311
- Nedrud, J.G., Liang, X.P., Hague, N. and Lamm, M.E. Combined oral/nasal immunization protects mice from sendai virus infection. *J. Immunol.* 1987, **139**, 3484-3492
- Wira, C. and Sandoe, C. Effect of uterine immunization and estradiol on specific IgA and IgG antibodies in uterine, vaginal and salivary secretions. *Immunology* 1989, **68**, 24-30
- Menge, A.C., Michalek, S.M., Russell, M.W. and Mestecky, J. Immune response of the female rat genital tract after oral and local immunization with keyhole limpet hemocyanin conjugated to the cholera toxin B subunit. *Infect. Immun.* 1993, **61**, 2162-2171
- Parr, E.L. and Parr, M.B. A comparison of antibody titres in mouse uterine fluid after immunization by several routes, and the effect of the uterus on antibody titres in vaginal fluid. *J. Reprod. Fertil.* 1990, **89**, 619-625
- Parr, E.L., Parr, M.B. and Thapar, M. A comparison of specific antibody responses in mouse vaginal fluid after immunization by several routes. *J. Reprod. Immunol.* 1988, **14**, 165-176
- Thapar, M.A., Parr, E.L. and Parr, M.B. The effect of adjuvants on antibody titers in mouse vaginal fluid after intra-vaginal immunization. *J. Reprod. Immunol.* 1990, **17**, 207-216
- McDermott, M.R., Brais, L.J. and Eveleigh, M.J. Mucosal and systemic antiviral antibodies in mice inoculated intravaginally with herpes simplex virus type 2. *J. Gen. Virol.* 1990, **71**, 1497-1504
- Haneberg, B., Kendall, D., Amerongen, H.M., Apter, F.M., Kraehenbuhl, J.P. and Neutra, M.R. Induction of specific immunoglobulin A in the small intestine, colon-rectum, and vagina measured by a new method for collection of secretions from local mucosal surfaces. *Infect. Immun.* 1994, **62**, 15-23
- Lehner, T., Berfmeier, L.A., Panagiotidi, C. *et al.* Induction of mucosal and systemic immunity to a recombinant simian immunodeficiency viral protein. *Science* 1992, **258**, 1365-1369
- Morin, J.E., Lubeck, M.D., Barton, J.E., Conley, A.J., Davis, A.R. and Hung, P.P. Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. *Proc. Natl Acad. Sci. USA* 1987, **84**, 4626-4630
- McDermott, M.R. and Bienenstock, J. Evidence for a common mucosal immunologic system I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. *J. Immunol.* 1979, **122**, 1892-1898
- Roux, M.E., McWilliams, M., Phillips-Quagliata, J., Weisz-Carrington, P. and Lamm, P. Origin of IgA-secreting plasma cells in the mammary gland. *J. Exp. Med.* 1977, **146**, 1311-1322
- Montgomery, P.C., Ayyildiz, A., Lemaitre-Coelho, I.M., Vaerman, J.R. and Rocky, J.G. Induction and expression of antibodies in secretions: the ocular immune system. *Ann. NY Acad. Sci.* 1983, **409**, 429-439
- Jackson, D.E., Lally, E.T., Nakamura, M.C. and Montgomery, P.C. Migration of IgA-bearing lymphocytes into salivary glands. *Cell Immunol.* 1981, **63**, 203-209
- Husband, A.J. and Gowans, J.L. The origin and antigen-dependent distribution of IgA-containing cells in the intestine. *J. Exp. Med.* 1978, **148**, 1146-1160
- Parr, M.B., Kepple, L., McDermott, M.R., Drew, M.D., Bozzola, J.J. and Parr, E.L. A mouse model for studies of mucosal immunity to vaginal infection by herpes simplex virus type 2. *Lab. Invest.* 1994, **70**, 369-380
- Eis-Hubinger, A.M., Schmidt, D.S. and Schneeweis, K.E. Anti-glycoprotein B monoclonal antibody protects T cell-depleted mice against herpes simplex virus infection by inhibition of virus replication at the inoculated mucous membranes. *J. Gen. Virol.* 1993, **74**, 379-385
- Whaley, K.J., Zeitlin, L., Barratt, R.A., Hoen, T.E. and Cone, R.A. Passive immunization of the vagina protects mice against vaginal transmission of genital herpes infections. *J. Infect. Dis.* 1994, **169**, 647-649
- Gallichan, W.S., Johnson, D.C., Graham, F.L. and Rosenthal, K.L. Mucosal immunity and protection after intranasal immunization with recombinant adenovirus expressing herpes simplex virus glycoprotein B. *J. Infect. Dis.* 1993, **168**, 622-629
