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A Single Injection of Recombinant Measles Virus Vaccines Expressing Human Immunodeficiency Virus (HIV) Type 1 Clade B Envelope Glycoproteins Induces Neutralizing Antibodies and Cellular Immune Responses to HIV

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The anchored and secreted forms of the human immunodeficiency virus type 1 (HIV-1) 89.6 envelope glycoprotein, either complete or after deletion of the V3 loop, were expressed in a cloned attenuated measles virus (MV) vector. The recombinant viruses grew as efficiently as the parental virus and expressed high levels of the HIV protein. Expression was stable during serial passages. The immunogenicity of these recombinant vectors was tested in mice susceptible to MV and in macaques. High titers of antibodies to both MV and HIV-Env were obtained after a single injection in susceptible mice. These antibodies neutralized homologous SHIV89.6p virus, as well as several heterologous HIV-1 primary isolates. A gp160 mutant in which the V3 loop was deleted induced antibodies that neutralized heterologous viruses more efficiently than antibodies induced by the native envelope protein. A high level of $CD8^+$ and $CD4^+$ cells specific for HIV gp120 was also detected in MV-susceptible mice. Furthermore, recombinant MV was able to raise immune responses against HIV in mice and macaques with a preexisting anti-MV immunity. Therefore, recombinant MV vaccines inducing anti-HIV neutralizing antibodies and specific T lymphocytes responses deserve to be tested as a candidate AIDS vaccine.

The vast majority of the 40 million people currently infected by human immunodeficiency virus (HIV) are living in developing countries (77a). In these areas, mother-to-child transmission, including via breast-feeding, accounts for half a million infections every year, and most cases of sexual transmission occur in individuals under the age of 20 years. Therefore, developing a preventive pediatric HIV vaccine is a major goal in the fight against AIDS. Such a vaccine must be easy to produce on a large scale and at low cost in developing countries. It must be safe and able to induce protective immunity after one or two injections.

Vaccines developed from replicating live attenuated RNA viruses, such as Sabin poliovirus, Schwarz measles virus (MV), or the 17D strain of yellow fever virus, have a longstanding safety and efficacy record. They are produced on a large scale in most developing countries and can be distributed at very low cost. These vaccines induce strong cellular and humoral immune responses after a single injection and are particularly efficient at stimulating long-lasting memory B- and T cells. Although live attenuated simian immunodeficiency virus (SIV) protects macaques efficiently (18), a live attenuated HIV vaccine is not envisioned at present for safety reasons (4). Therefore, a number of recombinant viral vectors such as modified vaccinia virus Ankara, canarypox virus, and adenovirus have been evaluated in preclinical or clinical trials (45). However, these replication-defective vectors require several high-dose injections in order to induce and maintain efficient responses. We propose to explore the possibility of using live attenuated MV as a polyvalent AIDS vaccination vector.

MV vaccine induces a very efficient, life-long immunity after a single low-dose injection [10^4 50% tissue culture infective dose(s) ($TCID_{50}$)] (26). Protection is mediated both by antibodies and by $CD4^+$ and $CD8^+$ T cells. The MV genome is very stable and reversion to pathogenicity has never been observed with this vaccine. MV replicates exclusively in the cytoplasm, ruling out the possibility of integration in host DNA. Furthermore, an infectious cDNA clone corresponding to the anti-genome of the Edmonston strain of MV, as well as a procedure to rescue the corresponding virus, has already been established (64). This cDNA has been adapted to create a vector to express foreign genes (63). It can accommodate up to 5 kb of foreign DNA and is genetically very stable (72, 78). Therefore, MV vaccine could be a vector to facilitate the induction of anti-HIV immunity. By taking advantage of the existing technology to produce and distribute large quantities of MV vaccine, recombinant MV-HIV could be used to mass immunize children and adolescents against both measles and AIDS. We describe here the production of live attenuated MV vaccines expressing different forms of clade B $HIV_{89.6}$ envelope glycoprotein (Env), and the induction of immune responses by these vaccines.

Neutralizing anti-HIV antibodies are directed at the envelope glycoprotein and can contribute to the control of HIV spread (9, 43, 56). Broadly neutralizing antibodies have been

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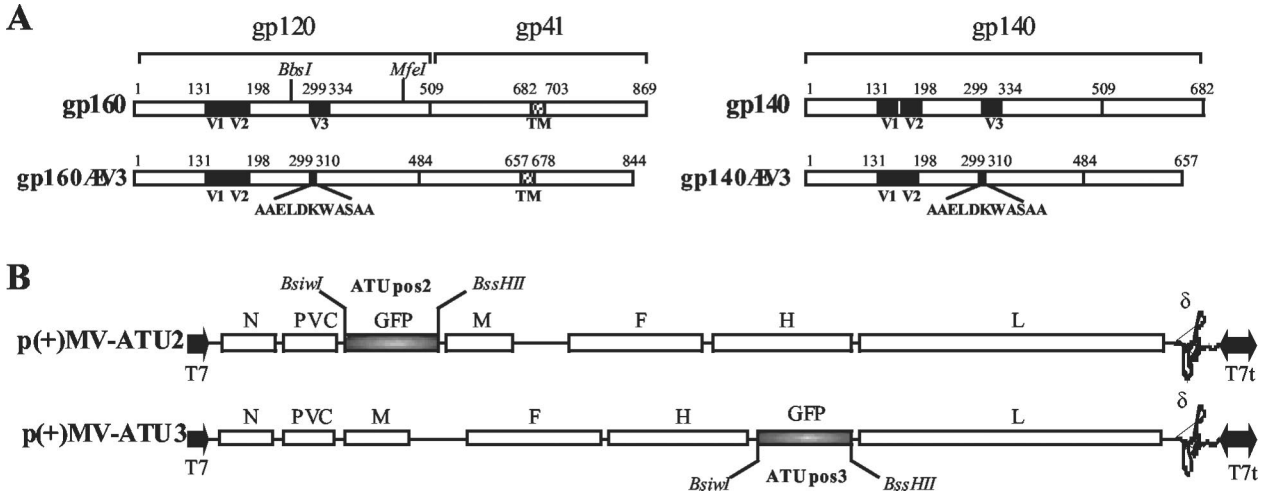

FIG. 1. Constructions of recombinant MV-$env_{HIV89.6}$. (A) Full-length gp160 and secreted gp140 HIV-1 Env proteins. Shaded boxes indicate V1, V2, and V3 regions; dashed boxes show the transmembrane domain. Amino acid positions are indicated. The *Bbs*I and *Mfe*I restriction sites used to introduce the ΔV3-AAELDKWASAA mutation are also indicated. (B) pMV(+) vectors with ATU containing a green fluorescent protein (GFP) gene in positions 2 and 3. The MV genes are indicated as follows: N, nucleoprotein; PVC, phosphoprotein and V C proteins; M, matrix; F, fusion; H, hemagglutinin; and L, polymerase. T7, T7 RNA polymerase promoter; T7t, T7 RNA polymerase terminator; ∂, hepatitis delta virus ribozyme.

detected in long-term nonprogressors (59). However, native gp120 is a poor inducer of cross-reactive neutralizing antibodies. As shown by X-ray crystallography, the variable V1 and V2 loops mask elements of the CD4 binding site, and the V2 and V3 loops mask the CD4-induced (CD4i) epitopes and the chemokine receptor binding site (38, 79, 81). Furthermore, some conserved epitopes can induce highly neutralizing antibodies, but they are buried in the three-dimensional structure of the envelope glycoprotein and become exposed only after binding to the receptor or coreceptor (52, 74, 75, 80). Neutralizing monoclonal antibodies (MAbs) have been obtained from patients' B cells (57). These are directed at gp41 linear or conformational epitopes (2F5) (52, 83) or at gp120 conformational epitopes (2G12, 17b, 48d, and b12) (39, 68, 75, 76). Used in synergy they can neutralize several primary isolates in vitro (44) and protect macaques against a mucosal challenge with simian/HIV (SHIV) (3). Although the role of neutralizing antibodies in the control of an established infection is unclear (61), several reports suggest that they could contribute to protective immunity (10, 45, 70). To be cross-reactive for primary isolates, antibodies must be directed at conserved epitopes that are critical for viral entry into host cells (24, 60, 77). However, in infected people, such antibodies appear slowly and are diluted in large quantities of antibodies directed at the accessible, highly variable gp120 loops (47, 58, 79). Most antibodies directed against the V3 loop are isolate specific and neutralize only genetically similar viruses (53). Likewise, the early neutralizing antibody response in SHIV-infected monkeys is strain specific and directed against epitopes of the V2 and V3 loops (23).

