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Induction of Genital Immunity by DNA Priming and Intranasal Booster Immunization with a Replication-Defective Adenoviral Recombinant

Zhi Quan Xiang, Susanna Pasquini, and Hildegund C. J. Ertl

Mice immunized through different routes such as i.m., intradermally, or intratracheally with a DNA vaccine to rabies virus developed high titers of serum Ab but only borderline levels of mucosal Abs determined from vaginal secretions. DNA vaccines given by either route enhanced vaginal IgA and IgG2a secretion upon a subsequent intranasal booster immunization with an E1-deleted adenoviral recombinant expressing the same Ag of rabies virus. DNA vaccine priming reduced the Ab response to the adenoviral Ags and counterbalanced the impaired B cell response to the rabies virus Ag expressed by the adenoviral recombinant in mice preimmune to adenovirus. The vaginal B cell response could further be enhanced by using the Th2-type cytokines IL-4 or IL-5 as genetic adjuvants concomitantly with the DNA vaccine before intranasal booster immunization with the recombinant vaccine. 


Vaccination is the most efficacious medical intervention to reduce human morbidity and mortality to infectious diseases. Currently, fewer than 20 vaccines are in use worldwide for the prevention of viral or bacterial infections in humans. Most of these, with some exceptions such as the live attenuated polio vaccine, a recently developed attenuated influenza virus vaccine, and an assortment vaccine to rotavirus, are applied systemically. Nevertheless, most infectious agents invade their host through the mucosal membranes of the airways, the digestive tract, or the urogenital tract, which are guarded by a separate and distinct immune system. Systemic immunization capable of protecting against a central infection will not necessarily guard against a mucosal infection, as was shown using SIV in monkeys (1).

Thus, for the prevention of many infectious diseases including sexually transmitted infectious diseases, vaccines must be developed that induce immune effector mechanisms at mucosal surfaces. The mucosal immune system that patrols the airways, intestines, and genital tract is constantly bombarded with "harmless" Ags, such as those present in food, that are best tolerated. The central immune system patrols the inner tissues and organs; this sterile environment warrants a more stringent control against foreign Ags. These two immune systems are thus faced with different challenges; consequently, they are distinct (2, 3) and require different routes of immunization for optimal activation of an Ag-specific immune response.

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We recently reported that an E1-deleted adenoviral recombinant derived from the human strain 5 expressing the rabies virus glycoprotein induces a strong secretory Ab response in the intestines and genital tract after i.n. immunization (4). Adenoviral recombinants are replication defective and thus exceptionally safe. Furthermore, they show high efficacy in inducing B, Th, and cytolytic T cell responses to the encoded transgene product (5–7). Nevertheless, one potential drawback for the use of human adenoviral recombinants as vaccine carriers in humans is the preexisting immunity to this common pathogen, which will interfere with the induction of an immune response to the transgene product.

DNA vaccines, one of the latest advances in vaccinology, are bacterial vectors that carry the gene of a pathogen under the control of a strong constitutively active promoter such as the one derived from CMV (8, 9). Inoculation of DNA vaccines into muscle or skin by a syringe or a propulsion device such as a gene gun has been shown to result in the uptake of DNA into cells followed by transcription and translation of the pathogen’s gene, resulting in an immune response composed of Abs, Th cells, and cytolytic T cells. Although some groups reported induction of mucosal immunity upon application of DNA vaccines to mucosal surfaces (10–13), such responses either were rather low or required the addition of adjuvants or other specific formulations. DNA vaccines were shown in several systems to prime the immune system to a subsequent booster immunization with a traditional vaccine (14, 15).

In these reports, the DNA vaccines as well as the Ags used for booster immunization were applied systemically. Considering the significant cross-talk between the central and the mucosal immune systems, we tested whether DNA vaccines given systemically could affect the vaginal Ab response to a subsequent i.n. inoculation of an adenoviral recombinant expressing the same transgene product. In this article we show that DNA vaccines to rabies virus given i.m. or i.d., or applied to mucosal surfaces induce strong systemic Ab responses but elicit only borderline responses at the genital mucosa. Nevertheless, after a second i.n. immunization with an E1-deleted adenoviral recombinant to rabies virus, mice primed with the DNA vaccines developed augmented and prolonged vaginal Ab responses. The responses could further be enhanced by using Th2 cytokines such as IL-4 or IL-5 as genetic adjuvants concomitantly with the DNA vaccine.
Materials and Methods

Mice

Female 6–10-wk-old C3H/He mice purchased from The Jackson Laboratory (Bar Harbor, ME) were kept in the Animal Facility of The Wistar Institute. They were used between 2 and 3 mo of age.

