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Identification of Viral Determinants of Macrophage Tropism for Simian Immunodeficiency Virus SIVmac

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Simian immunodeficiency virus (SIV), a lymphocytopathic lentivirus, induces an AIDS-like disease in rhesus macaques (*Macaca mulatta*). A pathogenic molecular clone of rhesus macaque SIV (SIVmac), SIVmac-239, replicates and induces cytopathology in T lymphocytes but is restricted for replication in macrophages. In contrast, a nonpathogenic molecular clone of SIVmac, SIVmac-1A11, replicates and induces syncytia (multinucleated giant cells) in cultures of both T lymphocytes and macrophages. SIVmac-1A11 does not cause disease in macaques. To map the viral determinants of macrophage tropism, reciprocal recombinant genomes were constructed between molecular clones of SIVmac-239 and SIVmac-1A11. Infectious recombinant viruses were rescued by transfection of cloned viral genomes into permissive lymphoid cells. Analysis of one pair of reciprocal recombinants revealed that an internal 6.2-kb DNA fragment of SIVmac-1A11 was necessary and sufficient for both syncytium formation and efficient replication in macrophages. This region includes the coding sequences for a portion of the *gag* gene, all of the *pol*, *vif*, *vpr*, and *vpx* genes, the first coding exons of *tat* and *rev*, and the external *env* glycoprotein gp130. Thus, the transmembrane glycoprotein of *env*, the *nef* gene, the second coding exons of *tat* and *rev*, and the long terminal repeats are not essential for in vitro macrophage tropism. Analysis of additional recombinants revealed that syncytium formation, but not virus production, was controlled by a 1.4-kb viral DNA fragment in SIVmac-1A11 encoding only the external *env* glycoprotein gp130. Thus, gp130 *env* of SIVmac-1A11 is necessary for entry of virus into macrophages but is not sufficient for a complete viral replication cycle in this cell type. We therefore conclude that gp130 *env* and one or more genetic elements (exclusive of the long terminal repeats, transmembrane glycoprotein of *env*, and second coding exons of *tat* and *rev*, and *nef*) are essential for a complete replication cycle of SIVmac in rhesus macaque macrophages.

Simian immunodeficiency virus (SIV), a virus in the lentivirus subfamily of retroviruses, causes an AIDS-like disease in rhesus macaques (30). Rhesus macaque SIV (SIVmac) shows 40 to 60% homology with human immunodeficiency virus type 1 (HIV-1) and 60 to 80% homology with HIV-2 (3, 12). Clinical manifestations of simian AIDS are similar to those of human AIDS; thus, SIVmac infection of rhesus macaques is a critically important animal model for pathogenesis studies (for reviews, see references 7, 14, and 31). Virus recovered from an infectious molecular clone of SIVmac, designated SIVmac-239, induces fatal immunodeficiency disease in rhesus macaques (23). Another molecular clone, SIVmac-1A11, produces seroconversion and transient viremia in experimentally inoculated animals and is nonpathogenic (36). Two other nonpathogenic molecular clones of SIVmac, SIVmac-142 and SIVmac-251, have been described previously (24, 38). A molecular clone derived from a distinct strain of SIV from sooty mangabey monkeys, designated SIVsmm-PBj14, is also fatal for pig-tailed macaques (*Macaca nemestrina*) (8).

Recently, we defined conditions for culturing macrophages from peripheral blood mononuclear cells (PBMC) of rhesus macaques (1). SIVmac-1A11 gave rise to a productive infection in these macrophage cultures as well as in cultures of rhesus macaque peripheral blood lymphocytes (1). SIV mac-239 replicated efficiently in cultures of peripheral blood lymphocytes but not in monocyte-derived macrophage cultures (1, 38). Although SIVmac-239 and SIVmac-1A11 show more than 98% sequence homology, several molecular features distinguish these two viruses (Fig. 1) (34). SIVmac-1A11 has a premature stop codon in the *vpr* gene, truncating the predicted protein molecule to 11 amino acids, while SIVmac-239 has an open reading frame for *vpr* (34, 43). The open reading frame corresponding to *vpr* is absent in SIVs that are indigenous to African green monkeys (SIVagm) (13, 49) but is present in SIV isolated from African mandrills (SIVmnd) (50). SIVmac-239 is predicted to encode a full-length transmembrane glycoprotein that is 354 amino acids in length (43). A premature stop codon in the transmembrane glycoprotein of SIVmac-1A11 is predicted to truncate the cytoplasmic tail of this molecule by 145 amino acids (34). Two additional molecular clones of SIVmac, SIVmac-251 and SIVmac-142, also have premature stop codons in the transmembrane envelope glycoprotein (3, 12, 18). The premature stop codon in *env* is a consequence of propagation of SIVmac in the human lymphoid cell line HuT-78 or in human PBMC (19, 26, 27). SIVmac-1A11 has an open reading frame for *nef*, whereas SIVmac-239 has a truncated *nef* (25, 34, 43). The significance of this truncation in *nef* is unknown. Viruses recovered from macaques infected with SIVmac-239 have a full-length *nef* (25).