- Hutchinson, L., Graham, F.L., Cai, W., Debroy, C., Person, S. and Johnson, D.C. Herpes simplex virus (HSV) glycoproteins B and K inhibit cell fusion induced by HSV syncytial mutants. *Virology* 1993, **196**, 514-531
- Allen, E. The oestrous cycle in the mouse. *Am. J. Anat.* 1922, **30**, 297-371
- Parr, M.B. and Parr, E.L. Mucosal immunity in the female and male reproductive tracts. In: *Handbook of Mucosal Immunology* (Eds Ogra, P.L., Strober, W., Mestecky, J., McGhee, J.R., Lamm, M.E. and Bienenstock, J.). Academic Press, Inc., San Diego, 1994, pp. 677-689
- Natuk, R.J., Lubeck, M.D., Chanda, P.K. *et al.* Immunogenicity of recombinant human adenovirus-human immunodeficiency virus vaccines in chimpanzees. *Aids Res. Hum. Retrovir.* 1993, **9**, 395-404
- Wu, H. and Russell, M.W. Induction of mucosal immunity by intranasal application of a streptococcal surface protein antigen with the cholera toxin B subunit. *Infect. Immun.* 1993, **61**, 314-322
- Bowen, J.C., Alpar, H.O., Phillipotts, R. and Brown, M.R.W. Mucosal delivery of herpes simplex virus vaccine. *Res. Virol.* 1992, **143**, 269-278
- Allardyce, R.A. and Rademaker, M. Female genital tract immunity and infertility after oral vaccination with sperm antigens in mice. *Adv. Exp. Med. Biol.* 1987, **216B**, 1807-1813
- Ogra, P., Okamoto, Y., Freihorst, J., Lascolea, L. and Merrick, J. Immunization of the gastrointestinal tract with bacterial and viral

- antigens: implications in mucosal immunity. *Immunol. Invest.* 1989, **18**, 559–570
- 33 Bergmann, K.-C. and Waldman, R. Oral immunization with influenza virus: experimental and clinical studies. *Curr. Top. Microbiol. Immunol.* 1989, **146**, 83–89
- 34 Marx, P.A., Compans, R.W., Gettie, A. *et al.* Protection against vaginal SIV transmission with microencapsulated vaccine. *Science* 1993, **260**, 1323–1327
- 35 Natuk, R.J., Chanda, P.K., Lubeck, M.D. *et al.* Adenovirus–human immunodeficiency virus (HIV) envelope recombinant vaccines elicit high-titered HIV-neutralizing antibodies in the dog model. *Proc. Natl Acad. Sci. USA* 1992, **89**, 7777–7781
- 36 Natuk, R.J., Davis, A.R., Chanda, P.K. *et al.* Adenovirus vectored vaccines. In: *Recombinant Vectors in Vaccine Development* (Ed. Brown, F.). Dev. Biol. Stand. Basel, Karger, 1994, pp. 71–77
- 37 Lande, I.J.M. Systemic immunity developing from intrauterine antigen exposure in the nonpregnant rat. *J. Reprod. Immunol.* 1986, **9**, 57–66
- 38 Tristram, D.A. and Ogra, P.L. Genital tract infection: implications in the prevention of maternal and fetal disease. In: *Handbook of Mucosal Immunology* (Eds Ogra, P.L., Strober, W., Mestecky, J., McGhee, J.R., Lamm, M.E. and Bienenstock, J.). Academic Press, Inc., San Diego, 1994, pp. 729–744
- 39 McDermott, M.R., Graham, F.L., Hanke, T. and Johnson, D.C. Protection of mice against lethal challenge with herpes simplex virus by vaccination with an adenovirus vector expressing HSV glycoprotein B. *Virology* 1989, **169**, 244–247
- 40 Rachman, F., Casimiri, V., Psychoyos, A. and Bernard, O. Immunoglobulins in the mouse uterus during the oestrous cycle. *J. Reprod. Fertil.* 1983, **69**, 17–21
- 41 Parr, E.L. and Parr, M.B. Secretory immunoglobulin binding to bacteria in the mouse uterus after mating. *J. Reprod. Immunol.* 1985, **8**, 71–82
- 42 Koff, W.C. The next steps toward a global AIDS vaccine. *Science* 1994, **266**, 1335–1337
- 43 Nakao, M., Hazama, M., Mayumi-Aono, A., Hinuma, S. and Fujisawa, Y. Immunotherapy of acute and recurrent herpes simplex virus type 2 infection with an adjuvant form of recombinant glycoprotein D-Interleukin-2 fusion protein. *J. Infect. Dis.* 1994, **169**, 787–791
- 44 Straus, S.E., Corey, L., Burke, R.L. *et al.* Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 immunotherapy of genital herpes. *Lancet* 1994, **343**, 1460–1463
- 45 Byars, N.E., Fraser-Smith, E.B., Pecyk, R.A. *et al.* Vaccinating guinea pigs with recombinant glycoprotein D of herpes simplex virus in an efficacious adjuvant formulation elicits protection against vaginal infection. *Vaccine* 1994, **12**, 200–209