HIV-1 Neutralizing Antibodies in the Genital and Respiratory Tracts of Mice Intronasally Immunized with Oligomeric gp160


http://www.jimmunol.org/content/160/4/2000
HIV-1 Neutralizing Antibodies in the Genital and Respiratory Tracts of Mice Intranasally Immunized with Oligomeric gp160


Because mucosal surfaces are a primary route of HIV-1 infection, we evaluated the mucosal immunogenicity of a candidate HIV-1 vaccine, oligomeric gp160 (o-gp160). In prior studies, parenteral immunization of rabbits with o-gp160 elicited broad neutralizing serum Ab responses against both T cell line-adapted HIV-1 and some primary HIV-1 isolates. In this study, nasal immunization of mice with o-gp160, formulated with liposomes containing monophosphoryl lipid A (MPL), MPL-AF, proteosomes, emulsomes, or proteosomes with emulsomes elicited strong gp160-specific IgG and IgA responses in serum as well as vaginal, lung, and intestinal washes and fecal pellets. The genital, respiratory, and intestinal Abs were determined to be locally produced. No mucosal immune responses were measurable when the immunogen was given s.c. Abs from sera and from vaginal and lung washes preferentially recognized native forms of monomeric gp120, suggesting no substantial loss in protein tertiary conformation after vaccine formulation and mucosal administration. Inhibition of HIV-1MN infection of H9 cells was found in sera from mice immunized intranasally with o-gp160 formulated with liposomes plus MPL, proteosomes, and proteosomes plus emulsomes. Formulations of o-gp160 with MPL-AF, proteosomes, emulsomes, or proteosomes plus emulsomes elicited strong gp160-neutralizing Ab in lung wash, and formulations with proteosomes, emulsomes, or proteosomes plus emulsomes elicited strong gp160-neutralizing Ab in vaginal wash. These data demonstrate the feasibility of inducing both systemic and mucosal HIV-1-neutralizing Ab by intranasal immunization with an oligomeric gp160 protein.

mucosal Ab responses in phase I human clinical trials (40). Intranasal administration of formalinized staphylococcal enterotoxin B (SEB) toxoid formulated with proteosomes elicited serum IgG and IgA, as well as bronchial IgA, and protected against lethal aerosolized SEB intoxication (8). Intranasal immunization of mice and guinea pigs with LPS of Brucella melitensis in proteosomes elicited serum and lung IgG/IgA responses (42). Intranasal immunization of mice with baculovirus-expressed gp160 formulated in proteosomes with or without emulsomes or cholera toxin B elicited strong serum, lung, intestinal, and vaginal IgG/IgA responses (43).

Functional HIV-1-neutralizing Ab responses after mucosal formulation of HIV-1 envelope glycoproteins or peptides have been assessed in several studies. An HIV-1 gp120 peptide in combination with lyosolphatidyl glycerol (LPG) administered i.v. to rats elicited serum and vaginal IgG/IgA but without functional HIV-1-neutralizing activity (44). Intranasal immunization with the HIV-1 C4/V3 peptide with cholera toxin in mice elicited serum and vaginal IgG/IgA anti-peptide responses with detectable serum, but not mucosal, HIV-1-neutralizing Ab responses (45). Oral immunization using a macromolecular peptide Ag with cholera toxin elicited strong fecal IgG and IgA responses that were capable of approximately 50% in vitro neutralization of HIV-1MN, HIV-1HIV, and HIV-1SR3 (46). A chimeric influenza virus that expresses a peptide from a conserved region within gp1 and administered i.n. with an i.p. booster immunization elicited peptide-specific IgA in respiratory, intestinal, and vaginal secretions and also elicited a serum Ab response capable of neutralizing HIV-1MN, HIV-1HIV, and HIV-1SR3 (47, 48).

We report here the induction of systemic and mucosal IgG and IgA responses in mice after i.n. immunization with an oligomeric gp160 protein (o-gp160) formulated using several adjuvants and vaccine delivery systems. Locally produced HIV-1 o-gp160-specific IgG and IgA responses were detected in vaginal, lung, and intestinal wash as well as fecal pellets. After i.n. immunization, serum responses were comparable to those obtained with parenteral o-gp160 administration with MPL adjuvant; however, mucosal IgG and IgA responses were obtained only in the i.n. immunized mice. The Abs elicited in serum and mucosal washes preferentially bound native forms of monomeric gp120, demonstrating that adjuvant formulation, i.n. administration, and local Ag uptake did not substantially alter gp120 tertiary structure. Sera from groups receiving o-gp160 with proteosomes with or without emulsomes, MPL-AF, and liposomes containing MPL neutralized HIV-1MN. Most significantly, lung and vaginal washes from groups receiving o-gp160 with proteosomes and/or emulsomes were also capable of neutralizing HIV-1MN. These are the first data demonstrating locally produced HIV-1 neutralizing Abs in the respiratory or genital tract resulting from immunization.

Materials and Methods

Proteins, peptides and Abs

Oligomeric gp160 was affinity purified from cell cultures infected with HIV-1MN (o-gp160MN) as described previously (49, 50). Briefly, chronically infected cell lines 6D5MN and 451MN were developed by infection of 6D5 cells (a subclone of HUT78 cell line) with the primary isolate HIV-1MN (51). Radioimmunoprecipitation analysis revealed that this cell line secreted both gp120 and gp160 into the media (49, 50). The gp160 protein was purified from the serum-free conditioned media by affinity chromatography using a mouse mAb to HIV-1 gp41 (49, 50). Structural analysis showed this gp160 to exist mostly as dimers and tetramers (approximately 75%) with some monomers (52). Baculovirus-expressed recombinant gp160Lum (Mrpg160Lum) was obtained from MicroGeneSys (Meridian, CT). Recombinant gp120Lum and gp120SN from Chinese hamster ovary cells and reduced, carboxymethylated (rcm) gp120Lum and rcmgp120SN was provided by Genentech Inc., South San Francisco, CA (53) for use in serologic assays.

