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Immunology

Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by preexisting poxvirus immunity

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ABSTRACT **Overcoming preexisting immunity to vaccinia virus in the adult population is a key requirement for development of otherwise potent recombinant vaccinia vaccines. Based on our observation that s.c. immunization with vaccinia induces cellular and antibody immunity to vaccinia only in systemic lymphoid tissue and not in mucosal sites, we hypothesized that the mucosal immune system remains naive to vaccinia and therefore amenable to immunization with recombinant vaccinia vectors despite earlier vaccinia exposure. We show that mucosal immunization of vaccinia-immune BALB/c mice with recombinant vaccinia expressing HIV gp160 induced specific serum antibody and strong HIV-specific cytotoxic T lymphocyte responses. These responses occurred not only in mucosal but also in systemic lymphoid tissue, whereas systemic immunization was ineffective under these circumstances. In this context, intrarectal immunization was more effective than intranasal immunization. Boosting with a second dose of recombinant vaccinia was also more effective via the mucosal route. The systemic HIV-specific cytotoxic T lymphocyte response was enhanced by coadministration of IL-12 at the mucosal site. These results also demonstrate the independent compartmentalization of the mucosal versus systemic immune systems and the asymmetric trafficking of lymphocytes between them. This approach to circumvent previous vaccinia immunity may be useful for induction of protective immunity against infectious diseases and cancer in the sizable populations with preexisting immunity to vaccinia from smallpox vaccination.**

Recombinant vaccinia virus vaccines are among the most potent for inducing protective immunity in animals against many viral infections and cancer, and they have been shown to be immunogenic in humans (reviewed in refs. 1 and 2). However, there are data in mice (3) and some data in humans (4) suggesting that preexisting vaccinia immunity, such as that occurring in a large proportion of the adult population because of smallpox vaccination, limits the effectiveness of recombinant vaccinia vectors as vaccines. Indeed, as described below, the detrimental effect of previous vaccinia immunity on vaccine efficacy was fully manifest in our own experimental model, in which previous immunization with vaccinia virus prevented induction of cytotoxic T lymphocytes (CTL) to HIV-1 gp160 by subsequent infection with recombinant vaccinia expressing HIV-1 gp160, extending the conclusion to CTL responses. Overall, the data indicate that the development of an effective recombinant vaccine based on vaccinia vectors, in particular one effective in prevention of HIV infection, depends on the discovery of ways to circumvent the problem of preexisting immunity.

A possible solution to this dilemma was suggested by our previous studies showing that systemic (s.c.) immunization of BALB/c mice with either replicating or nonreplicating recombinant vaccinia viruses induced CTL responses to the recombinant protein in systemic lymphoid tissue (spleen, SP), but did not induce antigen-specific CTL in mucosal sites (5). In addition, such s.c. immunization did not induce a significant HIV protein specific secretory IgA and IgG antibody response in rectal washes (5). We therefore hypothesized that after s.c. immunization with vaccinia virus, the inductive sites of the mucosal immune system may still be naive to the vaccinia antigens and thus mucosal routes of immunization can be used for the induction of recombinant protein-specific response in animals (or humans) with preexisting immunity to vaccinia. We also hypothesized that the same approach might be useful for boosting with a recombinant vaccinia vector more than once, or immunizing with a second recombinant vaccinia vaccine after another one had already been administered. Because vaccinia immunity in mice mimics that in humans, the mice should be a good model in which to test this hypothesis.

MATERIALS AND METHODS

Animals. Female BALB/c mice were purchased from Frederick Cancer Research Center (Frederick, MD). Mice used in this study were 6–12 weeks old.

Viruses. vSC8 is a recombinant vaccinia virus (strain WR) expressing β-galactosidase (with CTL epitope, Yew21: TPHPARIGL) (6); modified vaccinia Ankara (MVA)89.6, a recombinant replication-deficient vaccinia virus expressing the envelope protein gp160 of HIV-1 primary isolate strain 89.6 (containing the CTL epitope from the V3 loop of the HIV-1 89.6 Env protein: IGPGRAFYAR-P18–89.6R10 peptide; ref. 5); vPE16 (7), a recombinant vaccinia virus expressing the envelope protein gp160 of HIV-1 strain IIIB (containing the CTL epitope from the V3 loop of the HIV-1 IIIB Env protein: RGPGRAFVTI = P18-I10 peptide; refs. 8 and 9).

