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Nasal immunization of normal mice with HIVgp160-encapsulated hemagglutinating virus of Japan (HVJ)-liposome induced high titers of gp160-specific neutralizing IgG in serum and IgA in nasal wash, saliva, fecal extract, and vaginal wash, along with both Th1- and Th2-type responses. HIVgp160-specific IgG- and IgA-producing cells were also detected in mononuclear cells isolated from spleen, nasal cavity, salivary gland, intestinal lamina propria, and vaginal tissue of nasally immunized mice. In addition, CD8+ CTLs were induced in mice nasally immunized with gp160-HVJ-liposome. These findings suggest that two layers of effective HIV-specific humoral and cellular immunity, in mucosal and systemic sites, were induced by this nasal vaccine. In immunodeficient mice, nasal immunization with gp160-HVJ-liposome induced Ag-specific immune responses for the systemic and mucosal compartments of both Th1 (IFN-γ−/−) and Th2 (IL-4−/−). In vitro Ag-specific serum IgG Ab and vaginal wash samples possessing IgA and IgG Abs that had been induced by nasal immunization with gp160-HVJ-liposome were able to neutralize a clinically isolated strain of HIV-MN strain isolated from Japanese hemophiliac patients. Taken together, these results suggest that, for the prevention and control of AIDS, nasally administered gp160-HVJ-liposome is a powerful immunization tool that induces necessary Ag-specific immune responses at different stages of HIV infection. The Journal of Immunology, 2003, 170: 495–502.

To control HIV infection, it may be possible to develop vaccines that work to block the initial invasion of HIV or to eliminate the virus in patients infected with HIV. Such vaccines may be prophylactic or therapeutic or both. Given that HIV is disseminated mainly during human sexual activity, the mucosal surface of reproductive organs is the primary site of initial invasion and subsequent establishment of the infection in the systemic compartment (1). As a means of resisting HIV transmission through the epithelium of the reproductive organs, to provide a first line of defense against the invasion of HIV, an effective mucosal vaccine is an obvious candidate for development. Mucosal immunization has already been shown to induce Ag-specific immune responses in both the mucosal (e.g., sexual secretions) and systemic (e.g., serum) compartments (2). For inducing Ag-specific immune responses at the sites of sexual contact, nasal administration has been shown to be the most effective route for mucosal immunization (3, 4).

To effectively induce Ag-specific mucosal and systemic immune responses, in this study we used the viral-based hybrid Ag delivery system hemagglutinating virus of Japan (HVJ)5-liposome. The HVJ-liposome was developed by combining liposome with fusion proteins derived from the HVJ, also known as the Sendai virus, to enhance the efficiency of Ag delivery to targets (5, 6). In this system, vaccine Ags can be directly introduced into the cytoplasm by virus-cell fusion for the generation of Ag-specific CTLs (7). Moreover, this Ag delivery system has been shown to induce Ag-specific humoral immune responses (8). Thus, for the stimulation of two major arms of immune response including humoral (e.g., Ab) and cell mediated (e.g., CTL), HVJ-liposome is considered to be a useful vaccination Ag delivery vehicle. One interesting possibility entails the inclusion of HIV-surface glycoprotein gp160 into this HVJ-liposome. In a previous study, we demonstrated that, after nasal immunization, HVJ-liposome containing fluorescence-conjugated dextran is effectively taken up by epithelium-associated cells and APCs (8). Thus, HIV-liposome that contains gp160 could be effectively delivered into Ag-presenting M cells, epithelial cells, and APCs in nasopharyngeal-associated lymphoreticular tissue (NALT) and nasal passages (NP). Evidence that nasal immunization with HVJ-liposome containing OVA effectively induces both Ag-specific CTLs and IgA Ab responses (8) suggests that this novel mucosal Ag delivery vehicle could be implemented in the development of an HIV mucosal vaccine.
In this study, we examined whether nasal immunization with HIV-gp160-encapsulated HVJ-liposome (gp160-HVJ-liposome), in conditions in which normal and Th cell functions are deficient, can induce effective HIV-specific immunity in both the mucosal and systemic compartments. To characterize the quality and quantity of HIV Ag-specific immune responses, we tested mucosal gp160-HVJ-liposome with both normal and Th1 (IFN-γ−/−) and Th2 (IL-4−/−) immunodeficient mice. The gp160-HVJ-liposome induced Ag-specific IgG and IgA Abs in both types of mice. Postimmunization samples were also examined in vitro for isotype and subclass distribution as well as neutralizing activity against a clinically isolated strain of HIV. Furthermore, we also examined Ag-specific CTL responses at both mucosal and systemic sites after nasal immunization with gp160-HVJ-liposome. In short, the major aim of the present study was to evaluate the potential for application of the gp160-HVJ-liposome system in the development of a mucosal vaccine to control of HIV infection.