To favor the induction of cross-reactive neutralizing antibodies, we deleted the hypervariable V3 loop of the $HIV_{89.6}$ gp160 (anchored) and gp140 (soluble) proteins in order to eliminate this "immunological decoy" and to expose more conserved epitopes (5, 30, 67). Indeed, the conserved elements near the chemokine receptor-binding site are poorly exposed in native gp120 (81). Also, the N-linked glycosylation of a conserved amino acid of the V3 loop modifies the loop structure and obstructs access to the conserved CD4 binding and CD4i sites of the gp120 from several HIV-1 isolates (42). Deleting the V3 loop could redirect the antibody response against the chemokine receptor binding site, therefore inducing broadly neutralizing antibodies (25). In addition, we replaced the V3 loop with the ELDKWAS sequence flanked on both sides by two alanines to maintain the native conformation of this gp41 conserved epitope normally buried in the native protein and targeted by the broadly neutralizing 2F5 MAb (52). We investigated the immunogenicity of native and ΔV3 $Env_{HIV89.6}$ mutants expressed in live attenuated MV. We found that MV-HIV recombinant viruses induced cross-reactive neutralizing antibodies, as well as gp120-specific $CD4^+$- and $CD8^+$-T-cell responses, after a single injection in $CD46^{+/-}$ alpha/beta interferon receptor$^{-/-}$ (IFN-α/βR$^{-/-}$) mice. Furthermore, they induced anti-HIV antibodies in mice and macaques with preexisting anti-MV immunity.

MATERIALS AND METHODS

Plasmid constructions. All MV recombinant plasmids were derived from plasmid p(+)MV, which carries the antigenomic MV tag Edmonston B sequence (64). Two additional transcription units (ATU) containing unique restriction sites for easy insertion of open reading frames have been introduced in p(+)MV (63): one downstream from the P gene (position 2) and the other one downstream from the H gene (position 3). Both plasmids (kindly provided by M. A. Billeter, University of Zurich, Zurich, Switzerland) were used for inserting the SHIV89.6p *env* open reading frames.

The envelope glycoproteins used in the present study were derived from SHIV89.6p, a chimeric SHIV with the *tat*, *rev*, *vpu*, and *env* genes of HIV-1 on a SIVmac239 background (65). The *env* gene is derived from a cytopathic primary HIV-1 isolate, 89.6, which is tropic for both macrophages and T cells (15). The *env* sequence was amplified from plasmid pSHIV-KB9 (NIH-AIDS Research & Reference Reagent Program) that had been cloned after passage of the original virus in vivo (34). The full-length (gp160) and secreted (gp140) *env* sequences were amplified by PCR (*Pfu* polymerase) with primers that contain unique *Bsi*WI and *Bss*HII sites for subsequent cloning in MV vectors (Fig. 1). A start and a stop

codon were added to the ends of the genes. Nucleotides were also added after the stop codon in order to comply with the "rule of six," which stipulates that the number of nucleotides of MV genome must be a multiple of six (11). Both gp160 and gp140 *env* fragments were cloned in pCR2.1-TOPO plasmid (Invitrogen, Groningen, The Netherlands) and sequenced.

The V3 loop-deletion mutants were generated by using two overlapping DNA fragments flanking the V3 sequence to be deleted (amino acids 299 to 334, Fig. 1A) and containing the sequence coding for the AAELDKWASAA peptide. These fragments were annealed by PCR, cloned in pCR2.1-TOPO plasmid, and sequenced. After digestion with *Bbs*I and *Mfe*I, the DNA fragment encoding the ΔV3-AAELDKWASAA mutation was introduced in place of the corresponding fragment in the gp160 and gp140 sequences already cloned in pCR2.1-TOPO plasmids (Fig. 1A). After *Bsi*WI/*Bss*HII digestion, the native and ΔV3 gp160 and gp140 sequences were introduced into the pMV vector in ATU positions 2 and 3 (Fig. 1B). The resulting plasmids were designated pMV2-gp140$_{HIV}$, pMV2-gp160$_{HIV}$, pMV3-gp140ΔV3$_{HIV}$, and pMV2-gp160ΔV3$_{HIV}$.

Cells. Cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 5% fetal calf serum (FCS) for Vero cells (African green monkey kidney) or with 10% FCS and 1 mg of G418/ml for helper 293-3-46 cells (64) and for P4-CCR5 cells (HeLa-CD4-CXCR4-CCR5-HIVLTR-LacZ) (13).

Rescue of recombinant MV-Env$_{HIV89.6}$ viruses. Recombinant MV-HIV viruses were recovered from plasmids by using the helper-cell-based rescue system described by Radecke et al. (64) and modified by Parks et al. (54). Human helper cells expressing T7 RNA polymerase and measles N and P proteins (293-3-46 cells [kindly provided by M. A. Billeter) were cotransfected with the pMV-Env$_{HIV}$ plasmids (5 μg) and a plasmid expressing the MV L gene (pEMC-La, 20 ng [kindly provided by M. A. Billeter]). After overnight incubation at 37°C, the cells were heat shocked at 43°C for 3 h in fresh medium (54). Heat-shocked cells were incubated at 37°C for 2 days and then transferred onto a 70% confluent Vero cells layer (10-cm petri dishes). Syncytia appeared in Vero cells after 2 to 5 days of coculture. Single syncytia were harvested and transferred to Vero cells grown in 35-mm wells. The infected cells were expanded in 75- and 150-cm^3 flasks. When syncytia reached 80 to 90% confluence, the cells were scraped into a small volume of OptiMEM (Gibco-BRL) and then frozen and thawed once. After centrifugation, the supernatant, which contained virus, was stored at −80°C.

Virus titration. The titers of recombinant MV were determined by an end-point limit dilution assay on Vero cells. The $TCID_{50}$ values were calculated by the Kärber method (33).

Western blots. Monolayers of Vero cells (T-25 flasks) were infected at a multiplicity of infection (MOI) of 0.05 with the recombinant viruses. When syncytia reached 80 to 90% confluence, cells were lysed in 150 mM NaCl, 50 mM Tris (pH 8), 1% NP-40, 0.5 mM phenylmethylsulfonyl fluoride, and 0.2 mg of Pefabloc (Interbiotech)/ml. Chromatin was removed by centrifugation, and the concentration of protein in the supernatant was determined by a Bradford assay. Proteins (50 μg) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to cellulose membranes (Amersham Pharmacia Biotech). The blots were probed with a mouse monoclonal anti-HIV gp120 antibody (Chessie 13-39.1; NIH-AIDS Research & Reference Reagent Program) or with a monoclonal anti-MV N antibody (Chemicon, Temecula, Calif.). A goat anti-mouse immunoglobulin G (IgG)-horseradish peroxidase (HRP) conjugate (Amersham) was used as a secondary antibody. Peroxidase activity was visualized with an enhanced chemiluminescence detection kit (Pierce).

