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Recombinant Modified Vaccinia Virus Ankara Expressing the Surface gp120 of Simian Immunodeficiency Virus (SIV) Primes for a Rapid Neutralizing Antibody Response to SIV Infection in Macaques

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Neutralizing antibodies were assessed before and after intravenous challenge with pathogenic SIVsmE660 in rhesus macaques that had been immunized with recombinant modified vaccinia virus Ankara expressing one or more simian immunodeficiency virus gene products (MVA-SIV). Animals received either MVA-*gag-pol*, MVA-*env*, MVA-*gag-pol-env*, or nonrecombinant MVA. Although no animals were completely protected from infection with SIV, animals immunized with recombinant MVA-SIV vaccines had lower virus loads and prolonged survival relative to control animals that received nonrecombinant MVA (I. Ourmanov et al., J. Virol. 74:2740–2751, 2000). Titers of neutralizing antibodies measured with the vaccine strain SIVsmH-4 were low in the MVA-*env* and MVA-*gag-pol-env* groups of animals and were undetectable in the MVA-*gag-pol* and nonrecombinant MVA groups of animals on the day of challenge (4 weeks after final immunization). Titers of SIVsmH-4-neutralizing antibodies remained unchanged 1 week later but increased approximately 100-fold 2 weeks postchallenge in the MVA-*env* and MVA-*gag-pol-env* groups while the titers remained low or undetectable in the MVA-*gag-pol* and nonrecombinant MVA groups. This anamnestic neutralizing antibody response was also detected with T-cell-line-adapted stocks of SIVmac251 and SIV/DeltaB670 but not with SIVmac239, as this latter virus resisted neutralization. Most animals in each group had high titers of SIVsmH-4-neutralizing antibodies 8 weeks postchallenge. Titers of neutralizing antibodies were low or undetectable until about 12 weeks of infection in all groups of animals and showed little or no evidence of an anamnestic response when measured with SIVsmE660. The results indicate that recombinant MVA is a promising vector to use to prime for an anamnestic neutralizing antibody response following infection with primate lentiviruses that cause AIDS. However, the Env component of the present vaccine needs improvement in order to target a broad spectrum of viral variants, including those that resemble primary isolates.

Efforts to develop an AIDS vaccine have included the use of recombinant poxvirus vectors that are engineered to express one or more gene products of human immunodeficiency virus type 1 (HIV-1) (12, 15, 27). Vectors such as these have the potential to generate virus-specific $CD8^+$ cytotoxic T lymphocytes (CTL) and neutralizing antibodies (7) as two immune responses considered important for HIV-1 vaccine efficacy (14). Studies in macaques have shown that recombinant vaccinia virus vectors containing the Env glycoproteins of simian immunodeficiency virus (SIV) prime B cells to produce low levels of SIV-specific neutralizing antibodies and that subsequent boosting with subunit protein can dramatically elevate the levels of those antibodies (20, 21). A similar priming and boosting effect for neutralizing antibody production has been observed in phase I clinical trials of candidate HIV-1 vaccines consisting of recombinant vaccinia or canarypox virus vectors followed by Env glycoprotein inoculation (1, 5, 6, 41). These results suggest that recombinant poxviruses might prime for a similar secondary (anamnestic) neutralizing antibody response following virus infection. Hu et al. showed that a recombinant vaccinia virus vector containing HIV-1 gp160 (strain LAV) primed for anamnestic neutralizing antibody production in chimpanzees following challenge with homologous virus (22). Although it is currently unknown whether an accelerated neutralizing antibody response would provide a clinical benefit in HIV-1-infected individuals, the fact that many months are needed for neutralizing antibodies to rise to detectable levels following initial infection (24, 34, 40, 42) leaves open the possibility that it will.

