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Human and Simian Immunodeficiency Virus Capsid Proteins Are Major Viral Determinants of Early, Postentry Replication Blocks in Simian Cells

Christopher M. Owens,[1] Peter C. Yang,[1] Heinrich Göttlinger,[1] and Joseph Sodroski[1,2]*

Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute,[1] and Department of Pathology, Division of AIDS, Harvard Medical School, and Department of Immunology and Infectious Diseases, Harvard School of Public Health,[2] Boston, Massachusetts 02115

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The cells of most Old World monkey species exhibit early, postentry restrictions on infection by human immunodeficiency virus type 1 (HIV-1) but not by simian immunodeficiency virus of macaques (SIV_{mac}). Conversely, SIV_{mac}, but not HIV-1, infection is blocked in most New World monkey cells. By using chimeric HIV-1/SIV_{mac} viruses capable of a single round of infection, we demonstrated that a major viral determinant of this restriction is the capsid (CA) protein. The efficiency of early events following HIV-1 and SIV_{mac} entry is apparently determined by the interaction of the incoming viral CA and species-specific host factors.

Human immunodeficiency virus type 1 (HIV-1) infection is limited to humans and chimpanzees, whereas simian immunodeficiency viruses (SIV) naturally infect Old World monkeys (5, 13, 24, 28). The replication of HIV-1 and SIV depends on host cell factors, some of which potentially govern the species tropism of the viruses. HIV-1 and SIV entry into target cells is dependent on host cell receptors, CD4 and the chemokine receptors (2, 3, 11, 12, 14–16, 18, 19, 32, 38). Polymorphism in these receptors limits the ability of these viruses to enter cells of New World monkeys (34a).

CD4 and the chemokine receptors of several Old World monkey species are able to support HIV-1 entry (10, 39). However, HIV-1 replication is greater than 100-fold lower in the peripheral blood mononuclear cells (PBMC) of rhesus macaques, an Old World monkey species, than SIV_{mac} replication (27). The products of HIV-1 reverse transcription are significantly reduced in rhesus macaque cells relative to those in human PBMC, suggesting that a major block to productive HIV-1 replication in these monkey cells occurs following entry of the viral core into the host cell but prior to completion of reverse transcription (5a, 11a, 27). Studies of HIV-SIV chimeras indicate that HIV-1 regions in the 5′ half of the genome are responsible for the observed restricted replication in macaque cells (35, 46). However, HIV-SIV chimeras containing the HIV-1 reverse transcriptase replicate efficiently in rhesus macaques, indicating that this viral protein is not sufficient to mediate the postentry restriction (4, 50). Additionally, because the viral Vif protein exerts its phenotypic effects in a manner dependent on the virus-producing cell (21, 23), Vif is less likely to be involved in this species-specific block to HIV-1 infection, which is determined by the target cell rather than the virus-producing cell. Thus, by process of elimination, components of the HIV-1 Gag protein may be responsible for determining the species-specific restrictions (17, 44–46). The Gag proteins of HIV-1 and other retroviruses function at several different levels of the retrovirus life cycle, including assembly, maturation, uncoating of the virion core following entry, and reverse transcription (22). HIV-1 Gag is initially synthesized as a 55-kDa polyprotein, p55, and is subsequently cleaved by the viral protease into the four mature Gag proteins p17 matrix, p24 capsid (CA), p7 nucleocapsid (NC), and p6 (22). The CA and NC domains of primate immunodeficiency virus Gag polyproteins are joined by a p2 linker region (1). Each of the four Gag proteins interacts with viral and host cell factors at various points in the viral life cycle (21, 22, 33, 37, 47, 48, 53). For example, efficient viral replication requires the incorporation of cyclophilin A into HIV-1 but not SIV_{mac} virions (7–9, 20, 37, 49). It is thought that the prolyl isomerase function of cyclophilin A is necessary for inducing a conformational change in the CA protein, facilitating the uncoating of the viral core following entry (6, 25, 36, 37). However, cyclophilin A does not appear to be responsible for the species-specific block to HIV-1 infection (52).