Animal immunization. Mice susceptible for MV infection were obtained as described previously (50). FVB mice heterozygous for the CD46 MV receptor transgene (82) (kindly provided by F. Grosveld, Erasmus University, Rotterdam, The Netherlands) were crossed with 129Sv IFN-α/βR$^{-/-}$ mice (51) (kindly provided by M. Aguet, Swiss Institute for Experimental Cancer Research). The F_1 progeny were screened by PCR, and the CD46$^{+/-}$ animals were crossed again with 129Sv IFN-α/βR$^{-/-}$ mice. IFN-α/βR$^{-/-}$ CD46$^{+/-}$ animals were selected and used for immunization experiments. Six-week-old CD46$^{+/-}$ IFN-α/βR$^{-/-}$ mice were inoculated intraperitoneally with 5×10^6 $TCID_{50}$ of MV-HIV recombinant viruses. Control mice were immunized with 5×10^6 $TCID_{50}$ of empty MV vector (EdB-tag MV). Mice were euthanized at 7 days or 1 month postinfection. Spleen and whole blood were collected.

Two colony-bred rhesus macaques (i.e., *Macaca mulatta*) that were seronegative for simian type D retrovirus, simian T-cell lymphotropic virus, simian immunodeficiency virus, and MV were vaccinated subcutaneously with 10^4 $TCID_{50}$ of MV vaccine (Rouvax; Aventis Pasteur, Paris, France). The animals were boosted 1 year later with two injections of 5×10^6 $TCID_{50}$ of MV2-gp140 recombinant virus separated by a 1-month interval. Blood samples were collected at different time points, and the samples were tested for the presence of anti-MV and anti-HIV antibodies.

Characterization of humoral immune responses. Sera were collected 1 month after immunization and were heat inactivated. Anti-MV (Trinity Biotech) and anti-HIV Env (Sanofi Diagnostic Pasteur; Bio-Rad) antibodies were detected by using commercial enzyme-linked immunosorbent assay (ELISA) kits. An anti-mouse antibody-HRP conjugate (Amersham) was used as the secondary antibody. Titers were determined by limiting dilutions and calculated as the highest dilution of serum giving twice the absorbance of a 1/100 dilution of a mixture of control sera. The same ELISA kits were used for sera from macaque monkeys. An anti-monkey IgG secondary antibody was used to detect anti-HIV antibodies. Anti-MV antibodies were detected with an anti-human IgG in order to be able to calibrate the assay with standards supplied in the MV ELISA kit. Values were expressed in milli-international units per milliliter. A mixture of five samples from negative monkeys was used as the negative control. The titer of anti-ELDKWAS antibodies was determined by ELISA by using 96-well NeutrAvidin plates (Pierce) coated with the ELDKWAS biotinylated peptide (Neosystem; 5 μg/ml in 2 M $NaHCO_3$–2 M $Na_2CO_3 \cdot H_2O$ [pH 9.6]). Sera from mice immunized with standard MV were used as negative controls. Peptide-bound antibodies were detected by using an anti-mouse antibody-HRP conjugate.

HIV-1 neutralization assays. Seroneutralization was tested against SHIV89.6p (A. M. Aubertin, Université Louis Pasteur, Strasbourg, France); the clade B HIV-1 primary isolates Bx08 (8; C. Moog, INSERM, Strasbourg, H. Fleury, Bordeaux, France) and 92US660, 92US714, and 92HT593 (NIH-AIDS Research & Reference Reagent Program); and the clade A primary isolate 3253 (G. Pancino, Institut Pasteur, Paris, France). These viruses were propagated on phytohemagglutinin-stimulated human peripheral blood mononuclear cells as described elsewhere (8). As a control virus that should not be neutralized by the test sera, we used an HIV-1$_{LAI}$ (vesicular stomatitis virus [VSV]) pseudotype in which HIV Env is replaced by VSV-G Env (7). HIV-1 neutralization assays were performed by using the P4-CCR5 indicator cell line (13). P4-CCR5 cells were seeded into 96-well plates (20,000 cells per well) and then incubated at 37°C in DMEM–10% FCS for 24 h. The medium was replaced with 50 μl of DMEM–10% FCS–DEAE dextran (10 μg/ml), and the cells were incubated at 37°C for 45 min. Viruses (2 to 5 ng of p24) were incubated with serial serum dilutions in 50 μl of culture medium at 37°C for 45 min, and the virus-serum mixtures were added to the cells in triplicate. After 48 h of incubation, the β-galactosidase activity was measured by using a chemiluminescence reporter gene assay (Roche). The mean percent neutralization for each serum dilution tested was calculated in comparison with the value recorded in wells containing control serum from nonimmunized mice at the same dilution.

To determine whether the additional ELDKWAS epitope elicited specific antibodies important for neutralization, we performed an antibody-ELDKWAS peptide competition assay. Sera from immunized mice were diluted in culture medium and preincubated for 1 h at room temperature with increasing concentrations (30 to 100 μg/ml) of ELDKWAS peptide (Neosystem). Neutralization assays were then performed as described above, and values were compared to those obtained without serum-peptide preincubation. An unrelated SIV Nef peptide was included as a negative control. To control that the ELDKWAS peptide did not directly alter the viruses, infection was also performed in the presence of increasing doses of peptide without preincubation with mice sera. Competition between 2F5 (25 μg/ml) and ELDKWAS peptide was used as a positive control.

Cellular immune responses. Frozen splenocytes from immunized mice were thawed 18 h before functional assays and incubated in RPMI, 10% FCS, and 10 U of recombinant human interleukin-2 (rh-IL-2; Boehringer Mannheim). Their capacity to secrete IFN-γ upon specific stimulation was tested by enzyme-linked immunospot (ELISPOT) assays and flow cytometry. Cells were stimulated (18 to 36 h) by concanavalin A (5 μg/ml; Sigma) as a positive control, RPMI–IL-2 (10 U/ml) as a negative control, HIV-1 gp120 protein (1 μg/ml; AbCys), bovine serum albumin (BSA; 1 μg/ml; Sigma), or 5×10^5 $TCID_{50}$ of EdB-tag MV. For ELISPOT assays, Multiscreen-HA 96-well plates were coated overnight at 4°C with 6 μg of anti-mouse IFN-γ (R4-6A2; Pharmingen)/ml in phosphate-buffered saline (PBS), washed, and then incubated with 100 μl of RPMI and 10% FCS for 1 h at 37°C. The medium was replaced by 100 μl of cell suspension (5×10^5 splenocytes per well in duplicate) and 100 μl of stimulating agent. After 2 h at 37°C, heated-FCS (10%) was added, and the plates were incubated for 18 to 36 h at 37°C. After a washing step, biotinylated anti-mouse IFN-γ antibody (XMG1.2; Pharmingen) was added (100 μl; 4 μg/ml in PBS–0.1% FCS), and the plates were incubated for 2 h at room temperature. Streptravidin-alkaline phosphatase conjugate (Roche) was used as secondary step. Spots were developed with BCIP/NBT (Promega) and counted (ELISpot Reader; Bio-Sys).

For flow cytometry assays, 5×10^5 splenocytes in 100 μl of RPMI medium

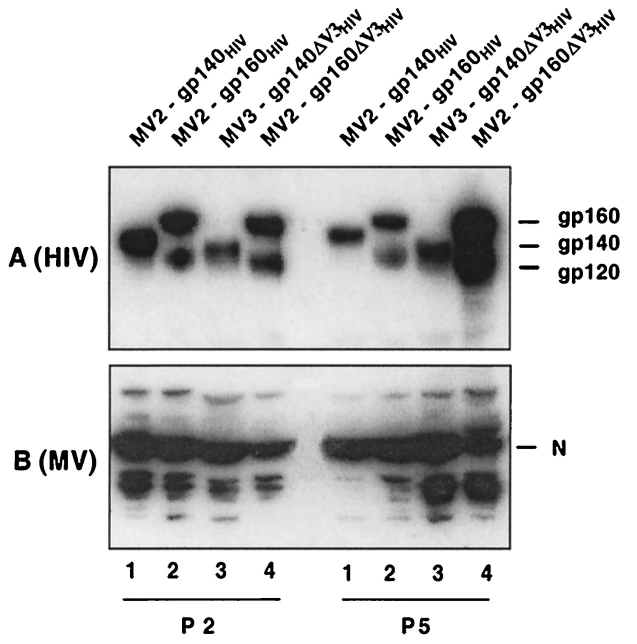

FIG. 2. Expression of HIV-$1_{89.6}$ envelope glycoproteins in recombinant MVs. gp160, gp140, and MV nucleoprotein (N) detected in lysates of Vero cells infected with MV-Env_{HIV} viruses. (A) HIV proteins were probed with mouse monoclonal anti-HIV gp120 antibody; (B) MV N protein was probed with monoclonal anti-MV N antibody.