This report describes the construction and analysis of recombinant viruses generated by reciprocal exchanges of specific regions of the SIVmac-1A11 and SIVmac-239 genomes. Infectious viruses were recovered from recombinant molecular clones by transfection. These recovered viruses were analyzed in cultures of rhesus macaque peripheral

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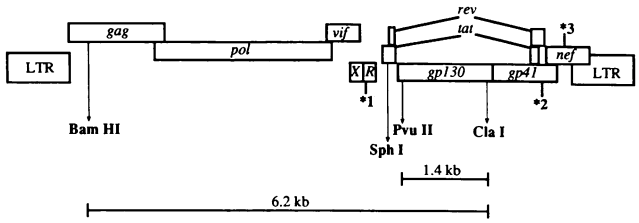


FIG. 1. Genetic organization of SIVmac. Genes coding for SIVmac proteins are shown. The complete nucleotide sequence for SIVmac is about 10.3 kb in length. *Bam*HI, *Sph*I, *Pvu*II, and *Cla*I restriction enzyme sites are conserved between SIVmac-1A11 and SIVmac-239; these sites were chosen to construct recombinant viral genomes. Details for the construction of these recombinant virus genomes are given in Materials and Methods. SIVmac-1A11 has a premature stop codon in the *vpr* gene (*1) and two premature stop codons in the transmembrane envelope glycoprotein (*2). SIVmac-239 has a premature stop codon in the *nef* gene (*3). LTR, long terminal repeat.

blood lymphocytes and macrophages. We mapped the ability of SIVmac-1A11 to form syncytia and to replicate in rhesus macaque macrophages to an internal 6.2-kb *Bam*HI-to-*Cla*I fragment. Subsequent analysis showed that a 1.4-kb fragment from SIVmac-1A11, contained within the 6.2-kb DNA fragment, was necessary for syncytium formation but was not sufficient for supporting a complete replication cycle in macrophages. This 1.4-kb DNA fragment encodes the external envelope glycoprotein gp130. Thus, we have mapped the viral determinants that are associated with macrophage tropism in the SIVmac system.

MATERIALS AND METHODS

Plasmid constructions to generate recombinant viral genomes. A schematic diagram of the genetic organization of SIVmac is shown in Fig. 1. Standard DNA cloning methods were employed to construct recombinant viral genomes (35). The proviral forms of both SIVmac-1A11 and SIVmac-239 were initially subcloned into a bacteriophage lambda vector (24, 36). All viral DNA fragments were subcloned into various bacterial plasmid vectors. The first set of recombinant viruses between SIVmac-1A11 and SIVmac-239, designated SIVmac-3C and SIVmac-4AB, was constructed by a reciprocal exchange of a 6.2-kb *Bam*HI-to-*Cla*I fragment (Fig. 1). These two restriction enzyme sites are conserved between SIVmac-1A11 and SIVmac-239.

The second pair of reciprocal recombinants was constructed to exchange the major envelope glycoprotein of SIVmac-239 and SIVmac-1A11 proviruses (Fig. 1). These two recombinant viruses were designated SIVmac-2311 and SIVmac-1A39. To make this exchange possible, two conserved sites (i.e., *Pvu*II and *Cla*I) between SIVmac-1A11 and SIVmac-239 clones were chosen. *Pvu*II cuts exactly 20 bp downstream from the start codon of gp130. *Cla*I cuts the envelope in a region that corresponds to about 35 amino acids upstream of the processing site between gp130 and the transmembrane envelope glycoprotein. Since *Pvu*II cuts many times within the provirus, the unique *Sph*I site upstream of the *Pvu*II site was used to make the replacement possible (Fig. 1).

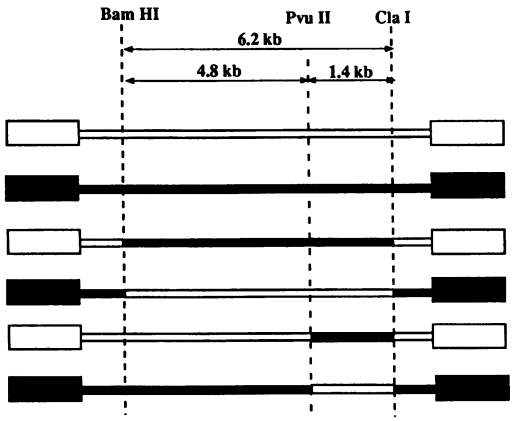
Mapping with the *Dra*I restriction enzyme was performed to validate the nature of the gp130 *env* recombinants. There are two *Dra*I sites in the *Pvu*II-to-*Cla*I fragment of SIVmac-1A11, resulting in a diagnostic fragment with an approximate size of 0.3 kb. The same fragment of SIVmac-239 has a single *Dra*I site. Further validation of these recombinant viruses came from Southern blot analysis of restriction enzyme digestions of total cellular DNA isolated from the infected cells (data not shown). Details of the construction procedures and plasmid maps of SIVmac subclones can be obtained from the corresponding author by written request.

