Comparison of Systemic and Mucosal Delivery of 2 Canarypox Virus Vaccines Expressing either HIV-1 Genes or the Gene for Rabies Virus G Protein

Peter F. Wright, Jiri Mestecky, M. Juliana McElrath, Michael C. Keefer, Geoffrey J. Gorse, Paul A. Goepfert, Zina Moldoveanu, David Schwartz, Paul W. Spearman, Raphaelle El Habib, Michele D. Spring, Yuwei Zhu, Carol Smith, Jorge Flores, Kent J. Weinhold, and the National Institutes of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group

Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee; Mucosal Immunology Laboratory, University of Alabama at Birmingham, Birmingham; University of Washington School of Medicine, Seattle; University of Rochester School of Medicine and Dentistry, Rochester, New York; Saint Louis Department of Veterans Affairs Medical Center and Saint Louis University School of Medicine, St. Louis, Missouri; Johns Hopkins University School of Medicine, Baltimore, and EMMES Corporation, Rockville, and Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland; Central Immunology Laboratory, Duke University Medical Center, Durham, North Carolina; Pasteur Aventis, Marcy l’Etoile, France

Background. Since the primary routes of human immunodeficiency type 1 (HIV-1) infection are across mucosal barriers, a randomized trial of canarypox virus–based vectors was conducted in 84 individuals, with delivery of vaccine by mucosal routes, and was accompanied by a detailed analysis of humoral, cellular, and mucosal immune responses.

Methods. Over the course of 6 months, HIV-1–specific (vCP 205) and rabies (vCP 65) canarypox virus vectors were delivered systemically and/or mucosally into the nose, mouth, vagina, or rectum in a 4-dose schedule, followed by 2 doses of HIV-1 MN recombinant glycoprotein (rgp) 120 or subunit rabies vaccine administered by the intramuscular route.

Results. Administration of vaccine and collection of samples were well tolerated. Serum IgG HIV-1–specific antibodies to rgp120 were rarely seen after either systemic or mucosal delivery of canarypox virus vaccine. In contrast, serum IgG antibodies and canarypox antibodies were detected in all individuals after systemic, but rarely after mucosal, delivery of vaccine. Suggestions of mucosal recognition of HIV-1 antigen included a cytotoxic T lymphocyte response in 4 of 8 individuals after administration of vaccine by the intrarectal route and a limited immunoglobulin A response at the same site.

Conclusions. Each of the routes of vaccine administration was feasible in the context of a phase 1 study with motivated individuals. However, with the doses and routes of administration used, canarypox virus was not an effective mucosal immunogen.

The current criteria for the development of an effective AIDS vaccine include demonstration of cytotoxic T lymphocyte (CTL) responses and induction of cross-reacting neutralizing antibodies against HIV-1 isolates [1, 2], although true correlates of protection are not well established. The roles that mucosal antibodies and local CTLs may play in what is usually a mucosally acquired disease are often neglected in consideration of protective immunity [3]. Results of animal models have suggested that mucosal immunity may be important in the prevention of infection [4, 5, 6]. At present, with 1 exception [7], all attempts at immunization against HIV-1 in humans have been made with systemically delivered vaccines.

HIV-1–specific IgA antibodies have been detected in secretions obtained from highly exposed but seronegative women [8, 9, 10]. These antibodies have been suggested as a correlate of protection against infection [11]. Local CTL responses can be detected in in vitro–expanded T cells from cervical scrapings of HIV-1–infected women [12], and systemic CTLs have been detected in an HIV-1–exposed but uninfected group [8].
However, other reports have demonstrated diminished IgA responses in HIV-1 infection and have suggested that IgA may not recognize the gp120 portion of the HIV-1 envelope [13, 14]. It has recently been shown that HIV-1–specific IgA responses are absent or are difficult to measure in HIV-1–infected patients [15]. With other viral pathogens, optimal induction of mucosal immunity has been achieved by direct mucosal delivery of live attenuated vaccines [16, 17]. Experience with respiratory viruses has convincingly demonstrated that local IgA antibody produced by topical delivery of vaccine can be a correlate of immunity [18]. To address the induction of immunity to HIV-1 by mucosal delivery of vaccine, a phase 1 safety and immunogenicity trial was conducted by use of live recombinant canarypox HIV-1 (ALVAC-HIV-1; vCP205) and rabies (ALVACRG; vCP65) vectors delivered mucosally, followed by booster injections of VaxGen MN recombinant glycoprotein (rgp) 120 or licensed rabies vaccine, by systemic delivery, in healthy adult volunteers. The study was designed to establish the safety of the canarypox preparations delivered mucosally and to determine whether delivery of vaccine by these routes stimulated or primed for mucosal or systemic humoral and cell-mediated responses.  