Peptides. P18–89.6R10 peptide (IGPGRAFYAR) (5) was synthesized on an automated peptide synthesizer (Symphony Multiplex, Rainin Instruments) by using fluorenylmethoxycarbonyl chemistry. This sequence corresponds to residues 311–320 of HIV-1 gp160 in the numbering of the Los Alamos database (10). The peptide was cleaved from the resin with trifluoroacetic acid and initially purified by preparative HPLC (P4 BioGel; Bio-Rad). Purification to a single peak was achieved by reversed-phase HPLC on μBondapack reverse-phase C_{18} analytical and preparative columns (Waters). The decapeptide P18-I10 from the V3 loop of the HIV-1 IIIB env

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Abbreviations: CTL, cytotoxic T lymphocytes; IN, intranasal(ly); IR, intrarectal(ly); MVA, modified vaccinia Ankara; pfu, plaque-forming units; PP, Peyer's patch(es); SP, spleen; rmIL-12, recombinant mouse IL 12.

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protein (RGPGRAFVTI) (8) was custom synthesized by Multiple Peptide Systems (San Diego), and was >95% pure by HPLC, mass spectrometry, and amino acid analysis.

Immunization. Mice were immunized s.c. with vSC8 vaccinia virus. One month later mice were reimmunized intrarectally (IR), intranasally (IN), or s.c. with MVA89.6 or vPE16. Viruses were diluted to the appropriate titer (plaque-forming units; pfu) in sterile PBS, and 150 μl of the virus inoculum was colorectally injected through an umbilical catheter inserted ≈4 cm within the colon while mice were under inhalation anesthesia (methoxyflurane; Pitman–Moore, Mundelein, IL). We used one single dose for s.c. immunization (5×10^7 pfu) and a single mucosal reimmunization with 1×10^7, 5×10^7, or 1×10^8 pfu. For IN immunizations, mice were lightly anesthetized with methoxyflurane and 10 μl of the virus inoculum was introduced into each nostril. Recombinant mouse IL-12 (rmIL-12) (a generous gift of Genetics Institute, Cambridge, MA) was delivered either i.p. (1 μg) or IR (1 μg) mixed with DOTAP (Boehringer Mannheim), a cationic lipofection agent, along with the recombinant vaccinia virus and also on days 5, 10, and 15.

Cell Purification. Antigen-specific T cells were isolated from Peyer's patches (PP), and SP. The PPs were carefully excised from the intestinal wall and dissociated into single cells by use of collagenase type VIII, 300 units/ml (Sigma) as described (5). Cells were layered onto a discontinuous gradient containing 75% and 40% Percoll (Pharmacia). After centrifugation (4°C, $600 \times g$, 20 min), the interface layer between the 75% and 40% Percoll was carefully removed and washed with incomplete medium. This procedure provided >90% viable PP lymphocytes with a cell yield of 1×10^7 lymphocytes/mouse. SPs were aseptically removed and single cell suspensions prepared by gently teasing them through sterile screens. The erythrocytes were lysed in Tris-buffered ammonium chloride and the remaining cells washed extensively in RPMI 1640 medium containing 2% fetal bovine serum.

CTL Assay. Immune cells from SP and PP were cultured at 5×10^6/ml in 24-well culture plates in complete T cell medium: RPMI 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml), and 5×10^{-5} M 2-mercaptoethanol as described (11). Three days later we added 10% Con A supernatant-containing medium as a source of IL-2. SP and PP cells were stimulated *in vitro* similarly for one or two 7-day stimulation cycles with 1 μM P18IIIB-I10 or P18–89.6R10 peptide together with 4×10^6 3,300-rad irradiated syngeneic SP cells. Cytolytic activity of CTL lines was measured by a 4-hr assay with ^{51}Cr-labeled targets. For testing the peptide specificity of CTL, ^{51}Cr-labeled P815 targets were pulsed or not for 2 hr with 1 μM peptide at the beginning of the assay. The percent specific ^{51}Cr release was calculated as 100 × (experimental release-spontaneous release)/(maximum release − spontaneous release). Maximum release was determined from supernatants of cells that were lysed by addition of 5% Triton X-100. Spontaneous release was determined from target cells incubated without added effector cells.