Viruses

Rabies virus of the Evelyn Rokitniki Abelseth (ERA) strain was propagated and titrated on baby hamster kidney (BHK)-21 cells and inactivated by β-propiolactone treatment (16). The E1-deleted adenovirus recombinant expressing the rabies virus glycoprotein of the ERA strain (Adrab.gp) was grown on E1-transfected 293 cells (7). An E1-deleted adenoviral recombinant expressing E7 of human papilloma virus type 16 (AdE7) was generated using previously described methods (7). The vaccinia virus recombinant expressing the glycoprotein of rabies virus strain ERA was propagated and titrated on HeLa cells (17). Purified adenovirus human strain 5 was purchased from the Vector Core Facility of the Gene Therapy Laboratory of the University of Pennsylvania.

Mammalian cell lines

BHK-21, HeLa, and 293 cells were grown in DMEM supplemented with 10% FBS and antibiotics.

Bacterial cell lines

Escherichia coli DH5α cells were grown in Luria-Bertani (LB) broth. Transformed cells were propagated in LB broth supplemented with ampicillin.

Plasmid vectors

The pSG5rab.gp vector expressing the glycoprotein of the ERA strain of rabies virus under the control of the SV40 promoter has been described previously (18). The pVR1012 vector (provided by Vical, San Diego, CA) was first modified by replacing the kanamycin resistance gene with the ampicillin resistance gene obtained from the pUC19 vector. The pVRamp.rab.gp vector was constructed by cloning the rabies virus glycoprotein gene into the multicloning site downstream of the CMV promoter. The resulting vector was subsequently analyzed by restriction enzyme digest and upon transient transfection of BHK-21 cells for appropriate expression of the viral glycoprotein using a specific mAb in an indirect immunofluorescence assay followed by analysis in a FACS (18). The mouse IL-4 cDNA and the β-galactosidase (lacZ) coding sequence were cloned into the pSG5 vector. The lacZ-expressing vector were identical with those of sera from naive mice. All groups of mice were boosted the day after harvesting starting for titrations.

Table 1. Viral recombinants and plasmid vectors

<table>
<thead>
<tr>
<th>Construct</th>
<th>Carrier</th>
<th>Transgene Product</th>
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<tr>
<td>Adrab.gp</td>
<td>E1-deleted adenovirus</td>
<td>Rabies virus glycoprotein</td>
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<tr>
<td>AdE7</td>
<td>E1-deleted adenovirus</td>
<td>E7 of HPV-16*</td>
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<td>Vaccinia virus</td>
<td>Rabies virus glycoprotein</td>
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<td>Rabies virus glycoprotein</td>
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<tr>
<td>pcDNA3IL-5</td>
<td>DNA (pCDNA3)</td>
<td>Mouse IL-5</td>
</tr>
</tbody>
</table>

* HPV, human papilloma virus.

Immunization of mice

Groups of five female mice were injected with 50 μg of vector diluted in 50 μl of either saline or water into both quadriceps muscles, dividing the dose equally to reduce variability. Injections were given i.d. at two sites into the skin of the lower back. Inoculation by the i.t. route was conducted in anesthetized mice after surgical exposure of the trachea. Adenovirus was either given s.c. in 200 μl of saline into the flank or i.n. by depositing 50 μl of saline containing varied amounts of virus onto both nostrils.

Preparation of samples

Serum was prepared from blood harvested by retroorbital puncture. For all titrations, sera were tested at serial dilutions starting with a 1:200 dilution. For isotype determination, sera were tested at a 1:800 dilution. Vaginal lavage was obtained by rinsing the vaginal cavity three times with 50 μl of sterile saline. Debris was removed by centrifugation. Vaginal lavage was most experiments tested immediately after harvesting starting for titrations, with a 1:2 dilution of the lavage fluid (total of 150 μl/mouse). A 1:8 dilution of the lavage fluids was used for isotyping tests. In some of the initial experiments, total Ig was determined in several different pooled lavage fluids with an ELISA using microtiter plates coated with a rabbit anti-mouse Ig to assess potential variability in the ratio of harvested lavage fluid to washing fluid. Using 4–5 mice/group, the amount of variability of total Ig in vaginal lavage fluids of different groups was negligible (data not shown).