**SUBJECTS AND METHODS**

**Populations studied.** A randomized, blinded, multicenter phase 1 safety and immunogenicity study was performed by the National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group (AVEG), a predecessor to the current HIV Vaccine Trials Network. The demographic characteristics of the population recruited are shown in table 1. Approval for this study was obtained from each of the investigator’s institutional review boards. Exclusion criteria were similar to those described in other phase 1 studies conducted by the AVEG and included any immunodeficiency or chronic illness, behavior that placed the individual at higher risk of HIV-1 infection, or poor compliance with the protocol [19]. All women were tested for pregnancy and counseled to not become pregnant during the study. Women with abnormal results of a pelvic examination or individuals with gastrointestinal symptoms or intrauterine bleeding were excluded. Individuals with a history of rabies vaccination were excluded, as were those who had recently received blood products, experimental agents, or vaccinations. Individuals were selected to be at low risk of acquiring HIV-1 and were counseled at each clinic visit about engaging in high-risk sexual behavior.

**Study design.** The 84 individuals (18–52 years old) in the study were divided into 7 study groups of 12 individuals, of whom 8 received the vCP205 construct and 4 received the vCP65 construct (table 2). The individuals were blinded to the product they received but not to the route of delivery. The mucosal routes of delivery included 4 doses of vCP vector administered by the oral, intranasal (inl), intravaginal, or intrarectal route, followed by 2 intramuscular (im) injections of subunit vaccine. Two study groups received a vCP vector by either the inl/im or intrarectal/im combined route, followed by subunit vaccine. In one group, im vector vaccinations alone were followed by inl vector vaccination. The dose of vCP205 (Pasteur Aventis) was $10^6.3$ TCID$_{50}$ and the dose of vCP65 (Pasteur Aventis) was $10^6.3$ TCID$_{50}$.

The inl vaccine was administered by use of a spray device (Becton-Dickinson) that delivers a large-particle aerosol to the upper airway. This device was successfully used in large-scale trials of influenza virus vaccine [16]. The oral vaccine was gargled for 15 s before being swallowed. The intrarectal vaccine was administered by use of a 3-mL plastic syringe pipette (Fisher Scientific Supply). The intravaginal vaccine was administered by use of the same type of pipette as above into the posterior fornix of the vagina, after which the woman remained supine with knees bent for 15 min.

The timing of vaccine administration is shown in table 2. The subunit products (Imovax diploid rabies vaccine [Pasteur Mérieux Connaught] and AIDSVAX MN rgp120 [VaxGen]) were originally scheduled to be administered at 9 and 15 months after initiation of the vaccination series, but there was a delay in the supply of MN rgp120 vaccine. The actual time of administration varied between 84 and 322 days after initiation of the vaccination series, with a median interval of 140 days between the end of the primary vaccination and the first booster injection. The second booster injection was administered as planned, 6 months after the first booster injection. Randomization and data and study management were performed by the EMMES Corporation (Rockville, MD).