Antibody ELISA. ELISA was used to determine the presence of anti HIV-1 gp120 antibody in serum samples. HIV-1 gp120 antibody (International Enzyme, Sallbrook, CA) was suspended in coating buffer (PBS) at a concentration of 10 μg/ml and plated in 96-well microtiter plates (Nunc) at 50 μl/well. After overnight incubation at 4°C, plates were washed three times with wash buffer (50 mM Tris, 0.2% Tween-20) and then the plates were coated with the HIV strain 89.6 recombinant gp120 protein (a gift of Patricia Earl, National Institute of Allergy and Infectious Diseases, Bethesda, MD) at 60 ng per well. The next day after incubating with blocking buffer (PBS with 2% BSA, 0.01% thimerosal, pH 7.2–7.4) at room temperature for 2 hr, the plates were washed three times with wash buffer before addition of the samples. All samples were diluted in PBS with 2% BSA, 0.01% thimerosal (pH 7.2–7.4), and added to the ELISA plates at 100 μl per well. After overnight incubation at 4°C, plates were washed three times with wash buffer. Peroxidase-conjugated goat anti-mouse IgG (Sigma) were diluted 1:2,000 (PBS with 2% BSA, 0.01% thimerosal, pH 7.2–7.4) and used as the detection antibody (100 μl per well). After 2 hr, plates were washed three times with wash buffer and reacted with ABTS [2,2′-azinobis (3-ethylbenzthiazolinesulfonic acid)] peroxidase substrate (Kirkegaard & Perry Laboratories). After a 10-min incubation, plates were read at 405 nm on a plate reader (Molecular Devices). For gp120-specific ELISA, sample dilutions were considered positive if the optical density recorded for that dilution was at least 2-fold higher than the optical density recorded for a control serum sample at the same dilution. For calculation of the average endpoint titer of anti-gp120 antibody responses, the mean log of the endpoint dilutions was determined and used to calculate the average endpoint titer.

RESULTS

Initially, to ask whether the problem of preexisting immunity applied to CTL induction, we immunized BALB/c mice s.c. with 5×10^7 pfu of vSC8, a replication-competent vaccinia virus that expresses β-galactosidase, and 1 month later, reimmunized these mice s.c. with either a replication-competent recombinant vaccinia expressing either gp160 HIV-1 IIIB Env protein (vPE16) or a replication-defective vaccinia virus expressing Env protein of HIV 89.6 strain (MVA89.6). (HIV-recombinant vaccinia viruses were chosen for reimmunization of mice with preexisting immunity because HIV is one of the major targets for recombinant vaccine development.) Three weeks later, we studied the recombinant protein-specific CTL-response in SP against target cells expressing the corresponding specific peptides (Fig. 1*a*). In both experimental groups, we found that immunization with vSC8 completely blocked the development of a HIV-1 gp160 P18 epitope-specific CTL response in SP ($P < 0.001$ for results of 10 individual SPs per group). Thus, we extended to CTL responses the finding that systemic vaccinia immunity interferes with immunization with recombinant vaccinia vector vaccines. In addition, after s.c. reimmunization with recombinant virus MVA89.6, neither mice s.c. preimmunized with vSC8 nor nonpreimmunized mice had P18-specific CTL in PPs (Fig. 1*b*).

To test our hypothesis that mucosal immunization would bypass the preexisting systemic vaccinia immunity, we immunized first s.c. with vSC8 at 5×10^7 pfu, and then challenged 1 month later IR with either vPE16 or MVA89.6, and finally studied P18-specific CTL response in the SP after 3 weeks. We found that immunization IR with MVA89.6 induced significant antigen-specific CTL responses in the SPs of mice with the preexisting immunity to vaccinia virus (Fig. 1*c*), which were almost as high as in mice without previous vaccinia exposure ($P < 0.001$ compared with Fig. 1*a*, 10 mice per group). This activity was dose-dependent, in that reimmunization with 1×10^8 pfu gave higher CTL activity than reimmunization with 1×10^7 or 5×10^7 pfu (data not shown). Similarly, significant ($P < 0.001$ compared with Fig. 1*a*) CTL activity was found after IR challenge with recombinant vPE16 vaccinia virus (expressing gp160 HIV-1 IIIB), albeit somewhat lower than in mice without preexisting immunity. Interestingly, in mice with preexisting immunity to vaccinia, reimmunization with vPE16 produced >8-fold lower CTL activity (lytic units) in the SP than did reimmunization with MVA89.6, consistent with our earlier study of mucosal immunity induced by IR MVA89.6 immunization (5). Of note, the lack of influence of preexisting vaccinia immunity on mucosally administered vaccinia vaccines was even more striking in the case of HIV-specific CTL activity in PP in the same groups of mice immunized IR with vPE16 and MVA89.6 (Fig. 1*d*). In this case, the level of this