Immunogen and adjuvants

The following MPL-containing adjuvant preparations were provided by Ribi ImmunocResChem Research, Inc. (Hamilton, MT) (54, 55); Ribi adjuvant system (Ras3c) composed of a 2.0% v/v squalene oil-in-water emulsion containing 250 µg/ml of 4’ monophosphoryl lipid A derived from LPS of Salmonella minnesota R595 (MPL), 250 µg/ml cell wall skeleton (CWS) from Mycobacterium phlei, and synthetic dicorynomycolate (S-TDCM); MPL-oe, a 1.0% v/v squalene oil-in-water emulsion containing 50 µg/ml MPL; and MPL-AF, MPL combined with the surfactant 1,2-dipalmityl-SN-glycero-3-phosphocholine (DPCC). Mixing of MPL with DPCC produces small micelles and solubilizes the MPL. Proteosomes, which form multimolecular protein complexes approximately 60 to 100 nm in diameter, were prepared from outer membrane protein preparations of group B serotyp 2 N. meningitides as described previously (43, 56). Emulsomes are oil in water submicron emulsions (50–500 nm) prepared by Pharmos Corp., Rehovot, Israel as described previously (43, 57) using a metabolizable and nontoxic oil, carbocel (B.F. Goodrich, Atlanta, GA), and Tribol surfactant. Walter Reed liposomes containing or lacking MPL were prepared from dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), and cholesterol as a stabilizing lipid (5–7, 58).

Immunizations

Female BALB/c mice (age 4–6 wk) were immunized at 0, 3, and 6 wk with o-gp160MN at various doses formulated in various adjuvants either by the s.c. or i.n. route (summarized in Table I). Systemic immunizations were performed by s.c. injection using 5 µg o-gp160 in a 0.2-ml volume. Intranasal immunizations were performed on halothane-anesthetized mice by placing 30 µl of vaccine on the mouse nares and allowing mice to inhale. This procedure was repeated 2 to 4 h later for a total dose volume of 60 µl. Serum, vaginal secretions, and fecal pellets were collected at two time-points before immunization and at 1 and 2 wk after each immunization. Lung and intestinal lavage were collected 2 wk after the final immunization at sacrifice (wk 8).

Collection of mucosal secretions

Estrus cycling of groups of female mice was synchronized by housing male mice adjacent to the females for 3 days before collection of mucosal washes. It has been previously demonstrated that specific IgA and IgG responses to mucosal immunizations in mice are effected by the estrous cycle, with higher IgA-specific responses elicited during estrus and higher IgG during diestrus (26).

Vaginal secretions. An amount equal to 25 µl of sterile PBS was instilled into the vaginal vault of female mice using a sterile 200-µl micropipette. A uniwick (10 mm x 3 mm, Polyfiltronics, Rockland, MA) (59) was then placed into the vaginal vault using sterile forceps and left in place for approximately 30 s. The wick was then removed, an additional 25 µl was instilled into the vaginal vault, and the opposite end of the same wick was inserted as described above. To assay vaginal washes, 800 µl of ELISA dilution buffer (PBS with 5% BSA, 5% casein, 0.01% Sodium azide, phenol red, pH 7.4) were added to each tube and then vortexed and centrifuged at 5,000 x g for 15 min at room temperature. For neutralization assays, vaginal wash samples were sterile filtered through a 0.2-µm filter (Fierce, Rockford, IL) and then dialyzed overnight against sterile PBS (Quality Biologic, Inc., Gaithersburg, MD).

Lung lavage. The lungs were flushed with 1 ml of lavage solution (sterile PBS with 0.2 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 1 µg/ml Aprotinin, 3.25 µM Bestatin, and 10 µM leupeptin) as described previously (9, 43). The procedure was repeated with a second 1-ml wash and the fractions combined. The lung lavage fluid collected (approximately 1.8 ml) was then centrifuged at 1,200 rpm for 10 min at 4°C to remove any tissue and cellular debris and stored at −70°C until assayed. For neutralization assays, lung lavage samples were further sterile filtered through a 0.2-µm filter.

Intestinal wash. A flexible plastic catheter (Baxter, Deerfield, IL) was inserted into one end of the small intestine containing the jejunum and ileum and a 3-ml syringe prefilled with 2 ml of lavage solution was then slowly instilled, and wash was collected as described previously (9, 43). The intestinal lavage fluid collected (approximately 1.8 ml) was then centrifuged at 1,200 rpm for 10 min at 4°C to remove any tissue, fecal matter, and cellular debris and stored at −70°C until assayed.
Fecal pellets. Processing of fecal pellets has been described previously (60). Approximately 0.1 grams of fecal pellets per cage of five mice, which is the equivalent of 12 to 15 individual pellets per group of five mice, were collected into a microcentrifuge tube. One milliliter of PBS/1% thimerosal solution was added to 0.1 g of fecal pellets, allowed to stand at room temperature for about 30 min, and intermittently vortexed vigorously until solution was homogenous. Samples were then spun in a microcentrifuge at 5000 × g for 10 to 15 min, and supernatants were stored at −70°C until assayed by ELISA.

Total Ig measurements by enzyme immunoassay

Total IgG and IgA concentrations were determined by use of a capture enzyme immunoassay (ELISA). Unlabeled goat anti-mouse (GAM) IgG (1:5000) or GAM-IgA (1:4000) (Southern Biotechnology Associates, Inc. Birmingham, AL) diluted in PBS (pH 7.4, 0.01% thimerosal) were coated overnight at 4°C onto Immulon 2 round-bottom 96-well microtiter plates. Plates were washed twice with wash buffer (PBS with 0.1% Tween-20 and 0.01% thimerosal, pH 7.4) before incubation with twofold dilutions of serum or mucosal samples diluted in sample diluent (wash buffer with 5% skim milk, pH 7.4) for 1 h at 37°C. Plates were subsequently washed four times with wash buffer and incubated with horseradish peroxidase (HRP)-conjugated GAM-IgA (1:10,000) or GAM-IgG (1:1000) (Kirkegaard and Perry, Gaithersburg, MD). After a 1-h incubation at 37°C, plates were washed four times, soaked for 10 min during the final wash, after which substrate (2′,2′-Azinobis(3-ethylbenzthiazoline-sulfonic acid), Kirkegaard and Perry) was added. After a 30-min incubation at 37°C, plates were read at 450 nm and 570 nm, the OD at 570 nm was subtracted from that at 450 nm. Sample endpoint dilutions were determined from the curves, using purified Ig standard mouse IgG and IgA (Chemicon, Temecula, CA) assayed in parallel; values were expressed in micrograms per milliliter. All assays were run at least in duplicate, and the results were averaged.