were incubated in V-bottom 96-well plates with either 1 μg of HIV-1 gp120 (AbCys)/ml or 5×10^5 $TCID_{50}$ of EdB-tag virus diluted in 100 μl of RPMI and 10 U of IL-2/ml. Control cells were incubated with RPMI with 10 U of IL-2/ml alone. After 2 h at 37°C, FCS was added (10%), and the plates were incubated overnight at 37°C. The medium was then replaced by 150 μl of RPMI and 10% FCS containing 10 U of rh-IL-2 and 10 μg of brefeldin A (Sigma)/ml. Cells were incubated for 4 h at 37°C, harvested, stained with anti-mouse CD8-APC (Pharmingen) and anti-mouse CD4-CyCr (Pharmingen) for 20 min at room temperature, washed with PBS-BSA (0.5%), and then fixed for 5 min at 37°C in CytoFix (Pharmingen). Cells were treated with 0.1% saponin (Sigma) in PBS-BSA (0.5%) and incubated for 30 min at room temperature with anti-mouse IFN-γ–PE (Pharmingen). Samples were analyzed by using a FACSCalibur cytometer (Becton Dickinson). The data were analyzed by using CellQuest software.

RESULTS

Recombinant MV expresses $Env_{HIV89.6}$ glycoproteins and replicates efficiently. The anchored (gp160) and soluble (gp140) forms of the HIV Env glycoprotein (strain SHIV89.6p), with or without deletion of the V3 loop and insertion of an additional ELDKWAS epitope, were inserted into one of the ATU of the p(+)MV vector (Fig. 1). Recombinant viruses MV2-gp140, MV2-gp160, MV3-gp140ΔV3, and MV2-gp160ΔV3 were obtained after transfection of the plasmids into the 293-3-46 helper cell line and propagation in Vero cells [MV2 and MV3 refer to the site of insertion, at position 2 or 3 of the p(+)MV vector, respectively, of the $Env_{HIV89.6}$ construction]. Expression of the $Env_{HIV89.6}$ was analyzed by Western blotting of infected-cell lysates (Fig. 2) and immunofluorescence (not shown). The MV2-gp140 and MV2-gp160 viruses gave a high level of expression of the $Env_{HIV89.6}$ protein (Fig. 2A, lanes 1, 2, and 4). As expected, the MV2-gp160 and MV2-gp160ΔV3 viruses expressed the env gp160 precursor, as well as the cleaved gp120 protein (Fig. 2A, lanes 2 and 4). In contrast, the MV2-gp140 and MV3-gp140ΔV3 viruses expressed only the secreted, uncleaved gp140 form. The MV3-gp140ΔV3 virus expressed slightly lower levels of transgene than viruses of the MV2 series, as expected, due to the transcription gradient observed in MV expression (Fig. 2A, lane 3). Taken together, these results indicate that $Env_{HIV89.6}$ and the ΔV3 mutants were efficiently expressed and correctly matured. The recombinant MV were passaged five times on Vero cells, and the expression of the transgene was compared to that of the MV nucleoprotein. Figure 2 shows that $Env_{HIV89.6}$ expression was similar for passages 2 and 5, confirming the stability of transgene expression in this system.

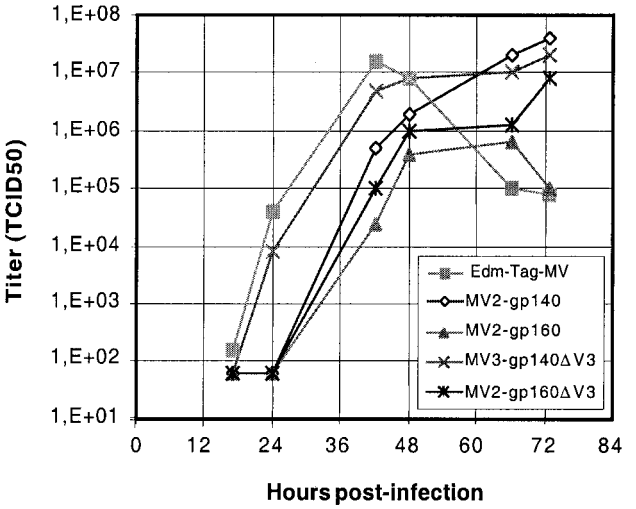

FIG. 3. Growth kinetics of recombinant MV-$Env_{HIV89.6}$ viruses on Vero cells. Vero cells were infected with EdB-tag MV and MV-$Env_{HIV89.6}$ viruses at an MOI of 0.01. At each time point, cells were collected, and cell-associated virus titers were determined by using the $TCID_{50}$ method on Vero cells.

The growth of MV-$Env_{HIV89.6}$ recombinant viruses was analyzed on Vero cells by using an MOI of 0.01 (Fig. 3). The growth of recombinant viruses was only slightly delayed compared to that of the standard EdB-tag MV rescued from p+(MV). Viruses expressing the secreted gp140 were less affected than viruses expressing the anchored gp160. The gp140ΔV3 recombinant grew at the same rate as control MV. The delay observed with viruses expressing the anchored gp160 may be due to a lower replication rate, to the larger size of the transgene, or to educed MV budding because of the insertion of gp160 at the surface of the infected cells. Nevertheless, the final yield of recombinant viruses was comparable to that of control MV, and peak titers of ca. 10^6 to 10^7 $TCID_{50}$/ml were obtained routinely.

Induction of humoral immune response to recombinant MV in susceptible mice. The immunogenicity of MV-$Env_{HIV89.6}$ viruses was tested in genetically modified mice expressing the human CD46 MV receptor and lacking the IFN-α/β receptor. Increasing doses of MV2-gp160 virus (10^3 to 10^7 $TCID_{50}$) were tested in five groups of three mice. ELISAs were performed to detect the presence of anti-MV and anti-HIV antibodies in sera collected 1 month after immunization (Fig. 4A). Both anti-MV and anti-HIV antibody titers increased when the dose