Materials and Methods

Mice

BALB/c mice were obtained from SLC Japan (Hamamatsu, Japan) and BALB/c strain mice with IFN-γ and IL-4 deficiency (IFN-γ−/− and IL-4−/−) between 6 and 12 wk of age were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in barrier-protected animal facilities under pathogen-free conditions using the ventilated microisolator cage in the experimental animal facility at the Research Institute for Microbial Diseases, Osaka University (Osaka, Japan).

Preparation of HVJ-liposome

The preparation of HVJ-liposome has been described in detail elsewhere (5–8). Briefly, the lipid mixtures (cholesterol, sphingomyelin, dioleoylphosphatidylcholine, phosphatidylglycerol, and phosphatidylserine) were deposited on the side of the flask by removing the chloroform solvent in a rotary evaporator. The dried lipid was hydrated in 200 μl of balanced salt solution (137 mM NaCl, 5.4 mM KCl, 10 mM Tris-HCl; pH 7.5) containing HIV gp160 (100 μg; Protein Sciences, Meriden, CT). A liposome suspension was prepared by vortexing, sonicating, and shaking to a final liposome concentration of 1 × 108 units/ml. The liposome suspension was heated to 65 °C for 10 min to inactivate the liposome. The liposome suspension was then centrifuged (20 000 × g) for 5 min at 4 °C and the liposome was removed by careful aspiration. The liposome-free supernatant was stored at −20 °C until use.

Immunization and sampling schedule

Mice (n = 4 per group) were nasally immunized with gp160-HVJ-liposome (1 μg per 10 μl per mouse). Each group of mice was immunized once a week for four consecutive weeks. To monitor the induction of IgG and IgA anti-gp160 specific Abs, serum, saliva, fecal extract, and vaginal wash were collected at day 0 as preimmunization samples and, after the immunization, were collected five times at weekly intervals.

Detection of Ag-specific Ab production by ELISA

HIV-specific Ab titers in serum, fecal extract, and vaginal wash were determined by ELISA using modified methods as described previously (9). ELISA plates were coated with 100 μl of 2 μg/ml gp160 in 0.1 M carbonate buffer and incubated overnight at 4 °C. The plates were then incubated with blocking solution (Block Ace; Dainippon Pharmaceuticals, Osaka, Japan) at 37 °C for 2 h. Dilutions of all mucosal secretions starting from 1/4 to 1/1000 PBS dilution condition medium according to a method described previously (10). After 1 h of incubation, the plates were washed, detection Abs consisting of 1/4000 PBS dilution of streptavidin HRP (Life Technologies, Grand Island, NY) (Pierce, Rockville, MD) for 1 h at room temperature. After incubation, color was developed with tetramethylbenzidine (Wako, Tokyo, Japan), stopped with 0.5 N HCl, and measured by OD492 on an ELISA reader (Lab System, Helsinki, Finland).

Isolation of mononuclear cells

Mononuclear cells from submandibular glands (SMG), NP, NALT, Peyer’s patch (PP), vaginal tissue (VT), and spleens (SP) were isolated as previously described (3, 8–12). In brief, mononuclear cells from NALT and SP were isolated by mechanical dissociation method using gentle teasing through stainless steel screens. NP, SMG, VT, and PP mononuclear cells were isolated by an enzymatic dissociation procedure using collagenase type IV (Sigma-Aldrich, St. Louis, MO).