Cell type-specific factors influence the infectivity of other retroviruses, such as the murine leukemia virus (MuLV). The early phase of MuLV infection can be dominantly blocked by a host cell factor, the product of the *Fv1* allele (41–43). Restriction by *Fv1* abrogates MuLV infection after reverse transcription but prior to entry of the preintegration complex into the nucleus (30, 31). *Fv1*-mediated resistance is not absolute and can be at least partially overcome by high multiplicities of infection (26). The *Fv1* allele is derived from a murine endogenous retroviral *gag* gene, the product of which can influence the function of the incoming MuLV CA protein during postentry events (26, 34).

In this study, we tested the hypothesis that the HIV-1 and SIV_{mac} CA proteins determine the early restrictions on infection by these viruses in Old World and New World monkey cells, respectively. This hypothesis is consistent with the previous observation that an SIV_{mac} mutant expressing the HIV-1 CA-p2 domain replicated in human cells but failed to replicate

* Corresponding author. Mailing address: Dana-Farber Cancer Institute, 44 Binney St.—JFB 824, Boston, MA 02115. Phone: (617) 632-3371. Fax: (617) 632-4338. E-mail: joseph_sodroski@dfci.harvard.edu.

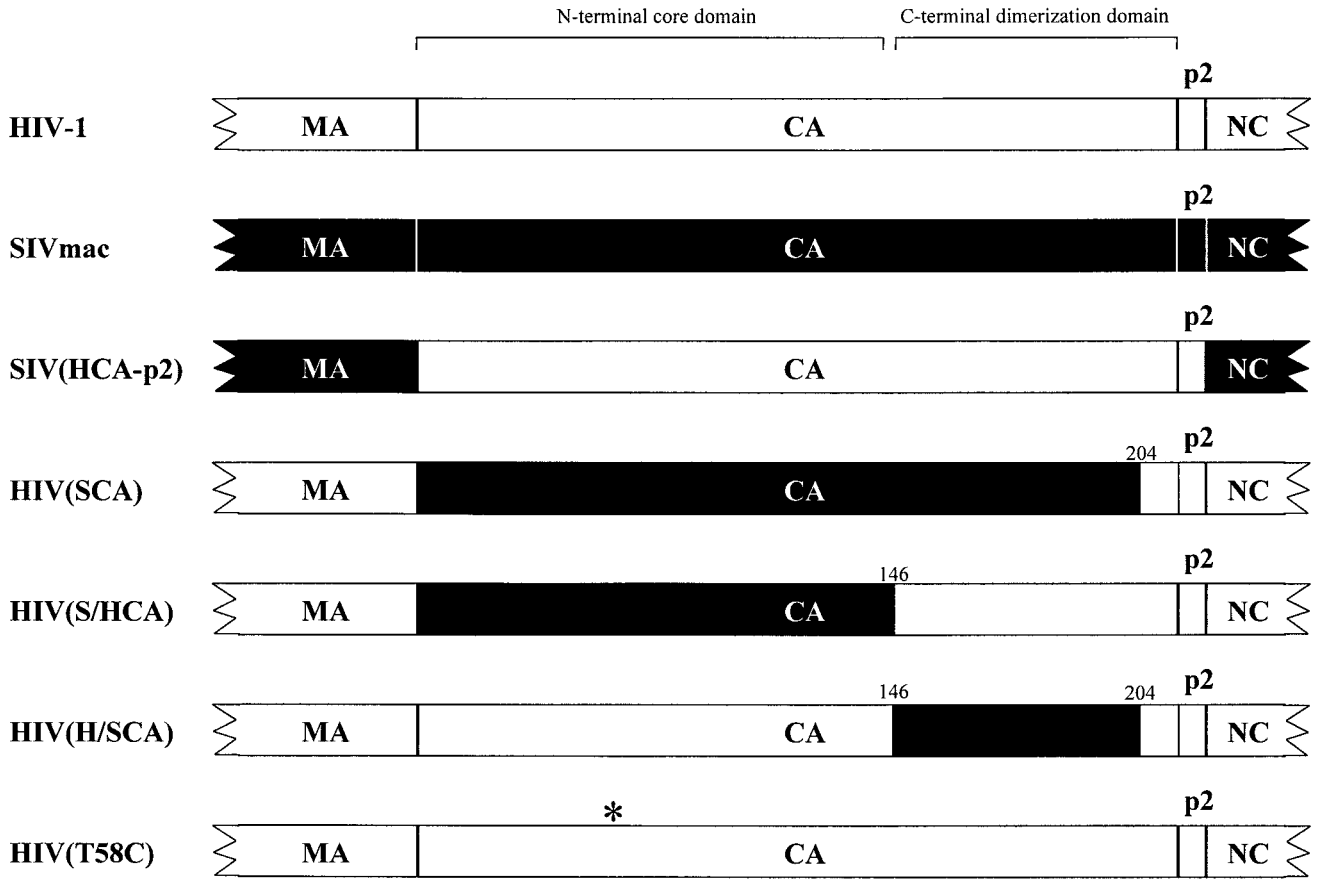

FIG. 1. HIV-1/SIV_{mac} recombinants. The name of the construct reflects the HIV-1 or SIV proviral backbone, and the names in parentheses designate the source of the CA or CA-p2 sequences (H = HIV-1; S = SIV_{mac}). The amino-terminal portions of the Gag polyproteins of the recombinant viruses used in this study are shown beneath the wild-type HIV-1 (white) and SIV_{mac}239 (black) sequences. The locations of the matrix (MA), CA, p2, and NC proteins are shown. The numbers represent the boundaries of the HIV-1 CA sequence that was replaced by the SIV_{mac} sequence. The locations of the N- and C-terminal domains of the CA proteins are shown. The asterisk represents the approximate location of a threonine-to-cysteine change at residue 58 of the HIV-1 CA protein.