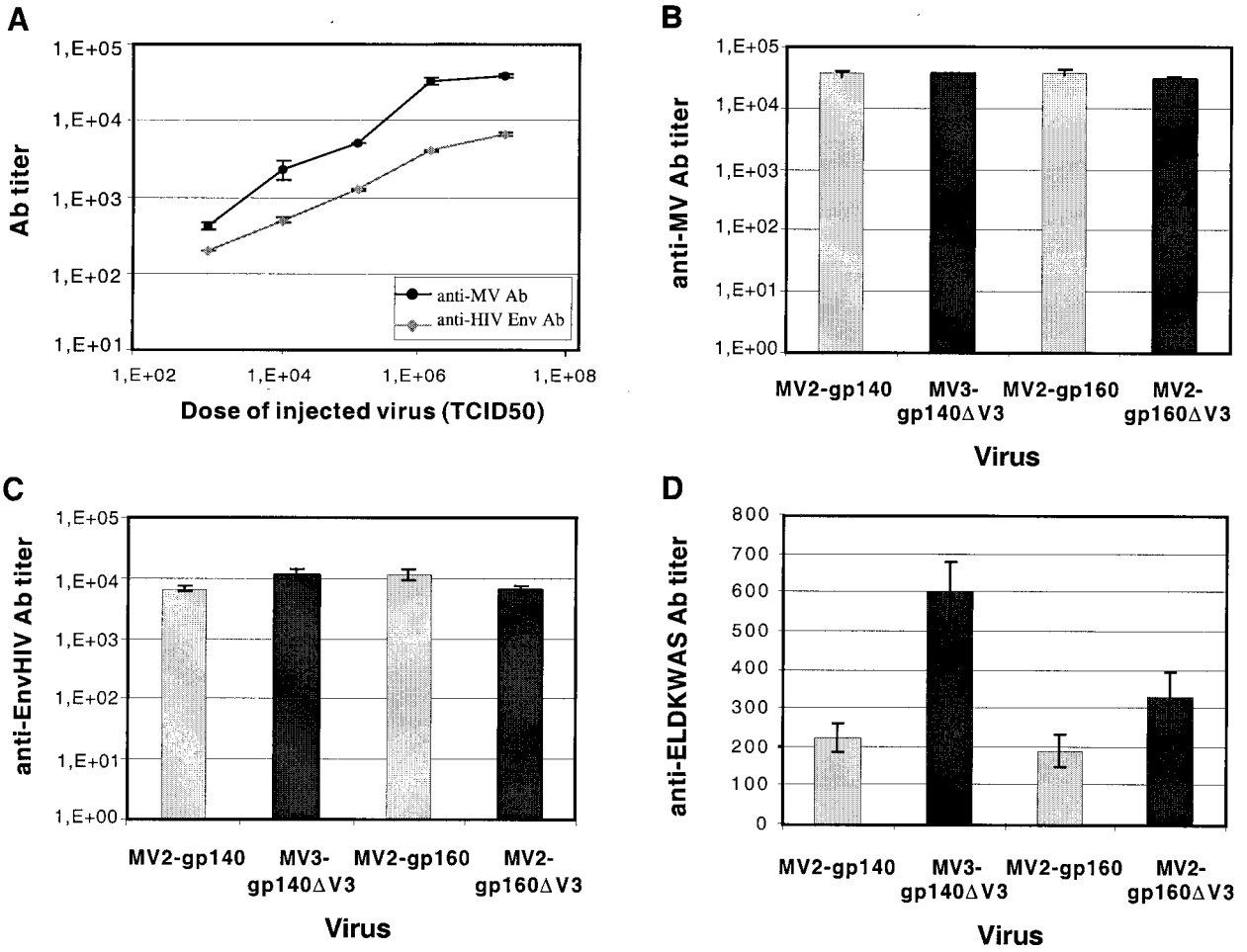

FIG. 4. Anti-HIV and anti-MV antibody titers in IFN-α/βR$^{-/-}$ CD46$^{+/-}$ mice immunized with MV-Env$_{HIV89.6}$ viruses. (A) Anti-MV and anti-HIV antibody titers detected 28 days after injection of increasing doses of MV-gp160 (three mice per group). (B to D) Anti-MV (B), anti-HIV (C), and anti-ELDKWAS (D) antibody titers were detected in sera from animals 28 days after the injection of 5×10^6 TCID$_{50}$ of MV-gp160/gp140 viruses (▨) and MV-gp160/gp140 ΔV3-ELDKWAS (■) viruses (six mice per group). The results are expressed as the mean values ± the standard deviation.

of recombinant MV increased. Since high anti-MV titers were obtained between 10^6 and 10^7 TCID$_{50}$, mice were immunized with 5×10^6 TCID$_{50}$ in all further experiments. At this dose, anti-MV antibody titers were sixfold higher than anti-HIV titers. One should keep in mind that immunization was against HIV Env only, whereas all MV proteins were expressed during infection. To compare the immunogenicity of the different Env$_{HIV89.6}$ constructs, four groups of six mice were inoculated intraperitoneally with various MV-Env$_{HIV89.6}$ viruses (Fig. 4B and C). All mice responded to MV (mean anti-MV titer = 5×10^4) and to HIV Env (mean anti-HIV titer = 8×10^3). No difference in anti-MV or anti-HIV titers was observed between the four constructs tested. Interestingly, expression from ATU position 2 or 3 of the MV vector did not affect the antibody response. Control mice that were immunized with empty MV (EdB-tag MV) raised similar anti-MV antibody titers (5×10^4) but remained negative for anti-HIV antibodies. Because the ΔV3 constructions expressed an additional ELDKWAS epitope, the antibody response against this gp41 epitope was examined separately by using a specific ELISA (Fig. 4D). The anti-ELDKWAS antibody titers were low for all of the different Env$_{HIV89.6}$ constructs. The ΔV3-ELDKWAS mutation caused only a twofold increase in anti-ELDKWAS antibody titers.

MV-Env$_{HIV89.6}$ viruses induce neutralizing anti-HIV antibodies. The capacity of sera from MV-Env$_{HIV89.6}$-immunized mice to neutralize either homologous SHIV89.6p virus or various heterologous primary HIV-1 isolates was tested by using a single-cycle virus infectivity assay on P4-CCR5 indicator cells (13). P4-CCR5 cells express the CD4, CXCR4, and CCR5 HIV-1 receptors and have been stably transfected with an HIV long terminal repeat LacZ construct. Therefore, these cells are susceptible to HIV-1 isolates and express β-galactosidase upon infection. The seroneutralization assay was validated by using a combination of anti-HIV immunoglobulin (HIVIG; 2.5 mg/ml) and MAbs (2F5 and 2G12, 25 μg/ml each) previously shown to synergistically neutralize primary HIV isolates (44). We also used sera from two infected patients at the same dilution previously shown to neutralize 90% of the infectivity of the Bx08 virus in a standard neutralization assay on human

peripheral blood mononuclear cells (8). To take into account the possibility of nonspecific inhibition of HIV-1 infection by mice sera, the 100% level of infectivity for each HIV-1 isolate was determined in the presence of the same dilution of control sera from nonimmunized mice. Thus, the neutralizing activity (Table 1) is expressed as the percentage of reduction of infection obtained in the presence of the serum tested compared to that obtained with a negative control serum used at the same dilution. Sera from HIV-negative individuals were used as negative controls for MAb and human HIV sera, and sera from nonimmunized mice were used as controls for mice sera.

Table 1 shows that the HIVIG/2F5/2G12 combination and sera from HIV-infected patients, used as positive controls, neutralized clade B and A viruses equally well in this assay. No significant inhibition of the infection of any of the viral isolate tested was observed with sera from mice immunized with empty MV (EdB-tag), showing that anti-MV immunity did not neutralize HIV nonspecifically. An HIV-1_{LAI} (VSV) pseudotype was used as a negative control that should not be neutralized by sera from MV-$Env_{HIV89.6}$-immunized mice. The infectivity of this VSV Env-pseudotyped HIV-1 was not inhibited by sera from MV-$Env_{HIV89.6}$-immunized mice, showing that the neutralization of HIV-1 primary isolates by these sera was due to antibodies specific for the HIV-1 envelope glycoprotein.

Serial dilutions of sera from MV-$Env_{HIV89.6}$-immunized mice were tested. The results obtained with 1:30 and 1:60 dilutions are presented in Table 1. Neutralizations with 1:120 dilutions were marginal except for the neutralization of SHIV89.6p. Antibodies induced by the four MV-$Env_{HIV89.6}$ viruses neutralized the homologous SHIV89.6p at both 1:30 and 1:60 dilutions. High neutralization activity (>90%) was observed except for sera from MV2-gp140-immunized mice. The ΔV3 mutants induced antibodies that neutralized SHIV89.6p slightly more efficiently. The gp160ΔV3 mutant elicited antibodies that neutralized 90% of infection by the SHIV89.6p at a 1:60 dilution and neutralized 50% of the infection at a 1:120 dilution. This suggests that the deletion of the V3 loop, known to contain type-specific neutralizing epitopes, had been compensated by the uncovering of other neutralizing epitopes.