We sought to determine whether prior inoculation with a recombinant attenuated poxvirus known as modified vaccinia virus Ankara (MVA) and containing the Env glycoproteins of SIV would prime B cells for an anamnestic neutralizing antibody response in rhesus macaques (*Macaca mulatta*) after intravenous challenge with pathogenic SIVsmE660 in cases where complete protection was not achieved. Four groups of six macaques received four inoculations intramuscularly with 10^8 PFU of either MVA, MVA-*gag-pol*, MVA-*env*, or MVA-*gag-pol-env*. Each of these poxvirus vectors has been described previously (17, 39, 45) and contained SIV components derived from nonpathogenic, molecularly cloned SIVsmH-4 (18). The animals were challenged intravenously with the related uncloned, highly pathogenic SIVsmE660 (19, 23) 4 weeks after final boosting. Although all animals became infected, those that received MVA-*gag-pol*, MVA-*env*, and MVA-*gag-pol-env* had lower plasma viral RNA ($P = 0.0016$) and prolonged survival relative to animals that received nonrecombinant MVA (39). There were no significant differences in the levels of plasma viremia between the three groups of animals receiving

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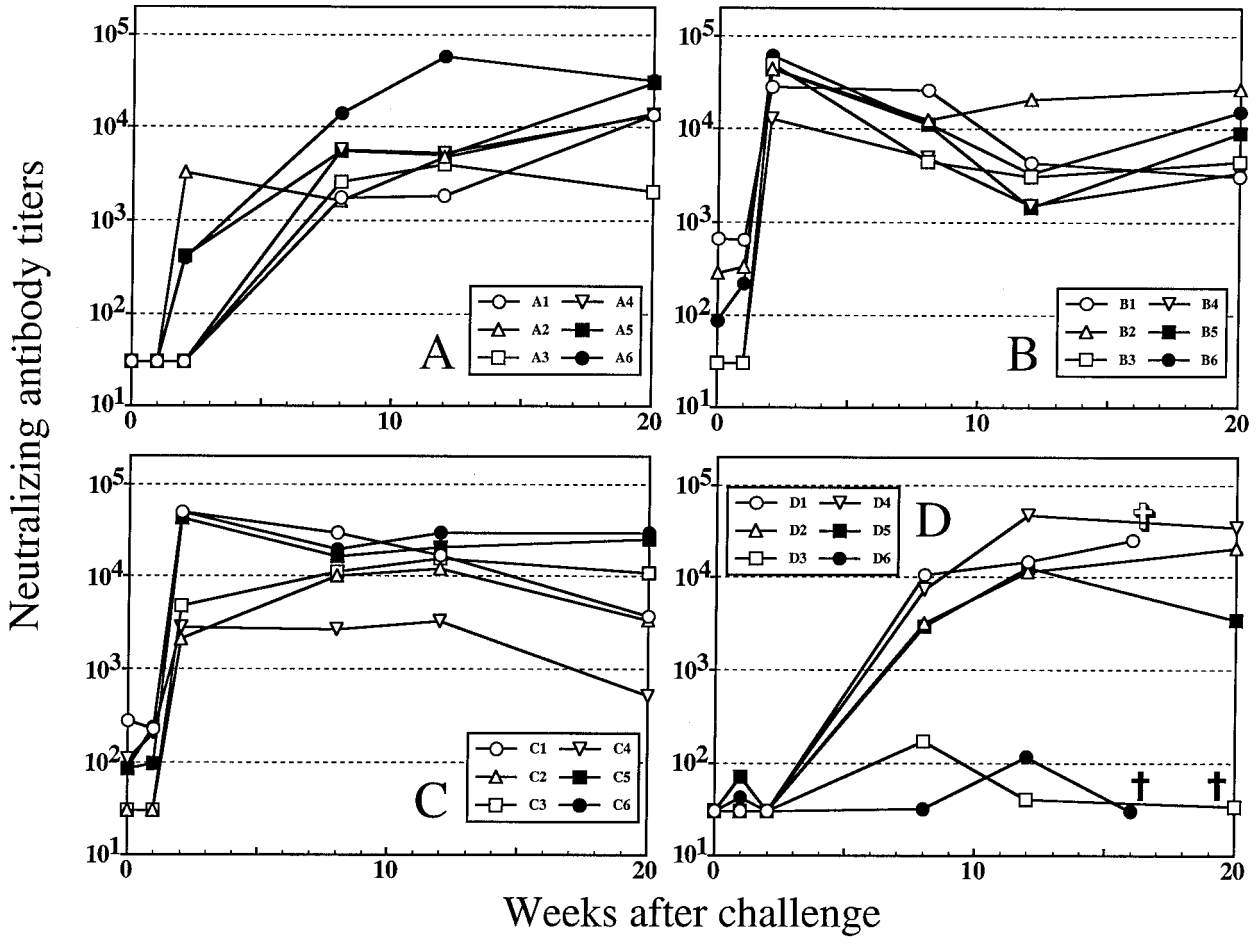