**Vaccine constructs.** vCP205 is a recombinant canarypox virus capable of entering mammalian cells, expressing its genes, and producing noninfectious virions. vCP205 expresses the gag gene portion of the Gag p55-polyprotein of the HIV-1 LAI strain, the protease gene expressing the p15 protein of the LAI strain and the rgp120 of the HIV-1 MN strain, with an anchoring transmembrane region of gp41 of the LAI strain. The vCP65 virus expresses the G protein of rabies virus under the control of the vaccinia virus H6 promoter. vCP65 has been shown to be safe in a variety of species and to induce antibody and protection in a dose-dependent fashion [20–22]. The subunit MN rgp120 (VaxGen) was produced in mammalian CHO cells and administered at a dose of 300 μg/mL in alum, and the inactivated rabies vaccine Imovax, which was prepared in human diploid cells, was administered at the standard potency of ≥2.5 IU of rabies antigen.

**Clinical safety.** Individuals were observed for 30 min after each vaccination. Individuals recorded their temperatures the evening of vaccination and were questioned about or were ex-
Table 1. Demographic characteristics and follow-up of individuals enrolled in the present study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rabies vaccine recipients</th>
<th>HIV vaccine recipients</th>
<th>Total</th>
<th>(n = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>25</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>31</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>25</td>
<td>47</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Native American/Alaskan Native</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>31 (18–52)</td>
<td>34 (18–50)</td>
<td>32 (18–52)</td>
<td></td>
</tr>
<tr>
<td>No. of vaccinations (time)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (month 0)</td>
<td>28</td>
<td>56</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>2 (month 1)</td>
<td>28</td>
<td>56</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>3 (month 3)</td>
<td>27 (96%)</td>
<td>56</td>
<td>83 (99%)</td>
<td></td>
</tr>
<tr>
<td>4 (month 6)</td>
<td>27 (96%)</td>
<td>52 (93%)</td>
<td>79 (94%)</td>
<td></td>
</tr>
<tr>
<td>5 (month 9)—booster injection</td>
<td>18 (64%)</td>
<td>44 (79%)</td>
<td>62 (74%)</td>
<td></td>
</tr>
<tr>
<td>6 (month 15)—booster injection</td>
<td>15 (54%)</td>
<td>40 (71%)</td>
<td>55 (65%)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. of individuals, unless otherwise noted.

amined for symptoms. Particular attention was paid to symptoms related to the route of vaccine administration. Hematologic, hepatic, and renal functions were monitored during the trial.

Samples were obtained from the first 4 individuals who received vaccine by each route, at 4–8 h and at 24 h after primary vaccination, to detect any residual virus as evidence of virus replication. Virus isolation was attempted in primary chick embryo fibroblast cells. No canarypox virus was recovered from 17 nasal, 11 oral, 9 vaginal, and 30 rectal samples obtained at either 4–8 h or 24 h after administration of vaccine.

Growth of canarypox virus on human mucosal epithelial cells. To confirm growth and protein expression of canarypox virus, a green fluorescent protein (GFP) insert in a canarypox vector was examined on primary human adenoid epithelial cells, in a model described elsewhere [23]. The GFP construct was a gift from James Tartaglia (Virogenetics, Troy, NY). The canarypox GFP construct expressed its encoded GFP protein, with a peak intensity of fluorescence seen at 48 h, and the number of fluorescent cells was proportional to the input virus, without spread to adjacent cells. As predicted, canarypox virus could initiate infection and express encoded proteins in human mucosal epithelium but was defective in production of infectious virus.

Systemic immunogenicity. Serum was collected for antibody responses after each dose of vaccine and finally at 9 months after the last dose of vaccine. HIV-1 antibody responses were measured by neutralization of the MN virus and by binding assays to the V3 loop of MN gp120 produced in CHO cells, as described in the standard AVEG protocols performed at the Central Immunology Laboratory (Duke University, Durham, NC) [24]. The data in the present study will focus on the immune response 2 weeks after the primary ALVAC series and after the first and second booster injections.

ELISPOT assays were performed at the Mucosal Immunology Laboratory (University of Alabama at Birmingham, Birmingham, AL) on heparinized peripheral blood samples obtained 1 week after the completion of the primary series and after the initial booster injection. IgG or IgA antibody–secreting cells were identified by use of the Chiron HIV-1 SF-2 gp120 produced in CHO cells, as a coating antigen [25]. Detection of >3 spots/10⁶ peripheral blood mononuclear cells was considered to be positive, since none of the 38 control subjects was found to have levels above this value.