Transfection of subcloned pieces of parental and recombinant clones to generate biologically active virus. Transfections of SIVmac plasmid subclones linearized by treatment with the appropriate restriction enzyme were performed by electroporation to rescue infectious SIVmac (1, 2, 24, 36). In some experiments, gel-purified SIVmac DNA fragments were ligated with T4 DNA ligase prior to transfection. For all transfections, 5 to 10 μg of DNA (at a 1:1 molar ratio) was cotransfected into CEM×174 cells by electroporation by using a Bio-Rad gene pulser and extender (Bio-Rad, Richmond, Calif.). Recovery of infectious virus was confirmed by several criteria that are described in Results.

Cell lines, viruses, and virus titration. CEM×174 cell line, a B × T somatic cell hybrid, is extremely sensitive to SIVmac-induced cytopathology (21). We used this cell line for all transfections of SIVmac DNA to recover biologically active virus because both SIVmac-1A11 and SIVmac-239 grow efficiently in CEM×174 cells. After transfection, culture supernatants known to contain infectious virus were used to infect new cultures of CEM×174 cells to generate virus stocks as described previously (1). CEM×174 cells were also used for titration of these virus stocks. The number of infectious units was quantitated by performing an end-point dilution assay in a 96-well plate format and monitoring the cells for both induction of cytopathology and virus production as detected by an enzyme-linked immunosorbent assay (ELISA) for SIV p27^{gag} (33). A stock of uncloned SIVmac isolate from which SIVmac-1A11 was molecularly cloned, referred to as SIVmac-BIO (biological), was also propagated in CEM×174 cells, and the titer of the virus was determined. This virus was used as a control in some experiments (1, 36).

Infection of rhesus macaque PBMC. PBMC were obtained from the venous blood of rhesus macaques housed at the California Regional Primate Research Center. These animals were seronegative for SIVmac, simian type D AIDS retrovirus, and simian T-cell leukemia virus. Gradient centrifugation with lymphocyte separation medium (Organon Teknika, Durham, N.C.) was used to obtain rhesus macaque PBMC. Cells were counted and plated in RPMI 1640 medium containing 0.5 μg of staphylococcal enterotoxin A (Toxin Technology, Madison, Wis.) and 20 U of interleukin-2 (Cellular Products, Buffalo, N.Y.) per ml and supplemented with 10% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM glutamine for 48 h prior to infection. Cells were infected with titered stocks of SIVmac at an approximate multiplicity of infection of 0.01 per cell. Cells were maintained in the same medium containing interleukin-2 but not staphylococcal enterotoxin A after infection with SIVmac. Culture supernatants were assayed regularly for the presence of SIV p27^{gag}.

Infection of primary cultures of rhesus macaque macrophages. Macrophage cultures were established from rhesus macaque PBMC by using conditioned medium from a human fibrous histiocytoma cell line, GCT, as described previously (1). Macrophages were infected at a multiplicity of infection of approximately 0.1 per cell. After infection, cultures were fed with fresh medium at intervals of 3 to 4 days, and culture supernatants were assayed for reverse transcriptase (RT)



	Macrophage Infection			PBMC Infection Kinetics
	Syncytial giant cells	p27 *gag*	RT Activity	
SIVmac-1A11	+	+	+	Delayed
SIVmac-239	-	-	-	Rapid
SIVmac-3C	-	ND	-	Rapid
SIVmac-4AB	+	ND	+	Delayed
SIVmac-1A39	-	-	-	Delayed
SIVmac-2311	+	+	-	Rapid

FIG. 2. Summary of results of virus replication in cultures of macrophages and PBMC. Open regions, SIVmac-1A11 sequences; closed regions, SIVmac-239 sequences. For macrophage infection, columns are as follows: Syncytial giant cells, formation of multinucleated giant cells; p27 *gag*, detection of SIV $p27^{gag}$ by immunocytochemical analysis of infected macrophages by using a mouse monoclonal antibody against SIV $p27^{gag}$; RT activity, particle-associated RT activity detected in the supernatant fluid recovered from infected macrophage cultures. ND, not done. The pattern of replication kinetics in PBMC is based on pairwise comparisons of the parental viruses (SIVmac-239 and SIVmac-1A11) and the two sets of recombinants (SIVmac-3C and SIVmac-4AB; SIVmac-1A39 and SIVmac-2311).

activity (20). Immunocytochemical analysis of macrophages with a monoclonal antibody to SIVmac $p27^{gag}$ was performed as previously described (1, 48).

Nucleotide sequence accession number. The complete nucleotide sequence of gp130 *env* of SIVmac-1A11 has been deposited in GenBank and is available under accession no. M74144 (41).

RESULTS

Construction and recovery of recombinant viruses by transfection. In a previous article, we reported that SIVmac-1A11 replicated efficiently in cultures of rhesus macaque macrophages but that SIVmac-239 did not replicate in this cell type (1). To map the genetic determinant(s) responsible for cytopathology and cell tropism, recombinant viruses were constructed from molecular clones of SIVmac-1A11 and SIV mac-239. Reciprocal recombinants between the cloned genomes of these two viruses were made to test the criteria of necessity and sufficiency. Limitations on the construction of recombinant viruses were imposed by the location of restriction enzyme sites that are conserved between the two viral genomes (Fig. 1).