HIV-1 gp160-specific activity and IgA measurements by ELISA

Affinity purified oligomeric gp160(451) at 1.25 μg/ml in PBS (pH 7.4, 0.01% thimerosal) was coated overnight at 4°C onto Immulon 2 round-bottom 96-well microtiter plates. Plates were washed twice with wash buffer before blocking the plates with BSA-casein (PBS with 0.5% casein, 0.5% BSA, 0.2% sodium azide, and phenol red, pH 7.4). After the 1-h blocking step, plates were washed three times with wash buffer and incubated with twofold serial dilutions of sera or mucosal samples (diluted in BSA-casein) overnight at room temperature. Plates were washed four times with wash buffer and incubated overnight at room temperature with HRP-GAM-IgG (Southern Biotechnology Associates) diluted 1:2000 in BSA-casein. Plates were washed four times (including a 10-min soak during the final wash), after which substrate (3, 3′, 5′-tetramethylbenzidine, TMB; Kirkegaard and Perry) was added. Reactions were stopped with 1 M H₂PO₄ after a 15-min incubation at 37°C. Plates were read at 450 nm and 570 nm as above. Sample endpoint dilutions were determined from the maximum dilution of Ab at which the OD signal was greater than twice the mean plus 2 SD of preimmune sera or wash.

Specific activity and coefficient of local Ig secretion calculations

HIV-1 gp160-specific activities were calculated by dividing the reciprocal endpoint titer for each individual serum or mucosal sample by the concentration of total Ig of the same isotype within that sample (26, 61, 62). For example, a specimen with an anti-o-gp160(451), IgG titer of 1:100 and total IgG concentration of 10 μg/ml would have a specific activity of 10. The coefficient of local Ig production (C₁) was determined by calculating the ratio of the specific activity (IgG or IgA) of a mucosal wash and the specific activity of the corresponding serum. C₁ values greater than one (C₁ > 1) indicate a higher relative composition of gp160-specific Abs within the mucosal wash than corresponding sera, demonstrating that the response cannot be attributed solely to serum transudate but rather a component of the immune response must be locally produced.

Antibody binding to native and denatured monomeric gp120

Native/denatured gp120, Ab-binding ratios were determined as previously described (63, 64). Monoclonal Abs and sera were diluted in HBS running buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.05% BSA) prior to injection. The wash buffer alone flowed through each flow cell. The net difference in signal, between baseline and approximately 20 s after completion of sample injection, was calculated to represent the binding value of that particular sample. The native/denatured gp120 Ab binding ratios were determined by dividing the amount of sample (serum or mucosal wash) bound to gp120 (binding in resonance units, RU) by the amount of sample binding to racgp120 binding (in RU) after normalization for any differences in the gp120 and racgp120 matrix concentrations. Control sera previously shown to bind specifically to denatured gp120 (R265) or native gp120 (US-B pool) were used as controls (65). Matrices were regenerated using 60 mM H₂PO₄ (trgp120, trcmpgp120) before injection of the next sample.

Virus neutralization assay

Virus isolate HIV-1MN was obtained from the National Institutes of Health AIDS Research and Reagent Repository. Unless otherwise specified, assessment of neutralizing Ab activity was performed as previously described with minor modifications (66, 67). H9 were used as target cells and virus growth kinetics and median tissue culture infective dose (TCID₅₀) were determined within the assay format. All sera were heat-inactivated at 56°C for 40 min before use.

Dilutions of test sera, or mucosal washes, were aliquoted in quadruplicate wells of a 96-well microtiter plate (25 μl per well). Culture media without Ab, pooled normal human serum (NHS, Sigma, St. Louis, MO), and preimmune sera or mucosal washes served as controls for baseline virus growth. An equal volume of virus stock (25 μl), representing 100 TCID₅₀, was added to each well. After 30 min at 37°C, 1 × 10⁵ H9 cells were added and incubated overnight at 37°C. Cells were then washed extensively and transferred to a 96-well microtiter plate with culture media containing 20 U/ml human IL-2. Inhibition of H9 infection was assessed by quantitative p24 measurement of cell supernatants during the early virus growth period and virus growth kinetics and median tissue culture infective dose (TCID₅₀) were determined within the assay format. All sera were heat-inactivated at 56°C for 40 min before use.

Dilutions of test sera, or mucosal washes, were aliquoted in quadruplicate wells of a 96-well microtiter plate (25 μl per well). Culture media without Ab, pooled normal human serum (NHS, Sigma, St. Louis, MO), and preimmune sera or mucosal washes served as controls for baseline virus growth. An equal volume of virus stock (25 μl), representing 100 TCID₅₀, was added to each well. After 30 min at 37°C, 1 × 10⁵ H9 cells were added and incubated overnight at 37°C. Cells were then washed extensively and transferred to a 96-well microtiter plate with culture media containing 20 U/ml human IL-2. Inhibition of H9 infection was assessed by quantitative p24 measurement of cell supernatants during the early virus growth period and virus growth kinetics and median tissue culture infective dose (TCID₅₀) were determined within the assay format. All sera were heat-inactivated at 56°C for 40 min before use.

