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Patterns of Viral Replication Correlate with Outcome in Simian Immunodeficiency Virus (SIV)-Infected Macaques: Effect of Prior Immunization with a Trivalent SIV Vaccine in Modified Vaccinia Virus Ankara

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The dynamics of plasma viremia were explored in a group of 12 simian immunodeficiency virus (SIV)-infected rhesus macaques (*Macaca mulatta*) that had received prior immunization with either nonrecombinant or trivalent (*gag-pol*, *env*) SIV-recombinant vaccinia viruses. Three distinct patterns of viral replication observed during and following primary viremia accounted for significant differences in survival times. High-level primary plasma viremia with subsequently increasing viremia was associated with rapid progression to AIDS ($n = 2$). A high-level primary plasma virus load with a transient decline and subsequent progressive increase in viremia in the post-acute phase of infection was associated with progression to AIDS within a year ($n = 6$). Low levels of primary plasma viremia followed by sustained restriction of virus replication were associated with maintenance of normal lymphocyte subsets and intact lymphoid architecture ($n = 4$), reminiscent of the profile observed in human immunodeficiency virus type 1-infected long-term nonprogressors. Three of four macaques that showed this pattern had been immunized with an SIV recombinant derived from the attenuated vaccinia virus, modified vaccinia virus Ankara. These data link the dynamics and extent of virus replication to disease course and suggest that sustained suppression of virus promotes long-term, asymptomatic survival of SIV-infected macaques. These findings also suggest that vaccine modulation of host immunity may have profound beneficial effects on the subsequent disease course, even if sterilizing immunity is not achieved.

A variety of studies have reinforced the theme that the pathogenesis and clinical course of human immunodeficiency virus type 1 (HIV-1) infection are linked to patterns of viral replication. First, highly sensitive nucleic acid amplification and quantification methods have demonstrated continuous viral replication throughout all clinical stages of HIV disease, with generally increasing levels during disease progression (14, 41, 44). Recently, the use of such methods, in conjunction with potent antiretroviral drug therapy, revealed extensive and rapid turnover of virus in plasma in patients with progressive HIV-1 infection (25, 53). Rapid emergence of drug-resistant virus in these patients also underscores the dynamic nature of HIV-1 replication in vivo (53). At the other end of the spectrum, a rare subset of HIV-infected individuals, designated long-term nonprogressors or survivors (6, 30, 41), also support a central role for active viral replication in determining AIDS pathogenesis. A common feature of these healthy, long-term-infected individuals is a relatively low level of apparent viral replication compared with that of individuals with actively progressing immunodeficiency. The pathogenesis and clinical outcome of HIV infection thus appear linked to the degree of viral replication in vivo. However, such studies of patients are limited by many factors, including frequent inaccessibility of early samples and the wide diversity of the viruses infecting these patients.

Simian immunodeficiency virus (SIV) infection of macaques is a useful animal model for studying the pathogenesis of human AIDS. SIV has a genetic organization and biology similar to those of HIV-1 and causes an immunodeficiency syndrome in macaques that recapitulates many of the features of HIV-1 infection in humans (2, 3, 7, 23, 24, 36, 46). The time course of SIV infection is compressed relative to that of HIV infection, and a defined inoculum of known pathogenic potential can be used, minimizing the role of viral factors in determining differences in viral dynamics. There is a growing accumulation of data suggesting that the pathogenesis of SIV infection of macaques is similar to that of human AIDS (2, 7, 23, 36, 46). Many previous vaccine trials have utilized the SIV/macaque model (11, 13, 17, 18, 21, 23, 26–28, 32, 34), and some of these demonstrated reductions in the extent of viral replication during the primary phase of infection (17, 27). However, since most of these studies were terminated prior to achievement of a disease endpoint or used minimally pathogenic virus challenges (27), the consequences of early modulation of the dy-

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namics of viral replication with respect to disease outcome remain unclear.

In a previous trial of psoralen-inactivated SIV as a sole immunogen prior to a cell-associated intravenous challenge, a prolongation of clinical latency was observed (23), and one of the five vaccinees has remained clinically healthy for over 4 years, with normal peripheral blood lymphocyte subsets (21a). Since this latter regimen generated primarily a humoral response, a strategy that included the use of live virus vectors, such as vaccinia virus, which additionally induce cell-mediated immunity, might result in more consistent and solid protection. Currently, the most successful vaccine strategy has involved the use of attenuated live SIV, either naturally occurring attenuated virus clones such as SIVmac1A11 (34) or HIV-2 (45) or engineered viruses such as SIVmac239 with a deletion of *nef* (13). Protection has varied from complete, apparently sterilizing immunity (13) to modification of subsequent disease course (34, 45). One of the few successful SIV subunit vaccine trials to date involved the use of vaccinia virus priming followed by a recombinant envelope glycoprotein boost (26, 27), a strategy designed to promote both humoral and cell-mediated immune responses. However, potential virulence of conventional vaccinia viruses makes use of these viral vectors in immunosuppressed individuals undesirable. A strategy employing either an attenuated vaccinia virus, such as NYVAC (50), modified vaccinia virus Ankara (MVA) (35, 37, 48, 49), or avian poxvirus (10) as a priming agent might provide good immunogenicity with improved safety.

In the present study, the immunogenicity and protective efficacy of the attenuated vaccinia virus MVA were compared with those of a conventional vaccinia virus, the New York Board of Health strain (Wyeth). Both vaccinia viruses expressed SIVsm *gag-pol* from the P7.5 early-late promoter and *env* from a synthetic early-late promoter; the optimal route of administration for each virus was used, intramuscular for MVA and intradermal for Wyeth. Although all macaques became infected following challenge, three different patterns of virus replication were observed. The consequences of these different patterns of viral replication were subsequently explored.

MATERIALS AND METHODS

Vaccinia virus recombinants. An expression cassette containing the *gag-pol* genes of SIVsmH4 under the control of the vaccinia virus early-late promoter (P7.5) and *env* of SIVsmH4 under control of the strong synthetic promoter (7a) was constructed and referred to as pSC59-g/p/e. In this plasmid, the expression cassette was flanked by the sequences for homologous recombination in the New York Board of Health (Wyeth) strain of vaccinia virus (33). The Ps-env/P7.5 *gag-pol* expression cassette was excised from pSC59-g/p/e by restriction digestion with *Not*I and *Sma*I and ligated into pIIIgptex.dsP (48) to construct the MVA transfer plasmid, pIIIgptex.SIV17A. The *gag-pol* and *env* coding regions of SIVsmH4 were flanked by MVA sequences to direct homologous recombination into the site of an existing deletion in the MVA genome (48). MVA was originally obtained from A. Mayr, Veterinary Faculty, University of Munich, Munich, Germany, and virus stocks were routinely propagated and their titers were determined by endpoint dilution in chicken embryo fibroblasts (CEF) to determine the 50% tissue culture infectious dose ($TCID_{50}$). To generate recombinant MVA virus, monolayers of nearly confluent CEF in six-well plates were infected with 0.05 $TCID_{50}$ of MVA per cell and at 90 min after infection were transfected with 10 μg of plasmid DNA by using Lipofectin reagent (Gibco BRL, Gaithersburg, Md.) as recommended by the manufacturer. At 48 h after infection, the cells were harvested and processed as described elsewhere (48). Recombinant MVA expressing the SIVsmH4 *env* and *gag-pol* genes (MVA-SIV) was detected by immunostaining of virus plaques using plasma from an SIV-infected rhesus macaque followed by incubation with peroxidase-conjugated protein A (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and *o*-dianisidine (Sigma, St. Louis, Mo.). After five consecutive rounds of plaque purification in CEF, virus stocks were prepared in CEF. Wyeth recombinant virus (Wyeth-SIV) was isolated as described previously (33).

To assess in vitro expression, BS-C-1 cell monolayers in six-well tissue culture plates were infected with 10 infectious units of recombinant MVA or Wyeth vaccinia virus per cell. After 24 h, infected cells were harvested, collected by centrifugation, and lysed in 0.5 ml of 0.5% Nonidet P-40 (Sigma)–0.1 M NaCl–0.1 M Tris-HCl (pH 8.0) on ice for 10 min. Total cell proteins were treated with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol, and lysate from the equivalent of 5×10^4 cells was electrophoresed on an SDS–10% polyacrylamide gel and electroblotted onto nitrocellulose for 2 h at 4°C in a buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol (pH 8.6). The blots were blocked in phosphate-buffered saline (PBS) containing 1% (wt/vol) nonfat dried milk and 0.1% Nonidet P-40 in PBS. The blots were incubated with ^{125}I-labeled protein A, washed in 0.1% Nonidet P-40–PBS, dried, and subjected to autoradiography.

Cells and viruses. Human HeLa cells, monkey BS-C-1 cells, and CEF were grown in minimal essential medium supplemented with 10% fetal calf serum. CEMx174 cells used for SIV rescue were grown in RPMI 1640 supplemented with glutamine and 10% fetal calf serum. Peripheral blood mononuclear cells (PBMC) were separated by centrifugation through lymphocyte separation medium (Organon Teknika, Inc., Durham, N.C.) and maintained for 4 days in RPMI 1640 supplemented with 10% interleukin-2 and 5 μg of phytohemagglutinin (PHA) per ml and subsequently in a similar medium lacking PHA.

The challenge virus used was a cell-free virus stock of uncloned SIV smE660 (18) which had been passaged in pigtailed macaque PBMC and titrated for infectivity by intravenous inoculation of 10-fold serial dilutions into rhesus macaques to determine monkey 50% infective doses (MID_{50}). This virus was highly pathogenic and highly related but not identical to the strain (SIVsmH4) used to construct the recombinant vaccinia viruses.

