Immunization of the Female Genital Tract with a DNA-Based Vaccine

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Vaccines are being sought for contraception and the prevention of sexually transmitted diseases. However, progress is slow in this area largely because of lack of information on induction of protective immune responses in genital tract mucosa. In this study, we investigated whether in vivo transfection with a model DNA-based antigen delivered by gene gun technology would induce an antibody response detectable in vaginal secretions. Female rats were immunized with plasmids encoding human growth hormone (HGH) under the control of a cytomegalovirus promoter (pCMV/HGH) via vaginal mucosa (V), Peyer’s patch (PP), and/or abdominal skin (S) routes. Localization of HGH in the target tissues demonstrated that all three sites can be transfected in vivo with pCMV/HGH. Vaginal tissues expressed roughly the same level of plasmid as skin. Antibodies to HGH were detectable in serum and vaginal secretions in rats immunized with pCMV/HGH. In the rats primed and boosted vaginally, vaginal immunoglobulin A (IgA) and IgG antibody titers to HGH were sustained for at least 14 weeks, whereas rats immunized via other routes and protocols (S/V, S/PP/PP, or PP/V) did not consistently sustain significant vaginal antibody titers beyond week 6. DNA-based immunizations administered by the gene gun may be an effective method of inducing local immunity in the female genital tract.

Vaccines for the female reproductive tract have potentially widespread applications, from the prevention of sexually transmitted diseases to contraception. No such vaccines, however, are currently available, largely because of lack of information about how best to stimulate a protective immune response in the genital tract mucosa.

The lower female genital tract is a recognized part of the common mucosal immune system (12). Classical studies in humans and primates have demonstrated that immunoglobulin A (IgA) is produced locally in the cervix and vagina upon immunization, is protective against genitally transmitted diseases to contraception. No such vaccines, however, are currently available, largely because of lack of information about how best to stimulate a protective immune response in the genital tract mucosa.

The lower female genital tract is an active inductive and effector site immunologically. In the human genital tract, dendritic cells (DCs) are abundant in the epithelia of the cervical transition zone, ectocervix, and vagina (4, 14, 28). Intraepithelial T lymphocytes, primarily of the CD8+ phenotype, and plasma cells, the majority of which secrete IgA and produce J chain (4, 10), populate the cervix and vagina.

Immunizing the lower female genital tract, however, is difficult. The activity of antigen-presenting cells (APCs) varies throughout the estrus cycle and is under the regulation of hormones (20, 23). DCs change in number, and the permeability of the epithelium to proteins is altered during the course of the estrus cycle (2, 9, 19).

Strong adjuvants and large, multiple doses of antigen are often required to induce a vigorous antibody response in the genital tract. Attempts to immunize the vaginas in several animal species by systemic and mucosal routes have yielded inconsistent results (1). No single regimen of priming and boosting appears most effective in raising antibody responses in vaginal fluids.

In the present study, we investigate the use of gene gun technology to transfect mucosal tissues and stimulate local antibody production in the genital tract of female rats. The gene gun is a helium gas pressure-driven device that delivers gold microparticles coated with plasmid DNA directly into tissues. This method of immunization may circumvent the problems of poor antigen penetration and the need for strong adjuvants to elicit an immune response in the lower female genital tract. Gene gun immunizations with plasmid-encoded antigens elicit protective humoral and cellular immune responses (5, 7, 22). The use of gene gun-administered DNA-based vaccines for mucosal surfaces has not been previously investigated.

The aim of our study was to test the ability of gene gun technology to induce a mucosal immune response in the female genital tract by using a reporter gene system, human growth hormone (HGH). HGH, a 161-amino-acid protein, is secreted by cells transfected with pCMV/HGH and is immunogenic in Lewis rats. Initial studies were done to confirm the expression of HGH in mucosal tissues (vagina and Peyer’s patches [PP]) compared to the current skin standard. Mucosal antibody studies using the HGH reporter gene system followed. Using this model system, we demonstrated that gene gun-administered plasmids transfected mucosal tissues in vivo and that vaginal immunization yielded higher titer cervicovaginal antibodies than the skin or PP route of immunization.