Table I. Ag, adjuvant, route, dose, and immunization schedule of BALB/c mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Ag</th>
<th>Adjuvant</th>
<th>No. of Mice</th>
<th>Route</th>
<th>Dose (μg)</th>
<th>Schedule (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1,A2,A3⁴</td>
<td>o-gp160(451)</td>
<td>RasC</td>
<td>5</td>
<td>s.c.</td>
<td>5</td>
<td>0.3,6</td>
</tr>
<tr>
<td>B1,B2,B3⁵</td>
<td>none</td>
<td>saline</td>
<td>5</td>
<td>i.n.</td>
<td>0</td>
<td>0.3,6</td>
</tr>
<tr>
<td>C1</td>
<td>o-gp160(451)</td>
<td>saline</td>
<td>5</td>
<td>i.n.</td>
<td>50</td>
<td>0.3,6</td>
</tr>
<tr>
<td>C2</td>
<td>o-gp160(451)</td>
<td>saline</td>
<td>5</td>
<td>i.n.</td>
<td>10</td>
<td>0.3,6</td>
</tr>
<tr>
<td>D1</td>
<td>o-gp160(451)</td>
<td>liposomes</td>
<td>5</td>
<td>i.n.</td>
<td>3</td>
<td>0.3,6</td>
</tr>
<tr>
<td>D2</td>
<td>o-gp160(451)</td>
<td>liposomes</td>
<td>5</td>
<td>i.n.</td>
<td>3</td>
<td>0.3,6</td>
</tr>
<tr>
<td>D3</td>
<td>o-gp160(451)</td>
<td>MPL-AF</td>
<td>5</td>
<td>i.n.</td>
<td>50</td>
<td>0.3,6</td>
</tr>
<tr>
<td>D4</td>
<td>o-gp160(451)</td>
<td>MPL-AF</td>
<td>5</td>
<td>i.n.</td>
<td>10</td>
<td>0.3,6</td>
</tr>
<tr>
<td>E1</td>
<td>o-gp160(451)</td>
<td>protosomes</td>
<td>5</td>
<td>i.n.</td>
<td>50</td>
<td>0.3,6</td>
</tr>
<tr>
<td>E2</td>
<td>o-gp160(451)</td>
<td>protosomes</td>
<td>5</td>
<td>i.n.</td>
<td>10</td>
<td>0.3,6</td>
</tr>
<tr>
<td>E3</td>
<td>o-gp160(451)</td>
<td>emulsomes</td>
<td>5</td>
<td>i.n.</td>
<td>50</td>
<td>0.3,6</td>
</tr>
<tr>
<td>E4</td>
<td>o-gp160(451)</td>
<td>emulsomes</td>
<td>5</td>
<td>i.n.</td>
<td>10</td>
<td>0.3,6</td>
</tr>
<tr>
<td>E5</td>
<td>o-gp160(451)</td>
<td>protosomes</td>
<td>5</td>
<td>i.n.</td>
<td>50</td>
<td>0.3,6</td>
</tr>
<tr>
<td>E6</td>
<td>o-gp160(451)</td>
<td>protosomes</td>
<td>5</td>
<td>i.n.</td>
<td>10</td>
<td>0.3,6</td>
</tr>
<tr>
<td>F1</td>
<td>Mrgp160(IIIb)</td>
<td>protosomes</td>
<td>5</td>
<td>i.n.</td>
<td>50</td>
<td>0.3,6</td>
</tr>
<tr>
<td>F2</td>
<td>Mrgp160(IIIb)</td>
<td>protosomes</td>
<td>5</td>
<td>i.n.</td>
<td>10</td>
<td>0.3,6</td>
</tr>
</tbody>
</table>

⁴ Three separate groups of BALBc mice (five mice per group) were immunized s.c. with o-gp160(451) in RasC3.

⁵ Three separate groups of BALBc mice (five mice per group) were immunized i.n. with saline only.
growth phase (day 4, 5, or 6). Average p24 Ag output in control wells was usually between 50 and 250 ng/ml. The serum or mucosal wash dilution causing 50% and 90% reduction in p24 Ag were calculated by linear regression analysis.

**Results**

**Systemic and mucosal Ab responses**

A summary of the vaccine groups analyzed in the current study is given in Table I. The various groups (A-F) are described by Ag, adjuvant, route, dose, and schedule. Group A mice received o-gp160<sub>451</sub> formulated in Ras3C and administered s.c. Group B were negative control groups receiving only saline i.n. Group C received o-gp160<sub>451</sub> in saline i.n. Groups D and E were designed to assess relative mucosal immunogenicity of several adjuvants including liposomes with or without MPL, MPL-AF, proteosomes, emulsomes, or proteosomes combined with emulsomes. Group F evaluated a baculovirus-expressed recombinant gp160<sub>100</sub> protein that in a previous study elicited strong systemic and mucosal immune responses when formulated with proteosomes combined with emulsomes and administered i.n. (43) and has been shown to elicit serum Abs with restricted TCLA and minimal neutralizing activity against primary HIV-1 (66). Both a high dose (50 µg) and low dose (10 µg) were included for most of the groups, with the exception of the liposome groups, where only a 3-µg dose was used due to the formulation restrictions of o-gp160<sub>451</sub> resulting from the small dose volume.

Serum, vaginal, and fecal IgG and IgA o-gp160-specific responses for s.c. and several i.n. immunized groups are shown in Figure 1. Doses of o-gp160<sub>451</sub> in the i.n. groups were 10 µg for the saline, proteosome, and proteosomes plus emulsomes groups and 3 µg for the liposome plus MPL group. Data are expressed as the mean endpoint titer (2-3 separate assays) of sera, vaginal wash, or fecal pellets pooled from the five individual mice in each group. O-gp160<sub>451</sub>-specific serum IgG responses were detected 2 wk after the first and 1 wk after the second immunization for the s.c. and i.n. groups, respectively (Fig. 1A). Maximum serum IgG titers of between 10<sup>4</sup> and 10<sup>7</sup> were attained after two or three immunizations. Strong responses were obtained in the Ras3C s.c. group as well as in the i.n. proteosomes with or without emulsomes, liposomes plus MPL, and saline adjuvant groups. Serum IgA responses were detected after a single immunization in the proteosomes with or without emulsomes and liposomes plus MPL i.n. groups after a single immunization, while a second Ras3C s.c. shot was required for serum IgA seroconversion. Peak serum IgA in adjuvanted i.n. and s.c. groups reached maximum titers of approximately 10<sup>5</sup>. Mice receiving saline-only (no o-gp160) had no detectable o-gp160<sub>451</sub> IgG or IgA responses at any timepoint evaluated (titers < 1:100) (data not shown).

Mucosal responses are shown in Figure 1, C to F. Despite the strong systemic IgG and IgA response to o-gp160 in the Ras3C s.c. group, there were no detectable responses within vaginal wash (Fig. 1, C and D) or fecal pellets (Fig. 1, E and F) at the lowest dilution tested (1:2), except a weak fecal IgA response (1:4) in one pool of the s.c. immunized groups (Fig. 1F). Mice receiving o-gp160 i.n., however, had detectable o-gp160-specific IgG and IgA responses in both vaginal wash and fecal pellets at the first immunization timepoint measured (1 wk post second immunization). In each case, both the proteosomes with or without emulsomes and liposome plus MPL formulations elicited strong IgA/IgG responses, with the proteosomes plus emulsomes group consistently yielding the strongest mucosal IgG/IgA responses. Responses in the groups with strong reactivity after two immunizations were not significantly boosted by a third immunization.