FIG. 1. Time course of SIVsmH-4-neutralizing antibody production following intravenous inoculation of vaccinated macaques with SIVsmE660. SIVsmH-4-neutralizing antibodies were measured in plasma samples obtained on the day of challenge and at multiple time points for 12 weeks postchallenge. Titers of neutralizing antibodies are the reciprocal plasma dilution at which 50% of CEMx174 cells were protected from virus-induced cell killing. Undetectable neutralization was given a value of 30, which was the lowest reciprocal plasma dilution tested. Panel A, MVA-*gag-pol*; panel B, MVA-*env*; panel C, MVA-*gag-pol-env*; panel D, MVA. †, animals sacrificed because of clinical manifestations of AIDS; ✞, animal that died of causes unrelated to AIDS.

recombinant MVAs. Plasma samples were obtained prior to vaccination, on the day of challenge, and at multiple times for up to 28 weeks postchallenge. Neutralizing activity against SIV was assessed in a CEMx174-cell-killing assay as described previously (32). Unless indicated otherwise, virus stocks were produced in either H9 cells (SIVsmH-4, SIVmac251, and SIV/DeltaB670), CEMx174 cells (SIVsmE660), or rhesus peripheral blood mononuclear cells (PBMC) (SIVmac239). An exception was one set of neutralization assays that was performed with the original animal challenge stock of SIVsmE660 grown in rhesus PBMC.

Neutralizing antibodies were first assessed with the vaccine strain of the virus, SIVsmH-4. The results are shown in Fig. 1. No SIVsmH-4-neutralizing antibodies were detected on the day of challenge in animals that received nonrecombinant MVA or MVA-*gag-pol*, which is consistent with the absence of *env* in these vaccines. Low titers of SIVsmH-4-neutralizing antibodies were detected on the day of challenge in three recipients of MVA-*env* (titers of 86 to 663) and four recipients of MVA-*gag-pol-env* (titers of 85 to 274). The titers remained essentially unchanged 1 week later for all animals. Titers of SIVsmH-4-neutralizing antibodies increased dramatically 2 weeks postchallenge in the MVA-*env* (average titer, 39,848) and MVA-*gag-pol-env* (average titer, 25,160) and remained low or undetectable in the MVA-*gag-pol* and nonrecombinant MVA groups at this time. These results suggest that MVA-*env* and MVA-*gag-pol-env* primed B cells sufficiently to permit a rapid and dramatic anamnestic neutralizing antibody response between 1 and 2 weeks postchallenge. A similar anamnestic antibody response was detected by SIVsmH-4 gp130 enzyme-linked immunosorbent assay (39). Nearly all animals had high titers of SIVsmH-4-neutralizing antibodies 8 weeks postchallenge (Fig. 1). Exceptions at 8 weeks were two animals in the nonrecombinant MVA group, whose neutralization titers were extremely low (animals D3 and D6). These two animals progressed to AIDS very rapidly (39). Early onset of virus-induced immune suppression in the two rapid progressors could account for their poor antibody-neutralizing response.