Animals and immunization schedule. Twelve juvenile simian retrovirus-seronegative rhesus macaques (*Macaca mulatta*) were immunized intramuscularly with 5×10^8 $TCID_{50}$ of MVA-SIV recombinant ($n = 4$) or nonrecombinant MVA ($n = 2$) or intradermally with 10^8 PFU of a vaccinia recombinant of the New York Department of Health (Wyeth-SIV) strain ($n = 4$) or nonrecombinant Wyeth ($n = 2$) at 0, 12, 20, and 28 weeks. Finally, animals primed with recombinant vaccinia viruses were given booster injections at 44 weeks of 250 μg of total protein of psoralen-inactivated SIV generated by propagation in CEMx174 cells (18, 28) which was administered without adjuvant. The animals were then challenged intravenously 4 weeks later with 50s MID_{50} of a cell-free, uncloned, related SIVsmE660 virus stock (24) generated in macaque PBMC (18) to avoid interference from antibodies to human cellular proteins present in the inactivated virus used for booster injections (1, 11). Blood samples (heparinized for PBMC) and plasma samples (obtained by use of acid citrate dextrose) were collected on the day of challenge, subsequently at 1, 2, 4, and 8 weeks, and monthly thereafter.

Quantitative PCR of SIV RNA and DNA. A system for HIV-1 DNA quantitation based upon an internally quality-controlled PCR assay (QC-PCR) was adapted for use in the SIV model (44). The internal-control template (pSGΔ83) and a corresponding wild-type template (pSG) were derived from the full-length pSIVmac/239 molecular clone. A 918-bp region of the *gag* coding region (positions 1193 to 2110) was generated by PCR amplification and cloned into the *Kpn*I to *Bam*HI sites of pBSII-SK(+) (Stratagene, La Jolla, Calif.). An 83-bp deletion was introduced to generate the internal control template. Primers S-GAG03 and S-GAG04 for the QC-PCR assay amplify 261- and 178-bp fragments from the wild-type and internal control templates, respectively. The primer sequences are as follows: S-GAG03, 5′ CAGGGAAiiAGCAGA TGAATTAG 3′ (1359); and S-GAG04, 5′ GTTTCACTTTCTCTTCTGCGTG 3′ (1873) ("i" represents inosine). For quantitation of viral DNA in PBMC or lymph nodes, DNA was extracted from viably frozen PBMC by using a commercially available kit (Schleicher & Schuell, Keene, N.H.) and glass bead adherence, resuspended in water, and denatured prior to analysis. Replicate aliquots of DNA were coamplified with different known copy numbers of the internal-control template with primers S-GAG03 and S-GAG04 through 45 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min. Products were resolved by electrophoresis and quantified by computerized video image analysis of ethidium bromide-stained gels as described previously (44). Copy numbers were calculated by interpolation on a plot of the log of the ratio of products for the internal-control template over the wild-type template versus the log of the copy number of the internal control added per reaction mixture and expressed as copies per microgram of DNA (determined by A_{260}), essentially as described previously for HIV-1 (44). Interassay variation was <25% (coefficient of variation).

A reverse transcription-PCR version of the QC-PCR procedure used for quantitating SIV DNA was employed for quantitation of viral RNA in plasma, using an in vitro runoff transcript from pSGΔ83 for the internal control template. Plasma samples for analysis were collected by using acid citrate dextrose as an anticoagulant and stored in a −70°C freezer until analysis. Virions were pelleted by ultracentrifugation and lysed with SDS-proteinase K and then subjected to serial organic extractions and precipitation using glycogen as a carrier. Replicate aliquots of the test RNA were subjected to reverse transcription with various known copy numbers of the in vitro transcript using random primers at 42°C for 30 min. The resulting cDNA was then amplified (45 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min) and the products were quantitated as for DNA QC-PCR analysis. Results were normalized to the volume of plasma extracted and expressed as SIV RNA copies per milliliter of plasma, as described for HIV-1 (43, 44). Interassay variation was <25% (coefficient of variation).

Flow cytometry, SIV isolation, and SIV neutralizing-antibody assay. Following virus challenge, a comprehensive virological analysis was performed with sequen-

tial plasma, PBMC, and lymph node biopsy specimens. Lymphocyte subsets (CD4, CD8, CD2, and CD20) were evaluated by fluorescence-activated cell sorting (FACS) of whole heparinized blood samples using methods previously described (22). Virus rescue was conducted by stimulation of 5×10^6 PBMC with 10% interleukin-2 and PHA (5 mg/ml) in RPMI 1640 media supplemented with glutamine, Pen-Strep, and 10% fetal calf serum for 4 days followed by cocultivation with an equal number of CEMx174 cells (18, 28). Rescue of virus from lymph nodes was accomplished by disruption of fresh lymph node tissue into a single-cell suspension by gentle Dounce homogenization and stimulation of 5×10^6 lymph node cells with PHA and interleukin-2 as done with PBMC, followed by cocultivation with CEMx174 cells. Other analyses included plasma p27 antigen levels (Coulter Corporation, Hialeah, Fla.), limiting-dilution isolation of virus from plasma, and lymph node histopathology with in situ hybridization analysis for expression of SIV RNA (15). Antibody to SIV antigens was assayed with a whole-virus HIV-2 enzyme-linked immunosorbent assay (ELISA) (Genetic Systems). Western blot (immunoblot) analysis of sequential sera was performed with pelleted virus from cell-free supernatants of CEMx174 cells infected with SIVsmE660 as the antigen as previously described (28). Neutralizing-antibody titers were assayed by using SIVsmH4 in CEMx174 cells as previously described (39).

Humoral vaccinia virus responses. Sequential plasma samples from immunized animals were assessed for antibodies to vaccinia virus proteins by ELISA and neutralizing-antibody assay. Vaccinia virus-specific binding titers were determined by an ELISA in which Immulon plates (Dynatech Lab, Inc., McLean, Va.) were coated with sucrose-purified vaccinia virus WR overnight at 37°C at a concentration of 5×10^5 PFU per well. The plates were then fixed in formaldehyde and blocked with PBS containing 4% bovine serum albumin. Serum samples were incubated for 1.5 h at 37°C, washed five times with PBS, and then incubated an additional 1.5 h with a 1:10,000 dilution of goat anti-monkey peroxidase conjugate (Organon Teknika). Following five washes, the plates were incubated with the peroxidase substrate 2,2′-azido-di-(3-ethylbenzthiazoline sulfonate) diammonium salt (ABTS) (Boehringer Mannheim Biochemicals) at 37°C, and the optical density was read on a Thermomax microplate reader (Molecular Devices, Sunnyvale, Calif.) at a wavelength of 405 nm at 30 min.

Vaccinia virus-specific neutralizing antibodies were assayed by a plaque reduction assay using vaccinia virus WR in BS-C-1 cells. Sera were diluted fivefold in PBS, and an equal volume of PBS containing approximately 100 PFU of vaccinia virus was added. The virus-plasma mixture was incubated for 30 min at 37°C before being plated in duplicate onto confluent monolayers of BS-C-1 cells. After a 1-h absorption, the virus-plasma mixture was replaced with fresh medium. At 48 h postinfection, the monolayers were stained with crystal violet. Neutralization was defined as a 70% reduction in plaque numbers.

Statistical methods. Repeated-measures analysis of variance, profile analysis, growth curve analysis, and other methods were used (12, 19, 29, 31, 40, 54). Repeated-measures and growth curve analyses were used to test for differences in the three distinct patterns of viral load patterns that emerged from the data. When appropriate, analyses were performed on both raw and log-transformed data. Ad hoc groups with differing patterns of viral replication were used to test for differences in survival time associated with these patterns. For simplicity, only the log rank statistic is reported for differences in survival times. Post hoc *t* tests and other measures were used to determine differences between groups receiving different immunization regimens.

RESULTS

Expression of SIV *env* and *gag-pol* by recombinant vaccinia viruses. A cassette containing the entire SIV *env* and *gag-pol* coding regions under the control of separate vaccinia virus promoters was inserted by homologous recombination into two strains of vaccinia virus, the fully replication-competent New York Board of Health strain (Wyeth vaccinia virus) and the highly attenuated and host cell-restricted MVA (Fig. 1). In both constructs, the *gag-pol* and *env* genes were regulated by the widely used P7.5 early-late promoter (9) and a strong synthetic early-late promoter (7a), respectively. The thymidine kinase (TK) locus was the insertion site in the genome of the Wyeth virus: previous studies had shown that disruption of the TK gene has a minimal effect on vaccinia virus replication in cultured mammalian cells but attenuates virulence in animal models (5). As previous experiments had shown that disruption of the TK gene of MVA impaired the ability to isolate recombinant viruses in permissive CEF, we used the site of an existing deletion in the MVA genome so as not to further impair replication of MVA (48, 49). After successive plaque purifications, the recombinant viruses were propagated and characterized by Southern blotting (data not shown) and Western blot

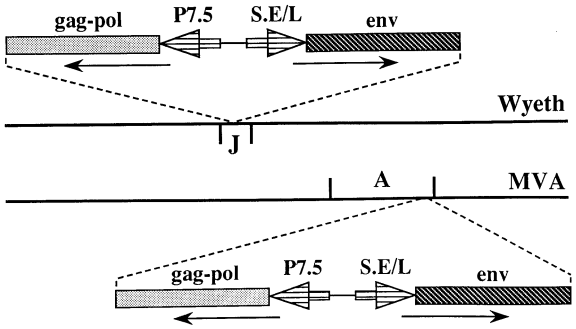

FIG. 1. Representation of recombinant MVA and Wyeth virus genomes. Expression cassettes consisting of the SIV *gag-pol* genes regulated by the vaccinia virus early-late P7.5 promoter and the SIV *env* gene regulated by the synthetic early-late promoter (S.E/L) are connected by dashed lines to the TK locus in the *Hin*dIII J fragment of the Wyeth virus genome and to the site of a deletion within the *Hin*dIII A fragment of the MVA genome. The directions of promoters (arrowheads) and open reading frames (arrows) are indicated.

analysis (Fig. 2). The defect in MVA replication in mammalian cells occurs at a late stage in virion morphogenesis and does not impair viral or recombinant protein synthesis (48). Accordingly, monkey cells infected with Wyeth and MVA double-recombinant viruses produced similar amounts of *env*- and *gag-pol*-derived proteins (Fig. 2, lanes 5 and 6). Cytoplasmic extracts of cells infected with recombinant viruses expressing *env* alone (Fig. 2, lane 3) or *gag-pol* alone (Fig. 2, lane 2) served as positive controls. Since the Gag-Pol precursor is processed after budding of virions from the cell surface, minimal amounts of CA and MA proteins were discerned in Western blots of cell lysates. The Env precursor, gp160, and cleaved gp120-gp41