The antibodies induced by the MV-$Env_{HIV89.6}$ recombinant also neutralized heterologous primary clade B isolates and a clade A virus. In each case, antibodies induced by the anchored gp160 were more neutralizing than antibodies induced by the secreted gp140. This result suggests that the gp160 protein was expressed by recombinant MV in a stable oligomeric form known to be more immunogenic. At the 1:60 dilution, none of the primary isolates could be neutralized by sera from MV2-gp140-immunized mice, whereas sera from MV2-gp160-immunized mice could still neutralize up to 50% of infectivity. The antibodies induced by the ΔV3-ELDKWAS $Env_{HIV89.6}$ neutralized heterologous viruses more efficiently than those induced by the native envelope. This was particularly striking with sera from MV2-gp160ΔV3-immunized mice at the 1:30 dilution, where neutralization reached 90% for most primary isolates tested. This neutralization was as efficient as that obtained with positive controls (human MAbs and sera).

The increased neutralizing activity of antibodies induced by the ΔV3-ELDKWAS mutants could be due either to the un-

TABLE 1. Neutralization of HIV-1 primary heterologous isolates by sera from MV-$Env_{HIV89.6}$-immunized mice[a]

Virus isolate (subtype)	% Reduction in infection in:								Positive controls	
	Mouse serum (1:30)				Mouse serum (1:60)				MAb[b] (2F5/2G12/HIV-1G)	Human HIV serum[c]
	MV	MV2 gp140	MV3 gp140 ΔV3	MV2 gp160	MV2 gp160 ΔV3	MV	MV2 gp140	MV3 gp140 ΔV3	MV2 gp160 ΔV3	4 (1:50)	33 (1:35)
SHIV 89.6	*<10*	**86**	**>90**	**>90**	**>90**	*<10*	55	**85**	**82**	**>90**	80	85
Bx08 (B)	*<10*	36	76	**80**	**>90**	*<10*	<10	55	50	73	**>90**	**>90**	**>90**
92 US 660 (B)	*<10*	20	50	75	**84**	*<10*	<10	20	35	68	**>90**	**>90**	83	80
92 US 714 (B)	*<10*	<10	33	71	**>90**	*<10*	<10	20	45	74	**>90**	**>90**	**>90**	**>90**
92 HT 593 (B)	*<10*	25	45	38	66	*<10*	<10	25	20	60	**>90**	**>90**	**>90**	68
3253 (A)	*<10*	<10	43	76	**80**	*<10*	<10	<10	20	70	**>90**	**>90**	65	45
HIV-1_{LAI} (VSV)	*<10*	<10	<10	<10	<10	*<10*	<10	<10	<10	<10	<10	<10	<10	<10

[a] Sera were evaluated for neutralizing antibodies at two dilutions. Values are the percent reduction in infection of primary HIV isolates on P4-CCR5 cells in the presence of sera from vaccinated mice. The 100% infection value was determined for each HIV isolate in the presence of sera from nonimmunized mice. Boldface numbers represent a neutralization percentage of >80%. Determinations were made in triplicate for three mice per point and the standard deviations were <10%.

[b] Mix of HIVIG (2.5 mg/ml) and MAbs 2F5 and 2G12 (25 μg/ml each).

[c] Numbers correspond to the nomenclature used by Burrer et al. (8).

covering of the chemokine receptor binding site or to the addition of a second ELDKWAS gp41 epitope. To evaluate the role of the anti-ELDKWAS antibodies in neutralizing activity, we performed an antibody-ELDKWAS peptide competition experiment. Mice sera were preincubated with increasing concentrations of the ELDKWAS peptide (10 to 100 μg/ml) before the neutralization assay was performed (with the Bx08 clade B virus). The preincubation of viruses with peptide did not alter their infectivity. The neutralizing activity of the 2F5 MAb (100 μg/ml) was completely abolished in the presence of 100 μg of ELDKWAS peptide/ml but not in the presence of an irrelevant Nef peptide. On the other hand, no reduction of neutralization was observed when sera from mice immunized with MV2-gp140, MV2-gp160, or ΔV3-ELDKWAS mutants were incubated with the ELDKWAS peptide. Thus, the native forms of $Env_{HIV89.6}$ did not induce detectable levels of 2F5-type neutralizing antibodies, and the additional ELDKWAS epitope did not increase this level. Rather, deletion of the V3 immunodominant region has probably unmasked conserved critical epitopes on the envelope glycoprotein that induced neutralizing antibodies of broader specificity. However, grafting an additional ELDKWAS peptide in place of the V3 loop may have contributed to conformational changes of the gp120 leading to the induction of broader neutralizing antibodies.

Taken together, these results show that deleting the V3 loop of $Env_{HIV89.6}$ and expressing the construct with a MV vector allowed the induction of antibodies with cross-neutralizing activity against clade A and B HIV-1 primary isolates, after a single injection in mice.

Induction of cellular immune responses against recombinant MV. The capacity of splenocytes from vaccinated mice to secrete IFN-γ was tested by ELISPOT assays and flow cytometry. Both MV-specific and HIV-specific responses were studied in splenocytes collected 7 days or 1 month after immunization. HIV gp120 native protein was used to stimulate both $CD4^+$ and $CD8^+$ T cells since it has been shown that exogenous antigens can be presented by major histocompatibility complex class I molecules (32). Representative results obtained with splenocytes from mice immunized with MV2-gp160 are shown in Fig. 5. A significant number of HIV gp120-specific cells (up to $600/10^6$ splenocytes) was detected by the ELISPOT assay 7 days or 1 month postinoculation (Fig. 5A). In a given mouse, the number of HIV gp120-specific spots corresponded to 15 to 20% of the number of MV-specific spots (not shown), indicating that MV vectors immunizes against the HIV proteins efficiently.

The phenotype of MV- and HIVgp120-specific cells collected 7 days after immunization and stimulated in vitro with exogenous HIV Env protein as described above was determined by three-color cytofluorometry (Fig. 5B). The frequency of HIV gp120-specific T cells (middle panel) in the $CD8^+$ and $CD4^+$ subsets was 1.76% (mean for three mice = 1.69%) and 0.92% (mean for three mice = 0.76%), respectively. The frequency of MV-specific T cells in $CD8^+$ and $CD4^+$ subsets (right panel) was 7.63% (mean for three mice = 7.03%) and 4.11% (mean for three mice = 3.50%), respectively. These frequencies are very close to those obtained by ELISPOT since $CD8^+$ and $CD4^+$ cells correspond to 6 to 10% and 15 to 20%, respectively, of total splenocytes of $CD46^{+/-}$ IFN-α/β$R^{-/-}$ mice (not shown). These results show that a single inoculation

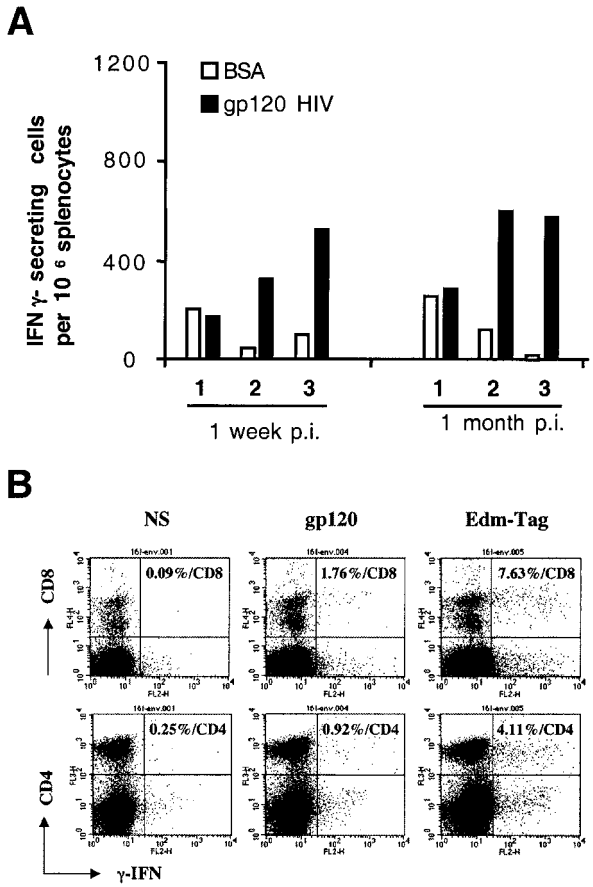

FIG. 5. Characterization of MV- and HIV-specific T cells in mice immunized by $MV2\text{-}gp160_{HIV89.6}$. (A) Enumeration of gp120-specific IFN-γ-ELISpots. Two groups of three mice were inoculated with 5×10^6 $TCID_{50}$ of MV2-gp160 virus and then euthanized 7 days or 1 month postinoculation. ELISPOT assays were performed with splenocytes from immunized mice. Stimulation with HIV-gp120 purified protein (■) or irrelevant BSA (□). (B) Fluorescence-activated cell sorting analysis of $CD8^+$ (upper panel) and $CD4^+$ (lower panel) T cells producing IFN-γ in mice splenocytes collected 7 days after immunization with 5×10^6 $TCID_{50}$ of MV2-gp160. Ex vivo splenocytes were stimulated either with medium (left panel), HIV gp120 (middle panel), or EdB-tag MV (right panel). The percentages are given according to the total $CD8^+$ and $CD4^+$ counts.

of MV2-gp160 virus induced high levels of HIVgp120- and MV-specific $CD8^+$ and $CD4^+$ lymphocytes.