Neutralizing antibodies were next assessed with SIVsmE660. This virus is an uncloned quasispecies of the same parental strain from which SIVsmH-4 was derived (18) and, therefore, is closely related to SIVsmH-4 genetically. As shown in Fig. 2, all animals were negative for SIVsmE660-neutralizing antibodies on the day of challenge. Also, no neutralization of this virus was detected 2 weeks postchallenge, and little neutralization was detected 8 weeks postchallenge despite the fact that most animals had very high SIVsmH-4 neutralization titers at one or both of these time points. It is important to note that preferential neutralization of SIVsmH-4 by week-8 plasma samples

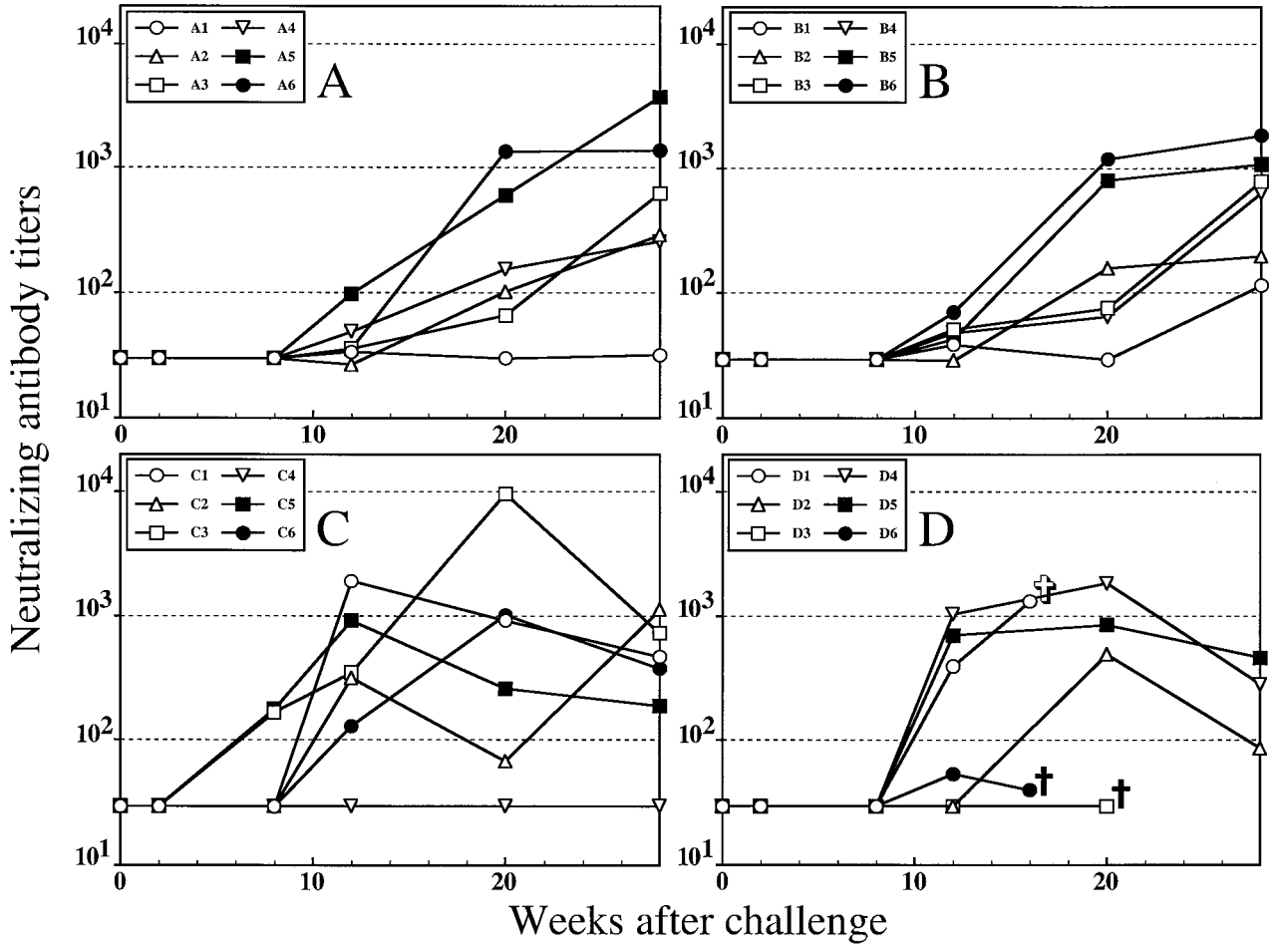

FIG. 2. Time course of challenge-strain-neutralizing antibody production following intravenous inoculation of vaccinated macaques with SIVsmE660. SIVsmE660-neutralizing antibodies were measured in plasma samples obtained on the day of challenge and at multiple time points for 28 weeks postchallenge. Titers of neutralizing antibodies are the reciprocal plasma dilution at which 50% of CEMx174 cells were protected from virus-induced cell killing. Undetectable neutralization was given a value of 30, which was the lowest reciprocal plasma dilution tested. Panel A, MVA-*gag-pol*; panel B, MVA-*env*; panel C, MVA-*gag-pol-env*; panel D, MVA. †, animals sacrificed because of clinical manifestations of AIDS; ✞, animal that died of causes unrelated to AIDS.