Detection of HIV-specific Ab-producing cells by ELISPOT assay

Mononuclear cells were analyzed for Ag-specific Ab production at single cell level by using Ag- and isotype-specific ELISPOT assay as previously described (8, 9, 12, 13). Briefly, 96-well filtration plates with a nitrocellulose base (Millititer HA; Millipore, Bedford, MA) were coated with 2 μg of gp160 per well. Single cell suspensions of mononuclear cells from different tissues were added at varying concentrations and then incubated at 37 °C for 4 h in air with 5% CO2 and 95% humidity. After incubation and washing, detection Abs consisting of 1 μg/ml of HRP-labeled goat anti-mouse-μ, -γ, or -α (Southern Biotechnology Associates) were added to the plate. The spots were developed by 3-amin-9-ethylcarbazole (Moss, Passadena, MD) and counted under a dissecting microscope.

Analysis of HIV-specific T cell responses

For analysis of gp160-specific T cell responses, CD4+ T cells were isolated from NALT, NP, PP, VT, and SP by a magnetic cell sorting system (MACS; Miltenyi Biotec, Auburn, CA) as previously described (14). Purified CD4+ T cells (>98%) were suspended in complete medium and cultured at a density of 1 × 106 cells/ml in the presence of gp160 (5 μg/ml) and T cell-depleted and irradiated (3000 rad) splenic feeder cells (1 × 106 cells/ml) in flat-bottom 96-well microculture plates (Costar, Cambridge, MA) (14). After 6 days of incubation, using cytokine-specific murine cytokine ELISA kits (Amersham Life Science, Arlington Heights, IL), culture supernatants of Ag-stimulated T cells were examined for the presence of IFN-γ, IL-2, IL-4, IL-5, IL-6, and IL-10.

Ag-specific CTL assay

Lymphocytes isolated from nasally vaccinated mice were restimulated in vitro for 5 days with gp160 gene-transfected synergistic SYLVAC/3T3 fibroblasts, termed 15-12, expressing an immunodominant epitope identified as a peptide composed of 15 aa (P18IIIB: RIQRGPGRAFVTIGK) (16). A long-term CTL line was also generated by repetitive stimulation of immune cells with nontoxic C-treatment gp160 gene-transfected fibroblasts and 10% Con A supernatant condition medium according to a method described previously (17). After culturing for 5 days, the cytolytic activity of the Ag-restimulated cells was measured using a 6-h CTL assay with various 51Cr-labeled targets as previously described (16–18). For testing the peptide specificity of CTLs, effectors and 51Cr-labeled targets were mixed with various concentrations of peptide at the beginning of the assay. Percent specific 51Cr release was calculated as follows: 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release). Maximum release was determined by evaluating supernatants of cells that were lysed by the addition of 5% Triton X-100. Spontaneous release was determined by evaluating target cells incubated without added effectors. SEMs of triplicate cultures were always within 5% of the mean. To confirm the surface phenotype and class I MHC restriction of the gp160-specific CTLs, the CTL effector cells were variously pretreated with anti-CD4 mAb (rat IgM, RL172.4 hybridoma) plus rabbit complement (Low-Tox Rabbit Complement; Cedarlane Laboratories, Hornby, Ontario, Canada), anti-CD8 mAb (rat IgM, 3.115 hybridoma) plus complement, or complement alone (16). These treated effector cell populations were then tested for killing activity on either fibroblasts transfected with gp160 gene (P18IIIB) or the control fibroblasts (18).

HIV neutralization activity assay

In vitro neutralizing assay of HIV was performed as previously described (19). Briefly, serum IgG Abs were purified from mice immunized with gp160-HVJ-liposome by using protein A-Sepharose (Amersham Biosciences, Little Chalfont, U.K.). Serum IgG was also purified from preimmunized and nonimmunized mice. In addition, vaginal wash was also tested for the presence of neutralizing activity. Appropriately, diluted serum and vaginal wash Abs were incubated with 100 μl of HIV IIIB (HIV-1 MPR) (kindly provided by Dr. T. Oda, Tokyo University, Japan) for 60 min at 37 °C, and the mixture was shaken with 1 × 106 PHA-activated PBMCs for 60 min at 37 °C.
bath (19). After washing, the cells were cultured in the presence of recombinant human IL-2 (40 U/ml; Shionogi, Osaka, Japan) for 7 days. After the incubation, culture supernatants were subjected to a p24 Ag ELISA (Dynabot, Tokyo, Japan) for the measurement of HIV. Data were expressed as percentage inhibition of p24 Ag production in the culture supernatants compared with results from cultures to which preimmunized or nonimmunized serum IgG or vaginal wash were added. Virus stocks were titrated on PHA-activated normal PBMCs, and the TCID50 of each virus was determined as described previously (19).