One pair of reciprocal recombinant viruses was designed to narrow the range of viral genes that would be included or excluded as determinants of cytopathology and cell tropism. This pair of recombinants involved an exchange of a large internal DNA fragment which is 6.2 kb long and encompasses sequences from the *Bam*HI site in the *gag* gene to the *Cla*I site close to the junction of the external and transmembrane domains of the envelope glycoprotein (Fig. 1). The 6.2-kb *Bam*HI-to-*Cla*I fragment includes a portion of the *gag* gene, all of the *pol*, *vif*, *vpx*, and *vpr* genes, the first coding exons of *tat* and *rev*, and the gene encoding external envelope glycoprotein gp130 (Fig. 1). These recombinant viruses are designated SIVmac-3C and SIVmac-4AB (Fig. 2).

The second pair of recombinant viruses was designed to focus on the gene for the external envelope glycoprotein (Fig. 1). In the HIV-1 system, the external envelope glycoprotein gp120 has been shown to determine macrophage tropism (5, 39, 45). This pair of SIV recombinant genomes involved a reciprocal exchange of a 1.4-kb *Pvu*II-to-*Cla*I DNA fragment in the gene for the external envelope glycoprotein gp130. These recombinant viruses are designated SIVmac-1A39 and SIVmac-2311. *Pvu*II cleaves SIVmac-1A11 and SIVmac-239 DNA at a site 20 bp downstream from the start codon of gp130 *env*. *Cla*I cleaves within the *env* gene of both viral genomes at a site 35 codons upstream from the processing site between gp130 and the transmembrane glycoprotein. The first 22 amino acids encoded by the *env* gene act as a signal peptide that is removed by a posttranslational cleavage (9). The predicted amino acid sequence of the signal peptides for SIVmac-1A11 and SIVmac-239 are identical. Thus, SIVmac-4AB and SIVmac-2311 encode the same envelope glycoprotein molecule (Fig. 2). Similarly, SIVmac-3C and SIVmac-1A39 code for an identical envelope glycoprotein molecule (Fig. 2).

A strategy identical to that of a previously reported procedure was employed to recover biologically active virus by transfection of plasmids containing cloned viral DNA into permissive human lymphoid cells (1, 53). Appropriate DNA fragments for the two sets of reciprocal recombinants and two parental viruses were introduced by transfection into CEM×174 cells. The presence of biologically active virus in transfected cultures was confirmed by (i) induction of cytopathic effects characterized by multinucleated giant cells, (ii) particle-associated RT activity and SIV $p27^{gag}$ in culture supernatant, (iii) passage of virus to new cultures by using cell-free supernatant, and (iv) restriction enzyme analysis and Southern blotting of whole-cell DNA from infected cultures (data not shown). Virus stocks in CEM×174 cells were prepared. Titers of these stocks were determined by an end-point dilution assay by testing for both induction of cytopathology and virus production by an ELISA for detection of SIV $p27^{gag}$ (33).

Replication of recombinant viruses in rhesus macaque PBMC. The replication efficiency of parental and recombinant viruses in rhesus macaque PBMC cultures infected at an approximate multiplicity of infection of 0.01 per cell was measured. Culture supernatants were monitored for the presence of SIV $p27^{gag}$ antigen at 2- to 3-day intervals. All recombinant viruses replicated in rhesus macaque PBMC, albeit with various degrees of efficiency (Fig. 3). In three independent experiments, SIVmac-1A11 consistently