Boosting the anti-HIV response in animals with preexisting anti-MV immunity. We first tested the possibility of boosting the anti-HIV response by performing a second injection of recombinant MV. Mice immunized with 5×10^6 $TCID_{50}$ of MV2-gp140 recombinant virus were boosted 1 month after the first injection with a second injection of the same recombinant virus at the same dose. The mean anti-MV and anti-HIV antibody titers at the time of boosting were 5×10^4 and 8×10^3, respectively. These titers increased 1 month after the boosting to, respectively, 5×10^5 and 5×10^4. Thus, anti-MV and HIV responses can be boosted 10 times by a second injection of the same dose of vaccine given 1 month after the first one.

We then tested the ability of recombinant MV to induce

anti-HIV antibodies in mice and monkeys in the presence of preexisting anti-MV immunity. Mice (three mice per point) were first immunized with 10^5 $TCID_{50}$ of EdB-tag MV (without an HIV insert). High levels of anti-MV antibodies were induced (Fig. 6A). The titer decreased slightly after 2 months and remained stable for the following 9 months. The mice were then inoculated with 5×10^6 $TCID_{50}$ of MV2-gp140 and boosted with the same dose 1 month later. The titer of anti-MV antibodies was increased 100 times, and high titers of anti-HIV antibodies (5×10^4) were induced. These titers were similar to those obtained after the immunization of naive animals with two injections.

The same experiment was performed with rhesus macaques (Fig. 6B). Two macaques were immunized with a standard dose (10^4 $TCID_{50}$) of MV vaccine (Rouvax). High anti-MV antibody levels were induced and remained stable for 1 year. Macaques were then inoculated with 5×10^6 $TCID_{50}$ of MV2-gp140 twice at 1-month intervals. Anti-MV titers were increased 150-fold after the first injection of MV-HIV, whereas the second injection had no or little effect. Anti-HIV antibodies were induced by the first MV2-gp140 injection despite the presence of preexisting anti-MV immunity. One month after the second MV2-gp140 injection, the anti-HIV antibody level had increased ~10-fold and had reached titers similar to those obtained in mice. This level remained stable for the following 5 months.

Taken together, these results show that the presence of preexisting MV immunity in mice and nonhuman primates did not prevent the induction of antibodies to HIV Env protein after immunization by MV-$Env_{HIV89.6}$ recombinant virus.

DISCUSSION

The main goal of the present study was to test the ability of live attenuated MV-$Env_{HIV89.6}$ recombinant viruses to induce anti-$Env_{HIV89.6}$ immune responses in transgenic mice susceptible to MV. Our results show that such recombinants are genetically stable, express the HIV Env protein at high levels, and induce high titers of antibodies to both MV and the HIV Env constructs. The anti-HIV antibodies titers were ca. 15 to 20% of those of the anti-MV antibodies. This corresponds roughly to the ratio of HIV to MV proteins expressed by the recombinant viruses. A high level of MV- and HIV-specific $CD8^+$ and $CD4^+$ cells was also induced. As much as 7% of the total $CD8^+$ T cells and 4% of the total $CD4^+$ T cells were MV specific, and 1.7% of the total $CD8^+$ T cells and 0.9% of the total $CD4^+$ T cells were HIV specific. Hence, MV-$Env_{HIV89.6}$ recombinant viruses were able to elicit humoral and cellular immune responses against both MV and HIV Env.

An important aspect of our results is that the anti-HIV antibodies induced were neutralizing for the homologous SHIV89.6p virus, as well as for several heterologous clade A and clade B HIV-1 primary isolates. Antibodies induced by MV expressing the native gp140 or gp160 more efficiently neutralized homologous compared to heterologous viruses. The V3 loop deletion increased the level of antibodies neutralizing homologous SHIV89.6p, as well as heterologous primary HIV-1 isolates. The effect was more pronounced against heterologous viruses. This observation suggests that removing the V3 region may have exposed some immunological ele-

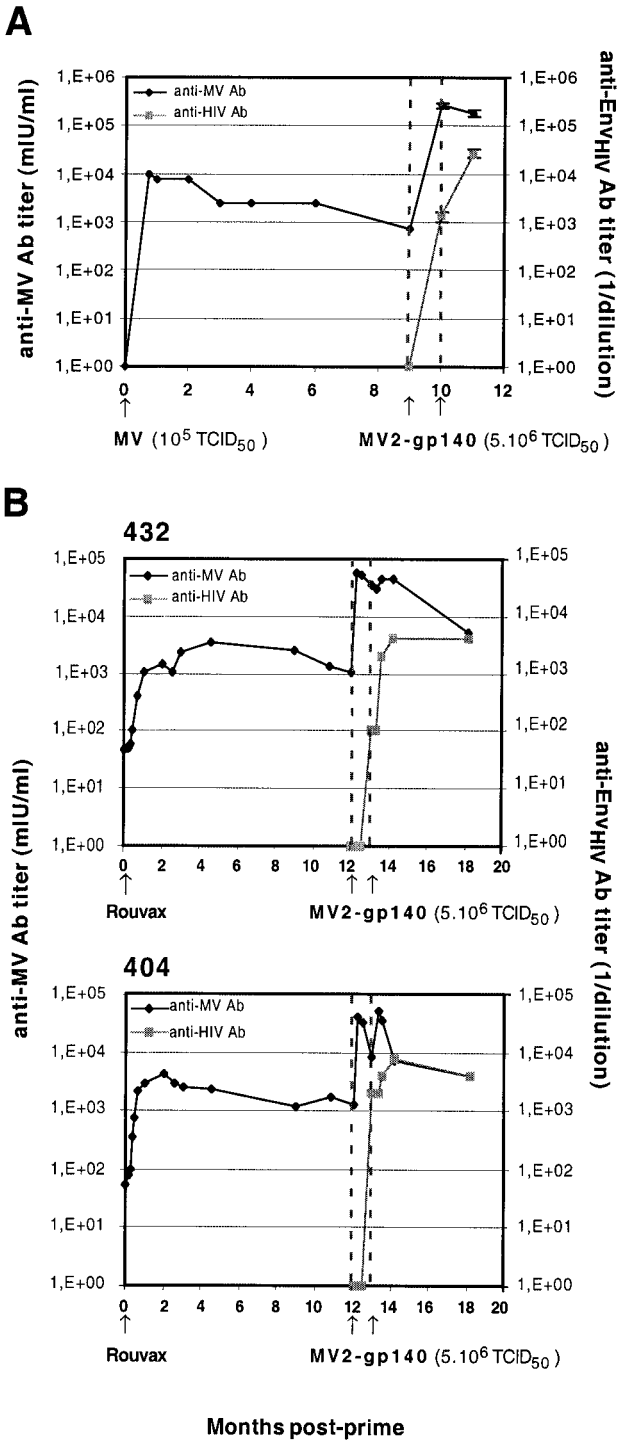

FIG. 6. Anti-MV and anti-HIV antibody titers in mice and macaques immunized with MV2-$gp140_{HIV89.6}$ virus months after MV priming. (A) Mice (three per group) were vaccinated with 10^5 $TCID_{50}$ of EdB-tag MV and then inoculated twice with 5×10^6 $TCID_{50}$ of MV2-gp140 virus as indicated (arrows). (B) Cynomolgus macaques (animals 432 and 404) were vaccinated with Rouvax and were then inoculated twice with 5×10^6 $TCID_{50}$ of MV2-gp140 virus as indicated (arrows).

ments conserved among several HIV-1 isolates. For the neutralization of SHIV89.6p, this exposure probably compensated for the elimination of V3 loop type-specific epitopes. It was previously shown that vaccines expressing the ΔV3 mutants of HIV-1_{IIIB} or HIV-$1_{HIV89.6}$ envelope glycoprotein induced broader $CD8^+$-T-cell activities than those induced by the wild-type counterparts (37). Hence, V3 loop deletion broadens both humoral and cellular immune responses.