in macaque PBMC, suggesting that this Gag region might play an important role in viral tropism (17). In the present study, we generated several HIV-1/SIV_{mac} Gag CA recombinants and mutants in the context of HIV-1 and SIV_{mac} vectors expressing green fluorescent protein (GFP). These recombinant viruses are capable of a single round of infection and allow assessment of the efficiency of the early phase of retrovirus replication. These viruses were analyzed for infection efficiency on a panel of cells, of which some are able to be infected comparably by HIV-1 and SIV_{mac} and others are restricted for infection by one of the viruses.

Construction of viruses with chimeric Gag proteins. To investigate the contribution of the HIV-1 and SIV_{mac} CA protein to infection of human and simian cells, viruses containing chimeric Gag proteins with HIV-1 (HXBc2 strain) and SIV_{mac}239 sequences were created. In the initial set of recombinants, the CA domain of either HIV-1 or SIV_{mac} was precisely substituted for the CA domain in the heterologous proviral vector. However, these chimeric vectors were found to be replication defective, even in human cell lines that support efficient HIV-1 and SIV_{mac} replication (data not shown). Further analysis revealed that the proteolytic processing of these chimeric Gag polyproteins at the CA-p2 junction was inefficient (data not shown). Therefore, additional chimeras were created or adapted for use in this study; in these chimeras, the C-terminal junction between HIV-1 and SIV_{mac} sequences is located either N terminal or C terminal to the CA-p2 cleavage site (Fig. 1). In the HIV(SCA) chimera, residues 1 to 204 of the HIV-1 CA were replaced by the corresponding SIV_{mac} CA residues, in the context of an HIV-1 vector. The HIV (H/SCA) construct contains most of the C-terminal CA domain of SIV_{mac}, replacing HIV-1 CA residues 146 to 204 in the HIV-1 vector. In both the HIV(SCA) and HIV(H/SCA) chimeras, the C-terminal SIV_{mac}/HIV-1 junction was positioned 26 amino acids N terminal to the CA-p2 cleavage site. The HIV(S/HCA) chimera contains the SIV_{mac} amino-terminal CA domain, replacing HIV-1 CA residues 1 to 146, in the HIV-1 vector. The SIV(HCA-p2) chimera contains the complete HIV-1 CA and p2 sequences in the context of an SIV vector, as previously described (17). Finally, an HIV-1 vector containing a single residue change (T58C) in CA was generated. Cysteine is found at the corresponding residue in the SIV, but not the HIV-1,