MATERIALS AND METHODS

Animals. Female Lewis rats approximately 9 weeks old were obtained from Harlan Sprague Dawley (Indianapolis, Ind.). Stage of the estrus cycle was determined by the use of vaginal smears and microscopic evaluation of vaginal cells (11). Anesthesia was performed with intraperitoneal (i.p.) injection of a mixture of ketamine (Parke-Davis, Morris Plains, N.J.) and xylazine (Miles, Shawnee Mission, Kan.). Rats were sacrificed by carbon dioxide asphyxiation.

DNA preparation. Purified plasmid DNA encoding HGH under the control of the cytomegalovirus immediate-early promoter, pCMV/HGH (Fig. 1), was obtained from Geniva, Inc. (Middleton, Wis.). The negative control vector, pCMV, was provided by James Arthos, Stanford University (Palo Alto, Calif.). To prepare DNA for gene gun delivery, 14 mg of 0.95-μm gold powder (Degussa,
South Plainfield, N.J.) and 35 μg of plasmid DNA were coprecipitated by the addition of 100 μl of 2.5 M CaCl₂ while vortexing. After vortexing, the precipitate was allowed to settle, washed with absolute ethanol, and resuspended in 2 ml of ethanol. The suspension was immersed in a sonication water bath for 2 to 5 s. One milliliter of the gold-DNA suspension was coated on a 7-in. piece of 3/32-in. Tefzel tubing (McMaster-Carr). After removal of the ethanol, the gold-DNA-coated tubing was dried with N₂. The tubing was cut into 1/2-in. pieces and stored at 4°C until use.

Antigen expression studies. (i) In vivo transfection. The Accell gene delivery instrument (Geniva) was used for all immunizations. The helium gas pressure used for motive force was 400 lb/in². For immunization by the skin route after anesthesia, the abdominal hair was clipped and four nonoverlapping sites were immunized on each rat with 1.25 μg of DNA per site. For vaginal immunization, two methods were used to obtain a diestrus vagina, which is optimal for immunization (20); the first one, which was used for the preliminary studies, employed timing the estrus cycle by inducing ovulation; the second suppressed the estrus cycle. To time the cycle, 5 days prior to immunization, 5 IU of pregnant mare’s serum (Sigma, St. Louis, Mo.) in phosphate-buffered saline (PBS) was injected i.p., followed 48 h later by an i.p. injection of 5 IU of human β-chorionic gonadotropin (Sigma) in PBS. To suppress the cycle, 7.5 mg of Depo-Provera (Upjohn, Kalamazoo, Mich.) was injected into the quadriiceps muscle 7 days prior to immunizations. For transfection, rats were anesthetized and the vaginal mucosa was exposed by making two small transverse incisions with blunt-tipped scissors inserted into the vagina. The anterior and posterior vaginal flaps were each immunized once with 1.25 μg of DNA, and incisions were closed with two to three 4.0 silk sutures (Ethicon, Somerville, N.J.) in the skin; the vaginal mucosa was not sutured. For PP immunization, rats were anesthetized and clipped, and a 2-cm left-of-midline incision was made in the skin and muscle layers to open the peritoneum. The intestinal loops were extracted, and PP were identified by visual inspection of the gut serosa. Each of four PP were immunized once with 1.25 μg of DNA. To minimize spiller DNA to the gut serosa, each immunization site was covered with a piece of Parafilm with a 0.5-cm hole cut in the center. The intestinal loops were replaced in the abdominal cavity, and the muscle and skin layers were sutured. (ii) Preparation of hyperimmune serum and vaginal secretions. The animals were sacrificed at 24, 48, 72, or 96 h posttransfection. Each skin transfection site was processed as a separate sample; three were dedicated to HGH radioimmunoassay (RIA) and one was preserved for immunohistochemistry. Each vagina, containing two target sites, was harvested whole. Two PP immunization sites were combined for one RIA sample, and one PP site was preserved for immunohistochemistry. Tissue lysates were prepared for samples dedicated to RIA for detection of HGH by mincing each sample in 0.5 ml of 0.5% Triton X-100 (Sigma) in sterile-filtered PBS. After centrifugation (5 min at 8,000 × g), the lysate supernatants were stored at −70°C until they were assayed. Samples dedicated to immunohistochemistry were placed in 10% formaldehyde for 24 to 48 h before embedding in paraffin. (iii) Detection of HGH antigen expression. An HGH RIA (Nichols Institute, San Juan Capistrano, Calif.) was used to detect HGH in skin, vagina, and PP lymph nodes. Lysate supernatants were thawed and diluted 1:50 in PBS. Samples were run according to kit instructions except that an extra wash step was added prior to counting radioactivity. Standards were run with each assay. After log-log transformation, the standard curve data were graphed and values for lysate samples were extrapolated from the curve. The final amount in each sample was scaled to estimate the amount of HGH in one target site containing 1.25 μg of DNA. Immunohistochemistry. Skin, vagina, and PP samples were cut into 5-μm sections from paraffin blocks, deparaffinized, and rehydrated. Normal serum block (Biogenix, San Ramon, Calif.) was applied for 1 h, followed by blocks for endogenous avidin and biotin (Biogenix). The sections were stained with 1:250 solution of rabbit anti-HGH antibodies (Dako, Carpinteria, Calif.) for 1 h. Anti-rabbit secondary antibodies (Biyogenix) were applied, followed by a streptavidin link (Biogenix) and then development with fast red (Biogenix) to give a red color to positively staining cells. Each step was followed by three washes in Tris buffer and performed at room temperature (RT). The slides were counterstained with hematoxylin and mounted with an aqueous medium. Two tissue types served as negative controls: sections immunized with pCMV/HGH and stained with normal rabbit serum instead of anti-HGH antibodies, and sections immunized with pCMV/JW4303 (blank) and stained with anti-HGH antibodies.