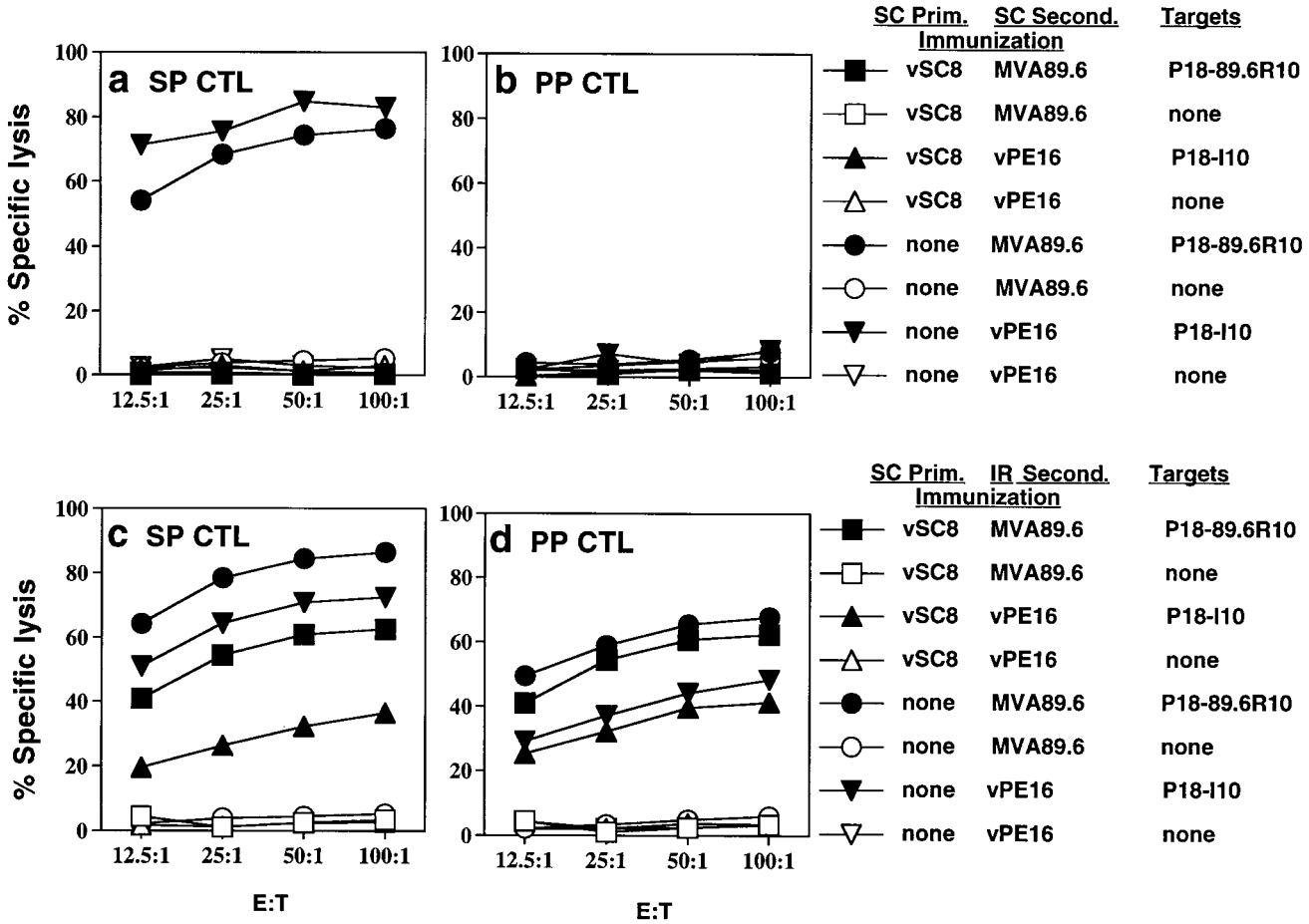

FIG. 1. Absence of P18–89.6R10- and P18-I10-specific CTL responses in the SP and PP in mice with preexisting immunity to vaccinia after s.c. reimmunization with recombinant viruses (*a* and *b*) and induction of P18–89.6R10- and P18-I10-specific CTL responses in the SP and PP in mice with preexisting immunity to vaccinia after IR reimmunization with recombinant viruses (*c* and *d*). BALB/c mice were immunized s.c. with vSC8 vaccinia virus at 5×10^7 pfu (*a–d*). One month later these mice and control naive mice were reimmunized either s.c. (*a* and *b*) or IR (*c* and *d*) with either recombinant vaccinia MVA89.6 or vPE16 at 1×10^8 pfu. Three weeks later the induction of P18–89.6R10- and P18-I10-specific CTL responses were studied in the SP (*a* and *c*) and PP (*b* and *d*). Results are means of 10 mice per group, with SP tested individually and PP pooled to obtain sufficient cell numbers. The percent specific ^{51}Cr release was calculated as described in *Materials and Methods.* E:T, effector-to-target ratio.

CTL activity was nearly the same as that in IR-immunized mice without previous vSC8 immunization.

Taken together, these studies showed that although systemic vaccinia immunity interferes with a subsequent immunization with a recombinant vaccinia vector vaccine, such interference can in fact be bypassed by reimmunization via the mucosal immune system. We speculate that the induction of an HIV-specific CTL response in the SP of mice with preexisting immunity to vaccinia was the result of the stimulation of naive cells in mucosal tissues and their subsequent migration from the mucosal inductive sites to the systemic lymphoid circulation. The alternative hypothesis, that CTL generation in SPs of mice with preexisting immunity to vaccinia depends on dissemination of vaccinia virus to the systemic lymphoid circulation, is unlikely because of the observed inability of systemic vaccinia to immunize under these circumstances (Fig. 1*a*).