Results

Nasally administered gp160-HVJ-liposome induced Ag-specific Abs in both serum and mucosal secretions

With high titers of gp160-specific serum IgG and IgA Abs already detected between the second and third week after initial immunization, gp160-specific Ab responses were clearly induced by nasal immunization with gp160-HVJ-liposome (Fig. 1). Furthermore, gp160-specific IgA Abs were present in different mucosal secretions including fecal, salivary, vaginal, and nasal (Fig. 2). Relevant to sexual activity, relatively high titers of HIV gp160-specific IgA and IgG Abs were present in the vaginal wash of mice nasally immunized with gp160-HVJ-liposome. Taken together, these results indicate that nasal vaccination with gp160-HVJ-liposome was an effective immunization procedure for the induction of HIV gp160-specific systemic IgG and IgA, as well as mucosal IgA and IgG, immune responses.

Induction of Ag-specific Ab-producing cells by nasal immunization with gp160-HVJ-liposome

The isolation of mononuclear cells from SP and different mucosal-associated tissues (e.g., VT, intestinal lamina propria (i-LP), NP, etc.) was performed as described previously (19). After washing, the cells were cultured in the presence of recombinant human IL-2 (40 U/ml; Shionogi, Osaka, Japan) for 7 days. After the incubation, culture supernatants were subjected to a p24 Ag ELISA (Dynabot, Tokyo, Japan) for the measurement of HIV. Data were expressed as percentage inhibition of p24 Ag production in the culture supernatants compared with results from cultures to which preimmunized or nonimmunized serum IgG or vaginal wash were added. Virus stocks were titrated on PHA-activated normal PBMCs, and the TCID50 of each virus was determined as described previously (19).
MUCOSAL HIV VACCINE WITH gp160-HVJ-LIPOSOME

SMG, NALT, and PP) of mice nasally immunized with gp160-HVJ-liposome provided further corroboration of the induction of Ag-specific Ab responses at the cellular level. Isotype- and Ag-specific ELISPOT assays revealed the presence of increased numbers of gp160-specific IgG and IgA Ab-forming cells in the SP and other parts of the systemic compartment (Fig. 3). In addition, Ag-specific IgG and IgA Ab-producing cells were detected in all of the mucosa-associated tissues, including VT, i-LP, NP, SMG, NALT, and PP (Fig. 3). As anticipated, high numbers of gp160-specific IgA Ab-producing cells were present in mucosal effector tissues, including VT, i-LP, SMG, and NP. These results show that nasal immunization with gp160-HVJ-liposome induced Ag-specific IgG- and IgA-producing cells in both the systemic and mucosal compartments.

In both Th1 and Th2 immunodeficient mice, nasal immunization with gp160-HVJ-liposome induced gp160-specific IgG in serum and vaginal secretions

To examine the potential for the application of nasal gp160-HVJ-liposome in the development of a clinical therapeutic vaccine, Th1 (IFN-γ−/−) and Th2 (IL-4−/−) deficient mice were nasally immunized with gp160-HVJ-liposome, after which increased levels of Ag-specific IgG Ab in serum were induced in these immunodeficient mice (Fig. 4). However, the levels of HIVgp160-specific IgG Abs induced in IFN-γ−/− and IL-4−/− mice were lower than in nasally immunized normal mice (Figs. 1 and 4). More specifically, in serum and vaginal wash, although the levels of HIVgp160-specific IgG Abs induced in IL-4−/− mice were lower than in nasally immunized normal mice (Figs. 1 and 4). These results suggest that nasal vaccination with gp160-HVJ-liposome might be an effective way to induce HIV Ag-specific immune responses even when the immunodeficient condition of HIV-infected patients varies during the different stages of progression of AIDS.