Enzyme-linked immunosorbent assay

Sera and vaginal lavage samples were tested for Abs to rabies virus on microtiter plate wells coated with purified β-propiolactone-inactivated ERA virus or adenovirus virus as described (7, 19). The x-axis units of the different graphs showing titrations reflect the relative dilution of samples on a logarithmic scale. Sera were tested in serial 2-fold dilutions starting with a dilution of 1:200; vaginal lavages, also tested at serial 2-fold dilutions, were started at a dilution of 1:2. Isotypes of specific Abs were determined using the Calbiochem Hybridoma Isotyping Kit (Calbiochem, La Jolla, CA) as described (19).

Statistical analysis

Data were analyzed by Student’s t test. Samples with p value <0.05 were considered to show a significant difference.

Results

Systemic Ab response to different combinations of vaccines expressing the same viral Ag

The first set of experiments was designed to compare the ability of DNA vaccines with that of traditional vaccines to prime the Ab response to a subsequent booster immunization with an E1-deleted adenoviral recombinant. Groups of five C3H/He mice were inoculated with 104 PFU of Adrab.gp virus, 106 PFU of vaccinia rabies virus, DNA vaccines with that of traditional vaccines to prime the Ab response to a subsequent booster immunization with an E1-deleted adenoviral recombinant. Groups of five C3H/He mice were inoculated with 106 PFU of Adrab.gp virus given s.c. Blood was collected 5 and 10 days later, and again serum Ab titers to rabies were determined by an ELISA. As shown in Fig. 1, 5 days after inoculating the serum with 104 PFU of Adrab.gp virus given s.c., or 50 μg of pSG5rab.gp vector or pSG5lacZ vector as a control, both given i.m. Mice were bled 10 wk later and tested for Abs to rabies virus (Fig. 1). The Ab response to rabies virus was statistically significantly highest in mice immunized with a low dose of the adenoviral recombinant that we had described previously to be an exceedingly potent vaccine (7). The DNA vaccine and the VRG recombinant induced comparable Ab titers, while Ab titers in sera of mice injected with the lacZ-expressing vector were identical with those of sera from naive mice. All groups of mice were boosted the day after harvesting the serum with 106 PFU of Adrab.gp virus given s.c. Blood was collected 5 and 10 days later, and again serum Ab titers to rabies were determined by an ELISA. As shown in Fig. 1, 5 days after booster immunization the adenoviral recombinant given twice resulted in the highest Ab titers followed by titers of sera from naive mice. Mice primed with the vaccinia recombinant showed at this early time point only a small increase in titers, while Abs in the pSG5lacZ-primed control mice were still below the level of detectability. Titers in mice immunized with the Adrab.gp recombinant remained stable 10 days after priming, while titers in VRG virus or pSG5rab.gp vector-primed...
mice increased further. At day 10, there was no statistically significant difference in Ab titers to rabies virus between any of the three groups of mice primed with either of the recombinant vaccines or the DNA vaccine. Titors of control mice primed with the pSG5lacZ vector remained well below those observed in Ag-primed mice. We next determined the isotype profile of mice primed with the different constructs and then boosted with the adenoviral recombinant. In all three groups, as well as in the control group primed with the pSG5lacZ construct, isotypes of Abs to rabies virus were mixed, i.e., composed of IgG1, IgG2a, and IgG2b with IgG2a being predominant (data not shown). These data confirm that DNA vaccines are highly suited to prime the immune system to a systemic booster immunization with a potent viral recombinant vaccine.