To determine whether other mucosal routes of immunization would bypass preexisting vaccinia immunity, we examined the CTL response of vSC8 s.c.-immunized mice that were reimmunized IN with either vPE16 or MVA89.6. Although significant HIV-specific CTL responses were induced in the SP after IN immunization with vaccinia virus expressing 89.6 env protein (MVA89.6) (Fig. 2*a*), the level of these responses was much lower (>10-fold in lytic units) than that in the IR-challenged groups of mice (at the same dose) ($P < 0.001$) (Fig. 2*b*). In addition, we did not find a significant level of P18-specific CTL responses in the SP after IN challenge with vPE16 (Fig. 2*c*). Thus, the IR route of mucosal immunization was more effective than the IN route, perhaps because of a difference in the inductive sites available.

In previous studies we showed that mucosal CTL responses to a peptide vaccine could be enhanced by coadministration of rIL-12 (12). We therefore asked whether the method of overcoming preexisting vaccinia immunity described above could be enhanced further by coadministration of rIL-12 with the recombinant vaccinia vaccine. For this purpose, we treated BALB/c mice preimmunized with vaccinia virus with 1 μg of rmIL-12 by the IP route on the day of s.c. reimmunization with the recombinant vaccinia viruses MVA89.6 or vPE16 and also on days 5, 10, and 15 after reimmunization. Alternatively, we treated the mice with the rmIL-12 (1 μg) + DOTAP (a cationic lipofection agent used as adjuvant) IR together with either MVA89.6 or vPE16 and also on days 5, 10, and 15 (Fig. 3*a*). We found that whereas coadministration of IL-12 i.p. had no effect on HIV-specific CTL responses in the SP elicited by systemic immunization, coadministration of IL-12 in DOTAP IR significantly enhanced the CTL level in the SP elicited by IR immunization ($P < 0.05$ for MVA and $P < 0.001$ for vPE16 groups) (Fig. 3*a*). The difference in efficacy of the two routes of administration of IL-12 may reflect the need to deliver the