Characterization of gp160-specific IgG subclass Abs in mice nasally vaccinated with gp160-HVJ-liposome

We next investigated the IgG subclass of gp160-specific Abs in serum isolated from immunodeficient (IFN-γ−/− and IL-4−/−) and wild-type mice nasally immunized with gp160-HVJ-liposome. In wild-type mice, gp160-specific IgG2a Ab level was highest, followed by Ab subclasses IgG1, IgG2b, and IgG3 (Fig. 5). As anticipated, Ag-specific IgG1, IgG2b, and IgG3 Abs, but not IgG2a Abs, were induced in IFN-γ-deficient mice (Fig. 5). In samples from IL-4−/− mice, the highest titers of Ag-specific subclass Ab were for IgG2a. Interestingly, high levels of gp160-specific IgG3 Abs were consistently induced in these two immunodeficient types and wild-type mice. The results obtained by the analysis of wild-type mice suggest that the gp160-HVJ-liposome system preferentially induced Th1-type responses based on the profile of the Ag-specific IgG subclass distribution. It is worth emphasizing that the
HVJ-liposome system can overcome Th1- and Th2-deficient conditions and induce selective Ag-specific IgG subclass Ab responses. These results further indicated that nasal vaccination with gp160-HVJ-liposome holds promise for inducing HIV Ag-specific Ab response in immunocompromised patients.

Nasal vaccination with gp160-encapsulated HVJ-liposome induced Ag-specific Th1- and Th2-type responses

To characterize the nature of gp160-specific CD4^+ T cell responses, Th1-specific (e.g., IFN-γ and IL-2) and Th2-specific (e.g., IL-4, IL-5, and IL-6) cytokine production was investigated. High levels of Th1-type cytokine (e.g., IFN-γ and IL-2) were detected in the culture supernatant harvested from in vitro gp160-stimulated CD4^+ T cells that were isolated from SP and mucosa-associated tissues including VT, NP, NALT, and i-LP from normal mice nasally immunized with gp160-HVJ-liposome (Fig. 6). The findings reflect the results of analysis of Ag-specific IgG subclass responses in wild-type mice nasally immunized with gp160-HVJ-liposome (Fig. 5). A similar Th1 cytokine profile was also apparent in samples from IL-4^-/- mice nasally immunized with gp160-HVJ-liposome. With IFN-γ^-/- mice, as anticipated, the only Th1 cytokine detected was IL-2. It is also worth noting that Th2-associated IgA-enhancing cytokines, such as IL-5 and IL-6, were induced by gp160-specific CD4^+ T cells isolated from systemic and mucosa-associated tissues of all of the mice tested. Taken together, these findings suggested that nasal immunization with gp160-HVJ-liposome induced both Th1- and Th2-type CD4^+ T cells in the mucosal and systemic compartments.

Induction of Ag-specific CD8^+ CTL responses by nasal immunization with gp160-HVJ-liposome

After confirming that Ag-specific Th1-type responses were elicited by nasal immunization with gp160-HVJ-liposome (Fig. 6), we next tested whether gp160-HVJ-liposome is able to induce MHC class I-restricted gp160-specific CTL responses. At first, CTL activity against gp160 was examined in splenic lymphocyte samples isolated from mice nasally immunized with gp160-HVJ-liposome. After in vitro restimulation of the immune splenic lymphocytes with gp160 gene-transfected 15-12 fibroblast stimulator cells, we found that the presence of gp160-specific CTL response was detected in mice nasally immunized with gp160-HVJ-liposome (Fig. 7).
FIGURE 8. Induction of gp160-specific neutralizing Ab responses by gp160-HVJ-liposome nasal vaccine. In vitro neutralization of HIV-MN was performed by a standard p24 release assay. Six weeks after the start of the nasal immunization regimen with gp160-HVJ-liposome, serum IgG samples and mucosal IgA samples from vaginal washes were prepared from wild-type mice (Δ) and immune-deficient mice (IFN-γ−/−, ○; IL-4−/−, □). These samples were analyzed for the presence of neutralizing Ab after testing against a clinically isolated strain (HIV-MNp). As a control, serum and mucosal samples from immunized mice and mice nasally immunized only with HVJ-liposome were obtained and tested (wild-type mice, ▲; IFN-γ−/−, ●; IL-4−/−, ▼). These control samples did not detect any Ag-specific neutralization activity. Results represent the values (mean ± SEM) of four mice in each experimental group.