was seen for all groups of animals, including those that received recombinant MVAs lacking Env (i.e., MVA-*gag-pol* and nonrecombinant MVA). This outcome indicates that the inability to neutralize SIVsmE660 was not related to vaccine-induced immune interference associated with SIVsmH-4 Env priming (38). The outcome is more likely explained by epitopes shared by both viruses that, although they are highly immunogenic in infected macaques, are not adequately exposed for antibody binding on the native SIVsmE660 Env complex relative to their exposure on the native SIVsmH-4 Env complex. On the basis of immunophenotype and implied differences in native envelope glycoprotein structure, SIVsmH-4 resembles a T-cell-line-adapted (TCLA) strain, whereas SIVsmE660 resembles primary isolates of HIV-1 (3, 35, 49).

Neutralization of SIVsmE660 was first detected 12 weeks postchallenge with plasmas from a subset of animals in each group, where the titers either peaked at this time or continued to rise for at least 20 to 28 weeks. Peak titers of SIVsmE660-neutralizing antibodies never reached the levels observed with SIVsmH-4 and, in fact, were always >10-fold lower in magnitude, again indicating that SIVsmE660 is much less sensitive to antibody-mediated neutralization than SIVsmH-4. It should be noted that the rapid progressors in the MVA group that developed low levels of neutralizing antibody to SIVsmH-4 also produced no antibodies that neutralized SIVsmE660.

The difficulty by which SIVsmE660 was neutralized relative to SIVsmH-4 suggests that detection of an anamnestic neutralizing antibody response targeting SIVsmE660 would be delayed relative to the response measured with SIVsmH-4. The MVA-*gag-pol-env* group of animals was the only case where an anamnestic neutralizing antibody response might have been detected with SIVsmE660. For example, two of six animals in this group had low titers of SIVsmE660-neutralizing antibodies 8 weeks postchallenge. By comparison, all animals in the remaining groups were negative (<30) at this time. In addition, five of six animals in the MVA-*gag-pol-env* group had titers of SIVsmE660-neutralizing antibodies that surpassed those in the MVA-*gag-pol* and MVA-*env* groups of animals 12 weeks postchallenge. However, because the magnitude of neutralization in the MVA-*gag-pol-env* animals was not much different from the nonrecombinant MVA group, any anamnestic response targeting SIVsmE660 was probably weak.

We next examined whether an anamnestic neutralizing antibody response could be detected with other strains of SIV. For this, plasma samples from three animals in each group were assessed for their ability to neutralize highly neutraliza-

TABLE 1. Magnitude and cross-reactivity of neutralizing antibodies detected 2 weeks post-virus challenge

Vaccine group and animal no.[a]	Nab titer to SIV of[b]:		
	smH-4	DeltaB670	mac251
MVA-SIV-*gag-pol*			
A4	<30	<20	<20
A5	420	31	<20
A6	396	30	<20
MVA-SIV-*env*			
B1	28,478	2,116	45
B5	43,680	5,947	831
B6	60,309	3,483	37
MVA-SIV-*gag-pol-env*			
C1	50,072	6,034	969
C5	42,297	3,700	958
C6	48,938	2,561	48
MVA			
D1	<20	<20	<20
D2	<20	<20	<20
D4	<20	<20	44

[a] Plasma samples from three animals in each of four immunization groups were obtained 2 weeks post-intravenous challenge with SIVsmE660.