CA protein. The T58C mutant was included in the study for comparison.

All of the chimeric plasmids in this study were constructed by separate PCRs that amplified the regions of the *gag* gene that extended from within the sequences encoding the CA protein to the flanking sequences. The ends of the primers used to amplify the initial products were designed to overlap at the junctions between the SIV_{mac} and HIV-1 sequences, thus facilitating their assembly. In a final reaction, the chimeras were assembled by adding the same amount of each initial PCR product to a new PCR mixture and using nested 5′ and 3′ primers to drive the assembly reaction. Each mutant was subsequently cloned into either HIV-1 Gag/Pol-expressing plasmid pCMVΔP1ΔenvpA (40) or SIV_{mac} GFP-expressing proviral vector pSIvec1.GFP (29) by using the restriction enzyme combination *Bss*HII and *Psp*OMI or *Dra*III and *Sbf*I, respectively. All constructs were sequenced to verify the position of the HIV-1/SIV_{mac} junction and to rule out unwanted changes. Single-round replication-competent HIV-1 virions pseudotyped by the vesicular stomatitis virus (VSV) G glycoprotein were made as previously described (29, 51), by cotransfecting 293T cells with the GFP-expressing HIV-1 vector (pHIvec2.GFP), the appropriate HIV-1 *gag/pol* construct, pHCMV-G (a VSV G envelope glycoprotein-expressing plasmid), and a Rev-expressing plasmid (psRev) in a 10:10:2:1 ratio. Single-round SIV_{mac} virions were made by cotransfecting pSIvec1.GFP (or chimeras), pHCMV-G, and psRev in a 20:2:1 ratio as previously described (29). 293T cells were plated at a density of 10^6/100-mm-diameter plate 24 h prior to transfection. Cells were transfected by calcium phosphate precipitation and incubated for 10 h before washing and replacement of the medium with RPMI 1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. Approximately 36 h following transfection, the supernatant containing recombinant viruses was harvested, filtered (0.45-μm pore size), and frozen in aliquots. To determine the amount of virus in the medium, an aliquot of frozen supernatant was thawed and analyzed by the reverse transcriptase assay (35). All of the constructs represented in Fig. 1 produced readily detectable quantities of virus (data not shown).

Virion proteins of HIV-1/SIV_{mac} chimeras. To examine the protein composition of virions produced by the chimeric *gag* constructs, the above-described transfections were repeated with 293T cells without the addition of an envelope glycoprotein-expressing plasmid. At approximately 20 h following transfection, the medium was removed and the cultures were metabolically labeled with [^{35}S]methionine (50 μCi/ml) for 16 h. Virions released into the supernatant were pelleted through a 20% sucrose cushion as described previously (17). The pelleted viral particles were lysed and resuspended in radioimmunoprecipitation assay buffer and analyzed directly by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis and autoradiography.