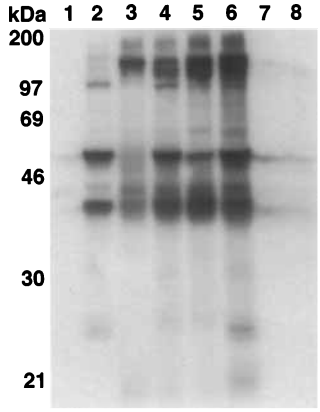

FIG. 2. Transient expression of SIV proteins expressed by recombinant MVA and Wyeth viruses. BS-C-1 monkey cells were either mock infected (lane 1) or infected with recombinant vaccinia virus WR expressing SIV *gag-pol* alone (lane 2), WR expressing *env* alone regulated by the P7.5 promoter (lane 3), Wyeth vaccinia virus expressing *env-gag-pol* regulated by the P7.5 promoter (lane 4), Wyeth vaccinia virus expressing *env* regulated by the synthetic early-late promoter and *gag-pol* regulated by the P7.5 promoter (lane 5), MVA expressing *env* regulated by the synthetic early-late promoter and *gag-pol* regulated by the P7.5 promoter (lane 6), nonrecombinant MVA (lane 7), or nonrecombinant Wyeth (lane 8). Proteins in cell lysates were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with polyclonal anti-SIV macaque serum followed by ^{125}I-protein A. An autogradiograph is shown, with the positions and molecular masses of protein standards indicated on the left. Lysates of cells infected with vaccinia viruses expressing *gag-pol* exhibit high-molecular-mass (>100-kDa) precursor proteins, the p55 Gag precursor, a major 41-kDa protein, and faint CA (26-kDa) and MA (16-kDa) protein bands. Lysates of cells infected with *env*-expressing vaccinia viruses exhibit gp160, gp120, and gp41 bands. The two patterns are superimposed in lysates infected with vaccinia viruses expressing *env* and *gag-pol*.

TABLE 1. Summary of reciprocal antibody titers to vaccinia virus or SIV in macaques immunized with vaccinia virus-SIV recombinant viruses

Antibody assay	Wk[a]	Reciprocal antibody titer in the indicated macaque immunized with:							
		MVA-SIV				Wyeth-SIV			
		H096	H102	H107	H118	H119	H120	H133	H145
Vaccinia virus ELISA									
	2	400	600	1,000	1,200	400	2,400	400	800
	14	25,600	19,200	19,200	19,200	19,200	19,200	19,200	38,400
	22	25,600	32,000	19,200	12,800	9,600	19,200	19,200	51,200
Vaccinia neutralizing antibody									
	2	<50	<50	<50	<50	<50	<50	<50	<50
	14	250	250	250	50	250	1,250	250	1,250
	22	250	250	250	50	250	250	250	1,250
SIV ELISA (HIV-2)									
	2	<100	<100	<100	<100	<100	<100	<100	<100
	14	800	400	400	800	<100	400	400	400
	22	1,600	800	400	200	<100	400	400	400
	30	400	400	100	100	<100	<100	<100	100
	48	25,600	1,600	400	400	<100	<100	<100	100
SIV neutralizing antibody									
	14	1,040	980	430	310	270	330	170	170
	22	220	870	150	170	420	1,100	280	290
	30	310	210	140	180	330	1,590	220	180
	48	220	420	270	180	240	1,680	120	610

[a] Week 0 designated as the time of the first vaccinia virus immunization. Subsequent immunizations were administered at 12, 20, and 28 weeks; thus, titers were determined 2 weeks after booster injections. Animals were given booster injections of inactivated SIV at 44 weeks and challenged at week 48.

bands were discerned, and several bands including *gag-pol* intracellular processing intermediates were detected (Fig. 2). Processed *env* and *gag-pol* proteins were also present in tissue culture medium (data not shown), consistent with production of pseudovirions.

Comparative immunogenicity of recombinant vaccinia viruses. The immunogenicity of vaccinia virus-encoded proteins and the SIV antigens expressed by the two vaccinia virus recombinants was assessed by Western blotting, ELISA, and neutralizing-antibody assay. Table 1 shows reciprocal antibody titers in macaques immunized with MVA-SIV and Wyeth-SIV recombinant viruses. Multivariate repeated-measures analyses and profile analyses (12, 40, 54) showed that the two groups of animals exhibited similar antibody responses to vaccinia virus proteins as assessed by both whole-vaccinia-virus ELISA and a plaque reduction neutralization assay. The values were relatively unchanged after the first and second booster injections. Most of the animals had a reciprocal neutralizing-antibody titer of 1:250 for vaccinia virus except for one MVA-SIV-immunized animal with a titer of 1:50 and a Wyeth-SIV-immunized animal with a titer of 1:1,250. All of the MVA-SIV- and Wyeth-SIV-immunized animals developed SIV-specific neutralizing antibody (and HIV-2 cross-reactive ELISA antibody) by the second booster; the titers of the Wyeth-SIV group all declined somewhat, whereas those of two animals of the MVA-SIV group increased with subsequent vaccinia virus booster injections. Although the intervening booster with inactivated SIV did not affect the neutralizing-antibody titers, at least a fourfold increase in ELISA titers was observed in animals which had previously received MVA-SIV.

These data suggested that the MVA and Wyeth recombinants were comparably immunogenic; however, Western blot analysis of SIV-specific antibodies (Fig. 3) in sequential samples indicated that the MVA-SIV recombinant generated a more sustained and broader response to SIV antigens. All animals developed antibody to envelope glycoproteins after the second immunization. Additional booster injections of the appropriate vaccinia virus recombinant resulted in a sustained antibody response in those animals receiving MVA-SIV, whereas Env-specific antibody responses in Wyeth-SIV vaccinees waned over the immunization period, despite boosting. Three of four MVA-SIV-immunized animals also developed antibody to Gag proteins (H096 by the second, H102 by the third, and H107 by the fourth immunization). In contrast, the antibody responses of the remaining MVA-SIV vaccinee (H118) and all of the Wyeth-SIV-immunized macaques were restricted to the envelope glycoproteins. Interestingly, MVA-SIV vaccinee H118, with the least robust Western blot response to SIV antigens, also showed the lowest levels of vaccinia virus-specific neutralizing antibody. A *t* test showed that neutralizing-antibody titers on the day of challenge were not significantly different between MVA-SIV and Wyeth-SIV recombinant-vaccinated groups.

Patterns of virus replication after SIV challenge. Following the immunization regimen, the animals were challenged intravenously with 50 MID_{50} of homologous, uncloned, cell-free SIVsmE660 stock generated in macaque PBMC. Examination of the dynamics of viremia in these 12 macaques revealed three distinct patterns: (i) uncontrolled viremia (pattern 1) with high primary levels that continued to increase; (ii) transient control of viremia (pattern 2) characterized by high primary levels with a transient decline in virus levels by 4 weeks postinfection and variable subsequent progressive increases; and (iii) sustained control of viremia in which low levels of primary plasma viremia were followed by sustained restriction of virus replication (pattern 3). Although absolute levels overlapped to some extent, a consistent pattern of replication was exhibited for animals within each group (Fig. 4).

Animals in the first group (pattern 1) were highly viremic during primary infection; levels of viral RNA in plasma con-

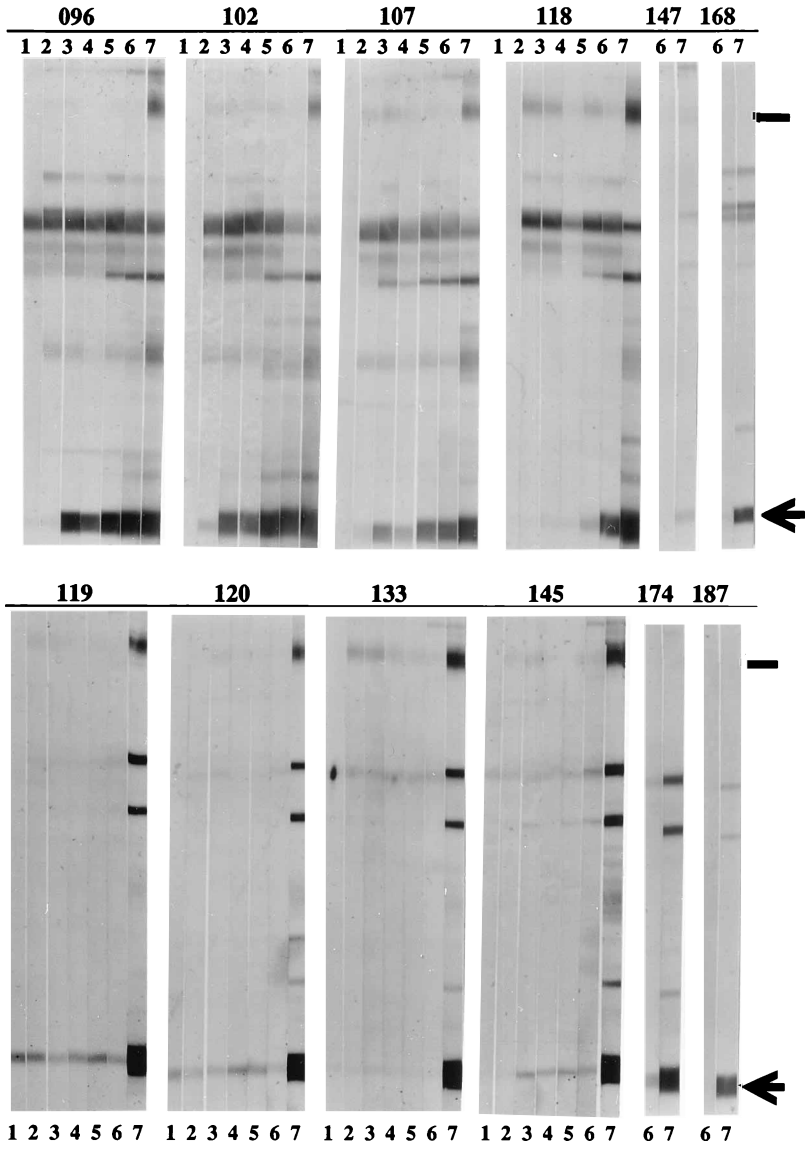

FIG. 3. SIV-specific Western blot analysis of sequential plasma samples through immunization and after challenge with SIV. Animals were immunized with MVA (096 through 168) or with Wyeth vaccinia virus (119 through 187). The first four panels (top and bottom) represent samples from the MVA-SIV- or Wyeth-SIV-immunized animals, whereas the two panels on the right represent animals immunized with nonrecombinant vaccinia virus with results for only the day of challenge (lanes 6) and 8 weeks postchallenge (lanes 7) shown. For each animal: lane 1, preimmunization plasma; lane 2, 2 weeks after second immunization; lane 3, 2 weeks after third immunization; lane 4, 2 weeks after fourth immunization; lane 5, 16 weeks after fourth immunization; lane 6, 4 weeks after inactivated SIV, day of SIV challenge; and lane 7, 8 weeks after SIV challenge. Animals 096, 102, and 107 all developed antibody to both gp120 (horizontal line) and gp40 by the second immunization (lanes 2) and Gag proteins (p26) (arrows) after the third immunization, whereas Gag reactivity was not observed in H118 samples until after inactivated SIV was administered. A cellular antigen which comigrates with p26-Gag can be observed in a number of the preimmunization samples in Wyeth-SIV vaccinees (H119, H120, and H133); however, the intensity of this band remains relatively constant until after challenge, consistent with the absence of a Gag-specific antibody response.