CTL assays were performed at the Central Immunology Laboratory, according to a standard protocol [26], by use of vaccinia vectors expressing epitopes of HIV-1 envelope, Gag, and protease, to stimulate the effector cells. Interleukin (IL)–7 and IL-2 were added during the 14-day stimulation. The target cells were Epstein Barr Virus–transformed B lymphoblastoid cell lines established from each individual. The cells were labeled with ⁵¹Cr and infected with vaccinia vectors. At 24 h, the effector cells were added for a 6-h ⁵¹Cr release assay. Background values against vaccinia were reduced by cold target competition, by use of vaccinia virus (vP1170)–infected targets in a 30-fold
excess. A positive result was scored as >10% release above background values. The responses were scored as positive only if they occurred on >1 assay. Assays were performed at effector :target ratios of 1:25 and 1:50.

Rabies virus antibody–binding assays were performed by use of the Sanofi rabies antibody kit (Pasteur Diagnostics), with a cutoff of 0.5 ELISA units/mL. The level of IgA antibody to rabies virus antigens in serum samples with detectable IgG antibody was defined by use of a kinetic ELISA previously developed for influenza virus [27]. IgA and IgG antibodies to canarypox virus were measured by use of a modification of the same kinetic ELISA, with purified canarypox virus supplied by Virogenetics. The cutoff for all kinetic ELISAs was 15 mOD/min, a measure of the maximum rate of change in optical density over time.

**Mucosal immunogenicity.** Samples were obtained according to protocols developed at the Mucosal Immunology Laboratory [28]. Emphasis was placed on specimens obtained from the site of vaccine administration and on samples obtained after completion of the primary canarypox series and after the first and second subunit booster injections. An initial blinded test of samples for HIV-1 antibodies was performed at the Mucosal Immunology Laboratory by use of chemiluminescence-enhanced Western blots [29]. Of 1800 Western blots run, only a few samples had >1 total heavy and light chain Ig bands to HIV proteins, and none could be confirmed by use of IgG- or IgA-specific reagents. Mucosal antibodies of the IgA and IgG classes to rabies virus antigen, HIV-1, and canarypox virus were all performed by use of variations of a kinetic ELISA originally developed for influenza virus [27]. The samples were not corrected for total IgA and IgG.

**Statistical analysis.** The χ² or Fisher’s exact test was used to compare data between the different routes. *P < .05* was considered to be statistically significant. The data analyses were performed by use of the statistical packages SPSS (version 11.0; SPSS).

## RESULTS

### Follow-up.
The 84 individuals enrolled in the trial, 5 did not complete the initial series of vaccinations with the vCP virus vectors: 2 individuals were lost to follow-up, 1 refused to continue, 1 underwent a hysterectomy, and 1 became pregnant. Fourteen additional individuals did not complete the delayed subunit booster injections. No individual withdrew because of the route of vaccine administration or because of mucosal samplings to measure immune responses.

**Vaccine safety.** The vaccines were well tolerated, with few local and systemic complaints. No temperatures >39°C were recorded during the trial. The only severe symptoms noted after vaccination with vCP 205 were 1 episode of malaise after administration by the oral route and 1 local reaction with erythema and induration ≥25 cm² after administration by the combined inl/im route. The number of individuals reporting moderate to severe reactions was somewhat greater for the combined inl/im and intrarectal/im routes (6/16) of vCP 205 than for a single route (4/40) of systemic or mucosal delivery (*P = .02*; table 3).