**DNA vaccines prime the mucosal immune system**

In the next set of experiments we tested whether DNA vaccines could induce mucosal immunity. Groups of 5 C3H/He mice were inoculated i.n., i.t., or i.m. with 50 µg of the pSG5rab.gp vector. Serum Ab titers determined 4 and 6 wk later showed that i.m. vaccination resulted in the highest Ab titers followed by i.t. immunization, whereas titers upon i.n. inoculation were only borderline positive, suggesting that vector DNA was preferentially taken up by the lower rather than the upper airways. (Fig. 2 shows data for the sera harvested after 6 wk; data for i.n. immunization are not shown.) Ab titers in vaginal lavage were determined 6 weeks after vaccination. Mice immunized i.t. had no detectable Abs to rabies virus in their vaginal secretions while low but statistically significant titers could be detected in i.m. immunized mice. We reported previously that an E1-deleted adenoviral recombinant to rabies virus given s.c. Mice were bled 5 (○) and 10 (■) days later. Normal mouse serum was used as a control (×).

As shown in Fig. 2, priming with the pSG5rab.gp vector enhanced Ab titers in both serum and vaginal lavage. Both routes of DNA vaccine priming, i.e., i.m. or i.t., were equally effective. To determine whether the different routes of priming had favored the development of distinct Ab isotypes, the isotype profiles of Abs to rabies virus in sera and vaginal lavage were determined. As shown in Fig. 3, the response in sera upon DNA vaccine priming by either route was clearly predominated by IgG2a. In vaginal lavage, primed as well as unprimed mice developed comparable levels of IgA; the difference in titers (Fig. 2) was mainly a reflection of levels of IgG2a which were significantly higher in DNA vaccine-primed mice (Fig. 3). These data show that DNA priming can augment the vaginal Ab response upon booster immunization with an adenoviral recombinant given i.n., mainly by enhancing IgG2a titers. Systemic priming is equally effective to prime through the lower airways.

The experiment was repeated to assess the duration of the DNA vaccine priming effect. Groups of five C3H/He mice were immunized with the pVRamp.rab.gp vector given i.m. at 50 µg. Control mice were inoculated with 50 µg of the empty pVRamp vector. DNA vaccine-immunized mice had readily measurable serum Ab titers to rabies virus 3 mo later (data not shown), whereas titers in vaginal lavage were below the level of detectability (Fig. 4). After booster immunization with the Adrab.gp recombinant given i.n., vaginal lavage titers in primed mice increased rapidly by day 5

**FIGURE 1.** Comparison of different vaccines for priming of a B cell response to rabies virus upon booster immunization with the Adrab.gp vaccine. Groups of female C3H/He mice were immunized with 10⁶ PFU of Adrab.gp virus, 10⁸ PFU of VRG virus, both given s.c., or 50 µg of pSG5rab.gp vector or 50 µg of pSG5lac2 vector, both given i.m. Serum Ab titers were tested 2 mo later (□). All mice were boosted with 10⁶ PFU of Adrab.gp virus given s.c. Mice were bled 5 (○) and 10 (■) days later. Normal mouse serum was used as a control (×).

**FIGURE 2.** Ab titers after administration of DNA vaccine priming by different routes. Groups of mice were immunized with 50 µg of pSG5rab.gp given i.m. (■), i.t. (□), or i.n. (○). Sera (left) and vaginal lavage (right) were tested 8 wk later for Abs to rabies virus (top). Samples from naive mice were used as control (×). Adrab.gp virus only. Mice were retested 4 wk after booster immunization with the Adrab.gp virus (bottom).

**FIGURE 3.** Ab isotypes upon different routes of DNA vaccine priming. The same samples described in Fig. 2 were tested 4 wk after Adrab.gp virus booster immunization for isotypes of Abs to rabies virus: □, Adrab.gp virus only; ■, pSG5rab.gp priming given i.d.; ○, pSG5rab.gp priming given i.m. Left, results for sera; right, vaginal lavage.
when titers in control mice were still undetectable. A further increase was observed by day 10 when titers in mice primed with the empty vector before the booster immunization with the Adrab.gp recombinant started to become positive. At later time points, i.e., 24 days and 3 mo after booster immunization, control mice had substantial levels of Abs to rabies virus in their vaginal lavage; nevertheless, titers of DNA vaccine-primed mice remained clearly superior. This was even more pronounced after 4 mo when titers in vaginal lavage of control mice had declined to levels below detectability, while titers of DNA vaccine-primed mice were still readily measurable (Fig. 4).