The virion components consisted of the CA protein (p24 for HIV-1 and p27 for SIV_{mac}) and smaller Gag products (Fig. 2). Proteolytic processing of the chimeric Gag precursor proteins was qualitatively and quantitatively similar to that of the wild-type HIV-1 or SIV_{mac} protein, except for the presence of an additional 23-kDa protein that appeared in the HIV(H/SCA) chimera. The presence of this additional band may reflect aberrant processing of this chimeric Gag protein. Comparison

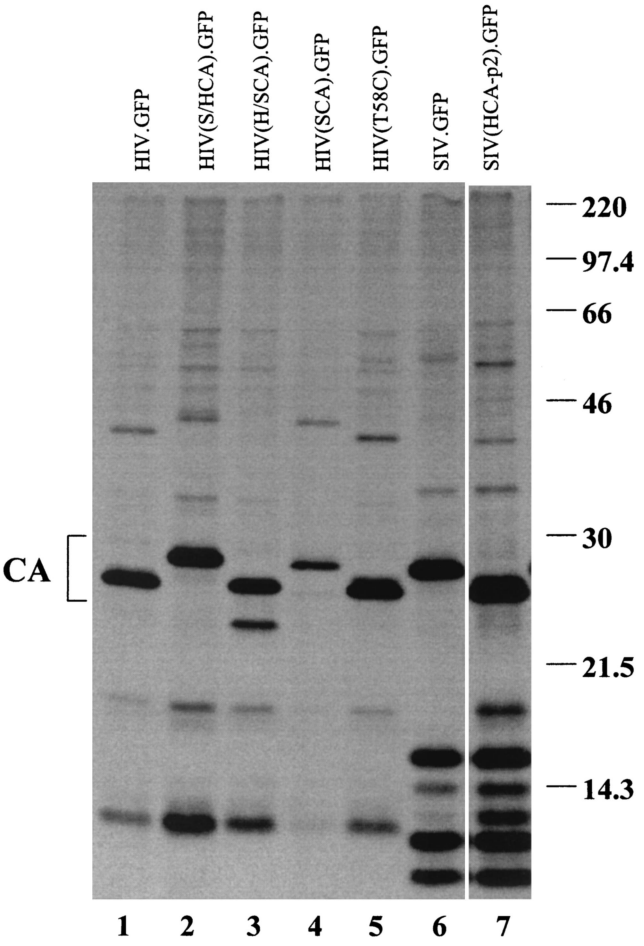

FIG. 2. Virion proteins produced by HIV-1/SIV_{mac} chimeras. 293T cells were transfected with plasmids HIV.GFP (lane 1), HIV(S/HCA).GFP (lane 2), HIV(H/SCA).GFP (lane 3), HIV(SCA).GFP (lane 4), HIV(T58C).GFP (lane 5), SIV.GFP (lane 6), and SIV(HCA-p2).GFP (lane 7). Following transfection, the cells were labeled with [^{35}S]methionine. Virions released into the cell supernatants were pelleted through 20% sucrose and resuspended in radioimmunoprecipitation assay buffer before analysis by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis and autoradiography. The location of the CA proteins is shown on the left. The values on the right are molecular sizes in kilodaltons.

of the migration of the HIV(S/HCA) CA protein (lane 2) with that of the HIV(H/SCA) (lane 3) CA protein suggests that the slower mobility of the SIV_{mac} CA protein compared with that of the HIV-1 CA protein is determined by the amino-terminal SIV_{mac} CA domain. Given the sequence similarities and equivalent expected sizes of the HIV-1 and SIV_{mac} amino-terminal CA domains, the basis for this migration difference is not apparent.