tinued to increase throughout the course of infection. As shown in Table 2, both animals (H147 and H168) exhibiting this pattern of viral replication were immunized with nonrecombinant MVA and thus were SIV naive. These macaques demonstrated high levels of viral RNA in plasma both within and subsequent to the primary phase of infection, with similarly high viral DNA levels in PBMC and lymph node samples, and exhibited an ineffective humoral response by 8 weeks postchallenge. This ineffective immune response was most evident in H147, which failed to develop measurable SIV-neutralizing antibody. Absolute CD4 lymphocyte counts for one of the animals (H168) declined precipitously by 16 weeks postchallenge. The CD4 numbers of the other animal (H147) also declined sharply after an initial increase but were still within a normal range at the time of euthanasia, despite severe lymphoid depletion demonstrated by histopathology.

The majority of study animals ($n = 6$) exhibited transient control of virus replication (pattern 2); an initial primary peak of viral RNA in plasma was followed by a transient decrease with subsequent rebound to levels approaching those seen in primary infection (Fig. 4). Peak levels of plasma viral RNA never exceeded the levels observed in animals with uncontrolled viremia (pattern 1), even in late stages of disease. The increase in plasma viral RNA following initial resolution of primary infection was associated with a gradual progressive decline in the numbers of circulating CD4 lymphocytes. This

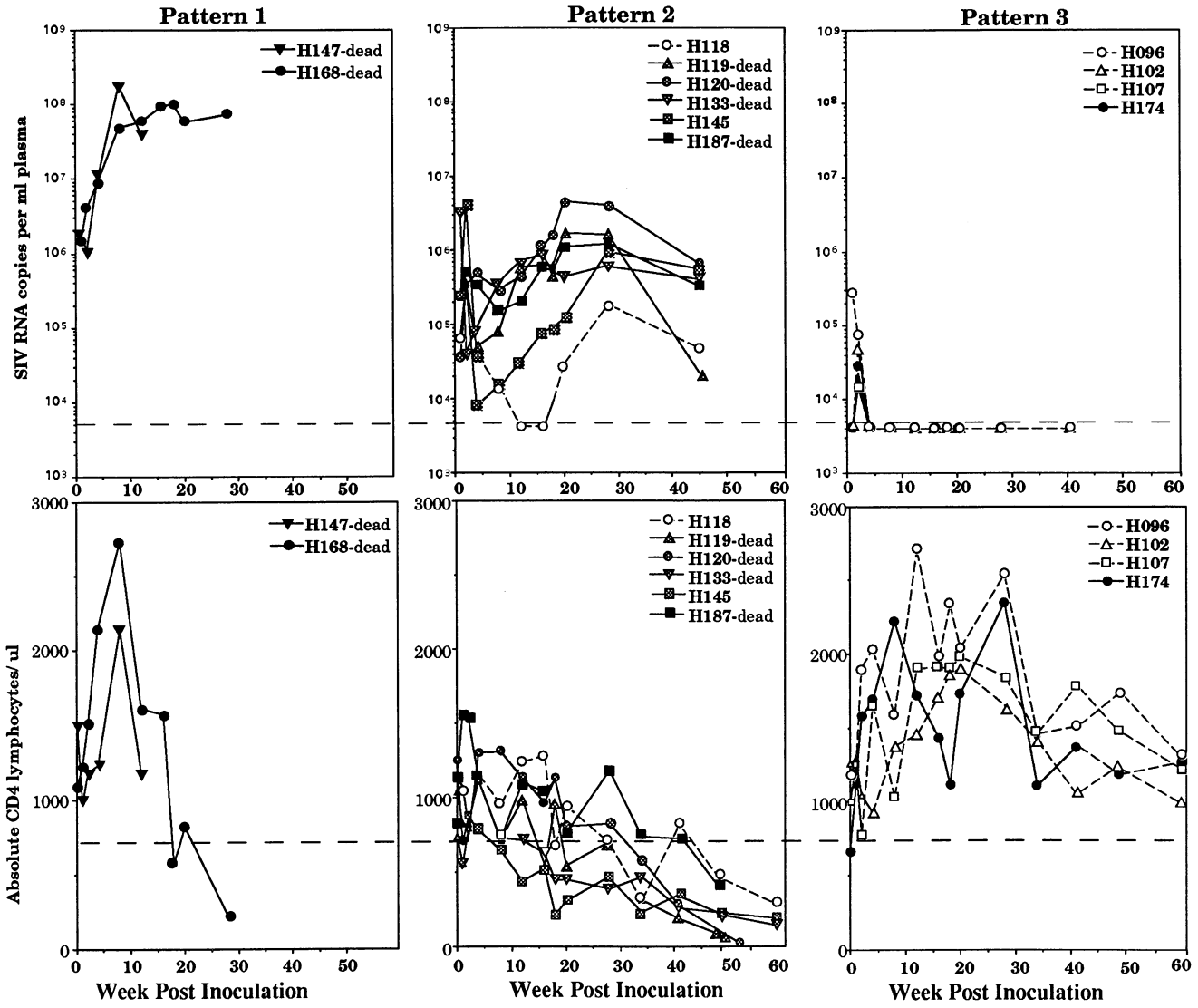

FIG. 4. Plasma viral RNA levels (top) and absolute numbers of circulating CD4 lymphocytes (bottom) in animals with different patterns of viral replication (patterns 1 to 3). Animals are grouped according to the pattern of viral replication rather than the immunogen received prior to challenge. Results for control animals (black symbols), Wyeth-SIV-immunized animals (shaded symbols), and the MVA-SIV vaccinees (dotted lines and open symbols) are shown. The horizontal dotted lines indicate the threshold sensitivity of the QC-PCR assay used (top panels) on the approximate lower limit of normal CD4 cells in macaques. Animals which were sacrificed because of clinical manifestations of AIDS at the time of the last plotted CD4 count are indicated as dead.

pattern was observed in all four animals immunized with Wyeth-SIV, one immunized with MVA-SIV (H118), and one immunized with nonrecombinant Wyeth (H187). These macaques had clear evidence of active viral replication as determined by RNA and DNA QC-PCR, and virus could be consistently isolated from PBMC; all exhibited strong anamnestic antibody responses after infection.

Pattern 3, controlled SIV replication, was observed in four animals and was characterized by a transient low-level primary plasma viremia followed by a rapid decline in viral RNA levels to below the threshold limit of quantitation (Fig. 4). In contrast to all other study animals, counts of absolute circulating CD4 cells for animals demonstrating this pattern of viral replication remained within the normal range throughout the study period. Three of four of the MVA-SIV-immunized animals (H096, H102, and H107) exhibited this pattern of curtailed viral replication. A fourth animal showing this pattern was immunized with the nonrecombinant Wyeth vaccine (H174). All four animals had overall lower levels of virus in plasma and PBMC than animals exhibiting pattern 1 or 2; good concordance between virus load data from plasma, PBMC, and lymph nodes was observed (Table 2). Whereas virus was consistently isolated from PBMC samples of other animals, virus isolation was frequently unsuccessful in these pattern 3 animals, consistent with low viral loads. An anamnestic antibody response was observed in the three pattern 3 MVA-SIV vaccinees.

The viral load data shown in Fig. 4 were subjected to repeated-measures and distribution-free growth curve analyses (19, 31) to determine whether the patterns of viral replication differed significantly from each other. Repeated-measure analyses showed that the three viral replication pattern groups were significantly different from each other, with probabilities beyond the 0.001 level of significance. As confirmation, distribution-free growth analyses, which accommodate comparisons

TABLE 2. Summary of virologic characteristics of SIV infection in immunized and naive rhesus macaques

Immunogen	Macaque	Reciprocal SIV neutralizing-antibody titer[a]		% PBMC virus isolation[b]	Results of QC-PCR							
					Primary plasma SIV viremia		Plasma viral RNA (log copies/ml)[c]		Viral DNA (copies/μg) in			
									PBMC	Lymph node biopsy		
		DOC	8 wk		p27[d]	TCID/ml[e]	Peak	Mean	Mean	5 wk	20 wk	50 wk
MVA-SIV	H096	310	8,880	41	−	0	5.4	4.5*	34	<27	<74	<6
	H102	170	2,270	40	−	0	4.6	3.6*	29	<12	<48	20
	H107	180	6,070	16	−	0	5.2	4.2*	51	<18	<90	<5
	H118	180	35,250	100	−	0	5.3	4.5	64	210	140	480
Wyeth-SIV	H119	170	48,630	100	−	0	6.2	5.8	390	190	940	340
	H120	290	52,490	100	−	0	6.7	6.1	250	240	570	220
	H133	180	21,280	100	+	100	6.2	5.7	510	340	230	90
	H145	610	52,490	100	−	0	6.1	5.7	200	950	530	300
Nonrecombinant MVA	H147	<24	<24	100	+	10,000	8.3	7.6	1,980	2,400	—[f]	—
	H168	<24	820	100	+	1,000	8.0	7.7	850	1,900	2,100	—
Nonrecombinant Wyeth	H174	<24	2,250	50	−	0	4.4	3.3*	30	7	<50	13
	H187	<24	2,940	100	+	10	6.6	5.8	520	330	1,100	790

[a] Reciprocal titer that inhibits cell killing by 50% (36). DOC, day of challenge; 8 wk, 8 weeks postchallenge.
[b] Percentage of a total of 12 sequential virus isolation attempts (at 1, 2, 4, 8, 12, 16, 20, 24, 32, 40, 48, and 56 weeks) that yielded virus, as determined by supernatant reverse transcriptase activity in cocultures.
[c] For values marked with an asterisk, the majority of samples from these animals were below the quantitative threshold of the procedure used (<5,000 copies per ml).
[d] +, presence of antigen in plasma at 1, 2, and 4 weeks postchallenge; −, antigen levels below the detection limit of the assay (<100 pg/ml).
[e] Values are log $TCID_{50}$ per milliliter of plasma, as determined by serial 10-fold dilutions, where 0 indicates <1 $TCID_{50}$/ml.
[f] —, animal dead.

with incomplete datum sets (in this case due to early deaths of some animals), demonstrated that the groups differed significantly from each other at the 0.01 level of significance.