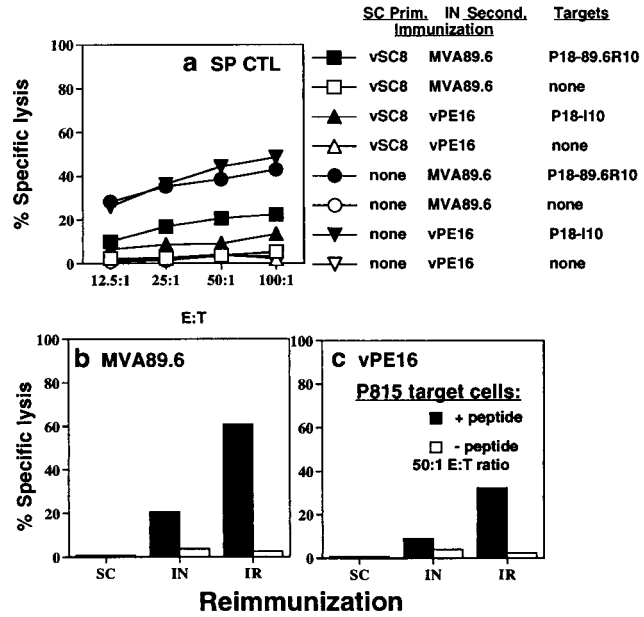

FIG. 2. Comparison of the induction of P18–89.6R10- and P18-I10-specific CTL responses in the SP of mice with preexisting immunity to vaccinia after either IR or IN reimmunization with recombinant viruses. (*a*) BALB/c mice were immunized s.c. with vSC8 vaccinia virus at 5×10^7 pfu. One month later the mice were reimmunized IN with either recombinant vaccinia vPE16 or MVA89.6 at 1×10^8 pfu. Three weeks later the induction of P18–89.6R10- and P18-I10-specific CTL responses were studied in the SP. The percent specific ^{51}Cr release was calculated as described in *Materials and Methods.* E:T, effector-to-target ratio. (*b* and *c*) Induction of the systemic HIV-specific CTL responses by different routes of reimmunization of mice with 5×10^7 pfu MVA89.6 (*b*) or vPE16 (*c*). Results are means of five mice per group tested individually. Killing of peptide-pulsed targets is compared with killing of unpulsed targets at an E/T of 50:1. Similar results were obtained at ratios of 25 and 12.5:1. In *a–c*, SEM of triplicate cultures were all <5% of the mean.

cytokine locally at the site of induction of the immune response (12), as well as the inhibitory effect of previous vaccinia immunity on systemic but not mucosal immunization. Thus, coadministration of IL-12 IR with the vaccinia enhanced the capacity to overcome preexisting systemic immunity to vaccinia by reimmunization via a mucosa route, but did not by itself overcome the effect of preexisting immunity on systemic immunization.

An important goal of vaccine development is the ability to boost the recombinant protein-specific immune response by a second immunization with the same vaccine. Thus, in view of the above results, we asked whether the mucosal route was more effective than the s.c. route for giving a second booster immunization with recombinant vaccinia viruses expressing HIV-1 gp160. First, we subjected groups of mice to a primary immunization with 5×10^7 pfu of MVA89.6 by the s.c. route, and then boosted them 1 month later with the same recombinant virus by either the s.c. or the IR route (1×10^8 pfu) (Fig. 3*b*). The response was boosted successfully only by the IR secondary immunization (resulting in a >8-fold increase in lytic units) ($P < 0.01$). Second, we subjected groups of mice to a primary immunization with 5×10^7 pfu of MVA89.6 by the IR route, and 1 month later boosted them with the same virus by either s.c. or IR routes (Fig. 3*c*). In this case, both routes of secondary immunization gave some boost, but the IR secondary was more effective (between 4- and 8-fold higher in lytic units) ($P < 0.01$). Thus, the results showed that both an initial s.c. and IR immunization could be boosted, but only by IR reimmunization. The reason why IR immunization can be boosted by IR reimmunization whereas s.c. immunization