The isotype profile of Abs to rabies virus in vaginal lavage showed that DNA-primed mice not only developed a robust IgG2a response but also had fairly stable titers of vaginal IgA that remained positive for the duration of the experiment (Fig. 5).

**Mucosal B cell response upon i.m. or i.d. priming with a DNA vaccine**

DNA immunization given i.m. results in a preferential Th1 response while, as was shown in some systems (20), i.d. injection favors activation of a Th2 response which is required to induce activation of IgA-secreting B cells. We therefore next compared the effect of i.d. vs i.m. priming with the pSG5rab.gp vector on the mucosal Ab response to i.m. booster immunization with the Adrab.gp vaccine. Groups of 5 C3H/He mice were inoculated either i.m. or i.d. with 50 μg of pSG5rab.gp or pSG5 vector. Vaginal lavage and sera were tested 8 wk later for Abs to rabies virus. While Ab titers in sera were substantial, with those achieved upon i.d. immunization being higher (data not shown), titers in vaginal lavage were comparable upon both routes of immunization. Mice were then boosted i.n. with 2 × 10⁶ PFU of the adenoviral recombinant and serum and vaginal lavage titers were measured 5 days as well as 4 wk later. Mice primed with the Ag-expressing vector exhibited clearly higher titers in sera (data not shown) and vaginal lavage compared with those inoculated with the control construct (data for vaginal lavage shown in Fig. 6). In this experiment, i.d. DNA vaccine priming resulted at both time points in slightly superior titers in sera and vaginal lavage compared with i.m. priming. This pattern was variable, and in subsequent experiments the difference in titers in either fluid upon i.m. or i.d. immunization was less pronounced or even reversed. Mice immunized i.d. with the control vector developed reproducibly very poor Ab responses compared with mice immunized i.m. with the same construct. We assume that this is in part a reflection of the immunomodulatory activity of bacterial DNA, which might have different effects depending on the anatomic site of vaccination. The isotypes of vaginal Abs to rabies virus tested after 5 days and 4 wk showed that i.m. DNA priming resulted in a preferential increase of IgG2a while i.d. priming strongly augmented an IgA response at the early time point that by 4 wk was in part replaced by IgG1 and IgG2a. This difference in isotype distribution was observed in all experiments.

**Effect of DNA vaccine priming on the Ab response to the vaccine carrier**

The effect of DNA vaccine priming on the Ab response to the adenoviral Ags of the Adrab.gp vaccine was investigated. Groups of C3H/He mice were primed with 50 μg of the pSG5rab.gp vaccine and boosted with the Adrab.gp construct given i.n. 6 wk later. Before the booster immunization, DNA-vaccinated mice still had serum Abs and low but detectable vaginal lavage Abs to rabies virus. Sera and vaginal lavage were tested 1 wk after the adenoviral boost for Abs to rabies virus and for Abs to adenovirus. As
expected, pSG5rab.gp-primed mice developed enhanced serum and vaginal lavage Ab titers upon booster immunization when compared with control mice. The magnitude of titers to the adenoviral Ags was reversed; mice that had been primed with the DNA vaccine to rabies virus developed only marginal serum and vaginal lavage titers to adenoviral Ags, while unprimed Adrab.gp-immunized mice developed substantial Ab titers at both sites, indicating that the preexisting immune response to the plasmid-encoded Ag had actively interfered with the Ab response to the vaccine carrier (Fig. 7).

**Effect of preexisting immunity to adenovirus on priming with a DNA vaccine followed by an adenoviral booster immunization**

Although the E1-deleted adenoviral recombinant to rabies virus is an exceedingly potent vaccine in rodents, humans will most likely show reduced efficacy due to preexisting immunity to wild-type adenovirus human strain 5, which forms the basis of the Adrab.gp recombinant. To test whether DNA vaccines could augment the Ab response to a transgene product expressed by an adenoviral recombinant in mice with preexisting immunity to the vaccine carrier, groups of 5 C3H/He mice were primed i.m. with 50 μg of pSG5rab.gp vector or pSG5 vector given i.m. After 2 mo, mice were boosted i.n. with 2 × 10^6 PFU of Adrab.gp virus. Naive control mice were immunized i.m. at the same days with Adrab.gp virus. Sera and vaginal lavage were harvested 10 days later and tested for Abs to rabies virus. As shown in Fig. 8, the Ab response to rabies virus as expressed by the adenoviral recombinant was reduced in sera of adenovirus pre-immune mice, while no difference was observed in vaginal lavage. In both fluids, DNA vaccine priming strongly augmented the Ab response above that seen in naive mice vaccinated with the Adrab.gp recombinant only, indicating that this combination vaccine regimen was suited to overcome the impairment of the Ab response to the rabies virus Ag due to preexisting immunity to the vaccine carrier.