Adverse events reported during the study included ankle pain, abdominal pain secondary to cholelithiasis, persistent lower back pain leading to anterior spinal fusion, and severe left temporal headaches attributed to migraines. These complaints were all considered to be unrelated to vaccination. One woman who had received vaccine by the intravaginal route complained of irregular menses, underwent hysterectomy, and was found on pathologic testing to have adenomatous hyperplasia, mild cervicitis, and leiomyomas. An intensive review of all reported menstrual irregularities, reported for 8 of the 38 women in the trial, showed no association with the intravaginal route of administration. All laboratory abnormalities were transient with no trends in lymphocyte populations or renal, hematologic, or hepatic profiles.

**HIV-1 humoral responses.** Total Ig HIV-1–specific antibody responses to MN rgp120, measured by ELISA, were rarely seen after the primary series of vCP205 delivered either by a combined (mucosal/systemic) route or a mucosal route (figure 1). After a single subunit booster injection of MN rgp120 vaccine, 10 of the 14 individuals who had received vaccine by a combined route of delivery had responses, compared with only 1 of the 32 individuals who had received vCP205 vaccine by

### Table 2. Schema for mucosal delivery of canarypox virus vaccines

<table>
<thead>
<tr>
<th>Groupa</th>
<th>Product</th>
<th>Route of administration</th>
<th>Groupa</th>
<th>Product</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A or RG</td>
<td>im</td>
<td>A</td>
<td>A or RG</td>
<td>im</td>
</tr>
<tr>
<td>B</td>
<td>A or RG</td>
<td>po</td>
<td>B</td>
<td>MN or IVX</td>
<td>im</td>
</tr>
<tr>
<td>C</td>
<td>A or RG</td>
<td>inf</td>
<td>C</td>
<td>MN or IVX</td>
<td>im</td>
</tr>
<tr>
<td>D</td>
<td>A or RG</td>
<td>intravaginal</td>
<td>D</td>
<td>MN or IVX</td>
<td>im</td>
</tr>
<tr>
<td>E</td>
<td>A or RG</td>
<td>inl/im</td>
<td>E</td>
<td>MN or IVX</td>
<td>im</td>
</tr>
<tr>
<td>F</td>
<td>A or RG</td>
<td>ir/im/ivag</td>
<td>F</td>
<td>MN or IVX</td>
<td>im</td>
</tr>
<tr>
<td>G</td>
<td>A or RG</td>
<td>ir/im/ivag</td>
<td>G</td>
<td>MN or IVX</td>
<td>im</td>
</tr>
</tbody>
</table>

**NOTE.** A, ALVAC-HIV vCP205 (10¹⁷ TCID₅₀); im, intramuscular; inf, intranasal; intravaginal; ivag, intravaginal; iv, intravenous; MN, Mucovax monovalent purified rabies vaccine; IVX, Imovax diploid cell rabies vaccine; ALVAC-RG, ALVAC-HIV vCP205 (10² TCID₅₀); po, oral; RG, ALVAC-RG vCP205 (10² TCID₅₀); inl, intrarectal; im, intramuscular.

a Each group included 12 individuals: 8 vaccine recipients and 4 control individuals. Control individuals received ALVAC-RG instead of ALVAC-vCP205 and Imovax instead of MN rgp120.

b The first booster injection was to be administered at month 9; a delay in vaccine availability, the vaccination was administered as soon as vaccine was available. The final vaccinations were administered 6 months later (see Subjects and Methods).
mucosal delivery ($P < .001$). Thus, systemically, but not mucosally, delivered vaccine led to priming for an HIV-1 response after a single dose of subunit vaccine. With the second dose of subunit vaccine, most (28/45) individuals, regardless of the initial route of delivery, had an immune response. The group of individuals who received vaccine by systemic delivery (table 2, group A) had no response to the initial im vCP205 vaccination or to subsequent im vCP205 vaccination.

HIV-1–specific serum antibody responses in the IgA isotype followed a pattern similar to that of the total Ig ELISA, with no responses seen in individuals after the primary vCP205 vaccinations. Responses after the first booster injection were more common ($P < .001$) after vCP205 vaccination by combined route of delivery (10/12) than after vaccination by mucosal delivery (2/22). To investigate whether a subunit vaccine alone could induce an IgA response, 5 individuals who had received $\geq 3$ doses of vaccine and participated in earlier AVEG trials were tested. All had easily measurable IgG responses, and 2 of the 5 individuals had HIV-1–specific IgA responses.