Infectivity of chimeric viruses in permissive and restricted target cells. HIV-1 and SIV_{mac} efficiently infect human PBMC and a number of human cell lines that express CD4 and coreceptors (2, 3, 10–12, 14–16, 18, 19, 38, 39). Furthermore, HIV-1 and SIV_{mac} vectors pseudotyped with the VSV G glycoprotein and capable of a single round of infection are able to infect many human cell lines and an occasional Old World monkey

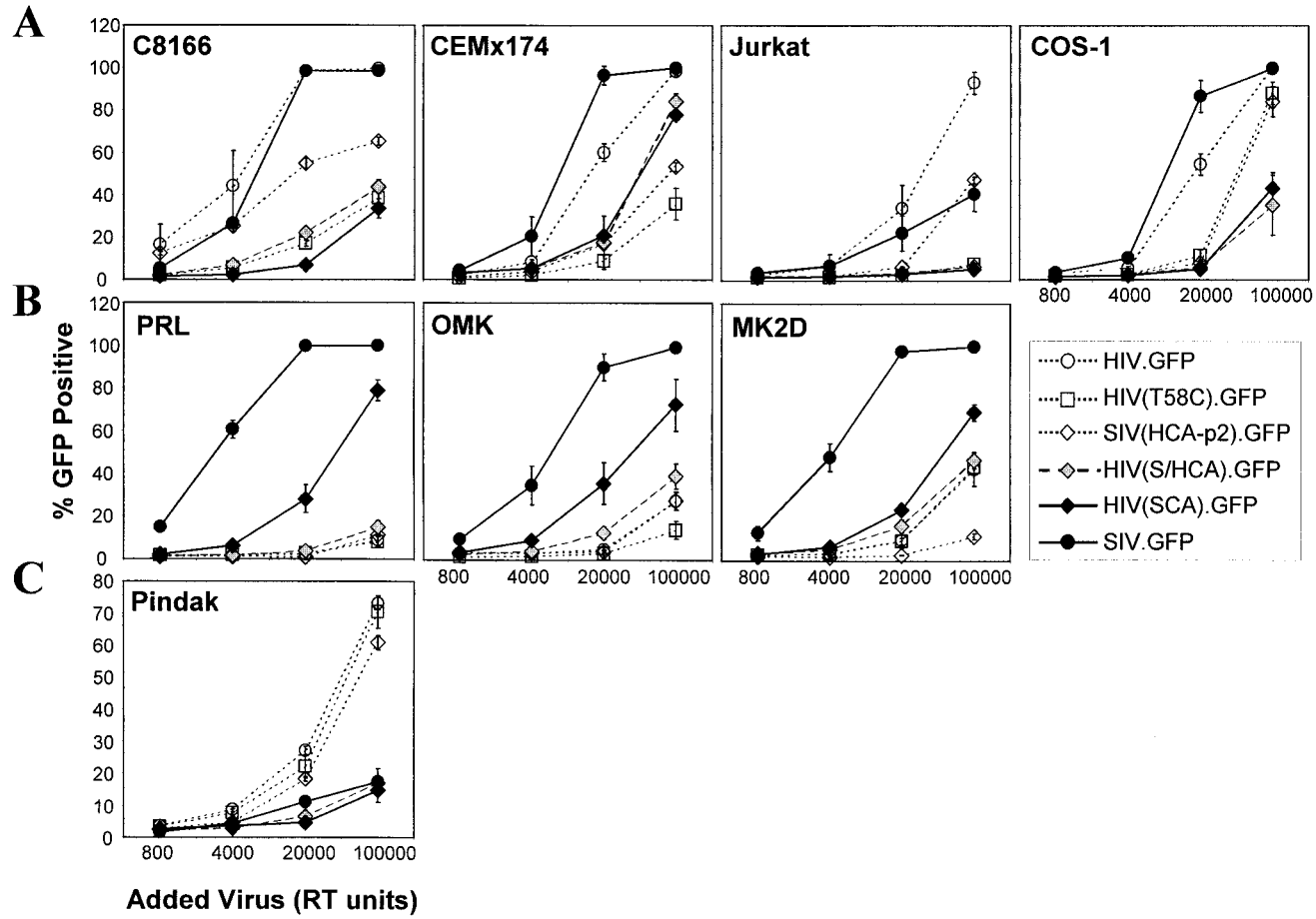

FIG. 3. Infection of cells by HIV-1/SIV_{mac} recombinants. Supernatants containing the indicated single-round HIV-1 and SIV_{mac} vectors, which encode GFP, were assessed for reverse transcriptase (RT) activity. The indicated number of RT units of virus was added to cells, which were incubated for 3 days prior to fluorescence-activated cell sorting analysis for GFP expression. The cells in the top row (A) are permissive for both HIV-1 and SIV_{mac}, the cells in the second row (B) are permissive for SIV_{mac} but restricted for HIV-1, and the cells in panel C are permissive for HIV-1 but restricted for SIV_{mac}. The symbols and lines used for the viruses are as follows: viruses with HIV-1 CA sequences, open symbols and dotted lines; viruses with SIV_{mac} CA sequences, black symbols and solid lines; virus with CA sequences derived from SIV_{mac} and HIV-1, gray symbol and dashed line.

cell line (e.g., COS-1) with comparable levels of efficiency (29). We assessed the ability of GFP-expressing vectors containing the chimeric *gag* genes to infect cell lines that had previously been shown to be permissive for both HIV-1 and SIV_{mac}: C8166 (human T lymphocytes), CEMx174 (human hybrid T/B lymphocytes), Jurkat (human T lymphocytes), and COS-1 (African green monkey kidney cells). Adherent cells in this study were plated 24 h prior to infection on 24-well plates at a density of 3×10^4 per well; suspension cells were plated on the day of infection at 7.5×10^4 per well. Thawed medium containing recombinant HIV-1 or SIV_{mac} was normalized on the basis of reverse transcriptase activity and added in fivefold serial dilutions to the cells, which were subsequently incubated for 3 days. Cells were then trypsinized if necessary, fixed in phosphate-buffered saline with 4% formaldehyde, and analyzed by fluorescence-activated cell sorting (Becton Dickinson FACScan). The average percentage of cells positive for GFP fluorescence and the standard deviation were calculated from at least four independent experiments.