Antibody studies. (i) Immunizations. The protocols for transfection of skin, vagina, and PP outlined above were used for immunization studies. Five groups of six animals each were immunized in the following combinations: skin (S), skin/vagina (SV), PP/PP (P/P), PP/vagina (PV), and vagina/vagina (VV) (Table 1). One endominal skin, 10 nonoverlapping sites were immunized. For PP immunizations, eight overlapping doses were delivered to four PP sites. One site on each anterior and posterior vaginal flap was immunized. The animals were boosted 1 month later. The rats immunized vaginally were pretreated with Depo-Provera as described above. Negative control animals were immunized by using the same protocols with the blank plasmid, pCMV/JW4303. This group consisted of 6 animals, two each immunized PP, PV, and VV.

(ii) Sample collection. Blood and vaginal secretions were collected with the rats under anesthesia prior to immunizations and at weekly or biweekly intervals thereafter. Blood obtained from the periorbital sinus was allowed to clot at RT for 4 to 6 h and refrigerated overnight. The samples were centrifuged, and the serum was stored at −70°C. Vaginal secretions were collected by first instilling 20 μl of saline into the vagina with a blunt-tipped feeding needle and then inserting preweighed wicks (Polyfiltronics, Rockland, Mass.) into the vagina, using a blunt-ended glass tube as an applicator (Biomedical Engineering Department, Children’s Hospital, Boston, Mass.) (6). The wicks remained in place for 5 to 10 min and were then removed, weighed, and stored at −20°C until extraction with 5% nonfat dry milk in PBS.

(iii) Preparation of hyperimmune serum and vaginal secretions. Positive control serum and vaginal secretion standards were prepared for assay in parallel with samples from DNA-immunized rats. For hyperimmune serum and vaginal secretions, six rats were immunized subcutaneously with 500 μg of purified HGH (from human pituitaries; Sigma) in PBS and complete Freund’s adjuvant (Cappel, Durham, N.C.) and then boosted 1 month later with 250 μg of HGH in PBS and incomplete Freund’s adjuvant (Cappel). Serum and vaginal secretions were collected every 7 to 10 days. Each sample was screened by enzyme-linked immunosorbent assay (ELISA). High-titer samples were pooled, aliquoted, and frozen at −70°C. Nonimmune serum and vaginal secretions were prepared by pooling samples from rats immunized with Freund’s complete and incomplete adjuvant and PBS and from unimmunized rats. Duplicate samples of hyperimmune serum and nonimmune serum were assayed with each ELISA. In addition, hyperimmune vaginal secretion samples and nonimmune vaginal secretion samples were assayed in duplicate as further control for ELISAs on vaginal secretions.