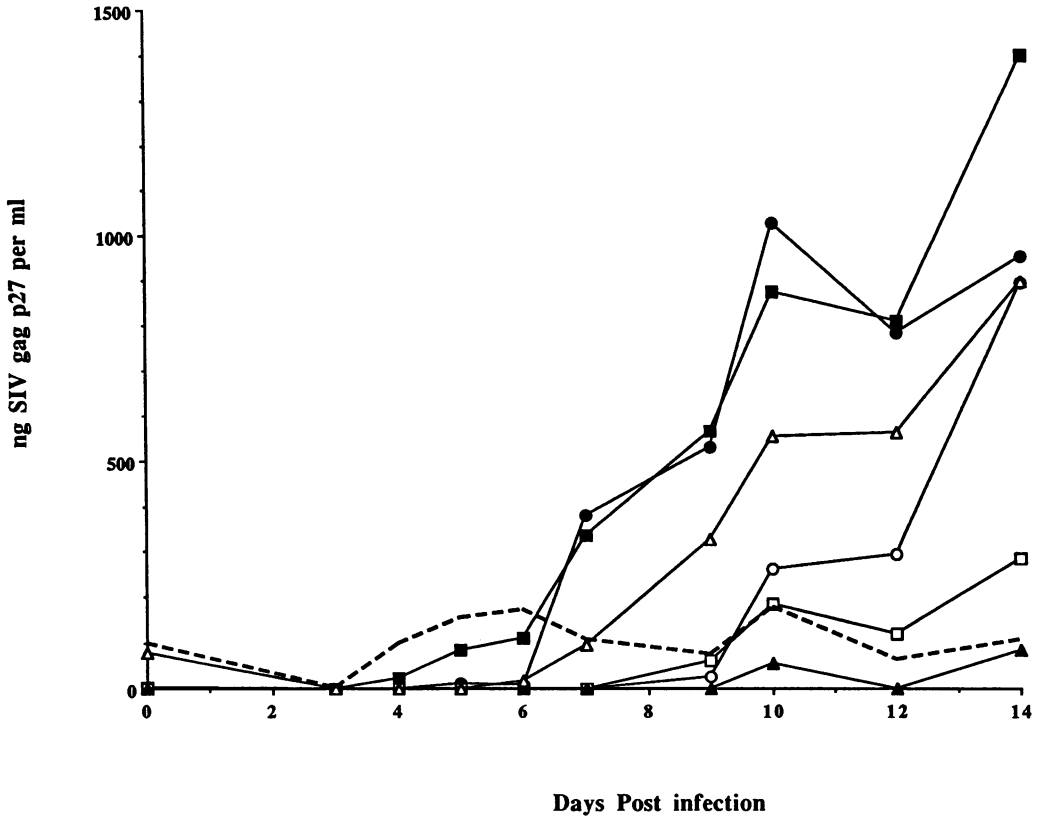


FIG. 3. Replication of viruses in rhesus macaque PBMC. PBMC from rhesus macaques were cultured and infected with stocks of SIVmac whose titers had been determined as described in Materials and Methods. Culture supernatants were sampled at various times and assayed for the presence of SIV p27^{gag}. Time zero represents the analysis of supernatant obtained from the final wash immediately following infection. Background obtained from the uninfected culture supernatant was subtracted from values for all time points. Structures of recombinant viral genomes are shown in Fig. 2. ○, SIVmac-1A11; ●, SIVmac-239; □, SIVmac-4AB; ■, SIVmac-3C; △, SIVmac-2311; ▲, SIVmac-1A39; – – –, SIVmac-BIO.

showed delayed growth kinetics in rhesus macaque PBMC compared with SIVmac-239 (Fig. 3). Replication of SIVmac-4AB was delayed with respect to the reciprocal recombinant, SIVmac-3C. Replication efficiency of SIVmac-1A39 was delayed with respect to the reciprocal recombinant, SIVmac-2311 (Fig. 3). In the experiment for which the results are shown in Fig. 3, SIVmac-1A39 did not replicate at a high level; however, in a separate experiment, this virus replicated more efficiently and its replication was delayed with respect to that of SIVmac-2311 (Fig. 2). Differences in levels of SIVmac replication are observed in PBMC from individual rhesus macaques (our unpublished results).

Replication of recombinant viruses in rhesus macaque monocyte-derived macrophages. In a previous study, we demonstrated that SIVmac-1A11, unlike SIVmac-239, induces syncytia and produces particle-associated RT activity in cultures of rhesus macaque monocyte-derived macrophages (1). Figure 4 is a composite of micrographs obtained from infected macrophage cultures at 10 days postinfection. SIVmac-1A11- and SIVmac-4AB-infected macrophages showed many syncytia (Fig. 4A and D). SIVmac-239- and SIVmac-3C-infected cultures did not show any syncytia (Fig. 4B and C). Thus, the genetic element responsible for macrophage tropism was provisionally assigned to the 6.2-kb internal *Bam*HI-to-*Cla*I fragment of SIVmac-1A11 (Fig. 1).

Supernatant fluids from infected macrophage cultures were analyzed for particle-associated RT activity. SIVmac-1A11 and SIVmac-4AB produced significant levels of RT activity in the medium, whereas significant RT activity was not detected in supernatant fluid from cultures infected with SIVmac-239 and SIVmac-3C (Table 1, experiment 1). The levels of RT activity detected after infection with SIVmac-1A11 were comparable to the levels of RT activity in cultures infected with SIVmac-4AB (Table 1, experiment 1). These results indicated that a protein (or proteins) encoded by the internal 6.2-kb *Bam*HI-to-*Cla*I fragment from SIVmac-1A11 was necessary and sufficient for conferring macrophage tropism.

The second set of recombinant viruses that involved exchange of DNA fragments encoding gp130 *env* were also evaluated for replication in macrophages. A panel of macrophage cultures infected with parental viruses (SIVmac-1A11 and SIVmac-239) and recombinant viruses (SIVmac-1A39 and SIVmac-2311), as well as the uncloned SIVmac (designated SIVmac-BIO), is shown in Fig. 5. Syncytia were observed in cultures infected with SIVmac-1A11, SIVmac-2311, and SIVmac-BIO. Similar syncytia were not induced either in cultures infected with SIVmac-239 and SIVmac-1A39 or in the mock-infected culture. Identical results were obtained when this experiment was repeated with macrophage cultures established from two other donor animals.