As shown in Table 3, the three distinct patterns of viral replication were generally associated with different immunization protocols; both pattern 1 animals were SIV naive at the time of challenge, both having been immunized with nonrecombinant MVA. This pattern was not observed in any of the SIV-immunized animals. Pattern 2 was observed in all of the Wyeth-SIV-immunized animals, as well as one SIV-naive control and one MVA-SIV vaccinee. Pattern 3 was associated primarily with MVA-SIV immunization; viral replication for three of four macaques immunized with MVA-SIV followed this pattern. Likely reflecting biologic variability in the response to SIV infection, one of the naive macaques also showed pattern 3, limited viral replication. In studies utilizing

TABLE 3. Outcome and pathologic findings for SIV-infected rhesus macaques through 62 weeks of follow-up

Immunogen	Macaque	Pattern of viremia[a]	SIV-specific in situ hybridization of lymph node biopsies						Survival (wk)	Clinical and pathologic finding(s)[b]
			SIV-positive cells[c]			FDC trapping[d]				
			5 wk	20 wk	50 wk	5 wk	20 wk	50 wk		
MVA-SIV	H096	3	0	0	0	0	0	0	62+	Healthy
	H102	3	0	0	0	0	0	0	62+	Healthy
	H107	3	0	0	0	0	0	0	62+	Healthy
	H118	2	1	1	1	0	0	1	62+	Lymphadenopathy, low CD4 count
Wyeth-SIV	H119	2	2	2	2	0	2	0	51	Disseminated *M. avium*, LD
	H120	2	2	2	2	0	2	0	54	Disseminated *M. avium*, LD, anemia
	H133	2	2	2	2	0	2	0	58	*P. carinii* pneumonia, LD, enteritis
	H145	2	2	2	2	1	2	2	62+	Lymphadenopathy, WL, low CD4 count (181/μl)
Vaccinia virus	H147	1	3	d	d	0	d	d	14	Protozoal colitis, granulomatous encephalitis and pneumonia, severe LD with syncytia
	H168	1	3	3	d	0	0	d	22	Granulomatous meningoencephalitis and pneumonia, severe LD with syncytia
	H174	3	1	0	0	1	0	0	62+	Healthy
	H187	2	2	2	2	0	2	0	54	Multifocal pyogranulomatous lymphadenitis, CMV testiculitis, LD

[a] Patterns of viremia: 1 = uncontrolled; 2 = transient control; 3 = controlled viremia as defined in the text.
[b] LD, lymphoid depletion; WL, weight loss, CMV, cytomegalovirus.
[c] Numbers indicate SIV-expressing cells per high-power field: 0, none detected; 1, 0 to 2 per field; 2, 5 to 10 per field; and 3, 30 to 50 per field. d, dead prior to the scheduled date of the biopsy.
[d] Numbers indicate the intensity of deposition of viral RNA in germinal centers: 0, none detected; 1, minimal deposition; and 2, heavy deposition.

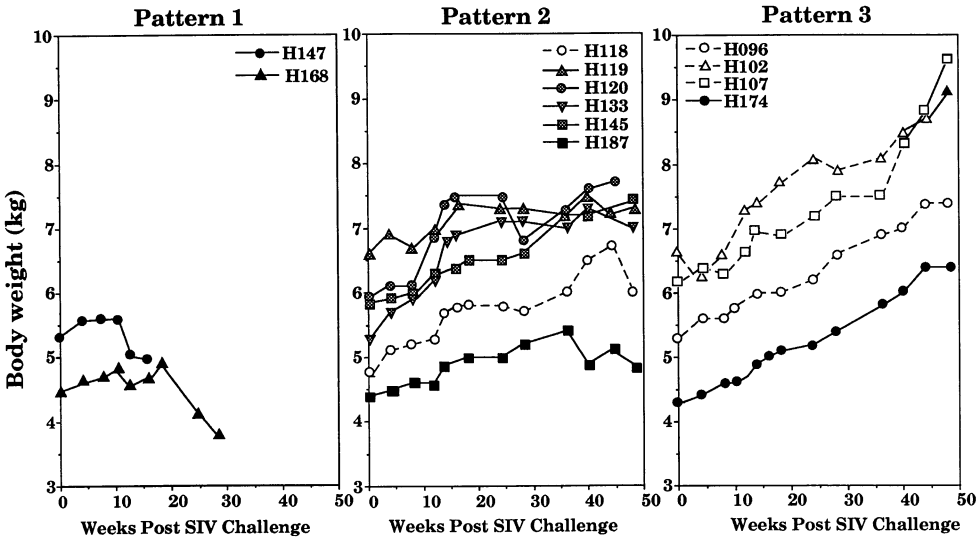

FIG. 5. Sequential body weight measurements of study macaques over a period of 1 year from challenge. The patterns of viral replication were correlated with weight gain as a measure of clinical condition. Animals are grouped according to the pattern of viral replication rather than the immunogen received prior to challenge. Results for control animals (black symbols), Wyeth-SIV-immunized animals (shaded symbols), and MVA-SIV vaccinees (dotted lines and open symbols) are shown.

the same challenge stock, 1 of 10 unimmunized animals shared a similar pattern of restricted viral replication (20). Thus, while one of four SIV-naive macaques exhibited limited viral replication, MVA-SIV immunization appeared to increase the proportion of animals showing restricted SIV replication. Comparison of viral-load parameters between the immunization groups with a *t* test showed statistically significant differences between MVA-SIV-immunized and Wyeth-SIV-immunized animals for peak plasma virus load by RNA measurements ($P = 0.0020$), mean plasma virus load by RNA measurements ($P = 0.0004$), PBMC viral DNA measurements ($P = 0.0006$), and percent frequency of virus isolation from PBMC ($P = 0.0296$), with the MVA-SIV-immunized animals having lower viral loads as determined by all parameters. Neutralizing-antibody titers were significantly higher in Wyeth-SIV-immunized animals following challenge ($P = 0.0296$), likely reflecting greater antigenic exposure during stimulation of the anamnestic response, because of levels of viral replication higher than those in the MVA-SIV-immunized animals.

Relationship of pattern of virus replication to survival. Virus load data from the three patterns of viral replication described above were subjected to standard nonparametric survival analysis (29). A global test showed that the three groups differed significantly with respect to survival (log rank chi-square = 16.30; 2 degrees of freedom; $P = 0.0003$), a highly significant result in light of the small number of animals studied. Thus, as shown in Table 3 and Fig. 4, animals with uncontrolled viremia deteriorated rapidly, necessitating euthanasia by 14 and 22 weeks postchallenge (pattern 1, rapid progressors), with a median survival of 18 weeks. Severe lymphoid depletion with massive expression of SIV within macrophages and multinucleated giant cells in lymphoid tissues, gastrointestinal-associated lymphoid tissue, and lung and brain tissues were observed in both animals. Animals with transient control of viremia (pattern 2, progressors) had a longer symptom-free survival, but four of these six animals succumbed to opportunistic infections (*Mycobacterium avium*, *Pneumocystis carinii*, and cytomegalovirus) by 62 weeks postchallenge. In these animals, the increasing levels of virus in plasma seen at various intervals, following the decrease associated with resolution of primary infection, were accompanied by progressive decreases in numbers of circulating $CD4^+$ cells. In subsequent anecdotal observations, one additional animal (H118) was euthanized, and the one survivor of this group shows evidence of disease progression with declining numbers of CD4 lymphocytes. In contrast, the four animals with restricted viremia (pattern 3, nonprogressors) are all clinically healthy nearly 2 years postchallenge. Three of these animals have no evidence of disease progression, as assessed by CD4 lymphocyte numbers, lymph node biopsies, and normal weight gain (Fig. 5), while one animal (H102) has recently exhibited declining CD4 numbers but remains clinically healthy.