(iv) ELISA for HGH-specific antibodies. IgG and IgA antibodies to HGH were assayed in serum and vaginal secretions. Ninety-six-well plates were coated overnight at 4°C with 5 μg of HGH per ml in carbonate coating buffer (pH 9.6) and frozen at −20°C. Prior to use, the plates were thawed at RT and blocked with 5% nonfat dry milk in PBS for 1 h. Serum samples were diluted in the blocking agent to 1:200 for IgG ELISAs and 1:50 for IgA. Vaginal secretions were diluted 1:5 in the blocking agent; 100 μl of sample was applied to each well in duplicate. After incubation for 1.5 h at RT, the plates were washed with PBS–0.05% Tween 20 (Sigma) and biotinylated anti-rat IgG or anti-rat IgA antibodies (The Binding Site, San Diego, Calif.) were applied, followed by incubation for 1 h and another wash step. Streptavidin-conjugated alkaline phosphatase (15 μg/ml in PBS–0.05% Tween 20; Jackson, West Grove, Pa.) was added, the mixture was incubated for 1 h at RT, and the plates were washed again. The alkaline phosphatase was developed with p-nitrophenyl phosphate (Pierce, Rockford, Ill.) for 30 min, and the optical density (OD) was read at 405 nm on a Titertek Multiskan plate reader.

(v) ELISA data analysis. One column (8 wells) on each 96-well plate was run without serum or vaginal secretion samples but with all other reagents to determine background from the secondary antibody. The ELISA plate reader subtracted background ODs from each sample. Mean ODs were calculated from duplicates for each sample. Duplicate positive control hyperimmune serum or vaginal secretion samples were run in tandem with test samples on each plate. The sample results were calculated as percentage of positive control (%PC). Using the OD of the positive control serum or vaginal sample from the same

### TABLE 1. Immunization groups

<table>
<thead>
<tr>
<th>Route (prime/boost)</th>
<th>No. of mice</th>
<th>Total DNA (μg)</th>
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<tbody>
<tr>
<td>S/S</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>S/V</td>
<td>4</td>
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</tr>
<tr>
<td>S/P</td>
<td>6</td>
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<tr>
<td>S/V</td>
<td>6</td>
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<tr>
<td>V/V</td>
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plate, a %PC was calculated for each test sample as follows: %PC = (mean test sample OD/mean positive control sample OD) × 100.

(vi) Statistics. StatView II computer software (Abacus Concepts, Berkeley, Calif.) was used for all statistical analyses. The results were logarithmically transformed to normalize the distributions and stabilize the variances of the groups. One-way analyses of variance were performed on the data. The HGH-immunized rat titers were compared to the negative control pCMV/JW4303-immunized rat titers by Fisher’s protected least significant difference set at the 5% level of significance. Titers between the different groups of HGH-immunized rats were then compared by the same method.

RESULTS

In vivo transfection studies. (i) RIA for HGH. HGH was detected in tissue lysates from vagina, PP, and abdominal skin samples immunized with plasmid pCMV/HGH. All rats transfected with the blank control plasmid, pCMV/JW4303, tested negative in the assay for HGH (data not shown). Each harvested sample of vagina and PP contained two DNA immunization sites, for a total of 2.5 µg of DNA. The measured values of HGH in each PP and vagina sample were halved to scale them down to the amount of HGH (1.25 µg) in one skin sample. The results from two experiments are combined in Table 2. Expression of the protein antigen was sustained in all three tissue types for 96 h. There was no significant difference between vagina and skin. Unlike the case for vagina and skin, there was no detectable antigen in PP at 24 h.

(ii) Immunohistochemistry of tissue sections. Immunostaining for HGH on paraffin sections of skin and vagina from immunized animals revealed HGH expression (Fig. 2 and 3). The transfected cells appeared to be epithelial by morphology. In both skin and vagina, the transfected cells were one to four cell layers deep from the uppermost layer of epithelium. A few positively stained cells also contained gold microparticles (Fig. 3). The two types of negative control skin and vagina (HGH-transfected tissue stained with rabbit serum and blank plasmid-transfected tissue stained with anti-HGH antibodies) did not show positive staining for HGH-containing cells.

Antibody studies. (i) Systemic response to HGH plasmid immunization. All groups developed anti-HGH IgG and IgA antibodies in serum. Titers were considered positive if they were significantly higher than titers for the negative control group. Negative control titers remained at roughly preimmune levels throughout, with a mean (± 1 standard error [SE]) serum IgG titer of 0.7 ± 0.1 %PC and a mean (± 1 SE) serum IgA titer of 4.8 ± 0.9 %PC.