In contrast to cultures infected with SIVmac-1A11 and SIVmac-4AB (Table 1, experiment 1), significant RT activity could not be detected in cultures infected with SIVmac-2311

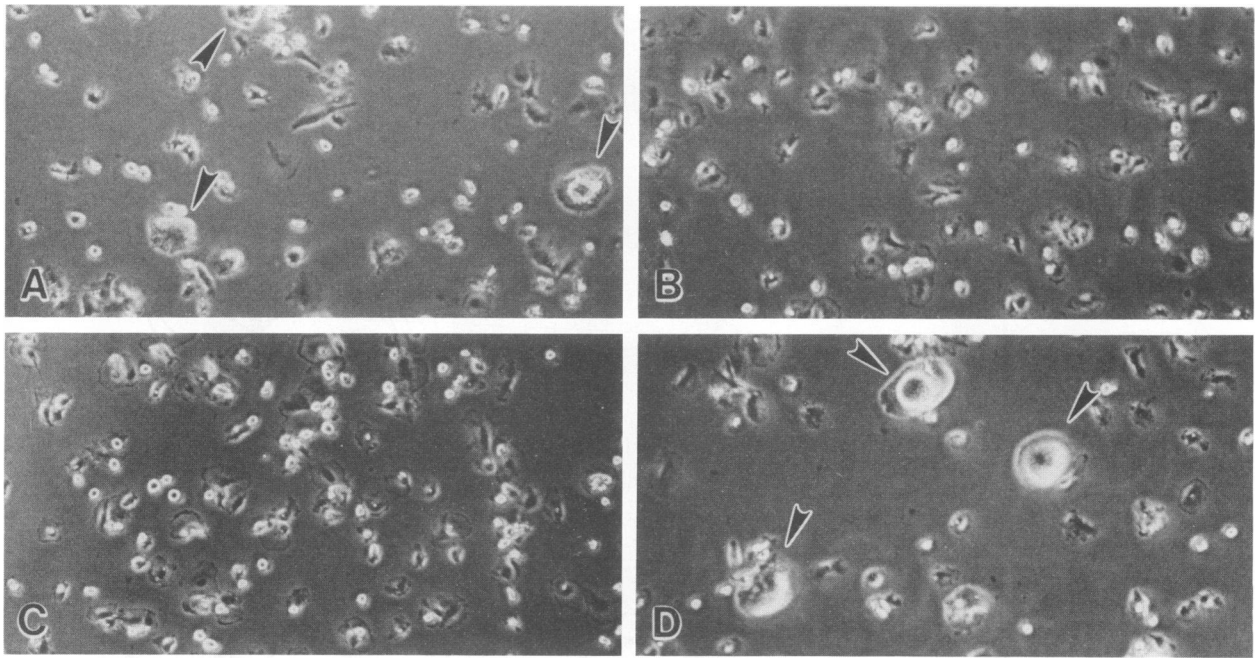


FIG. 4. Photomicrograph of cytopathology in infected macrophage cultures. Macrophage cultures were established as previously described from healthy, seronegative donor macaques (1). Infections with virus stocks that were previously titered on CEM×174 cells were carried out as described in Materials and Methods. Photomicrographs were taken at 10 days postinfection. (A) SIVmac-1A11; (B) SIVmac-239; (C) SIVmac-3C; (D) SIVmac-4AB. Arrowheads indicate syncytia. Magnification, ×100.

(Table 1, experiment 2). We therefore analyzed macrophages infected with SIVmac-2311 and SIVmac-1A39 for the presence of SIV viral antigen. A mouse monoclonal antibody raised against SIV $p27^{gag}$ was used for immunocytochemical analysis of the infected cells (48). Multinucleated giant cells in SIVmac-2311 cultures stained positive with the anti-SIV $p27^{gag}$ antibody (data not shown). No multinucleated giant cells or staining in cultures infected with SIVmac-1A39 was observed (Fig. 5C). These observations suggest that the release of SIVmac-2311 into culture supernatants might be blocked even though viral structural proteins were expressed. SIVmac-2311 productively infected cultures of rhesus macaque PBMC (Fig. 3) as well as CEM×174 cells (data not shown). Taken together, these results indicate that the 1.4-kb *Pvu*II-to-*Cla*I fragment from SIVmac-1A11 is necessary and sufficient for the induction of syncytia in macrophages (Fig. 1). Thus, the external envelope glycoprotein (i.e., gp130) of SIVmac-1A11 is essential for syncytium formation in this cell type.