Pathology and virus expression in sequential lymph node biopsies. Histopathologic analysis and in situ hybridization for SIV of sequential lymph node biopsies from these animals were highly indicative of disease progression (Table 3 and Fig. 6) and agreed well with other virus load parameters. High numbers of SIV-expressing cells were observed in all sequential lymph nodes biopsied from the rapid progressors (Fig. 6B and D). Lymph nodes remained relatively quiescent without evidence of the formation of secondary follicles and showed marked lymphoid depletion in biopsies taken at 20 weeks (Fig. 6C and D). Lower numbers of SIV-expressing cells were observed in lymph nodes biopsied from the progressors and remained relatively constant throughout the disease course. Progressors developed prominent follicular hyperplasia by 20 weeks postchallenge (Fig. 6E to H). Hybridization was observed within germinal centers in a pattern consistent with association of viral particles with follicular dendritic cells (FDC), as seen in HIV-1-infection (4, 15, 40, 51) and recently in SIV infection (2, 7). In contrast, lymph nodes biopsied at 50 weeks (late in disease) from four of these macaques revealed dissolution and hyalinization of germinal centers (H119, H120, H133, and H187; Fig. 6) and an associated loss of FDC virus trapping. This finding was highly predictive of the onset of opportunistic infections within the ensuing weeks. Lymph node biopsies collected at 50 weeks from the then-surviving progressors (H118 and H145) demonstrated marked follicular hyperplasia and trapping of virus within germinal centers, suggestive of a similar pattern of infection with slower kinetics. One of the

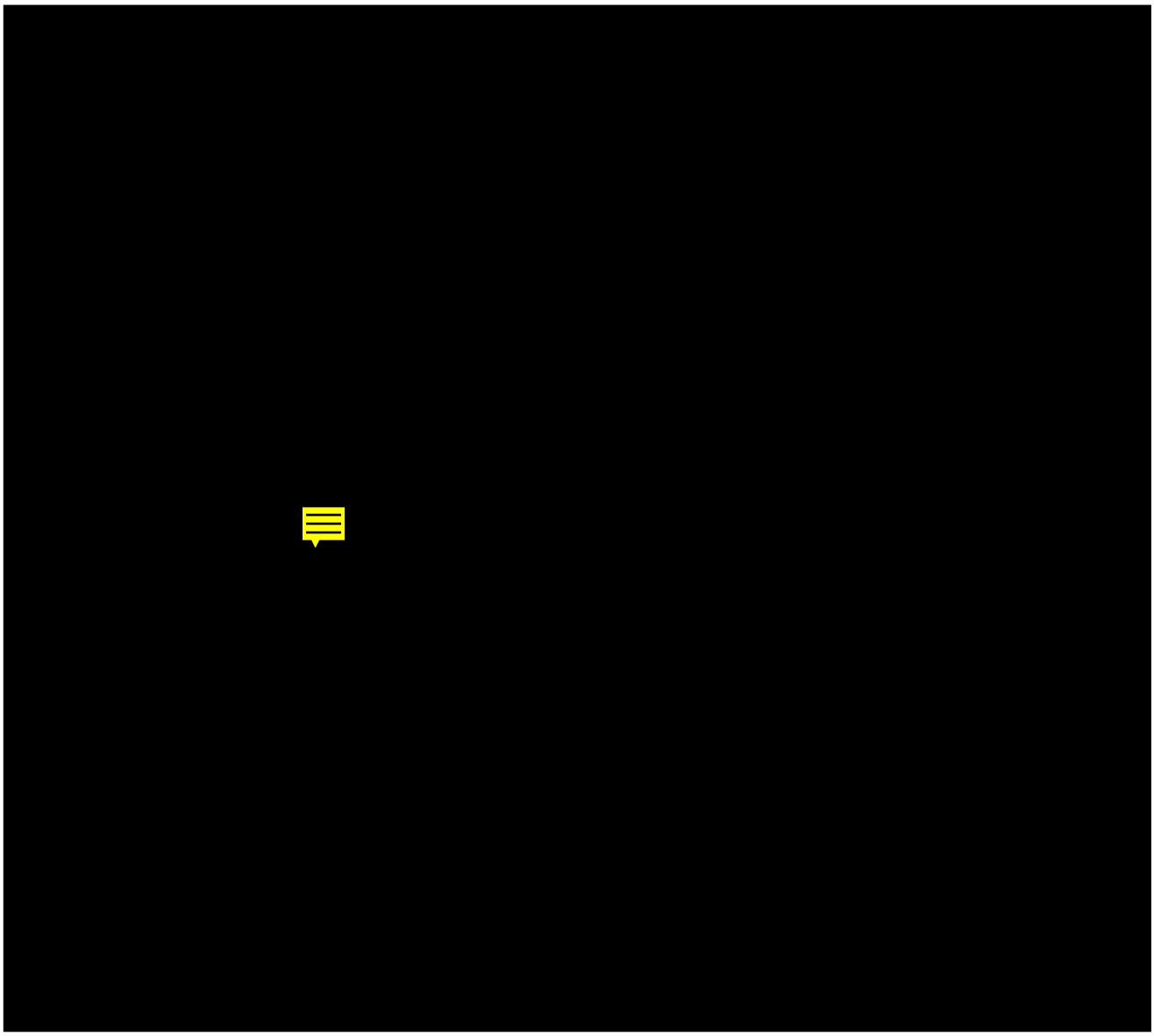

FIG. 6. Histopathology and SIV-specific in situ hybridization of sequential lymph node biopsies from representative animals which exhibited different patterns of viral replication. (A to D) Pattern 1. (A) Hematoxylin and eosin (H&E)-stained section of lymph node biopsied at 5 weeks postinfection from H147, showing quiescent nature of lymph node at low magnification (×5). (B) In situ hybridization for SIV RNA of same-section lymph node biopsies, demonstrating the large number of SIV-expressing cells (×5). (C) H&E-stained section of lymph node biopsied at 20 weeks postinfection from H168, showing lymphoid depletion (×5). (D) In situ hybridization for SIV RNA of the section in panel C, demonstrating large numbers of SIV-expressing cells (×5). (E to H) Pattern 2. (E) H&E-stained section of lymph node biopsied from H119 at 20 weeks showing marked follicular hyperplasia (note that this lymph node was photographed at the same magnification as that in panel A [×5]). (F) Same lymph node at higher magnification (×20). (G) H&E-stained section of lymph node biopsied from H187 at 50 weeks, showing dissolution of the germinal centers and replacement with hyalinized material. (H) In situ hybridization for SIV RNA of lymph node biopsy from H120 at 20 weeks postchallenge, showing extensive signal within germinal centers in a distribution characteristic of FDC (×2). (I to L) Pattern 3. (I) H&E-stained section of lymph node biopsy obtained from H102 at 5 weeks postchallenge, showing the quiescent nature of lymphoid architecture and absence of lymphoid hyperplasia (×5). (J) H&E-stained section of lymph node biopsy obtained from H096 at 20 weeks postchallenge (×5). (K) H&E-stained section of lymph node biopsy obtained from H102 at 50 weeks postchallenge (×8). (L) In situ hybridization for SIV of lymph node biopsy obtained from H096 at 5 weeks postchallenge, showing the lack of SIV-expressing cells (×3.5).

most striking findings was the lack of apparent viral expression in all but one early lymph node biopsy from the nonprogressors. The lymphoid architecture of biopsies obtained from these animals remained intact and quiescent (Fig. 6I to L), with no evidence of deposition of virus within germinal centers.

DISCUSSION

The findings of this study underscore the central significance of the extent and patterns of viral replication in determining the course and outcome of infection with SIV. Prompt, substantial, and sustained suppression of viral replication following primary infection was associated with prolonged symptom-free survival. The patterns of viral replication over time were often more informative than absolute or mean levels of viral RNA in plasma, particularly during the primary phase of infection. Thus, the absolute peak of primary viremia was most closely associated with subsequent disease course at the high and low extremes. The patterns of replication over time were

more informative than the mean levels; for example, although similar mean plasma viral RNA levels were observed in H096 (pattern 3) and H118 (pattern 2), viremia in the latter animal was only temporarily controlled. The subsequent increase in viral RNA levels in plasma of H118 was associated with declining CD4 lymphocyte numbers. Failure to control primary viremia (pattern 1) and transient control of viremia with increasing levels after an initial decrease (pattern 2) were both associated with progressive disease, irrespective of the absolute peak levels reached during the viremia of primary infection. Plasma viremia can be considered a crude reflection of the overall level of viral replication in tissues in anatomic continuity with the plasma compartment. The quasi-steady-state level of plasma viremia established in the post-acute phase of infection appears to provide an indication of the outcome of the initial interaction between the virus and host with profound prognostic implications. An unexpected finding was the tendency for plasma viremia to plateau or decline as the animals progressed towards AIDS. Thus, absolute plasma viremia in animals such as H119 declined by as much as 100-fold as they approached the terminal stages of disease, presumably because of severe lymphoid depletion. This finding contrasted with the extremely high-level viremia observed preterminally in animals which progressed rapidly.

Overall, the MVA-SIV vaccination protocol appeared to affect the extent and pattern of SIV replication following challenge. Despite small group sizes, the MVA-SIV-immunized animals showed statistically significantly lower levels of viral replication than the Wyeth-SIV-immunized animals. In the absence of immunization or other intervention, this profile of restricted viral replication is observed at the frequency of about 1 in every 10 inoculated macaques (reference 20 and unpublished data). Therefore, the significantly lower levels of viral replication and the predominance of three of four animals exhibiting this rare pattern in the MVA-SIV-immunized group are suggestive of a protective role of prior immunization. Although the psoralen-inactivated whole-virus booster may have contributed to the protective effect, the difference in outcome between the Wyeth-SIV and MVA-SIV groups suggests that the MVA-SIV immunogen was the essential component of the protocol. In terms of host factors, it will be important to identify the immunological correlates of the more favorable outcome seen in the MVA-SIV-immunized animals. Since all animals had low levels of SIV-neutralizing antibody at the time of challenge, preexisting neutralizing antibody, as measured in vitro, is an unlikely mediator of the curtailed viral replication seen in the MVA-SIV-immunized animals. Following infection, anamnestic antibody responses mirrored the degree of active viral replication, with the lowest titers seen in animals with sustained control of viremia. An exclusive feature of the three MVA-SIV-immunized animals with restricted replication was a detectable Gag-specific antibody prior to challenge. While antibody to Gag is an improbable mediator of the protective effect, it may be an indirect indicator of greater overall immunogenicity perhaps leading to other responses, such as a Gag-specific cytotoxic T-cell response. These animals were not evaluated for cell-mediated immune responses. The evaluation of cytotoxic T-cell responses and CD8 cell-mediated virus-suppressive activity (8, 52), as well as other host factors that may affect viral replication, will be critical in dissecting underlying immune mechanisms.