**FIGURE 6.** Comparison of i.m. and i.d. routes of DNA vaccine priming. Mice were immunized i.m. or i.d with 50 μg of pSG5rab.gp or 50 μg of pSG5 vector. Vaginal lavage was harvested 8 wk later and tested for Abs (data before boost shown for pSG5rab.gp immune mice and naive mice). Mice were then boosted with Adrab.gp virus given i.n. Vaginal lavage Ab titers (left) and isotypes (right) were tested 5 days and 4 wk later. Tiers (left) and isotypes (right) (pSG5rab.gp i.d. + Adrab.gp); pSG5rab.gp i.m. + Adrab.gp; pSG5 i.d. + Adrab.gp; pSG5 i.m. + Adrab.gp; naive mice.

**FIGURE 7.** Ab response to adenoviral Ags in DNA vaccine-primed mice. Groups of C3H/He mice were immunized i.m. with 50 μg of pSG5rab.gp or pSG5 as a control. After 2 mo, they were boosted with 2 × 10^6 PFU of Adrab.gp virus given i.n. Serum and vaginal lavage samples were tested 1 wk later for Abs to rabies virus or adenovirus. Vaginal lavage: pSG5rab.gp + Adrab.gp; pSG5 + Adrab.gp; samples from naive mice. Sera: pSG5rab.gp + Adrab.gp; pSG5 + Adrab.gp; normal mouse serum.
The effect of IL-5 and IL-4 on priming with the DNA vaccine

The immune response to a DNA vaccine can readily be enhanced by the use of genetic adjuvants in the form of plasmid vectors expressing mouse cytokines given concomitantly with the DNA vaccine (21, 22). In the next set of experiments, we tested the effect of IL-4 and IL-5, two cytokines known to promote mucosal IgA responses, on the Ab response upon DNA vaccine priming followed by i.n. booster immunization with the adenoviral recombinant. Mice vaccinated with the DNA vaccine in the presence of IL-4 had increased serum Ab titers before booster immunization, IL-4 had no such effect on titers in vaginal lavage (data not shown). IL-5 in combination with the pSG5rab.gp vector did not increase serum Ab titers but enhanced vaginal lavage titers tested before boosting. Mice were boosted 6–8 wk later with the Adrab.gp recombinant given i.n. Adrab.gp was used at $2 \times 10^6$ PFU in the initial experiments. The IL-5-encoding plasmid, as shown in Fig. 9, markedly increased Ab titers in vaginal lavage, serum Ab titers were not enhanced. IL-5 augmented mainly Th2-type isoforms such as IgG1 in vaginal lavage. IL-5 also caused a slight but statistically significant increase in secretion of IgG2a, an isotype associated with Th1 responses. IL-5 given without the DNA vaccine had only a marginal effect on Ab titers in vaginal lavage after booster immunization with the Adbag.gp recombinant. IL-4 also augmented Ab titers in vaginal lavage following a booster immunization with $2 \times 10^6$ PFU of Adbag.gp virus (data not shown). Nevertheless, the effect was not as striking as that observed with IL-5. Upon using a lowered dose of Adbag.gp virus given at $5 \times 10^3$ PFU i.n., the IL-4-encoding plasmid given concomitantly with the DNA vaccine strongly increased the vaginal Ab response (Fig. 10). The IL-4 vector given alone, i.e., without the pSG5rab.gp vector, had no such effect (data not shown). In contrast to IL-5, IL-4 mainly increased Th1-related Abs of the IgG2a isotype in vaginal lavage.