Neutralizing antibodies to the MN strain were not seen after primary vCP205 vaccinations (figure 2). With the first booster injection of MN vaccine, there was evidence that the vaccination by the combined route of delivery had primed the humoral response: 10 of 12 individuals responded, compared with 2 of 22 individuals who had received initial vaccination by mucosal delivery ($P < .001$).

When IgA and IgG antibody–secreting cells were examined by ELISPOT 1 week after individuals had received the last vCP205 vaccination, 0 of 52 had IgA and 2 of 52 had IgG HIV-1–specific antibody–secreting cells in their peripheral blood samples. One week after the second booster injection of MN rgp120, IgG antibody–secreting cells were frequently seen (i.e., in 31 of 39 individuals). IgA antibody–secreting cells were seen in 8 of 39 individuals. With a different measure of humoral immunity, MN rgp120 vaccination by systemic delivery enhanced the very limited HIV-1–specific humoral response to primary vCP205 vaccination. That response was mainly in the IgG isotype.

**HIV-1 cellular responses.** HIV-1–specific CTL responses with specific $^{51}$Cr release $>10\%$ were seen in 10 (19\%) of 53 individuals receiving the vCP205 construct and in 1 (4\%) of 25 individuals receiving the vCP65 construct ($P = .09$). All bulk CTL responses were mediated by CD8 T cells, as demonstrated by depletion of CD8 T cells. The frequency of CTL responses by route of administration of did not differ significantly: 1 of 6 occurred after systemic delivery, 3 of 15 occurred after delivery by the combined routes, and 6 of 32 occurred after mucosal delivery. However, it was of interest that 4 of 8 individuals who received vCP205 vaccine by the intrarectal route had a demonstrable CTL response.

**Rabies systemic responses.** Serum rabies antibody responses to vCP65 were measured after the primary series of vaccinations. The rabies response to vCP65 was greater than the HIV response to vCP205. All tested individuals who had received vCP65 by systemic or combined delivery had a serum IgG (figure 3A) and IgA (figure 3B) antibody response. Responses were detected as early as the second dose of vaccine. Significantly fewer (2/14) individuals who had received vCP65 by mucosal delivery had rabies IgG antibody response ($P < .001$). One of these had preexisting antibody, presumably as a result of prior, unreported rabies vaccination. After a single booster injection of the Imovax rabies vaccine, 6 of 12 individuals who received vCP65 by mucosal delivery had rabies IgG response. This is an expected response rate to a single dose of Imovax and is not evidence of priming [30]. However, all 7 individuals who received vCP65 by mucosal delivery had a high IgA rabies virus serum antibody response after the first booster injection of Imovax by systemic delivery, suggesting that mucosal priming had occurred (figure 3B).

**Canarypox virus systemic responses.** Canarypox virus–specific IgG and IgA antibodies were measured at the completion of the primary vaccinations in 34 individuals. The data for vCP65 and vCP205 are combined. IgG immune responses in 16 individuals who received vaccine by either systemic or combined delivery were measured; all individuals responded (figure 4A). Limited IgG antibody responses to mucosal delivery of vaccine were seen (i.e., in 5 of 18 individuals). Serum IgA

---

**Table 3. Moderate or severe systemic reactions to administration of HIV-1 and rabies canarypox vectors.**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>vCP 205 (HIV-1)</th>
<th>vCP 65 (rabies), delivery by all routes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mucosal delivery</td>
<td>Systemic delivery</td>
</tr>
<tr>
<td>Malaise</td>
<td>3 (9)</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>4 (13)</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of individuals.
responses were seen in 6 of 16 individuals who received vaccine by either systemic or combined delivery and in 3 of 18 individuals who received vaccine by mucosal delivery (figure 4B). IgA responses were detected in 5 of 9 individuals who received intrarectal vaccine.