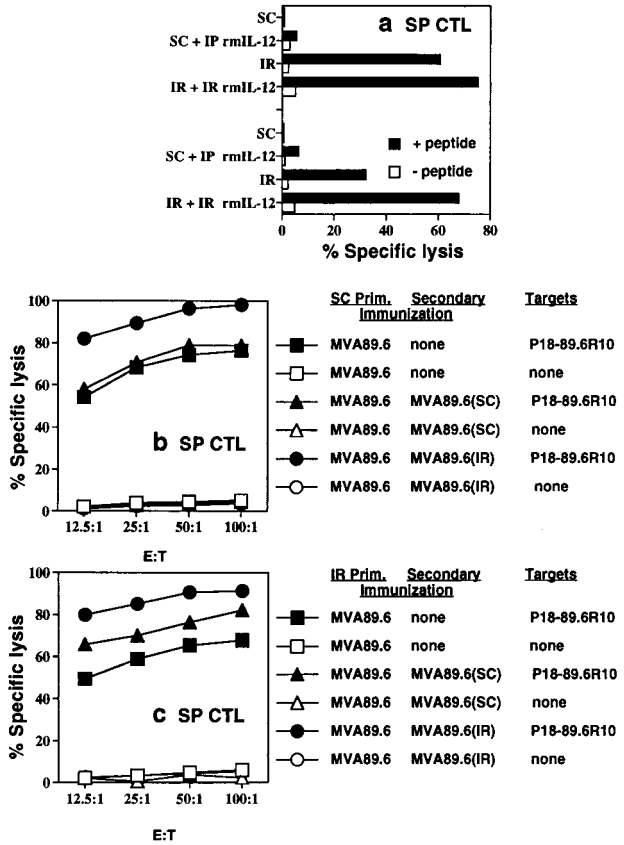

FIG. 3. Enhancement of the splenic HIV-specific CTL response by mucosal (not systemic) treatment with rmIL-12 in mice with preexisting immunity to vaccinia. BALB/c mice (five mice per group) with preexisting immunity to vaccinia were immunized by the s.c. or IR route with recombinant vaccinia virus with or without 1 μg of rmIL-12 and also on day 5,10 and 15 (*a*). For s.c. immunization, the IL-12 was given i.p., whereas for IR immunization, the IL-12 was given IR in DOTAP with the virus. Boosting of P18–89.6R10-specific CTL responses in the SP of BALB/c mice (*b* and *c*). BALB/c mice (5/group) were immunized either s.c. (*b*) or IR (*c*) with MVA89.6 vaccinia virus at 5×10^7 pfu. One month later the mice were reimmunized either s.c. or IR with the same recombinant vaccinia MVA89.6 at 1×10^8 pfu. Three weeks later the induction of P18–89.6R10-specific CTL responses were studied in the SP. The percent specific ^{51}Cr release was calculated as described in *Materials and Methods*. E:T, effector-to-target ratio.

cannot be boosted by s.c. reimmunization is unclear; however, it may relate to the patterns of cell traffic in the mucosal immune system and the presence of inductive sites (e.g., PPs) in the mucosal case containing naive cell populations despite the initial IR exposure to vaccinia virus.

Finally, to determine the ability of mucosal reimmunization of mice having preexisting immunity to vaccinia to induce recombinant protein-specific IgG serum antibody responses, we immunized vaccinia-immune and control nonimmune BALB/c mice either s.c., IR, or IN with 5×10^7 pfu MVA89.6. We found that previous s.c. immunization with vaccinia substantially reduced the titer of gp120-specific antibody induced by s.c. or IN immunization with MVA89.6 but did not affect the titer induced by IR immunization with MVA89.6 (Fig. 4). Thus, the IR route of immunization circumvents previous vaccinia immunity for antibody production as well as for CTL induction. Of note, the IR treatment of mice with preexisting immunity to vaccinia with rmIL-12 along with recombinant vaccinia vaccine did not substantially increase the titer of gp120-specific IgG antibody in the serum (data not shown).