Safety issues constituted the main reason for testing recombinant MVA in this study. The high degree of attenuation of this vector was documented in humans during its use as a smallpox vaccine (35). After more than 500 passages in CEF, the virus has multiple deletions and is host restricted (35). Since the replication block of MVA in mammalian cells occurs at a late stage in viral morphogenesis, after the synthesis of early and late proteins, MVA should be a safe expression vector (48). The vaccine potential of MVA was demonstrated in influenza virus murine challenge studies (49), in which the MVA vector was as good as or better than a replication-competent vector in inducing humoral and cell-mediated immunity and in protecting mice from lethal influenza virus challenge. Additionally, in cancer therapy studies modeled with the murine tumor antigen, MVA proved to be superior to a replication-competent vaccinia virus (6a). In the present study, we chose the intradermal route for Wyeth virus since previous studies had suggested the superiority of this site for smallpox vaccination (16), whereas the intramuscular route was used most commonly with MVA. The monkeys had no evident side effects following the inoculations of MVA-SIV, whereas typical pox lesions occurred at the inoculation site after the first injections of Wyeth-SIV. The two groups of animals had similar low vaccinia virus and SIV neutralizing-antibody titers, although the MVA-SIV-immunized animals appeared to have made a broader immune response to Env and Gag-Pol proteins, as determined by Western blotting. Interestingly, the monkeys receiving the MVA recombinants were better able to resist the immunosuppressive effects of SIV infection than those receiving the Wyeth virus vector. The superior result achieved with MVA is counterintuitive, since the virus is replication incompetent. Nevertheless, poxviruses, including vaccinia virus, have acquired a large number of genes that dampen the host immune response (47). Although the MVA genome has not been sequenced, it is evident from the large number of deletions (35) that many so-called "nonessential" genes, including some required for inhibition of host defense, are missing. Perhaps the immune response to SIV was enhanced in the MVA-SIV-immunized animals compared with that in the Wyeth-SIV-immunized animals because of the absence of one or more such immune response-dampening genes in MVA.

Although a reduction in primary viremia has been observed in previous vaccine studies in which sterilizing immunity has not been achieved (17, 27), the significance of this reduction with regard to clinical outcome has remained unclear. While the small group sizes and the duration of follow-up to date do not allow us to address ultimate clinical outcomes in the surviving animals, the differences after 1 year of follow-up and the associations between distinct patterns of viral replication and survival are highly significant. On the basis of historical experience with macaques infected with the isolate used in this study, survival trends after more than 1 year are likely to be sustained (24; unpublished observations). Indeed, all animals with curtailed viral replication (three of the MVA-SIV-immunized animals and one animal immunized with nonrecombinant Wyeth vaccinia virus) remain alive and healthy after a follow-up of 2 years. This pattern of viral replication and the corresponding clinical outcome are highly reminiscent of the profile that typifies long-term human nonprogressors of HIV-1 infection (6, 41). Limited viral replication during primary infection followed by sustained control of viral replication was associated with long-term, symptom-free survival and maintenance of normal levels of $CD4^+$ T cells. However, intermediate degrees of viral suppression, such as those observed in one MVA-SIV vaccinee and all of the Wyeth-SIV vaccinees, were associated with only a marginal survival benefit.

The ability to model the full spectrum of human HIV infection, from rapidly progressive immunodeficiency to apparent long-term nonprogression, all within 1 year is an extremely powerful feature of this model. The results of the present study reaffirm the pivotal importance of viral replication in the

pathogenesis of primate lentivirus infection. The association between patterns of viral replication and progression and the suggested role of immunization in apparent nonprogressive SIV infection provide a hopeful basis for therapeutic and vaccine intervention for human AIDS.

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REFERENCES

1. **Arthur, L. O., J. W. Bess, R. C. Sowder, R. E. Benveniste, D. L. Mann, J.-C. Chermann, and L. E. Henderson.** 1992. Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. Science **258:**1935–1938.
2. **Baskin, G., L. N. Martin, M. Murphey-Corb, F.-S. Hu, D. Kuebler, and B. Davison.** 1995. Distribution of SIV in lymph nodes of serially sacrificed rhesus monkeys. AIDS Res. Hum. Retroviruses **11:**273–285.
3. **Baskin, G. B., M. Murphey-Corb, E. A. Watson, and L. N. Martin.** 1989. Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV/Delta). Vet. Pathol. **25:**456–467.
4. **Biberfeld, P., K. J. Chayt, L. M. Marselle, G. Biberfeld, R. C. Gallo, and M. Harper.** 1986. HTLV-III expression in infected lymph nodes and relevance to pathogenesis of lymphadenopathy. Am. J. Pathol. **123:**436–440.
5. **Buller, R. M. L., G. L. Smith, K. Cremer, A. L. Notkins, and B. Moss.** 1985. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase negative phenotype. Nature (London) **317:**813–815.
6. **Cao, Y., L. Qin, L. Zhang, J. Safrit, and D. Ho.** 1995. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. N. Engl. J. Med. **332:**201–208.

6a. **Carroll, M. W., W. W. Overwijk, R. Chamberlain, S. A. Rosenberg, B. Moss, and N. P. Restifo.** Unpublished data.

7. **Chakrabarti, L., M.-C. Cumont, L. Montagnier, and B. Hurtrel.** 1994. Variable course of simian immunodeficiency virus infection in lymph nodes: relation to disease progression. J. Virol. **68:**6634–6642.

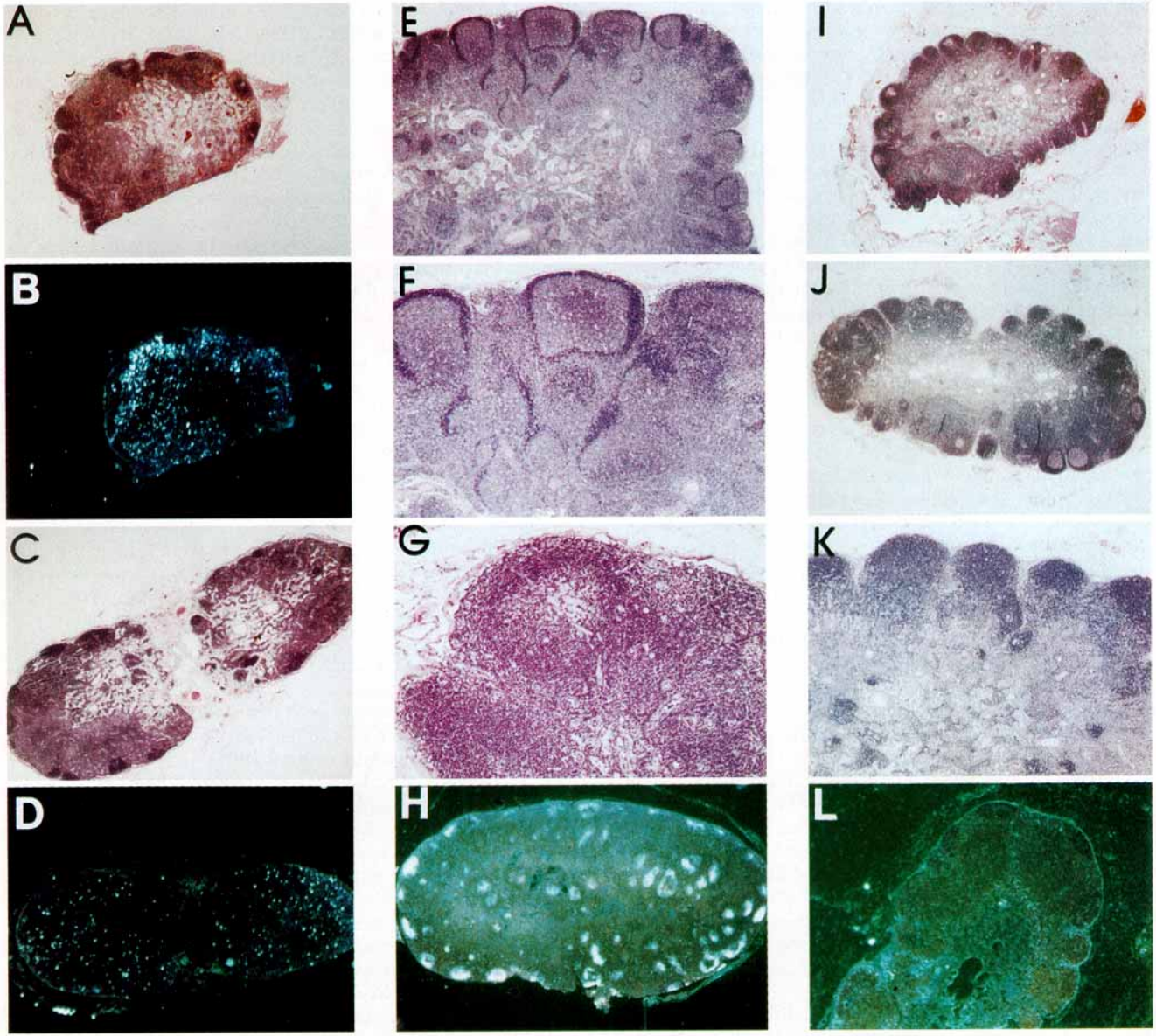
7a. **Chakrabarti, S., and B. Moss.** Unpublished data.