Serum IgG titers show a classical antibody response; for all groups, titers began to rise at week 2 and increased after boosting at week 4 (Fig. 4). Serum IgG titers increased by 1.8 to 3 times maximum preboost values for all groups except V/V, the titers for which increased by 5 times preboost values. V/V serum IgG dropped to preimmune levels at the week 4 time point, prior to boosting.

The serum IgA titers against HGH were significant for all groups by week 3 but dropped off for V/V and P/P groups at week 4 (Fig. 5). Highest titers were attained at week 5 for

![FIG. 2. Gold microparticle coated with pCMV/HGH visible in abdominal skin epithelium cell stained with antibodies to HGH. Magnification, ×1,125.](http://iai.asm.org/ on October 14, 2017 by guest)
(ii) **Vaginal response to HGH plasmid immunization.** All groups developed significant vaginal IgG titers to HGH, and all but the P/P group raised significant vaginal IgA titers. There was more intragroup variation in vaginal titers compared to serum titers. Negative control vaginal titers remained at pre-immune levels throughout the experiment. Mean (± 1 SE) negative control vaginal IgG and IgA titers were 0.3 ± 0.1 and 2.7 ± 0.4 %PC, respectively.

At weeks 2 and 3, low vaginal IgG titers were present in one to two rats per group, but intragroup variation prevented these
from achieving significance over control titers (Fig. 6). At week 4, S/V, P/P, and P/V titers were all significantly higher than for controls; S/S and V/V titers were not. At week 5, 1 week postboost, the mean titer for the V/V group was 37 ± 11 %PC, which was significantly higher than that for controls. Other groups did not achieve significance because of intragroup variation. At week 6, all groups had mean titers significantly greater than those for controls, with the V/V (52 ± 38 %PC) and S/V (47 ± 28 %PC) groups having mean titers that were significantly higher than those for the P/P (3 ± 2 %PC) and P/V (10 ± 9 %PC) groups. At weeks 8 and 10, only the V/V group had significant vaginal IgG titers (mean titers, 41 ± 13

FIG. 5. Serum IgA antibodies to HGH. Data are presented as means ± SE. All titers were significantly higher (P < 0.05) than negative control serum from the same time points from week 2 to week 14, except S/S at week 2 and P/P and V/V at week 4.

FIG. 6. Vaginal IgG antibodies to HGH. Data are means ± SE. An asterisk indicates that the titer was significantly higher (P < 0.05) than negative control vaginal titers from the same time point. At week 6, V/V and S/V titers were significantly higher than P/P and P/V titers.
and 18 ± 7 %PC, respectively). V/V and P/V IgG vaginal titers were significantly higher than those for controls at week 14, with mean titers of 16 ± 6 %PC for V/V and 4 ± 2 %PC for P/V.

Through weeks 1 to 4, all groups showed low vaginal IgA titers, but these were not significantly higher than titers for controls (Fig. 7). At week 5, 1 week postboost, the V/V and P/V group achieved significant titers. At week 6, the P/V group titers decreased and S/V, S/S, and V/V vaginal IgA titers were significantly higher than those for controls; there was no significant difference in titers between groups. In the P/P group, at weeks 1, 3, 4, 5, 6, and 10, one rat (different rat each time) achieved high vaginal IgA titers, but the titers were so low for the rest of the group that overall the group mean never achieved significance. At week 8, the highest mean vaginal IgA titer, 41 ± 13 %PC, was seen in the V/V group. At week 10, the V/V mean titer was 18 ± 13 %PC, and at week 14 it was 19 ± 7 %PC. In summary, the P/V, V/V, S/S, and S/V groups achieved significant vaginal IgA titers after boosting, and only the V/V group was able to sustain these titers at 8, 10, and 14 weeks. A comparison of serum and vaginal IgA titers over time for the different immunization groups suggests that a majority of the IgA isotype immunoglobulins in vaginal secretions of the V/V immunization group at the late time points (8, 10, and 14 weeks) were locally produced or concentrated (e.g., serum/vaginal titer ratios of 6 for the S/S group and 1.5 for the V/V group at the 8-week time point). A similar effect was observed for IgG.