7). When these splenic lymphocytes were pretreated with mAb anti-CD4 or anti-CD8, the removal of gp160-specific CTL activity was subsequently achieved by anti-CD8 mAb treatment (Fig. 7). These findings suggest that nasal immunization with gp160-HVJ-liposome resulted in the priming of Ag-specific CD8+ CTL responses.

Neutralization of HIV-1 by gp160-specific Abs from mice nasally immunized with gp160-HVJ-liposome

To better characterize virus neutralization activity, a well-characterized, clinically isolated strain of HIV-MNp that expresses IHIG-PGRAFY at the core sequence of the HIV principal neutralizing determinant (19, 21) was used as the viral source. Serum IgG and vaginal secretory IgA (S-IgA) Ab samples obtained from wild-type mice nasally immunized with gp160-HVJ-liposome possessed neutralizing activity against HIV-MNp (Fig. 8). Similar neutralization activity was observed with IgG immune serum purified from both IFN-γ- and IL-4-deficient mice that had been nasally vaccinated with gp160-HVJ-liposome (Fig. 8). Preimmunized IgG and vaginal wash samples containing S-IgA, however, showed no neutralizing activity (data not shown). These results indicated that nasal immunization with gp160-HVJ-liposome was capable of strongly inducing gp160-specific IgG and S-IgA Abs with virus-neutralizing activity.

Discussion

An effective and safe prophylactic HIV vaccine that targets both systemic and mucosal immunity could play a decisive role in combating the spread of HIV infection through the epithelium of reproductive organs. Research has already shown that vaginal secretions containing locally produced S-IgA and serum-derived IgG Abs contribute to the formation of first line of defense at the epithelium of reproductive organs (22, 23). For example, Ag-specific S-IgA and IgG in vaginal wash samples have been shown to possess neutralizing activity against viruses or bacteria associated with sexual transmission (3, 24). One of the significant findings of the present study is the demonstration of the induction of HIV-specific IgA and IgG Ab responses in both the systemic and mucosal compartments at sites of sexual contact. We also found that these Ag-specific Abs that were induced by nasal immunization with HVJ-liposome containing HIV gp160 possessed neutralizing activity against a clinically isolated strain of HIV-MNp. In generating these effective mucosal IgA and systemic IgG Abs, nasally administered gp160-HVJ-liposome induced appropriately balanced Th1- and Th2-type CD4+ T cell responses. In addition, HVJ-liposome that contained HIV gp160 elicited Ag-specific CTL responses. These findings indicated that HVJ-liposome is an effective nasal Ag delivery system for the induction of both humoral (e.g., S-IgA and serum IgG) and cell-mediated (e.g., CTL) immunity in the mucosal and systemic compartments.

Nasal administration has been shown to be as effective at inducing Ag-specific immune responses, including Ab production and CTL activity, in both the mucosal and systemic compartments as oral immunization (15, 22, 23, 25, 26). Our own previous studies and work by other researchers has shown that coadministration of mucosal adjuvant is essential for the generation of Ag-specific mucosal and systemic immune responses via the respiratory and gastrointestinal immune system (3, 9, 13, 15). Thus, our previous report has shown that nasal vaccination with the fimbrial protein of Porphyromonas gingivalis and a well-known mucosal adjuvant cholera toxin (CT) induces protective immunity via the generation of Th2 cell-mediated Ag-specific mucosal IgA Ab responses (15). Nasal vaccine containing fimbrial protein alone, however, did not lead to the induction of Ag-specific Th and B cell responses (15). Furthermore, when female rhesus macaques were nasally immunized with p55 of SIV in the presence or absence of CT as a mucosal adjuvant (3), nasal vaccine containing both p55 and CT induced Ag-specific IgA and IgG Abs in mucosal secretions (e.g., cervicovaginal, rectal, and salivary), but similar Ag-specific immune responses were not detected in primates nasally immunized solely with p55 (3).