A summary of all o-gp160-specific IgG/IgA immunogenicity data for both the high and low dose groups for samples collected 2 wk after the third immunization (wk 8) is given in Table II. Mice receiving o-gp160 s.c. (Ras3C/5) responded with strong serum IgG/IgA-specific responses but no detectable responses in any of the mucosal washes. Control mice (saline/0) had no response at the lowest dilution of serum (1:100) or mucosal wash (1:2) tested. Mice receiving i.n. o-gp160 both high (saline/50) and low (saline/10) dose in saline had detectable gp160-specific IgG and IgA responses in most compartments though these responses were weaker than the majority of the other adjuvanted groups. Mice receiving o-gp160 formulated in liposomes plus MPL, MPL-AF, proteosomes, emulsomes, and proteosomes plus emulsomes had detectable IgG and IgA in each of the mucosal compartments evaluated. The strongest gp160-specific IgG and IgA responses across compartments were obtained in the proteosomes with or without emulsomes groups.

Ratios of IgG/IgA gp160-binding titers and specific activities were evaluated in systemic and mucosal compartments (Fig. 2). Absolute IgG and IgA titers cannot be compared due to potential differences in IgG and IgA secondary Ab sensitivities, but relative IgG and IgA levels across compartments and between adjuvant groups can be evaluated. Values above the x-axis indicate higher IgG-specific titers relative to IgA, while values below indicate higher IgA-specific titers. IgG responses to o-gp160 were 10- to 1000-fold higher relative to IgA within the serum and intestinal wash, while vaginal wash and fecal IgG and IgA gp160-specific titers were more comparable with IgG/IgA ratios closer to one (Fig. 2A). Lung responses among the groups were mixed with the proteosomes with or without emulsomes groups yielding 10-fold higher IgG titers while the liposomes plus MPL and MPL-AF groups had higher relative IgA titers. These data indicate that adjuvant formulation may impact on the Ig class of vaccine-specific mucosal responses. Different IgG/IgA ratios within sera and some mucosal washes support the presence of locally produced vaccine-specific Ab. Alternatively, IgG/IgA ratios for serum and mucosal samples were calculated based upon specific activities (Fig. 2B). The use of specific activity corrects for differences in total IgG and IgA concentrations (listed in Table III) within each mucosal compartment. Data are comparable for serum, vaginal, and intestinal washes due to similar concentrations of total IgG and IgA at these sites. Intestinal wash and fecal pellet IgG/IgA-specific activity ratios were 10- to 1000-fold higher than the IgG/IgA-binding titer ratios attributable to the higher relative concentration of total IgA present in these samples.

**Local mucosal IgG and IgA production**

To determine whether the IgG and IgA o-gp160-specific responses were attributable to local Ab production or due to serum transudate, specific activities were calculated for serum and each of the mucosal compartments (25, 61, 62). This was accomplished by dividing the o-gp160-specific endpoint titer by the total Ig concentration within the compartment. Significant contribution from serum transudate of systemic Abs would result in similar specific activities for the serum and mucosal wash compartments. The absence of detectable IgG or IgA within vaginal, lung, and intestinal washes or fecal pellets from s.c. immunized mice indicates that serum transudation into mucosal compartments and contamination of mucosal washes with blood during collection procedures were minimal.

Specific activities for serum and mucosal washes for both the s.c. and i.n. groups are listed in Table III. Also listed in Table III legend are the total IgG and IgA concentrations in serum and the various mucosal samples. Specific activities for IgG and IgA in serum ranged...
FIGURE 1. HIV-1 env-specific serum and mucosal IgG and IgA. Serum and mucosal IgG/IgA responses from mice immunized at 0, 3, and 6 wk s.c. with o-gp160 formulated in Ras3C (group A) or mice immunized i.n. with o-gp160 formulated in either saline (group C2), liposomes plus MPL (group D2), proteosomes alone (group E2), or proteosomes plus emulsomes (group E6). Sera or mucosal washes were pooled from five mice in each group and screened for endpoint titers of serum IgG (A), serum IgA (B), vaginal IgG (C), vaginal IgA (D), fecal IgG (E), and fecal IgA (F) by ELISA at the timepoints indicated. Note the different y-axis for serum, vaginal, and fecal studies.
from 41 to 5920 and 1 to 84, respectively. Since the mucosal washes for the s.c. groups were negative (at a 1:2 dilution) for gp160-specific IgG and IgA, the values listed for these groups in Table III indicated the maximum specific activity assuming a positive signal in undiluted mucosal washes. Specific activities with an asterisk in Table III indicate a greater than threefold increase over the corresponding serum value. Nasal administration of α-gp160 in saline elicited significant locally produced lung IgA and intestinal IgG in the high dose group and vaginal IgA in the low dose group. Locally produced IgG and IgA were more prevalent in the adjuvanted groups, where local IgG and

### Table II. IgG and IgA titers against α-gp160(451) in serum and mucosal washes from mice immunized s.c. or i.n. with α-gp160

<table>
<thead>
<tr>
<th>Adjuvant/Dose a</th>
<th>Serum b</th>
<th>Vaginal</th>
<th>Lung</th>
<th>Intestinal</th>
<th>Fecal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgA</td>
<td>IgG</td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>s.c. α-gp160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-Ras3c/5</td>
<td>26,700,000</td>
<td>5,300</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>i.n. α-gp160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-saline/0</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>C1-saline/50</td>
<td>820,000</td>
<td>3,200</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>16</td>
</tr>
<tr>
<td>C2-saline/10</td>
<td>51,200</td>
<td>800</td>
<td>32</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>D1-lipo/MPL/3</td>
<td>205,000</td>
<td>3,200</td>
<td>8</td>
<td>128</td>
<td>&lt;2</td>
</tr>
<tr>
<td>D2-MPL-AF/50</td>
<td>820,000</td>
<td>51,200</td>
<td>2.048</td>
<td>512</td>
<td>8</td>
</tr>
<tr>
<td>D3-MPL-AF/10</td>
<td>12,800,000</td>
<td>51,200</td>
<td>2.048</td>
<td>2.048</td>
<td>128</td>
</tr>
<tr>
<td>E1-prot/50</td>
<td>6,400,000</td>
<td>25,600</td>
<td>16,380</td>
<td>16,400</td>
<td>16,380</td>
</tr>
<tr>
<td>E2-prot/10</td>
<td>6,400,000</td>
<td>6,400</td>
<td>4,996</td>
<td>2,048</td>
<td>8,190</td>
</tr>
<tr>
<td>E3-emul/50</td>
<td>3,200,000</td>
<td>25,600</td>
<td>1,024</td>
<td>2,048</td>
<td>8,190</td>
</tr>
<tr>
<td>E4-emul/10</td>
<td>3,200,000</td>
<td>12,800</td>
<td>1,024</td>
<td>2,048</td>
<td>16,400</td>
</tr>
<tr>
<td>E5-prot-emul/50</td>
<td>12,800,000</td>
<td>51,200</td>
<td>131,000</td>
<td>65,500</td>
<td>32,770</td>
</tr>
<tr>
<td>E6-prot-emul/10</td>
<td>3,200,000</td>
<td>3,200</td>
<td>32,770</td>
<td>16,400</td>
<td>8,190</td>
</tr>
</tbody>
</table>