The infectivity of HIV(H/SCA).GFP was negligible in all of the cells tested (data not shown). The aberrant proteolytic processing of the HIV(H/SCA) Gag precursor protein (Fig. 2, lane 3) may contribute to this poor level of infectivity. Figure 3A illustrates the results obtained with the other recombinant viruses in this panel of permissive cells. All four cell lines were infected efficiently by the HIV.GFP and SIV.GFP vectors. In general, the infectivities of the Gag chimeras were lower than that of either the HIV.GFP or the SIV.GFP vector. Apparently, some aspect of the postentry function of Gag is deleteriously affected in these chimeras, despite efficient virion production and processing of the Gag polyprotein. None of the chimeras was consistently more defective than the others in the permissive cell lines studied.

The infectivity of the GFP-expressing viruses was then studied in cell lines exhibiting postentry restrictions to HIV-1 but not SIV_{mac}. Primary rhesus lung (PRL) and MK2D cells were derived from rhesus macaques, which are Old World monkeys (29). Owl monkey kidney (OMK) cells are unusual among New World monkey cells in that they allow SIV_{mac} infection but restrict HIV-1 infection (29). OMK cells were included in this

study to compare the results obtained with atypical New World monkey cells with those obtained with representative Old World monkey cells. As expected, SIV.GFP infected each of these lines more efficiently than did HIV.GFP (Fig. 3B). HIV(T58C).GFP infected all three cell lines comparably to HIV.GFP. Notably, SIV(HCA-p2).GFP infected PRL, OMK, and MK2D cells with an efficiency equivalent to or less than that of HIV.GFP. Thus, the presence of the HIV-1 CA-p2 region in an SIV_{mac} background confers on the virus susceptibility to the early-phase restrictions operative in PRL, OMK, and MK2D cells. HIV(SCA).GFP infected all three HIV-1-restricted cell lines more efficiently than did HIV.GFP, in contrast to the more attenuated phenotype of HIV(SCA).GFP in permissive cells. This result suggests that the presence of the SIV_{mac} CA in the context of HIV-1 allows the virus to bypass restrictions on HIV-1 replication operative in PRL, OMK, and MK2D cells. HIV(S/HCA).GFP did not infect the HIV-restricted cell lines as efficiently as HIV(SCA).GFP, indicating that the amino-terminal SIV_{mac} CA domain is not sufficient to allow bypass of the HIV-1 restriction.