[b] Plasma samples were assessed for antibodies neutralizing SIVsmH-4, SIV/DeltaB670, and SIVmac251 in CEMx174 cells as described in the text. Titers of neutralizing antibodies are the reciprocal plasma dilution at which 50% of cells were protected from virus-induced killing.

TABLE 2. Magnitude and cross-reactivity of neutralizing antibodies detected 8 weeks post-virus challenge

Vaccine group and animal no.[a]	Nab titer to SIV of[b]:				
	smH-4	E660	DeltaB670	mac251	mac239
MVA-SIV-*gag-pol*					
A4	5,788	<30	2,011	476	<20
A5	5,539	<30	1,978	343	<20
A6	14,168	<30	3,713	503	<20
MVA-SIV-*env*					
B1	26,347	<30	1,968	162	<20
B5	11,279	<30	1,599	756	<20
B6	11,546	<30	506	<20	<20
MVA-SIV-*gag-pol-env*					
C1	29,281	<30	4,127	858	<20
C5	16,310	180	2,950	1,015	<20
C6	19,422	<30	2,494	<20	<20
MVA					
D1	10,633	<30	4,689	661	<20
D2	3,206	<30	1,118	345	<20
D4	7,405	<30	2,465	337	<20

[a] Plasma samples from three animals in each of four immunization groups were obtained 8 weeks post-intravenous challenge with SIVsmE660.

[b] Plasma samples were assessed for antibodies neutralizing SIVsmH-4, SIVsmE660, SIV/DeltaB670, SIVmac251, and molecularly cloned SIVmac239 in CEMx174 cells as described in the text. Titers of neutralizing antibodies are the reciprocal plasma dilution at which 50% of cells were protected from virus-induced killing.

tion-sensitive, TCLA stocks of SIV/DeltaB670 (50) and SIVmac251 (25, 30), and a neutralization-insensitive stock of molecularly cloned SIVmac239/nef-open (30, 32). Because the anamnestic responses detected with SIVsmH-4 were fairly uniform, we selected plasma samples randomly from a subset of animals in each group for these assessments. The results shown in Tables 1 and 2 include titers measured with SIVsmH-4 and SIVsmE660 derived from Fig. 1 and 2 for comparison. Table 1 shows that an anamnestic neutralizing antibody response was detectable with SIV/DeltaB670 and, to a lesser extent, with SIVmac251 2 weeks postchallenge in the MVA-*env* and MVA-*gag-pol-env* groups of animals. Table 2 shows that the anamnestic response was no longer evident 8 weeks postchallenge, which was similar to the results obtained with SIVsmH-4. Table 2 also shows that all 8-week plasma samples failed to neutralize SIVmac239. We conclude that the neutralizing activity of the antibodies produced during the anamnestic phase, and shortly thereafter, were highly specific for SIVsmH-4 and heterologous TCLA strains of SIV.

The fact that passively administered neutralizing antibodies have proven effective against AIDS viruses in macaques (4, 8, 13, 28, 33, 46) and hu-PBL-SCID mice (9) suggests that neutralizing antibody induction would benefit an HIV-1 vaccine. How much of a benefit the antibodies provide may depend on their ability to neutralize diverse genetic variants of the virus, including primary isolates (9, 33, 46). Antibodies produced during the anamnestic phase in our vaccinated animals did not neutralize SIVsmE660, making it uncertain that the antibodies contributed to the partial efficacy observed (39). The fact that equal levels of efficacy were achieved regardless of whether *env* was present in the recombinant MVA vaccine (39) is further evidence that neutralizing antibodies probably contributed little. Nonetheless, it was possible that our assay stock of SIVsmE660 produced in CEMx174 cells was less sensitive to neutralization than the animal challenge stock produced in rhesus PBMC and, therefore, underestimated neutralization potency. To address this possibility, we performed neutralization assays with the animal challenge stock of SIVsmE660 without further passage. Because this stock was limited in supply and is valuable for animal challenges, our assessments were made on a subset of plasma samples. We selected five samples obtained during the anamnestic phase (2 weeks postchallenge) that contained high titers of SIVsmH-4-neutralizing antibodies (animals B1, B5, B6, C1, and C3), and three samples obtained 28 weeks postchallenge that neutralized our assay stock of SIVsmE660. As can be seen in Table 3, titers of neutralizing antibodies obtained with the 28-week-postchallenge samples were similar for both stocks of virus in two of

TABLE 3. Comparative neutralization of the assay and animal challenge stocks of SIVsmE660

Animal no.[a]	Nab titer measured with SIVsmE660[b]:		
	Cell viability		Reduction in p27
	Assay stock	Challenge stock	Challenge stock
B5	1,082	1,340	2,118
B6	1,841	7,502	7,405
C1	470	384	181

[a] Plasma samples were obtained 28 weeks post-intravenous inoculation with SIVsmE660.