In contrast with the previous investigations discussed above, HVJ-liposome effectively induces Ag-specific Th1 and Th2 cells together with the associated IgG and IgA Ab responses in both mucosal and systemic sites without coadministration of mucosal adjuvant (e.g., CT or Escherichia coli heat-labile enterotoxin). It is pertinent to consider why HVJ-liposome induces mucosal and systemic immune responses without mucosal adjuvant. One reason may be its ability to directly deliver the Ag to cells associated with the mucosal immune system. It was shown that HVJ-liposome directly attaches to the cell membrane using envelope glycoproteins, the so-called fusion proteins of the Sendai virus (27–29), which function during natural infection via the respiratory epithelium (30, 31). Furthermore, in a previous study we found that HVJ-liposome is able to preferentially attach to nasal epithelia and deliver encapsulated Ag directly to the surface of epithelial cells, M cells, and APCs (8). The immunoenhancing activity of envelope glycoproteins composed with F and H proteins may bypass the need for adjuvant. For example, it has been shown that virus-associated fusion proteins possess immunoenhancement or adjuvant activity (8, 29). Other reports have shown that the liposome inherently possesses adjuvant activity (32, 33). Moreover, there is evidence that, after Sendai virus infection of an epithelial cell line (NIH 3T3), overexpression of NF-kB gene is induced, which leads to the up-regulation of IFN-β mRNA induction (34). Therefore, it is also possible that HIV-liposome is involved in the activation of cytokine synthesis that enhances immune response. Taken together, this evidence of the multiple immunoenhancing activities of HIV-liposome is encouraging for the development of a novel and effective Ag delivery vehicle that generates HIV Ag-specific mucosal and systemic immune responses without administration of any additional mucosal adjuvant.
HVJ-liposome, a novel viral-based hybrid consisting of liposome with Sendai virus fusion proteins, has proved effective as a delivery vehicle for introducing DNA into a host (5–7). Not only is HVJ-liposome capable of delivering the encapsulated oligodeoxynucleotide DNA into cell cytoplasm (5–8), this delivery also results in presentation of MHC class I-mediated peptide that induces cell-mediated immune responses (29). Nasal immunization with HVJ-liposome also induces Ag-specific CTL responses at systemic lymphoid tissues in an MHC class I-dependent manner (8). Direct intracellular delivery of Ag via the fusion process demonstrated an encapsulated Ag to the MHC class I pathway (8, 29). In addition to these MHC class I-mediated CTL responses, nasal immunization with gp160-HVJ-liposome induced high levels of gp160-specific CD4+ Th1 and Th2 cell-mediated Ab responses in both systemic and mucosal tissues. This result suggests that mucosally administered HIV-liposome can activate MHC class II-mediated CD4+ T cell responses for the subsequent induction of gp160-specific IgG and IgA Abs. To this end, our previous study demonstrated that HVJ-liposome is capable of presenting peptide Ag together with MHC class II molecules of epithelial cells and macrophages (8). Other evidence from previous studies has also shown that mucosal epithelial cells are able to present Ags (35, 36). Thus, nasally administered HIV-liposome appears to use the mucosa-associated Ag presentation pathway for the maximum induction of gp160-specific IgA immune response. These different findings, taken together, provide evidence that gp160-HVJ-liposome effectively enhances gp160-specific MHC class I-mediated CD8+ CTL and class II-regulated CD4+ Th1/Th2 cell activities.

The established cytokine profile of Ag-specific Th1 and Th2 cells predicts the presence of IFN-γ and IL-4-dependent IgG subclass Ab responses in mice nasally immunized with gp160-HIV-liposome, and our findings show a profile of Th1 and Th2 cytokines in line with anticipated IgG subclass Ab responses, which indicates that nasally administered HVJ-liposome causes simultaneous induction of Ag-specific serum IgG1 and IgG2a Ab responses. It was long ago shown that IFN-γ is the key cytokine for generation of IgG2a Abs and that IL-4 is the key cytokine for IgG1 Abs (37). Our current results corroborate our previous finding that nasal immunization with OVA-fusogenic liposomes results in the generation of both the Th1 and Th2 cytokine-mediated IgG2a and IgG1 responses (8). Even so, our previous and current investigations of the induction of Ag-specific IgG3 subclass Ab responses differ. Whereas in the previous study Ag-specific IgG3 Abs were not induced in wild-type mice nasally immunized with HIV-liposome containing OVA (8), in the current study, using an identical Ag delivery vehicle, HIV Ag-specific IgG3 Ab responses were in evidence. We do not have any conclusive explanation, other than that the discrepancy may be due to the differing nature of the antigenicity of OVA and gp160.