**HIV-1 mucosal responses.** No mucosal antibody responses to HIV-1 were seen after the initial vCP205 vaccinations by mucosal delivery (0/19) or by combined delivery (0/10), when measured at the site of vaccine administration. Even after boosting with subunit vaccine, only 2 vaginal and 2 nasal samples were positive for HIV-1-specific IgG. A rectal sample from 1 individual was found to be positive for IgA.

**Rabies mucosal responses.** A limited number of samples, chosen to match the route of administration, were examined for rabies virus–specific antibodies. IgG antibodies to rabies were seen at the site of vaccine administration in the 1 individual who had preexisting serum antibody. IgA antibodies were detected in 13 samples from the site of vaccine administration. At the time of the first booster injection of subunit rabies vaccine, IgG rabies antibodies were seen in only 3 cervical secretions. All cervical samples were obtained 5–20 days after the last menstrual period, making blood contamination unlikely.

**Canarypox virus mucosal responses.** A limited number of samples, chosen to match the route of administration, were examined for canarypox virus–specific antibodies. We did not differentiate between individuals who received vCP65 and those who received vCP205. After the primary vaccinations, IgG canarypox antibodies were seen in only 6 of 52 individuals tested; 5 of the positive responses were in individuals who had received vaccine administered by the intravaginal route. This, again, suggested a local IgG response at this site, since intravaginal canarypox vaccine had not induced a systemic response. Thirteen of 52 individuals tested had a mucosal IgA response, and 8 of the responders were among the 11 tested who received vaccine administered by the intrarectal route, suggesting that a local IgA mucosal response was stimulated by vaccine delivered by this route.

**DISCUSSION**

Mucosal delivery of vaccines and targeted stimulation of mucosal immune responses is justified for infections that are acquired by mucosal routes, such as HIV-1. Vaccination by the inl route with a canarypox vector expressing a canine distemper hemagglutinin and fusion proteins fully protected ferrets from canine distemper and were as immunogenic as those with the same vector administered by the im route [31]. Attenuated poxviruses have been administered mucosally to animals and
Figure 2. Serum neutralizing antibody to HIV-1 MN strain, in individuals receiving primary vCP205 or subunit intramuscular (im) MN recombinant glycoprotein 120 vaccine. Individuals receiving vaccine by mucosal delivery received vCP205 by 1 of the 4 mucosal routes (intranasal [inl], oral, intravaginal, or intrarectal). Individuals receiving vaccine by combined (mucosal/systemic) delivery received vCP205 by either the inl/im or the intrarectal/im route.

humans, to provide protection against variola and rabies [32], and modified vaccinia Ankara administered by the intrarectal route has been shown to induce mucosal CTLs [33].

In primate models, protection against infection with simian immunodeficiency virus (SIV) and the chimeric simian-human immunodeficiency virus (SHIV)–1 constructs has been shown to result from mucosal delivery of peptide-based vaccines [34], vaccinia-derived vectors [35], and orally administered Sabin poliovirus vectors expressing overlapping peptides of the SIV genome [36]. In the primate model, passive vaccination with monoclonal antibody also protected against SHIV infection [37, 38]. With modified vaccinia Ankara delivered systemically, protection has been demonstrated with a mucosal SIV challenge [6].

The delivery of canarypox vaccines by all of the mucosal routes in the present study was well tolerated and accepted. There were no safety issues posed by mucosal or systemic delivery of the canarypox virus–vectored vaccines, confirming the strong safety record of this product [39, 40]. The lack of recovery of canarypox virus at mucosal sites indicates that, as would be expected, this is a replication-defective virus and that the original virus inoculum is not retained at the mucosal site for an extended period of time.

Although the HIV-1–specific responses to the vaccine delivered systemically were disappointing, they were consistent with those seen in other recent studies of HIV-1 canarypox products [19, 41]. There was priming for the first subunit booster injection of subunit canarypox vaccine. With the first booster injection of gp120, a serum IgA response was detected. In previous studies, an IgA response was seen in 7 of 12 individuals after 2 doses of rgp120 subunit vaccine alone [40].