DISCUSSION

We have demonstrated the effectiveness of in vivo transfection with DNA-based antigens as a vaccination strategy for the female genital tract. The use of gene gun-administered DNA-based vaccines to stimulate immunity at mucosal surfaces has not previously been investigated. Indeed, there is only one previous account in the literature of the use of DNA-based vaccines at mucosal surfaces. Mouse nasal mucosa was immunized with hemagglutinin-encoding plasmids, and the mice were protected upon challenge with influenza virus (5). The site and type of tissue expressing the DNA were not identified, and the mucosal immune response to the immunization was not characterized in this report.

There are two published studies of DNA immunization to elicit protection against a genital tract pathogen. In the first, by Bourne and associates (2), DNA immunization by intramuscular injection with a plasmid encoding a herpes simplex virus type 1 (HSV-1) glycoprotein resulted in IgG antibody to the glycoprotein in serum and vaginal secretions and undetectable IgA titers in the vaginal secretions. On vaginal challenge with HSV-2, the animals became infected locally, but the magnitude of viral replication was reduced. The immunized animals were protected from lethal neurologic disease, which normally follows HSV-2 infection in mice. From these results, it appears that the intramuscular injection did not result in a mucosal immune response, but there was some partial local protection, either from T cells or from the vaginal IgG antibodies. The immunized mice were completely protected from the systemic manifestations of HSV-2. In the second study, by Kuklin and associates (9), mice were immunized intranasally or intramuscularly with plasmid DNA encoding HSV-1 antigen. The intranasal approach induced a mucosal antibody response in vaginal secretions but was inferior to intramuscular DNA delivery in providing protection against lethal HSV challenge via the vaginal route.

DNA vaccines administered by gene gun to the female genital tract may represent a method to stimulate a local immune response, an important first line of defense. We demonstrated that it is possible to transfect mucosal tissues (vaginal mucosa and PP) in vivo. Our tissue sections of skin and vagina stained...
with antibodies to HGH showed discrete HGH-secreting cells in the epithelial cell layers. The majority of positive cells had an epithelial morphology, but double-labeling studies were not performed and this population may have also included DCs. By RIA for HGH, we demonstrated that mucosal tissues are capable of good levels of expression of plasmid comparable to skin. Vagina and skin expression of pCMV/HGH were detected as early as 24 h posttransfection and did not differ significantly at any time point. Expression in skin and vagina had not tapered off by our last experimental time point, 96 h.

While the mechanism by which DNA-based antigens activate the immune system has not yet been elucidated (18), recent work done on APCs in skin sheds light on a potential mechanism for our immunizations (3). Condon and coworkers demonstrated that gene gun immunizations to skin transfected cutaneous DCs and that these cells migrate to draining lymph nodes within 24 h postimmunization (3). A similar mechanism might function for vagina: transfected DCs in the vaginal epithelium might migrate to genital nodes and present antigen in a major histocompatibility complex class I-restricted manner along with the appropriate costimulatory signals. Transfection of DCs might be the key to successful immunization in the genital tract, as these cells are potent APCs (24). It would be of interest to positively identify the transfected cells and determine whether they migrate to regional lymph nodes.

In addition to DCs, other transfected cells in the epithelium (e.g., epithelial cells and fibroblasts) might also function in the initiation of an immune response. Epithelial cells, functioning as nonprofessional APCs, might present antigen locally to intraepithelial T lymphocytes, as they do in the gastrointestinal tract (17). Vaginal epithelial cells produce cytokines when naturally infected by chlamydiae (21). It is possible that gene gun transfection also induces cytokine production in epithelial cells, which might affect local antigen presentation and function of immune-competent cells. Since the HGH produced by transfected cells is also secreted, it is likely that the secreted protein travels via lymphatics back to genital nodes, where it might be phagocytosed by other APCs (e.g., macrophages and B cells) and presented through the major histocompatibility complex class II pathway.

In our experiments, combinations of systemic and mucosal immunization protocols were effective in raising serum and mucosal antibodies to a DNA-based antigen. All routes of immunization tested stimulated antibodies to HGH in both serum and vaginal secretions. The serum antibody response to HGH followed classical antigen exposure-antibody response kinetics. There were no differences in serum IgG titers among the different systemic and mucosal groups except at the week 4 time point, when the V/V serum IgG titers were undetectable. This lack of difference in serum IgG titers was surprising, given the differences in doses of DNA among the groups. One possible explanation is that even the lowest immunization dose (total of 5 lg for V/V) was higher than the range where one might see a dose-response relationship. In previous studies, 0.04 to 0.4 lg of gene gun-administered DNA-based antigen was enough to protect animals from lethal challenge with influenza virus (5). There was more variation between the different groups in serum IgA titers. S/S and V/V in general raised higher serum IgA titers than the other mucosal (P/P and P/V) and systemic (S/V) groups. The reason for this is unknown.