TABLE 1. Virus production in rhesus macaque macrophage cultures infected with parental and recombinant viruses

Clone	RT activity (10^3) at days postinfection[a]					
	0	5	8	11	15	19
Experiment 1						
SIVmac-1A11	0.1	166.3	1,062.2	765.3	432.4	488.7
SIVmac-239	0.1	10.7	9.6	9.2	10.0	11.0
SIVmac-3C	0.4	10.0	8.4	8.3	7.1	11.3
SIVmac-4AB	0.1	156.3	641.0	436.1	479.3	316.4
Experiment 2						
SIVmac-1A11	0.2	20.5	245.9	864.3	895.1	793.3
SIVmac-239	<0.1	9.4	9.7	9.1	8.8	8.7
SIVmac-1A39	<0.1	9.2	9.3	7.5	12.0	12.3
SIVmac-2311	<0.1	9.8	10.7	10.4	10.3	8.8

[a] Cultures of rhesus macaque macrophages were infected with SIVmac clones as described in Materials and Methods. Supernatant fluid recovered from each macrophage culture was collected at each time interval indicated and assayed for particle-associated RT activity as previously described (20). Time zero represents RT activity in the supernatant fluid obtained from the final wash immediately following infection. Each RT value is given as counts per minute per milliliter of culture supernatant and represents an average of duplicate samples collected at the indicated times. Background counts were subtracted from values at all individual time points.

DISCUSSION

Identification of the viral determinant(s) of cell tropism is an important step for elucidating molecular mechanisms of viral pathogenesis. This report presents the construction and in vitro analysis of recombinant viruses generated between molecular clones of SIVmac, SIVmac-1A11 and SIVmac-239, which show differential tropism for rhesus macaque macrophages in cell culture systems (1). The ability of SIVmac-1A11 to form syncytia and to replicate in macrophages was initially mapped to an internal 6.2-kb *Bam*HI-to-*Cla*I fragment (Fig. 2). This observation excluded the *nef* gene, the transmembrane envelope glycoprotein, and the long terminal repeat from playing essential roles in macrophage tropism of SIVmac. Further mapping showed that the 1.4-kb viral DNA fragment contained within the larger 6.2-kb fragment was responsible for the property of syncytium formation and, thus, entry of SIVmac-1A11 into rhesus macaque macrophages (Fig. 2). This 1.4-kb DNA fragment exclusively encodes the external envelope glycoprotein gp130 (Fig. 1).

To our surprise, macrophage cultures infected with SIV mac-2311 did not produce a significant level of RT activity compared with cultures infected with SIVmac-1A11 or SIV

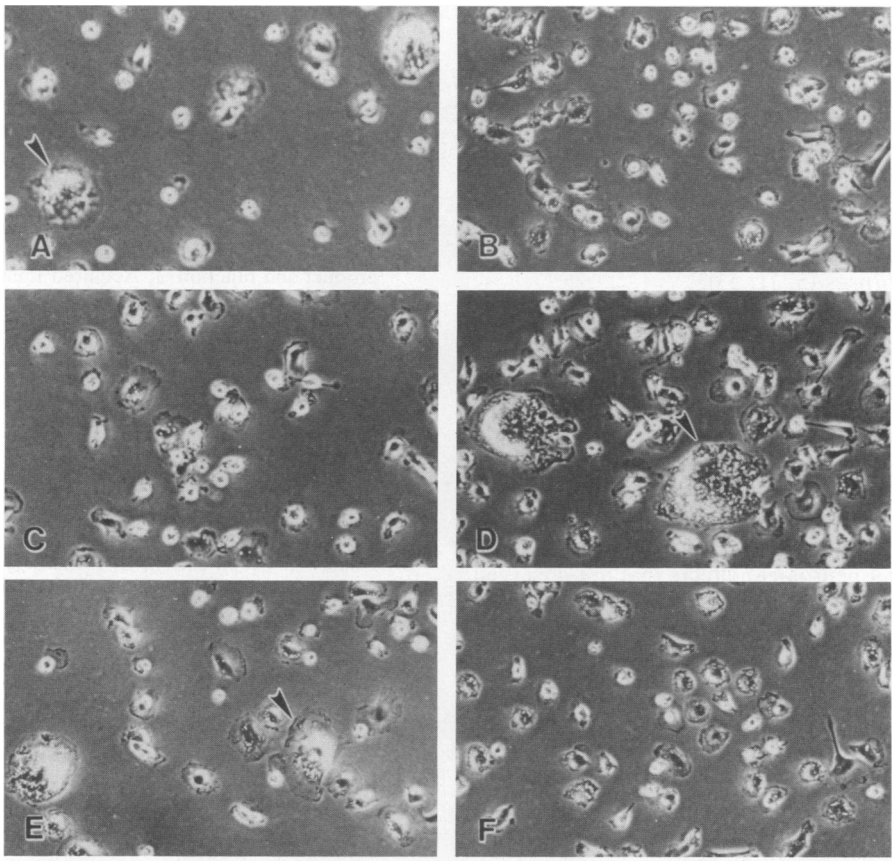


FIG. 5. Photomicrograph of cytopathology in infected macrophage cultures. Macrophage cultures were established and infected as described in Materials and Methods. Photomicrographs were taken at 10 days postinfection. (A) SIVmac-1A11; (B) SIVmac-239; (C) SIVmac1A39; (D) SIVmac-2311; (E) SIVmac-BIO; (F) mock-infected culture. Arrowheads indicate syncytia. Magnification, ×100.