An effort was made in a single group of vaccine recipients to determine whether canarypox virus vaccinations by systemic delivery might prime for mucosal delivery of the same vaccine. There was no evidence of this occurring; when measured, canarypox virus–antibody responses continued to decline through the mucosal booster injections. However, because of the small number of individuals included, the present study does not definitively answer the question concerning the importance of systemic priming that precedes mucosal boosting, or vice versa.

This is the first study performed in the evaluation of AIDS vaccines that has systematically addressed the mucosal and systemic responses to the control rabies virus antigen insert and to the canarypox vector itself. The canarypox virus responses proved to be the most sensitive way of detecting a vaccine take. It has suggested that, in some, but not in the majority, of individuals who received vaccine by mucosal delivery, the vector induced the expression of protein sufficiently to initiate an im-
A hierarchy can be established of humoral immunogenicity, with the vector itself being the most immunogenic, followed by the rabies insert, and finally by the HIV-1 insert. The rabies construct induced more humoral immunity than did vCP205, after the primary series of vaccinations by systemic delivery. This could be a result of the protein expression induced by this construct or the sensitivity of the detection assay but also may be an indication that the gp120 is a less immunogenic glycoprotein than the rabies G glycoprotein. vCP205 routinely induced antibodies to the canarypox vector when ad-

Figure 3. Serum IgG (A) and IgA (B) antibody to rabies, in individuals receiving vCP65 or intramuscular (im) inactivated Imovax rabies vaccine. Individuals receiving vaccine by mucosal delivery received vCP65 by 1 of the 4 mucosal routes (intranasal [inl], oral, intravaginal, or intrarectal). Individuals receiving vaccine by combined (mucosal/systemic) delivery received vCP65 by either the inl/im or the intrarectal/im route.
ministered systemically. A dose effect has been postulated for the canarypox product. The consistent induction of a response to the vector indicates that any dose effect is not a function of a “nontake” of the vector at the dose used in the present study—$10^{6.7}$ TCID$_{50}$. Conversely, the lack of a systemic canarypox virus—antibody response by mucosal delivery suggests that a problem with the mucosal delivery might be a lack of initial infectivity of the vector due to nonpermissiveness of the cells lining the mucosa.
or innate defense mechanisms of the mucosa, including particle clearance by ciliary action or blockage of adherence by mucin. However, when primary epithelial cells derived from adenoids were used as a target, a canarypox GFP was expressed, indicating that there is no intrinsic resistance to infection with canarypox virus by cells from this site.

Mucosal responses were much more difficult to analyze, and they pose risks of overinterpretation of the limited positive responses seen. However, 2 responses were notable and may be significant as the field advances. In agreement with other studies (for review see [42]), the intrarectal route was the most likely to induce a local IgA response, both to the rabies and to the canarypox antigens. This route also led to CTL responses in 4 of 8 individuals, which is better than the response to vaccine delivered systemically. A cervicovaginal IgG response to the same antigens was seen in some of the individuals who received intravaginal vaccine at a time chosen to be remote from their menstrual periods.

The overall conclusion must be that very limited HIV-1–specific mucosal responses were seen with systemically or mucosally delivered vaccine. However, a template has now been established for the mucosal delivery of HIV-1 vaccines. Other vaccines, after establishment of preliminary data on immunogenicity in animal models and a theoretical basis for presuming mucosal effectiveness, should be evaluated using protocols such as the one used in the present study.

References

vaccination with recombinant poxvirus vaccines protects ferrets against symptomatic CDV infection. Vaccine 1999; 17:308–18.


40. Gorse GJ, Corey L, Patel GB, et al. HIV-1 \textsubscript{MN} recombinant glycoprotein 160 vaccine induced cellular and humoral immunity boosted by HIV-1 \textsubscript{MN} recombinant glycoprotein 120 vaccine. AIDS Res Hum Retroviruses 1999; 15:115–32.