a Groups are listed as receiving vaccine s.c. or i.n.; the group designation is given first followed by the adjuvant and dose of α-gp160(451).
b Sera and washes were collected 2 wk after the third immunization. Mucosal washes were assayed starting at a 1:2 dilution, and sera were assayed starting at a 1:100 dilution.
c Sera or mucosal washes from five mice per group were pooled and assayed for IgG and IgA responses against α-gp160(451) by ELISA; results are given as endpoint titers.

**FIGURE 2.** Ratios of HIV-1 env-specific IgG and IgA. Analysis of the relative levels of α-gp160 451-specific IgG and IgA titers (A) or specific activities (B) in serum and mucosal samples (vaginal, lung, and intestinal wash, and fecal pellets). IgG/IgA titer ratios were calculated by dividing the ELISA IgG endpoint titer by the IgA endpoint titer; IgG/IgA-specific activity ratios were calculated by dividing the IgG-specific activity by the IgA-specific activity.
IgA were detected in four of four and three of four mucosal compartments, respectively, in the proteosomes with or without emulsomes groups (both high and low dose) and one of four and two of four, respectively, in the high dose MPL-AF group. Additionally, local IgG and IgA responses were detected in one of four and one of four, respectively, in the low dose (3 μg) liposome containing MPL group.

**Ab in serum and mucosal washes preferentially bind native forms of monomeric gp120**

We have previously demonstrated that clinical trials using several candidate HIV-1 envelope subunit vaccines administered i.m. elicited a serum Ab response preferentially reactive with denatured forms of gp120 (64). This type of response was elicited despite the immunogen itself being considered native or CD4-binding competent. Recently, formulation of o-gp160 protein in some, but not all, adjuvants elicited serum responses preferentially reactive with native forms of gp120 and with neutralizing activity against some primary HIV-1 isolates, and these responses were impacted by adjuvant formulation and number of immunizations, as well as the route of vaccine administration (65). In the current study, we were particularly interested to determine whether o-gp160 formulated could be formulated with selected mucosal adjuvants and administered i.n. while preserving protein structure for recognition by the immune system.

 Serum Ab binding to native and denatured forms of gp120MN is shown in Figure 3A. Pooled HIV-1 sera served as an assay control for preferential binding to natively folded gp120, as shown previously (63, 64, 68). R265 is a polyclonal serum from a rabbit receiving o-gp160451 formulated in CFA/IFA and administered s.c. and served as an assay control for preferentially binding to denatured gp120. Subcutaneous administration of o-gp140451 in Ras3C (s.c.-Ras3C) elicited Abs that preferentially bound native gp120. Nasal administration of o-gp160451 formulated in MPL-AF (i.n.-MPL-AF), liposomes plus MPL (i.n.-Lipo/MPL), proteosomes (i.n.-Prot), emulsomes (i.n.-Emul), and proteosomes plus emulsomes (i.n.-Prot/Emul) similarly elicited Abs preferentially reactive with native gp120. Sera from mice receiving baculovirus recombinant gp160 immunized with i.n.-Mrgp160 preferentially bound denatured gp120 compared to data obtained when this immunogen was administered i.m. in alum (64, 65). Serum native/denatured gp120 binding ratios were comparable in the s.c. and i.n. groups. Vaginal wash (Fig. 3B) and lung wash (Fig. 3C) from i.n. o-gp160-immunized mice preferentially bound native gp120, yielding native/denatured gp120 Ab binding profiles comparable with those observed with the corresponding sera. Vaginal wash, but not lung wash, from Mrgp160-immunized mice preferentially bound denatured gp120 consistent with the sera. No gp160-specific vaginal Ab was detected from s.c. immunized mice in agreement with the ELISA data (Table II). These data demonstrate that a multimeric HIV-1 gp160 can be formulated and mucosally administered to elicit local mucosal Ab responses preferentially reactive with epitopes on native gp120, dependent on proper tertiary (conformational) structure.

**HIV-1-neutralizing Ab responses in sera and mucosal washes from i.n. immunized mice**

Sera from s.c. and i.n. immunized mice were tested for neutralizing capacity against the heterologous T cell line-adapted HIV-1MN. HIV-1MN is approximately 85% homologous to HIV-1451 within gp160 and was chosen for the neutralization assays since HIV-1451 was not available at the time of this study. Previous studies, however, have demonstrated the ability of anti-ogp160-immune sera to neutralize multiple TCLA HIV-1 isolates (65). Pooled sera from mice immunized s.c. with o-gp160451 in Ras3C and i.n. with proteosomes with or without emulsomes after three immunizations reduced HIV-1MN infectivity greater than 90% as compared with the preimmune sera (Fig. 4A). Neutralizing responses were undetectable after the 1:200 serum dilution. The most potent neutralizing responses were obtained with sera from mice immunized s.c. consistent with the higher serum IgG-specific binding titers. US9 and US18, individual HIV-1 sera with moderate and strong neutralizing capacities, respectively, are included for comparison purposes. Neutralizing activity of sera from i.n. immunized mice was comparable with that of the moderate to strong neutralizing HIV-1 sera.
Neutralizing activity of vaginal and lung washes from i.n. immunized mice is shown in Figure 4, B and C, respectively. Vaginal wash pooled from three separate groups of five mice each immunized nasally with saline without o-gp160 (i.n.-saline) had no effect on HIV-1MN growth, whereas vaginal wash from mice immunized with proteosomes (i.n.-prot) (50-μg dose) or proteosomes plus emulsomes (i.n.-prot/emul) at both the 50- and 10-μg o-gp160 dose reduced HIV-1 MN growth by >90%. Similarly, lung washes from the same three groups of mice immunized i.n. with saline (i.n.-saline) had no effect on HIV-1MN growth, whereas lung washes from mice immunized with proteosomes (i.n.-prot) (50-μg dose) or proteosomes plus emulsomes (i.n.-prot/emul) at both the 50- and 10-μg o-gp160 dose reduced HIV-1MN growth by >90%.