Antibodies elicited by MV2-gp160ΔV3 neutralized heterologous isolates more efficiently than those induced by MV2-gp140ΔV3. Given that the neutralizing capacity of an antibody is linked to its ability to bind to the oligomeric envelope (69), the gp160ΔV3 expressed by MV appears to be a better antigenic mimic of the envelope glycoprotein trimeric complex present on virions than the gp140ΔV3 is. Even when expressed by a recombinant MV, gp140 does not seem able to form stable trimeric immunogenic complexes. Indeed, the immunogenicity of gp140 is increased after stabilization of oligomers either by an intramolecular disulfide bond (6) or by elimination of the gp120-gp41 cleavage site (5).

The broader neutralizing capacity of antibodies induced by MV2-gp160ΔV3-ELDKWAS was not due to the addition of a second ELDKWAS gp41 epitope, as shown by antibody-peptide competition experiments, but rather to the exposure of previously masked conserved neutralizing epitopes. Several groups have inserted the ELDKWAS epitope into various immunogenic molecules and failed to induce neutralizing antibodies (14, 21, 40). These studies suggest that the conformation of the grafted epitope is essential for the induction of neutralizing antibodies. A β-turn-like constraint was shown to be the most likely conformation of the ELDKWAS epitope recognized by the 2F5 neutralizing MAb (29). We inserted the short AAELDKWASAA sequence in place of the V3 loop, which is flanked by β-strands (38, 39), with the hope of mimicking the structure of the 2F5 epitope. Our results indicate that this was probably not achieved. Thus, as already suggested, the conformation of the 2F5 epitope must depend largely on flanking residues and the local conformation of the protein (83).

Several studies used variable loop deletions to reduce the immunodominant response and to expose conserved epitopes. The results of these studies were often contradictory. In some of them, deletion of the variable loops did not improve the induction of neutralizing antibodies (36, 41). This was the case when the immunogenicities of different V1-V2-, V3-, or V1-3-deleted envelopes (i.e., envelopes from which the V1-V2, V3, or V1-3 regions, respectively, had been deleted) were tested in rabbits injected with naked DNA (gp120, gp140, and gp160) (41) and in mice primed with gp160 recombinant vaccinia virus and boosted with soluble gp120 (36). In other cases, neutralizing antibodies with broad specificity were obtained after multiple injections of large amounts of soluble gp120 protein from which V1-3 had been deleted or after a DNA prime-protein boost regimen by using gp140 from which V2 had been deleted (5, 73). In our study, similar titers of antibodies able to neutralize heterologous isolates were obtained in mice after a single injection of MV-gp160 ΔV3-ELDKWAS. Several hypotheses may explain these discrepancies. First, epitope exposure and conformational flexibility of HIV-1 envelope are essential for the induction of cross-reactive neutralizing antibodies (49, 69). Hence, depending on the deleted region, the Env processing and its resulting structure, as well as epitope exposure, may be altered. For example, it was shown that deleting the V2 region of HIV-1_{SF162} Env did not directly increase the immunogenicity of the CD4-binding site but modified the antigenic structure of some already immunogenic regions and made additional regions immunogenic (73). Interestingly, these data suggest that the antibody composition and the relative ratio of each antibody are responsible for the differential ability of sera to neutralize HIV-1 isolates. Second, immunogenic determinants in gp120 can differ according to the virus strain (48). Actually, different HIV-1 strains were used in the different studies cited above. Third, different vaccination methods can induce different patterns of immune responses (12), especially regarding the induction of neutralizing antibodies (20). Good immunogenicity in our system is likely due to good levels of expression and maturation of HIV Env in cells infected by live recombinant MV, resulting to an efficient presentation to the immune system.

What are the advantages of MV among the existing viral vector systems developed for HIV immunization? Practical and logistics aspects have already been exposed. In addition, replicating live attenuated MV has the advantageous capacity to induce long-lasting immunity (27). Moreover, MV and HIV have several properties in common, including the fact that they both infect monocytes, macrophages, and dendritic cells (22, 28). Therefore, an MV vector that targets the HIV proteins in the same compartment as HIV itself may provide advantages, particularly in the induction of "danger signals" (46). This is considered to be one of the reasons for the protection induced by attenuated strains of pathogenic viruses. Indeed, live attenuated SIV provides strong protection against pathogenic SIV (18, 31), even if the correlates of protection remain unclear in this model (16). A live attenuated poliovirus vaccine has been engineered to express foreign genes (2, 62), and a recombinant Sabin poliovirus/SIV protected macaques from a virulent SIV challenge (17). However, poliovirus is less genetically stable than MV, and reversion to pathogenicity may occur (35). On the contrary, the Edmonston-derived live attenuated MV strains differ from the Edmonston wild-type isolate by at least 45 coding mutations (55) and from wild-type field isolates by even more mutations, making reversion almost impossible. VSV, another negative-strand RNA virus, has been made into a live attenuated vector expressing HIV proteins and is able to protect macaques from a SHIV challenge (66). However, whether VSV is safe for humans is unknown at this point, whereas live MV vaccines have a very long safety track record.

The presence of anti-MV immunity in nearly the entire adult human population would seem to restrict the use of MV recombinants to infants, an already worthy goal in any event. However, several studies showed that revaccinating previously immunized individuals results in a boost of anti-MV antibodies, suggesting that the attenuated live vaccine replicated and expressed its proteins in spite of preexisting immunity (19). Under such circumstances one might hope to be able to vaccinate adults against a foreign antigen with an MV recombinant. Indeed, our results demonstrate, in both mice and macaques, that high levels of anti-HIV neutralizing antibodies can be obtained in the presence of preexisting anti-MV immunity.

Various priming-boosting regimens, with combinations of

naked DNA and viral vectors such as modified vaccinia virus Ankara (1) or adenovirus (71) in macaques, led to a good containment of viremia and $CD4^+$ loss after a challenge with pathogenic SHIV89.6p. In the present study, we show that a single injection of MV-$Env_{HIV89.6}$ was able to induce humoral and cellular responses. It will be important to determine the ability of these recombinant MV-$Env_{HIV89.6}$ viruses, alone or in combination with recombinant MVs expressing other SHIV89.6p genes, to protect macaques against a SHIV89.6p challenge.

In conclusion, our study shows that a single injection of live attenuated MV vaccine expressing a $gp160_{HIV89.6}$ with a deletion of V3 induces antibodies in mice that neutralize several primary HIV-1 isolates, as well as high levels of specific $CD4^+$- and $CD8^+$-T-cell responses. Anti-HIV antibodies were induced even in animals with preexisting anti-MV immunity. Although the correlates of immune protection against HIV-1 infection are still not completely understood, a preventive immunization should, ideally, induce both specific T lymphocytes and antibodies that neutralize primary isolates. The results presented here indicate that this might be possible by using an already existing live MV vaccine as a vector for expressing HIV proteins.

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