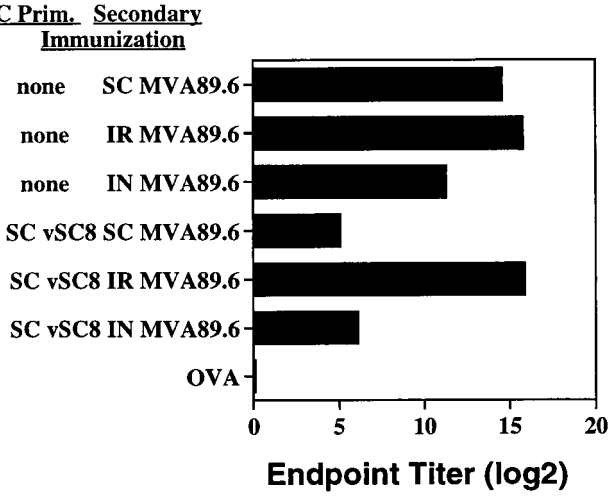

FIG. 4. Serum anti-gp120 IgG titer in BALB/c mice (5/group) with preexisting immunity to vaccinia after mucosal reimmunization with recombinant replication-deficient vaccinia virus expressing gp160 HIV-1 89.6 (MVA89.6) by different routes. Serum was assayed for the presence of anti-gp120 IgG by ELISA. Results are reported as the reciprocal of last dilution giving a positive ELISA response.

DISCUSSION

These studies have been aimed at finding a solution to one of the principal problems in using recombinant vaccinia vector vaccines. Indeed, the two main concerns that have hindered application of this potent vaccine vector are the studies showing interference with recombinant vaccinia immunization in people previously immunized with a vaccinia vaccine for smallpox (4), and the issue of safety in a potentially immunodeficient target population, despite the safe administration of the smallpox vaccinia vaccine to millions of people around the world (13). These concerns have encouraged the use of avian poxvirus vectors as alternative delivery systems. However, although these vectors are considered safer because they do not replicate in mammalian cells, and are not affected by previous vaccinia immunity (2), they appear to be less potent than vaccinia vectors (14–16). The problem of safety has been overcome with the introduction of highly attenuated vaccinia virus strains, such as NYVAC (17) and MVA (18–21), as vectors. MVA is replication incompetent in most mammalian cells, but recombinants are as or more immunogenic than replication competent strains (5, 14, 18–20, 22–28). In addition, both MVA and NYVAC were reported to be more immunogenic than avian poxvirus vectors (14–16). Thus, replication-defective vaccinia virus vectors appear to have the safety of avian poxvirus vectors without a decrement in immunogenicity. A remaining problem for the use of this potent vaccine approach, previous vaccinia immunity, was overcome by our current study. The data indicate that this strategy should be useful for boosting or administering a second vaccinia-based vaccine as well, and it may be applicable to other recombinant viral vectors besides vaccinia. Whether this immunization strategy will succeed in humans as well as in mice remains to be determined.

An additional conclusion from this study, together with our previous results (5, 11, 12), is that migration of immune T cells between the mucosal and systemic immune systems is asymmetrically restricted in the sense that cells traffic from the mucosal system to the systemic system but not vice versa. Thus, systemic infection with vaccinia virus does not induce CTL that migrate to the mucosal immune system, and apparently the virus does not infect mucosal tissues sufficiently under these circumstances to induce mucosal immunity. On this basis, the mucosal immune system remains naive to vaccinia virus. In contrast, mucosal infection with recombinant vaccinia virus induces not only CTL in the mucosa, but CTL that traffic out to the systemic immune system. The results cannot be explained by dissemination of the virus from the site of mucosal inoculation for two reasons. First, this dissemination would not account for the induction of immunity in the face of the barrier of preexisting vaccinia immunity in the systemic immune system. Second, MVA is replication incompetent, so it is unlikely to spread beyond the site of inoculation. The conclusion that trafficking of CTL from mucosal sites to the systemic immune system is efficient in contrast to migration in the reverse direction may have implications beyond the application described here.

In summary, this study establishes that mucosal routes of immunization can be used to induce systemic recombinant protein-specific CTL and antibody responses with vaccinia vectors in the face of preexisting systemic immunity to vaccinia. Furthermore, mucosal coadministration of rmIL-12 can substantially increase this systemic CTL response in vaccinia immune animals. The ability to circumvent preexisting vaccinia immunity should greatly improve the utility of this otherwise very promising viral vector for vaccines against a variety of pathogens, including HIV.

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