A summary of serum and mucosal Ab neutralizing data from all groups tested at 1:8 and 1:40 dilutions against 100 TCID<sub>50</sub> input HIV-1<sub>MN</sub> is shown in Table IV. It is important to note that dilutions of 1:8 represent a further dilution of the mucosal washes, as follows. For example, to extract Ig from vaginal wicks, the wicks were diluted approximately 16-fold in buffer (see Materials and Methods). Data are presented as the percent reduction in viral growth in the presence of sera or mucosal wash collected 2 wk after the third immunization as compared with the preimmune sample. A reduction of fivefold (80%) in viral growth was considered significant in this assay. For each assay, HIV-1 serum controls, US9, US10, and US18 neutralized HIV-1<sub>MN</sub> ≥ 85%. Sera, but not mucosal washes, from s.c. immunized mice had >80% neutralizing activity at the 1:8 dilution. Sera from several of the i.n. groups also had neutralizing
titers >80%, including liposomes plus MPL, proteosomes and proteosomes plus emulsomes. Adjuvant MPL-AF yielded weak neutralization, 65% and 79%, at the two doses tested despite high serum IgG o-gp160 binding titers. Neutralizing activity (>80%) in vaginal wash was detected only in the groups receiving proteosomes alone, emulsomes alone, or proteosomes plus emulsomes, with the strongest responses in the proteosomes with or without emulsomes group. Neutralizing activity in lung wash was detected in the groups receiving MPL-AF, proteosomes alone, emulsomes alone, and proteosomes plus emulsomes, with the strongest responses in the proteosomes with or without emulsomes group. Neutralizing activity in mucosal washes correlated with the presence of high titer mucosal o-gp160,451 IgG- and IgA-specific responses. Although strong serum IgG and IgA o-gp160 binding titers were elicited in the Mrgp160imm groups (data not shown) and serum neutralizing Abs were present, no neutralizing Abs were detected within the mucosal washes, demonstrating the importance of both adjuvant and protein structure in eliciting mucosal neutralizing Ab responses.

Discussion

Intramuscular immunization with an HIV-1 oligomeric gp160 protein has previously been shown to elicit Abs preferentially reactive with native, monomeric gp120 and with neutralizing activity against T cell line-adapted and primary HIV-1 isolates (65). Targeting the induction of these neutralizing Ab responses to local mucosal sites using mucosal adjuvants and routes of administration was the goal of the present study. Nasal immunization with o-gp160 in several adjuvant formulations including liposomes, proteosomes, emulsomes, and MPL-AF was able to elicit local IgG and IgA responses at multiple mucosal sites studied. These systemic and mucosal Ab responses preferentially bound native monomeric gp120. Furthermore, formulation in proteosomes or the combination of proteosomes and emulsomes elicited neutralizing Ab (>90%) in the genital tract (vaginal wash) and respiratory tract
Potent mucosal immunogenicity of liposomes containing MPL and proteosomes (in saline or with emulsomes) may be related to enhanced uptake of liposome, proteosome, and emulsomes at mucosal surfaces. Liposomes can be taken up by rat microfold cells (M-cells) and transcytosed (69). Additionally, proteosomes, composed of outer membrane neisserial porins, have been found to up-regulate B cell expression of costimulatory molecules B7-2 and to increase the T lymphocyte costimulatory ability (70). Formulation of o-gp160 with alhydrogel or MPL adjuvants has been found to up-regulate B cell expression of costimulatory molecule B7-2 and to increase the T lymphocyte costimulatory ability of B lymphocytes (70).

Local production of HIV-1-specific Ig was demonstrated in the genital tract, intestinal tract, and lungs of mice immunized i.n. with o-gp160 in proteosomes, emulsomes, or proteosomes combined with emulsomes. These data are in contrast to specific IgA activities within the genital tract of HIV-1-infected women and SIV-infected macaques. Ab from vaginal wash and cervical secretions from HIV-1-infected women (61, 62, 71), suggesting a deficiency in the local IgA response to natural HIV-1 infection. IgA responses to pneumococcal vaccine were also found to be decreased in HIV-1-infected patients with <500 CD4 circulating cells/mm$^3$ (72). Precedently IgG and IgA responses were also detected in HIV-1-infected chimpanzees (vaginal wash, rectal, saliva, urethral, and semen) (73). Study of SIV chronically infected, but healthy, macaques showed significantly higher IgG and IgA in serum, vaginal washes, and saliva, but showed comparable amounts in rectal washes with a reduced number of IgA plasma cells within the lamina propria of the genital tract as compared with uninfected macaques, suggesting an abnormal genital tract mucosal immune response to SIV (74, 75). Minimal local HIV-specific IgA and predominant IgG responses were also found within parotid saliva and nasal wash of HIV-1-infected women (61 and our unpublished data). HIV-1 gp160-specific IgG and IgA responses at multiple mucosal sites in the present study indicate that a strong local mucosal IgA response to the highly glycosylated HIV-1 envelope glycoprotein can be induced in mice.

Prevention of degradation of protein tertiary structure upon mucosal formulation and immunization may be important for the induction of an optimal functional HIV-1 envelope-specific Ab response. Formulation of o-gp160 with alhydrogel or MPL adjuvants and administered systemically to mice and rabbits elicited Abs preferentially reactive with native forms of gp120, resulting in enhanced neutralizing activity against TCLA and primary HIV-1 isolates (65). This was in contrast to elicited Ab responses to several IIIB monomeric HIV-1 env candidate vaccines that predominantly bound to linear epitopes not accessible on native gp120, resulting in a restricted HIV-1 neutralization profile (64, 66). Inclusion of Abs to native gp120 was found to be dependent upon the tertiary structure. o-gp160 formulated with MPL and proteosomes, emulsomes, or proteosomes plus emulsomes was capable of inhibiting HIV-1MN infection of PBMC in vitro. It remains unknown whether this neutralization was mediated by local IgG or sIgA although it is