Discussion

DNA vaccines, which were initially described in 1992 (8, 9), have been shown since then in many species to induce humoral and cell-mediated immunity to viruses, bacteria, parasites, and tumor Ags. DNA vaccines have certain advantages; they stimulate cytolytic T cells not induced by protein vaccines (9), they induce at least in rodents exceptionally long-lasting immune responses (23), they have marginal side effects (24), they provide their own adjuvant in form of unmethylated CpG sequences present in the bacterial part of the vector (25), and they do not induce immunity to the vaccine carrier (26), which restricts the repeated use of viral or bacterial recombinant vaccines. Their disadvantages include that they are not as potent as optimized recombinant vaccines or live attenuated pathogens, and furthermore that they are unsuited to induce strong mucosal immune responses in their naked form. We observed in some experiments stimulation of low titers of rabies virus Ag-specific IgG2a in the vaginal secretions of i.m. DNA...
vaccine-immunized mice. Other investigators have shown that incorporation of DNA vaccines into polymers (11), QS-21 saponin (12), or formulations containing adjuvants such as cholera toxin (10) augments the mucosal immune response upon i.n. inoculation suggesting that such modifications might boost the efficacy of mucosal DNA immunization.

The comparatively low immunogenicity of DNA vaccines can be reversed by using DNA vaccines for priming followed by a traditional, more potent vaccine for booster immunization (14, 15). We tested in the experiments detailed here whether DNA vaccines, which are known to prime the systemic immune response, were also able to augment the mucosal immune response upon i.n. booster immunization with a replication-defective E1-deleted adenoviral recombinant.

E1-deleted adenoviral recombinants of the human strain 5 were initially developed for gene therapy, especially for the treatment of lung disorders such as cystic fibrosis (27). Such recombinants expressing reporter proteins were found, during preclinical trials, to infect cells of the Airways efficiently, leading to high levels of transgene expression (28). Side effects but for a mild inflammatory reaction were not observed (29) even upon application of the high doses of recombinant virus needed to achieve eventually therapeutic levels of the missing protein. Nevertheless, expression of the transgene product was transient (6, 27). In immunocompetent individuals, the E1-deleted adenoviral recombinants induced cytolytic T cells to the structural proteins of adenovirus as well as to the transgene product, which rapidly eliminated the infected cells. A concomitantly induced CD4+ T cell response sponsored activation of B cells including those secreting neutralizing Abs to adenovirus. The Abs reduced uptake of the adenoviral gene therapy vehicle upon readministration. Although the strong immune response to the adenoviral recombinant restricted their use for permanent replacement of missing or faulty genes, it suggested their suitability as vaccine carriers. We previously described that the E1-deleted adenoviral recombinant expressing the glycoprotein of rabies virus induces a potent rabies virus-neutralizing Ab response after s.c. inoculation. The efficacy of the replication-defective adenoviral recombinant exceeded that of a vaccinia recombinant expressing the same Ag of rabies virus by a factor of at least 100; while 10^4 PFU of the adenoviral recombinant induced complete protection to challenge with rabies, the vaccinia recombinant used at 10^6 PFU induced protection in less than one-half of the animals. Adenoviral recombinants also have the advantage of inducing both central and mucosal immunity upon i.n. application.

The mucosal immune system differs from the systemic immune system with regard to a number of parameters; nevertheless, there appears to be substantial cross-communication between those two systems. Systemic immunization commonly results in low but measurable immune responses at mucosal surfaces while vice versa mucosal immunization causes activation of central immune responses. Furthermore, previous studies have shown that s.c. priming with a protein-based vaccine to influenza virus followed by a systemic booster immunization with the same vaccine augmented the local mucosal immune response (30). Vice versa mucosal priming with a vaccine to HIV has been shown to augment the mucosal immune response to an i.d. booster immunization with the same recombinant (31). Although it is currently unclear whether the two immune systems communicate by lymphocyte trafficking or by migration of APC, these data nevertheless strongly suggest that prime boost regimens using alternating central and mucosal routes of immunization might enhance the immune response at both sites.