All immunized groups developed anti-HGH IgG and IgA antibodies in vaginal secretions, and the V/V group sustained vaginal antibody titers for at least 14 weeks. Prior to boosting, P/V, P/P, and S/V groups had significant vaginal IgG titers; no group had significant IgA titers. At 1 to 2 weeks postboost, all of the groups had significant IgG titers, and all but the P/P group had significant IgA titers. After the 1- to 2-week postboost period, vaginal antibodies fell off to undetectable levels in all of the groups except for the V/V group. The V/V group was the only group that consistently sustained IgG and IgA titers out to the final time point; serum levels of IgG and IgA dropped off more gradually for all groups. This result suggests that the vaginal immunizations induced local antibody production of IgG and IgA at this site.

Our results indicate that DNA-vaccines can elicit mucosal immunity in the female genital tract and that vaginal immunization is an effective regimen to induce immunity at this site. However, our study was limited and points to further directions for research in this field. For one, we did not ascertain the origin of the antibodies detected in vaginal secretions. The vaginal antibodies measured in the 1- to 2-week postboost period may have been serum transudate, as serum titers were also highest at this time. That the vaginal antibodies were sustained beyond the immediate postboost period in the V/V group and not in other groups even though serum titers remained high suggests local production. Measurement of the proportion of IgA antibodies with secretory component in vaginal secretions would provide further evidence for local production. We attempted this, but the reagents available were not sufficiently specific to provide a clear answer.

Oral immunizations have previously been found to be effective in raising mucosal immune responses in the genital tract. Our experiments, however, did not show that PP immunizations conferred any advantage over parenteral immunizations in raising antibody titers in the genital tract. Plasmid expression measured by RIA in PP was significantly lower than in skin or vagina, and no expression was detected in the first 24 h. This may have been an artifact of our immunization protocol. To prevent spillover of DNA onto the serosa of the gut, we covered each PP with a piece of paraffin with a 5-mm hole cut in the center prior to immunizing. While the gold microprojectile beads are often concentrated in the center of the immunization site, the total DNA dose to each PP was lowered by an unknown amount. Also, immunization from the serosal side of the PP may have limited the contact that the antigen had with local APCs. The low expression of plasmid DNA in PP needs to be investigated further before it can be concluded that the gastrointestinal tract is a poor inductive site for DNA immunizations for the genital tract. Other promising routes of antigen administration that should be tested in subsequent studies include intragastric and intranasal approaches.

The prospect of plasmid-based DNA vaccines is exciting. There are several advantages to this approach: plasmids can be easily manipulated by using molecular techniques, the DNA is stable and resistant to temperature extremes (25), and the use of naked plasmids avoids the problems of a vector-mediated approach (8). Several DNA vaccine animal models exist, some of which can be used for diseases caused by viruses that infect mucosal surfaces, such as human (and simian) immunodeficiency virus, influenza virus, and HSV. Most of the vaccination studies use muscular needle injection of DNA to transfet cells, and the remaining few use the gene gun approach (25). None of the protocols to date have been aimed specifically at inducing mucosal immunity.

Further studies that unite the work done on mucosal vaccines with the DNA vaccine method would be fruitful in developing protective vaccines for the genital tract. Characterization of the T-cell response to DNA-based antigens administered mucosally would be valuable in assisting the development of protective vaccines to pathogens that require a cell-mediated immune response for protection (e.g., HSV) or...
that induce damage through cell-mediated mechanisms (e.g., chlamydiae). The local cytokine environment of the vaginal epithelium may affect the type of immune response generated at this site. Manipulation of the cytokine environment by cotransfection with cytokine-encoding plasmids has potential for tailoring a DNA vaccine to ensure that a protective immune response is initiated (26).

The work presented here explores a new area in vaccine development: DNA vaccines to induce mucosal immunity. We have demonstrated that mucosal tissues are capable of expressing DNA-based antigens and that the vaginal mucosal route of immunization is more effective than systemic (skin) immunization in inducing a local vaginal immune response. Further studies are needed to determine whether this approach stimulates long-acting protective immunity at mucosal sites.

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