mac-4AB (Table 1, experiment 2, and Fig. 2). SIVmac-2311 readily induced syncytia in rhesus macaque macrophages, and these cells stained positive with an anti-SIV *gag* monoclonal antibody. The envelope glycoprotein molecules encoded by SIVmac-4AB and SIVmac-2311 are identical. SIVmac-2311 readily replicates in both CEM×174 cells and rhesus macaque PBMC (Fig. 3). Thus, gp130 *env* of SIVmac-1A11 is necessary for entry of the virus into macrophages but is not sufficient for a complete viral replication cycle in these cells. A gene product other than gp130 *env* that is encoded within the 4.8-kb *Bam*HI-to-*Pvu*II DNA fragment appears to be responsible for differential control of virus production in macrophages (Fig. 2). Recently, the HIV-1 Vif has been implicated in processing of the viral envelope glycoprotein (16). Previous data had also suggested that Vif acts at a posttranslational level (10, 47). Analysis of additional SIV recombinants will be necessary to determine whether Vif plays a role in virus production in macrophages.

In the HIV-1 system, Vpr has been implicated in accelerating virus replication in cultures of T cells (6, 40). SIVmac-239 encodes a full-length *vpr*, but the SIVmac-1A11 *vpr* gene is truncated such that a peptide of only 11 amino acids is encoded (34, 43). In our tissue culture assessments, SIVmac-1A11 and recombinant viruses which contained the truncated *vpr* gene (i.e., SIVmac-4AB and SIVmac-1A39) showed delayed kinetics of replication in cultures of rhesus macaque PBMC, whereas SIVmac-239 and recombinant viruses with an intact *vpr* (i.e., SIVmac-3C and SIVmac-2311) showed a relatively rapid replication compared with their counterparts (Fig. 2 and 3). Further studies will be necessary to establish the precise function of Vpr in the life cycle of SIVmac. The observation that SIVmac-1A11 consistently showed delayed kinetics of replication in cultures of rhesus macaque PBMC compared with SIVmac-239 may have implications for virus infection in macaques. It is possible that the slow replication kinetics of SIVmac-1A11 in the initial stages of infection in macaques would give the host an ample opportunity to mount an effective antiviral immune response. In contrast, SIVmac-239 may replicate more rapidly in animals and thus outpace the host immune responses. These ideas are supported by the observation that SIVmac-1A11-infected animals were transiently viremic but SIVmac-239-infected animals were persistently viremic (23, 36–38).

In the HIV-2 system, mutational inactivation of the *vpr* gene (sbl/isy clone) abrogated the ability of this virus to replicate in macrophage cultures (17). Our data are not consistent with the contention that *vpr* alone is the major determinant for macrophage tropism of SIVmac, because *vpr* of SIVmac-1A11, a virus that is macrophage tropic, is truncated after the first 11 amino acids. A role for the HIV-1 Vpr with respect to macrophage tropism remains to be explored.

The envelope glycoprotein of both HIV and SIV is the major determinant for T-cell tropism; it is necessary and sufficient for induction of cytopathology in T-lymphoid cells (28, 32, 46). Recombinant viruses constructed between mo-

lecular clones of HIV-1 that showed a differential cell tropism for macrophages have been used to map viral determinants of macrophage tropism. In vitro analysis of these HIV-1 recombinants has demonstrated that the viral determinant for macrophage tropism is located within the external envelope glycoprotein gp120 exclusive from the principal CD4 binding domain (39, 45). Other in vitro properties of HIV-1 such as efficiency of replication in T cells and the ability of certain isolates to down-modulate surface expression of the CD4 molecule have also been mapped by using recombinant viruses (5, 11, 53).

In the HIV system, a strong correlation between neuropathogenesis and recovery of macrophage-tropic virus strains from infected patients has been established (4). Virus isolates recovered from the brain tissue of HIV-infected individuals with neurologic symptoms replicate efficiently in cultures of primary macrophages. In concordance with these in vitro observations, macrophages represent the predominant cell type that makes up the lesions and harbors HIV in the central nervous system (52). However, in some cases, the neuropathologic lesions appear to be insufficient to account for the global neurologic effects that are observed. Thus, central nervous system disease may be an indirect consequence of infection of cells in the macrophage lineage. Recent reports suggest that HIV-infected macrophages produce soluble factors that are toxic to central nervous system tissue (15, 42, 51). The use of SIV infection of rhesus macaques as an animal model for HIV infection and AIDS offers an opportunity to test the relevance of in vitro properties of viruses to pathogenesis. In the SIV system, 50 to 60% of infected macaques show encephalopathy and signs of neurologic involvement (29, 44). All of the recombinant viruses described in this report have been inoculated into rhesus macaques to analyze clinical symptoms and tissue distribution of the virus. These animal studies will be critical for evaluation of the significance of macrophage tropism for pathogenesis in the SIVmac system.

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ADDENDUM

A recent report by Kestler et al. suggests that the *nef* gene of SIVmac does not play a role in macrophage tropism (22); this finding is consistent with results presented in this report.

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