One of the most significant findings of this study is that nasal immunization with gp160-HVJ-liposome is capable of inducing high levels of gp160-specific serum IgG and mixed S-IgA and IgG in vaginal wash samples isolated from both in immunodefi cient Th1 type (IFN-γ) or Th2 type (IL-4) mice and in wild-type mice (Fig. 4). In human AIDS, it has been debated whether Th1- and Th2-type CD4+ T cells are predominantly depleted in HIV-infected individuals. It has been reported that, during the development of HIV infection, there is a change from Th1 to Th2 responses (38). However, another study suggested that a switch from Th1 to Th2 responses does not occur during the progression of HIV infection (39). Meanwhile, it has been shown that HIV preferentially replicates within CD4+ T cells with phenotypical Th0 and Th2 cytokine synthesis (40). Although the exact state of CD4+ T cell deficiency is unknown, we are making provisions for this in the development of a mucosal vaccine that can offer induction of effective Ag-specific immune responses in HIV-infected patients at different stages of CD4+ T cell deficiency. At the same time, we are concerned with developing a therapeutic vaccine system that is able to effectively counteract different Th-type cell deficiency profiles in AIDS. Because nasal administration of the gp160-HVJ-liposome vaccine results in the induction of Ag-specific mucosal IgA and IgG as well as serum IgG Abs in both Th1- and Th2-type immunodefi cient mice, our current findings show promising prospects for the application of gp160-HVJ-liposome in the development of a therapeutic mucosal vaccine (Figs. 4 and 6).

This study has also shown that nasal vaccination with gp160-HVJ-liposome is capable of inducing high levels of Ag-specific neutralizing Abs in both serum and mucosal secretions in both wild-type mice and Th1- and Th2-type immunodefi cient mice (Fig. 8). The rapid clearance of HIV from the peripheral blood after primary HIV infection is generally thought to be the result of the development of neutralizing Abs or the formation of Ag-Ab complexes (41). The majority of neutralizing Abs detected in HIV-infected individuals react with epitopes that are present on HIV gp120 and gp41 (42). These Abs have been shown to neutralize either a single particular isolate (type specific) or several isolates with varying degrees of sequence homology (group specific). One recent report has in fact demonstrated in vitro that the vaginal wash obtained from mice vaginally immunized with inactivated HIV-1 captured Con A-immobilized polystyrene nanospheres contained an in vitro neutralizing activity against the strain used for the immunization (43). Furthermore, other reports suggest that nasal immunization of mice with gp160 protein is capable of inducing systemic and mucosal HIV-1-neutralizing Abs (25, 44, 45). These results have shown that, after mucosal immunization with Ags and adjuvant, vaginal wash secretions are capable of neutralizing HIV. However, in fact, mucosal Ag-specific Abs were not induced by systemic immunization with different forms of HIV Ags, including gp160 and gp41 (44, 45). The current study found that nasal immunization with gp160-HVJ-liposome in the absence of coadministered adjuvant induces, in vaginal secretions, neutralizing Abs against a clinically isolated strain of HIV-MNP. Because vaginal wash samples contained both S-IgA and IgG Abs, it would be interesting to know which isotype of Ag-specific Abs preferentially provides protective immunity at the reproductive epithelium. To directly address this issue, it is important to purify individual isotypes of Ag-specific Abs from vaginal washes. We were unable to do this in the current study, because the amount of sample was limited. A future task remains: the elucidation of whether a particular isotype or both have pronutralizing activity in the reproductive compartment of mice nasally immunized with gp160-HVJ-liposome.

In summary, for the prevention and control of HIV infection, our present investigation has demonstrated the potential prophylactic and therapeutic usefulness of a nasal vaccine based on gp160-HVJ-liposome. It is especially significant that, as well as systemic CTL activity, gp160-HVJ-liposome can induce high levels of gp160-specific mucosal IgA or IgG and systemic IgG Abs with neutralizing activity.

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References
MUCOSAL HIV VIRUS WITH gp160-HVJ-LIPOSOME


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