The infectivity of the chimeric viruses in a cell line restricted for SIV_{mac} but permissive for HIV-1 was examined. A cell line, Pindak, derived from squirrel monkeys, a New World species, was used as a representative target cell. As expected, HIV.GFP was significantly more efficient than SIV.GFP at infecting Pindak cells (Fig. 3C). The viruses with the HIV-1 CA domain, HIV(T58C).GFP and SIV(HCA-p2).GFP, infected Pindak cells almost as efficiently as did HIV.GFP. By contrast, viruses with SIV_{mac} CA components, HIV(SCA).GFP and HIV(S/HCA).GFP, inefficiently infected these cells. Thus, the CA protein appears to be a major viral determinant of the early-phase SIV_{mac} restriction in Pindak cells. The amino-terminal domain of the SIV_{mac} CA protein is apparently sufficient to render the virus susceptible to the Pindak cell restriction.

In this study, we created HIV-1 and SIV_{mac} vectors containing chimeric Gag proteins. The data obtained with the subset of these chimeric viruses competent for a single round of infection indicate that the CA protein is a major determinant of the early-phase, postentry HIV-1 or SIV block observed in nonhuman primates. HIV(SCA).GFP, which contains the SIV_{mac} CA protein in an HIV-1 background, exhibited some decrease in the ability to infect permissive human and COS-1 cells, compared with HIV.GFP or SIV.GFP. Despite this generalized decrease in infectivity, HIV(SCA).GFP efficiently infected three cell types, including PRL cells, that are restricted for HIV-1 infection. In each case, HIV(SCA).GFP infected the cells more efficiently than did wild-type HIV.GFP. In these same cells, SIV(HCA-p2).GFP, which contains the HIV-1 CA-p2 region in an SIV_{mac} background, was as inefficient as HIV.GFP. Thus, even though SIV(HCA-p2).GFP exhibited a reasonable ability to infect cells permissive for HIV-1 infection, infection by this virus was inefficient in Old World monkey cells in which HIV-1 is restricted. These results strongly implicate the viral CA as a determinant of this restriction.

SIV_{mac}, but not HIV-1, encounters early-phase, postentry restrictions in many New World monkey cells. The chimeric viruses with the HIV-1 CA domain efficiently infected a New World monkey cell line, Pindak. By contrast, chimeric viruses with the SIV_{mac} CA domain exhibited much less efficient infection of these cells. Thus, it appears that viral CA determinants mediate early-phase blocks to primate immunodeficiency viruses in cells derived from Old World and New World monkeys.

Further research is required to determine whether the mechanisms of viral restriction are similar in Old World and New World monkeys. This line of inquiry will be assisted by a precise definition of the CA region that specifies the restriction. The HIV-1 and SIV_{mac} CA proteins are composed of N-terminal and C-terminal domains. Only one of the chimeras containing heterologous CA domains was replication competent. HIV(S/HCA).GFP, in contrast to the HIV-1 variant containing the complete SIV_{mac} CA protein, behaved more like HIV-1 in cells restricted for HIV-1 infection and more like SIV_{mac} in a cell line restricted for SIV_{mac} infection. This could indicate differences in the precise CA determinants for the restrictions in these cell types. Alternatively, the CA structural requirements that govern the molecular interactions relevant to the restriction may involve both the N- and C-terminal domains. The results reported herein should assist efforts to elucidate the basis of the species-specific blocks to primate immunodeficiency virus infection.

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