### Table IV. HIV-1(MN) neutralization activity in serum, vaginal wash, and lung wash from mice immunized s.c. or i.n. with o-gp160(451)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Immunogen</th>
<th>Dose</th>
<th>Adjuvant</th>
<th>Serum</th>
<th>Vaginal</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>o-gp160(451)</td>
<td>5</td>
<td>Ras3C</td>
<td>100</td>
<td>95</td>
<td>&gt;50</td>
</tr>
<tr>
<td>A1</td>
<td>o-gp160(451)</td>
<td>5</td>
<td>Ras3C</td>
<td>100</td>
<td>89</td>
<td>&lt;50</td>
</tr>
<tr>
<td>A2</td>
<td>o-gp160(451)</td>
<td>5</td>
<td>Ras3C</td>
<td>98</td>
<td>62</td>
<td>&lt;50</td>
</tr>
<tr>
<td>l.n.</td>
<td>none</td>
<td>0</td>
<td>saline</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>C1</td>
<td>o-gp160(451)</td>
<td>50</td>
<td>saline</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>C2</td>
<td>o-gp160(451)</td>
<td>10</td>
<td>saline</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>D1</td>
<td>o-gp160(451)</td>
<td>3</td>
<td>lipo</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>D2</td>
<td>o-gp160(451)</td>
<td>3</td>
<td>lipo/MPL</td>
<td>89</td>
<td>50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>D3</td>
<td>o-gp160(451)</td>
<td>50</td>
<td>MPL-AF</td>
<td>65</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>D4</td>
<td>o-gp160(451)</td>
<td>10</td>
<td>MPL-AF</td>
<td>79</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>E1</td>
<td>o-gp160(451)</td>
<td>50</td>
<td>prot</td>
<td>94</td>
<td>&lt;50</td>
<td>93</td>
</tr>
<tr>
<td>E2</td>
<td>o-gp160(451)</td>
<td>10</td>
<td>prot</td>
<td>99</td>
<td>51</td>
<td>55</td>
</tr>
<tr>
<td>E3</td>
<td>o-gp160(451)</td>
<td>50</td>
<td>emul</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>E4</td>
<td>o-gp160(451)</td>
<td>10</td>
<td>emul</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>E5</td>
<td>o-gp160(451)</td>
<td>50</td>
<td>prot/emul</td>
<td>99</td>
<td>93</td>
<td>96</td>
</tr>
<tr>
<td>E6</td>
<td>o-gp160(451)</td>
<td>10</td>
<td>prot/emul</td>
<td>99</td>
<td>68</td>
<td>87</td>
</tr>
<tr>
<td>F1</td>
<td>rgp160(IIIB)</td>
<td>50</td>
<td>prot/emul</td>
<td>100</td>
<td>86</td>
<td>&lt;50</td>
</tr>
<tr>
<td>F2</td>
<td>rgp160(IIIB)</td>
<td>10</td>
<td>prot/emul</td>
<td>100</td>
<td>91</td>
<td>&lt;50</td>
</tr>
<tr>
<td>HIV-1 sera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values in bold font indicate greater than 80% reduction in HIV-1(MN) growth.

* Baculovirus expressed rgp160(IIIB).
possible that both contributed, since similar HIV-1 env-specific IgG and IgA titers were detected. Serum IgA has previously been shown to possess HIV-1 neutralization capacity (76, 77) although another study also demonstrated that serum IgA was capable of enhancing HIV-1 infection (78). However, it is difficult to determine whether IgA-mediated neutralization of TCLA HIV-1 of PBMC will have any significance in preventing mucosal HIV-1 infection since cells other than PBMC may be targets for initial infection or adherence.

HIV-1 has been shown to infect epidermal Langerhans cells in vitro (79), and acute SIV vaginal infection of rhesus macaques resulted in an initial infection of dendritic cells within lamina propria beneath mucosal epithelium (80), consistent with findings of SIV-infected cells in the submucosa of the genital tract and within the vaginal epithelium of SIV chronically infected female macaques (81). In vitro, primary HIV-1 isolates were found to cross epithelial cells from the apical side via transcytosis and infect basolateral mononuclear cells without infecting epithelial cells themselves (82). HIV-1 has also been found to adhere to M-cells within the mouse and rabbit intestine and to be present within endosomes of M-cells and also present in intraepithelial pockets (83). It is not known which receptors or proteins on the surface of mucosal epithelia or on HIV-1 may be important in viral infection, and it is possible that different regions or epitopes within gp120 may be critical in adherence to epithelial or M-cells. Monkey and guinea pig sera raised against gp120 peptides included three conserved regions that were able to neutralize infection of vaginal epithelial cells (84).

It may be important to determine the ability of HIV-1-specific mucosal Ig (IgG or IgA) to inhibit infection of mucosal epithelium and to determine whether sIgA or IgM may be more efficient at preventing adherence or infection of mucosal epithelium or dendritic cells. Studies designed to determine the relative efficiencies of sIgA or IgM in inhibiting HIV-1 infection using perhaps more relevant target cell such as dendritic/T cell conjugates may provide additional information regarding the role of mucosal Ab. These studies are particularly important due to the observations that an in vitro non-neutralizing hybridoma producing IgA specific for rotavirus VP6 was found to protective against rotavirus infection, while a neutralizing IgA mAb specific for outermost protein coat (VP4) was ineffective, suggesting that the protective effect of non-neutralizing IgA occurred during IgA transcytosis rather than extracellularly in the intestinal lumen (85), perhaps providing an example of sIgA-mediated intracellular viral neutralization (86, 87). Studies to determine the immunogenicity of gp120ON, administrated mucosally to rhesus macaque with subsequent vaginal challenge, are ongoing to determine the ability of produced HIV-1 envelope-specific IgG/IgA responses to prevent simian human immunodeficiency virus mucosal infection.

Acknowledgments

The authors thank Drs. P. Berman and T. Gregory of Genentech Inc. (South San Francisco, CA) for the native conformation and reduced carboxymethylated gp120MAB reagents used in the study; Dr. G. Smith (MicroGeneSys, Inc, Meriden, CT) for recombinant gp160MAB; Dr. S. Amselem for emulsomes; S. Veit, C. Ettore, M. Berger, M. Louder, and S. Surman for excellent technical assistance; Dr. J. VanCott for helpful discussions; the technical staff at Biocon, Inc. for care of the laboratory animals; and the nursing staff and Dr. C. Oster and the Military Medical Consortium for Applied Retroviral Research for sera from HIV-infected volunteers and for care of these individuals.

References