8. **Cocchi, F., A. L. DeVico, A. Garzino-Demo, S. K. Arya, R. C. Gallo, and P. Lusso.** 1995. Identification of RANTES, MIP-1α, and MIP-1β as major HIV-suppressive factors produced by $CD8^+$ T cells. Science **270:**1811–1815.
9. **Cochran, M. A., C. Puckett, and B. Moss.** 1985. In vitro mutagenesis of the promoter region for a vaccinia virus gene: evidence for tandem early and late regulatory synthesis. J. Virol. **54:**30–37.
10. **Codoz, M., A. Stradt, B. Meignier, J. Taylor, J. Tartaglia, E. Paoletti, and S. Plotkin.** 1992. Immunization with canarypox expressing rabies glycoprotein. Lancet **iii:**1529–1532.
11. **Cranage, M. P., N. Polyanskaya, B. McBride, N. Cook, L. A. E. Ashworth, M. Dennis, A. Baskerville, P. J. Greenway, T. Corcoran, P. Kitchin, J. Rose, M. Murphey-Corb, R. C. Desroisiers, E. J. Stott, and G. H. Farrar.** 1993. Studies on the specificity of the vaccine effect elicited by inactivated simian immunodeficiency virus. AIDS Res. Hum. Retroviruses **9:**13–22.
12. **Crowder, M. J., and D. J. Hand.** 1990. Analyses of repeated measures. Chapman & Hall, New York.
13. **Daniel, M. D., F. Kirchoff, S. C. Czajak, P. K. Seghal, and R. C. Desrosiers.** 1992. Protective effects of live attenuated SIV vaccine with a deletion in the *nef* gene. Science **258:**1938–1941.
14. **Embertson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. Haase.** 1994. Massive covert infection of helper T lymphocytes by HIV during the incubation period of AIDS. Nature (London) **362:**359–362.
15. **Fox, C., C. Tenner-Racz, P. Racz, A. Firpo, P. A. Pizzo, and A. S. Fauci.** 1991. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. J. Infect. Dis. **164:**1051.
16. **Galasso, G. D., D. T. Karzon, S. L. Katz, S. Krugman, J. Neff, and F. C. Robbins.** 1977. Smallpox vaccines comparing variations in dose and route of administration. J. Infect. Dis. **135:**131–186.
17. **Giavedoni, L. D., V. Planelles, N. L. Haigwood, S. Ahmad, J. D. Kluge, M. L. Marthas, M. B. Gardner, P. A. Luciw, and T. D. Yilma.** 1993. Immune response of rhesus macaques to recombinant simian immunodeficiency virus gp130 does not protect from challenge infection. J. Virol. **67:**577–583.
18. **Goldstein, S., W. R. Elkins, W. T. London, A. Hahn, R. Goeken, J. E. Martin, and V. M. Hirsch.** 1994. Immunization with whole inactivated vaccine protects from infection by SIV grown in human but not macaque cells. J. Med. Primatol. **23:**75–82.
19. **Guo, S., R. Simon, J. E. Talmadge, and J. E. Klabansky.** 1987. An interactive computer program for the analysis of growth curves. Comput. Biomed. Res. **20:**37–48.
20. **Haigwood, N., A. Watson, J. McClure, W. F. Sutton, J. Ranchalis, B. Travis, S.-L. Hu, V. M. Hirsch, G. Voss, N. Letvin, A. Lewis, and P. R. Johnson.** 1995. Passive immune globulin therapy in the SIV/macaque model reduces viremia and delays disease, p. 149–158. *In* R. Chanock, F. Brown, H. Ginsberg, and E. Norby (ed.), Vaccines 95. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Hirsch, V., S. Goldstein, R. Chanock, W. R. Elkins, G. Sutter, B. Moss, J. Sisler, J. Lifson, and T. Fuerst.** 1995. Limited virus replication following SIV challenge of macaques immunized with attenuated MVA vaccinia expressing SIVsm *env* and *gag-pol*, p. 195–200. *In* R. Chanock, F. Brown, H. Ginsberg, and E. Norby (ed.), Vaccines 95. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

21a. **Hirsch, V. M.** Unpublished data.

22. **Hirsch, V. M., G. Dapolito, P. R. Johnson, W. R. Elkins, W. T. London, R. J. Montali, S. Goldstein, and C. Brown.** 1995. Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with extent of in vivo replication. J. Virol. **69:**955–967.
23. **Hirsch, V. M., S. Goldstein, N. A. Hynes, W. R. Elkins, W. T. London, P. M. Zack, D. Montefiori, and P. R. Johnson.** 1994. Prolonged clinical latency and survival of macaques given a whole inactivated simian immunodeficiency virus vaccine. J. Infect. Dis. **170:**51–59.
24. **Hirsch, V. M., and P. R. Johnson.** 1994. Pathogenic diversity of simian immunodeficiency viruses. Virus Res. **32:**183–203.
25. **Ho, D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature (London) **373:**123–126.
26. **Hu, S.-L., K. Abrams, G. Barber, P. Moran, J. M. Zarling, A. J. Langlois, L. Kuller, W. R. Morton, and R. E. Benveniste.** 1992. Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. Science **255:**456–459.
27. **Israel, Z. R., P. F. Edmondson, D. H. Maul, S. P. O'Neil, S. P. Mossman, C. Thiriart, L. Fabry, O. Van Opstal, C. Bruck, F. Bex, A. Burny, P. N. Fultz, J. I. Mullins, and E. A. Hoover.** 1994. Incomplete protection, but suppression of virus burden, elicited by subunit simian immunodeficiency virus vaccines. J. Virol. **68:**1843–1853.
28. **Johnson, P. R., D. C. Montefiori, S. Goldstein, et al.** 1992. Inactivated whole-virus vaccine derived from a proviral molecular clone of simian immunodeficiency virus induces high levels of neutralizing antibodies and confers protection against heterologous challenge. Proc. Natl. Acad. Sci. USA **89:**2175–2179.
29. **Kalbfleish, J. D., and R. L. Prentice.** 1980. The statistical analysis of failure time data. John Wiley & Sons, Inc., New York.
30. **Kirchoff, F., T. C. Greenough, D. B. Brettler, J. L. Sullivan, and R. C. Desrosiers.** 1995. Brief report: absence of intact *nef* sequences in a long-term survivor with nonprogressive HIV-1 infection. N. Engl. J. Med. **332:**228–232.
31. **Koziol, J. A., and D. A. Maxwell.** 1981. A distribution-free test for tumor growth analyses with application to an animal tumor immunotherapy experiment. Biometrics **37:**383–390.
32. **Lohman, B., M. McChesney, C. J. Miller, E. McGowan, S. M. Joye, K. K. A. Van Rompey, E. Reay, L. Antipa, N. C. Pederson, and M. L. Marthas.** 1994. A partially attenuated simian immunodeficiency virus induces host immunity that correlates with resistance to pathogenic virus challenge. J. Virol. **68:**7021–7029.
33. **Mackett, M., G. L. Smith, and B. Moss.** 1984. General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. J. Virol. **49:**857–864.
34. **Marthas, M., S. Sujipto, J. Higgins, B. Lohman, J. Torten, P. A. Luciw, P. A. Marx, and N. C. Pedersen.** 1990. Immunization with a live attenuated simian immunodeficiency virus (SIV) prevents early disease but not infection in rhesus macaques challenged with pathogenic SIV. J. Virol. **64:**3694–3700.
35. **Mayr, A., V. Hochstein, and H. Stickl.** 1975. Abstammung, Eigenschaften und Verwendung des attenuierten Vaccinia-stammes MVA. Infection **3:**6–14.
36. **McClure, H. M., D. C. Anderson, P. N. Fultz, A. A. Ansari, E. Lockwood, and A. Brodie.** 1989. Spectrum of disease in macaques monkeys chronically infected with SIVsmm. Vet. Immunol. Immunopathol. **21:**13–24.
37. **Meyer, H., G. Sutter, and A. Mayr.** 1991. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. J. Gen. Virol. **72:**1031–1038.
38. **Michael, N., M. Vahey, D. S. Burke, and R. R. Redfield.** 1992. Viral DNA and mRNA expression correlate with the stage of human immunodeficiency virus (HIV) type 1 infection in humans: evidence for viral replication in all stages of HIV disease. J. Virol. **66:**310–316.
39. **Montefiori, D. C., W. E. Robinson, S. S. Schuffman, and W. M. Mitchell.** 1988. Evaluation of antiviral drugs and neutralizing antibodies against human immunodeficiency virus by a rapid and sensitive microtiter assay. J. Clin. Microbiol. **26:**231–235.

40. **Morrison, D. F.** 1976. Multivariate statistical methods, 2nd ed. McGraw-Hill, New York.
41. **Panteleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature (London) **362:**355–358.
42. **Panteleo, G., S. Menzo, M. Vaccarezza, C. Graziosi, O. J. Cohen, J. F. Demarest, D. Montefiori, J. M. Orenstein, C. Fox, L. K. Schrager, J. B. Margolick, S. Buchbinder, J. V. Giorgi, and A. S. Fauci.** 1995. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. N. Engl. J. Med. **332:**209–216.
43. **Piatak, M., K.-C. Luk, B. Williams, and J. D. Lifson.** 1993. Quantitative competitive polymerase chain reaction for accurate quantitation of HIV DNA and RNA species. BioTechniques **14:**70–77.
44. **Piatak, M., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson.** 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science **259:** 1749–1754.
45. **Putkonen, P., L. Walther, Y.-J. Zhang, S.-L. Li, C. Nilsson, J. Albert, P. Biberfeld, R. Thorensson, and G. Biberfeld.** 1995. Longterm protection against SIV-induced disease in macaques vaccinated with a live attenuated HIV-2 vaccine. Nature Med. **1:**914–918.
46. **Reimann, K., K. Tenner-Racz, P. Racz, D. Montefiori, Y. Yasutomi, W. Lin, B. J. Ransil, and N. L. Letvin.** 1994. Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. J. Virol. **68:**2368–2370.
47. **Smith, G. L.** 1994. Virus strategies for evasion of the host response to infection. Trends Microbiol. **2:**81–88.
48. **Sutter, G., and B. Moss.** 1992. Nonreplicating vaccinia vector efficiently expresses recombinant genes. Proc. Natl. Acad. Sci. USA **89:**10847–10851.
49. **Sutter, G., L. S. Wyatt, P. L. Foley, J. R. Bennick, and B. Moss.** 1994. A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. Vaccine **12:**53–59.
50. **Tartaglia, J., M. E. Perkus, J. Taylor, E. K. Norton, J. C. Audonnet, W. I. Cox, S. W. Davis, J. Vanderhoeven, B. Meigner, M. Riviere, B. Languet, and E. Paoletti.** 1992. NYVAC—a highly attenuated strain of vaccinia virus. Virology **188:**217–232.
51. **Tenner-Racz, K., P. Racz, S. Gardner, et al.** 1988. Ultrastructural analysis of germinal centers in lymph nodes of patients with HIV-1-induced persistent generalized lymphadenopathy: evidence for persistence of infection. Prog. AIDS Pathol. **1:**29–40.
52. **Walker, C., A. Erikson, F. Hseuh, and J. Levy.** 1991. Inhibition of human immunodeficiency virus replication in acutely infected $CD4^+$ cells by $CD8^+$ cells involves a noncytotoxic mechanism. J. Virol. **65:**5921–5927.
53. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowalk, B. H. Hahn, M. S. Saag, and G. M. Shaw.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature (London) **373:**117.
54. **Winer, B. J., D. R. Brown, and D. Michels.** 1991. Statistical principles in experimental design, 2nd ed. McGraw-Hill, New York.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