[b] Neutralization was measured with the assay stock of SIVsmE660 grown in CEMx174 cells and the animal challenge stock of the same virus grown in macaque PBMC. Assays were performed with CEMx174 cells as described in the text with one modification: cells exposed to the animal challenge stock of virus were washed three times with growth medium 1 day after the addition of cells in order to remove the virus inoculum and residual anti-p27 antibodies. Culture supernatants were harvested for p27 quantification at the time cells were stained for viability. Neutralization titers are the reciprocal plasma dilution where either 50% of cells were protected from virus-induced killing or p27 synthesis was reduced 90% relative to virus control wells (no plasma sample). The average amount of p27 present in the virus control wells was 95 ng/ml.

three cases. Also, we showed that 50% protection from virus-induced cell killing corresponds very closely to a 90% reduction in p27 Gag antigen synthesis in these assays (Table 3). Although the titer was approximately 4 times higher with the animal challenge stock in one case (animal B6), we do not consider this to be a major difference with respect to occasional assay-to-assay variation. No neutralization of the challenge stock of SIVsmE660 was detected with plasmas obtained during the anamnestic phase, which agreed with results obtained with our assay stock of the virus.

We conclude that SIVsmH-4 Env did not prime for a secondary neutralizing antibody response to SIVsmE660 in these studies. However, there are a number of cases where antibodies lacking detectable neutralizing activity in vitro were nonetheless capable of preventing infection by other viruses (10, 26, 29, 36, 37, 43, 44), and at least one example exists in the SIV-macaque model (48). With this in mind, we do not wish to exclude the possibility that nonneutralizing antibodies, particularly those involved in antibody-dependent cytotoxicity (47) and immune complex clearance (31), played a role in our studies.

The ability to prime for a more-broadly cross-reactive neutralizing antibody response with this vaccine candidate will most likely depend on the nature of the immunogen(s) incorporated into the vector. One example would be to use multiple recombinant MVA vectors, each containing the Env glycoproteins from a different strain of virus as a single vaccination modality. An alternate strategy would be to incorporate the Env glycoproteins from a single strain of virus after modifying them to present conserved neutralization epitopes in a highly immunogenic configuration. An important first step will be to determine whether greater efficacy can be achieved in the present model by priming for a neutralizing antibody response that is capable of targeting the challenge virus. This can be tested by incorporating the Env glycoproteins from a strain of virus that is closely matched to the challenge virus in terms of antigenicity and quasispecies complexity. We are in the process of pursuing this goal by using the highly pathogenic, molecularly cloned SIVsmE543-3 as both the vaccine and challenge strain. This virus exhibits a neutralization-resistant phenotype reminiscent of primary HIV-1 isolates (16).

With the majority of HIV-1 transmissions occurring in developing countries that have limited financial resources, the high cost of Env glycoprotein production, especially in the case of a polyvalent vaccine, will be a major economic challenge for global immunization (2). Recombinant vectors offer a cost-effective and feasible alternative. Although inoculation with recombinant poxviruses without Env glycoprotein boosting has not induced high levels of neutralizing antibodies, efficient B-cell priming by these vectors should facilitate an anamnestic neutralizing antibody response to infection. An appropriate anamnestic B-cell response might exert sufficient pressure on the virus during early stages of infection as the CTL response matures. One of the vectors used here (MVA-*gag-pol*) was previously shown to elicit potent SIV-specific CTL in macaques (45). Together, these immune responses might be capable of controlling virus replication to an extent that would limit immune suppression and virus transmission better than either response alone.

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