We initially tested the effect of i.m., i.t., and i.d. DNA vaccine priming on the vaginal Ab response to an i.n. booster immunization with the Adrab.gp recombinant to establish whether any of these routes was superior in priming vaginal Ab secretion. The i.t. immunization was tested as a mucosal immunization route. Immunization by the i.d. route was explored, because in some systems this route was shown to generate a preferential Th2 response compared with i.m. DNA vaccine immunization (20), which due to the IL-12 and IFN-γ induction by the CpG sequences of the vector backbone causes a strongly biased Th1 response (25). Furthermore, T cells residing in the skin show phenotypic similarities with T cells in the vaginal epithelium (32), suggesting not only common ancestors but potentially also interchangeable homing patterns. All three routes of DNA vaccination induced vaginal Ab responses upon i.n. Adrab.gp booster immunization that were comparable in magnitude; the only reproducible difference was that i.d. priming induced higher IgA responses compared with i.m. or i.t. priming which is consistent with the notion that this route of DNA vaccination sponsors Th2 type immunity. The route chosen for all of the subsequent experiments, i.m. DNA vaccine priming, not only enhanced the vaginal Ab response following i.n. Adrab.gp booster immunization but also extended the longevity of the vaginal Ab response.

Although our data show that the systemic and mucosal Ab response in experimental animals to a single i.n. immunization with the Adrab.gp recombinant is not only substantial but also long-lasting, in humans, the natural host for wild-type adenovirus human strain 5, preexisting Abs to structural proteins of the virus that can be demonstrated in >95% of the population are likely to affect the efficacy of the vaccine. We had shown previously that this could be counterbalanced by increasing the vaccine dose, an approach that might not be practical for clinical use as this would augment the cost of the vaccine and enhance the risk of side effects. The use of DNA vaccine priming also rescued the Ab response to the rabies virus glycoprotein expressed by the Adrab.gp recombinant in mice preimmune to adenoviral Ags, suggesting that such a vaccine regimen might be highly suitable for use in humans with previous exposure to wild-type adenovirus. Conversely, DNA vaccine priming reduced the Ab response to the adenoviral Ags after i.n. booster immunization with the Adrab.gp recombinant. This phenomenon, which we cannot yet fully explain, might reflect
cytokine competition between already activated B cells to rabies virus and naive B cells to the adenviral Ags. Regardless of the mechanisms, this observed reduction in the Ab response to the vaccine carrier is very encouraging in that it suggests that using a combination of DNA vaccine priming before a booster immunization with a viral recombinant vaccine might allow for repeated use of the recombinant. Nevertheless, these experiments remain to be extended to additional recombinant vaccines based on carriers other than adenovirus to ensure that DNA vaccine priming in general impairs the B cell response to the Ags of the recombinant vaccine.

In an attempt to further augment the vaginal Ab response, we tested two Th2 cytokines, i.e., IL-4 and IL-5 which both promote stimulation of IgA-secreting B cells, as genetic adjuvants given concomitantly with the DNA vaccine. Numerous studies have shown that most cytokines expressed by plasmid vectors enhance or modulate the systemic immune response to a DNA vaccine (33). We had reported previously that IL-4 augmented the central B and T cell response to a DNA vaccine given i.m. IL-4 did not switch the immune response toward the Th2 pathway but rather augmented Th1-linked immunity including cytolytic T cells and B cells secreting IgG2a Abs (21). At the mucosa, the effect of IL-4 given with the DNA vaccine priming could best be demonstrated upon lowering the dose of the Adrab.gp recombinant used for i.n. booster immunization. As we had observed previously, IL-4 augmented secretion of IgG2a, a Th1-type isotype, rather than spon-soring Th2-related isotypes. IL-5 was more efficacious as a mu-coval genetic adjuvant and strongly augmented vaginal Ab secretions even before boost or upon the use of high doses of the Adrabgp vaccine for reimmunization. In contrast to IL-4, IL-5 favored stimulation of Th2 type Ab isotypes such as IgA and IgG1.

In summary, our data, using a simple and well-defined viral test system in mice, show that DNA vaccines given i.m. can prime a vaginal Ab response including secretion of specific IgA to i.n. immunization with an adenoviral recombinant expressing the same Ag. The vaginal Ab response could further be augmented by using IL-4 and especially IL-5 as genetic adjuvants concomitantly with the DNA vaccine. These results demonstrate that such a vaccine regimen, which does not require the use of toxic adjuvants, might be suitable for prevention of diseases, such as sexually transmitted diseases, where the pathogens infect through mucosal surfaces.

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References