Serological and Structural Comparison of Immunodeficiency Viruses from Man, African Green Monkey, Rhesus Monkey and Sooty Mangabey

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We have studied the serological relationship among the human immunodeficiency virus type 1 (HIV-1), and three simian immunodeficiency viruses (SIV). SIVagm was isolated from African green monkeys *(Cercopithecus aethiops),* and compared with the previously described isolates of SIVmac from a rhesus macaque *(Macaca mulatta)* and SIVsm from a sooty mangabey *(Cercocebus atys)*. With respect to the glycoproteins, the simian viruses represent a subgroup apparently different from HIV. To classify HIV and SIV isolates further, we compared tryptic peptide maps of the core polypeptides p18 and p24 of HIV-2, three HIV-1 and five SIV isolates. Each peptide map was distinguishable, and differences are most prominent between the HIV-1 group and the SIVmac/SIVsm group. HIV-2 is very similar to SIVmac and SIVsm. The three SIVagm isolates form a more heterogeneous group. The p24s of all SIVagms are more similar to the p24s of HIV-1, but with respect to p18, one isolate is similar to HIV-1, while the two others are more related to SIVmac, SIVsm, and HIV-2.

The human immunodeficiency virus (HIV) is the infectious agent causing AIDS, the aquired immunodeficiency syndrome [1–3]. Related simian viruses (SIV) are found in rhesus monkeys [4], African green monkeys [5, 6], and sooty mangabeys [7]. The viruses from rhesus monkey [8] and sooty mangabey [7] induce immunodeficiency when transmitted to the rhesus monkey; furthermore, HTLV-IV and HIV-2, two African isolates obtained from healthy humans or AIDS patients, respectively, are more similar to SIVmac, than to HIV-1 [9, 10]. Thus, simian viruses related to HIVs are important in revealing the origin of the HIVs and in the study of pathogenesis of human AIDS in animal model systems. Previous work on the SIVs describes the relationship between each SIV and HIV-1 [5–7]. Recently, Kornfeld *et al.* [11] have found by restriction enzyme analysis that six SIVagms from West Africa and two HTLV-IV isolates are very similar, but the identity of the SIVagm and HTLV-IV isolates remains questionable [12, 13]. Here we report on the serological and structural similarities between three simian viruses, SIVmac [8], our SIVagm isolates and SIVsm [14], and their relationships to HIV/HTLV-IIIB [15] and HIV-2 [10].

Our data suggest a high degree of variability among the core polypeptides of our three independent isolates of SIVagm from East Africa. These isolates varied more from each other than three HIV-1 isolates.

The serological cross-reactivities between the virus polypeptides were tested by immunoprecipitation. H9/HTLV-III cells [15], HUT-78/SIVmac cells [4], H9/SIVsm cells and MT-2 cells infected with SIVagm-a were labelled with [^{35}S]cysteine. Cell extracts were used as a source of virus antigens in the immunoprecipitation assay [16].

The polyacrylamide gel electrophoresis (PAGE) analyses of the immunocomplexes revealed a relatively simple pattern of cross-reactivities of the glycoproteins (Fig. 1). The two antisera to HIV-1 recognized gp120 of HIV-1 whereas none of the antisera to SIVs recognized this glycoprotein. In contrast, all sera of SIV infected monkeys reacted with the homologous and heterologous gp130 glycoproteins of the three simian viruses SIVagm-a, SIVsm and SIVmac. In addition, weaker cross-reactions were observed between human *anti*-HIV-1 sera and gp130 of SIVagm and SIVsm. However, the core polypeptide p24 and the core polypeptide precursor Pr55 of HIV-1 and SIVs contained epitopes that

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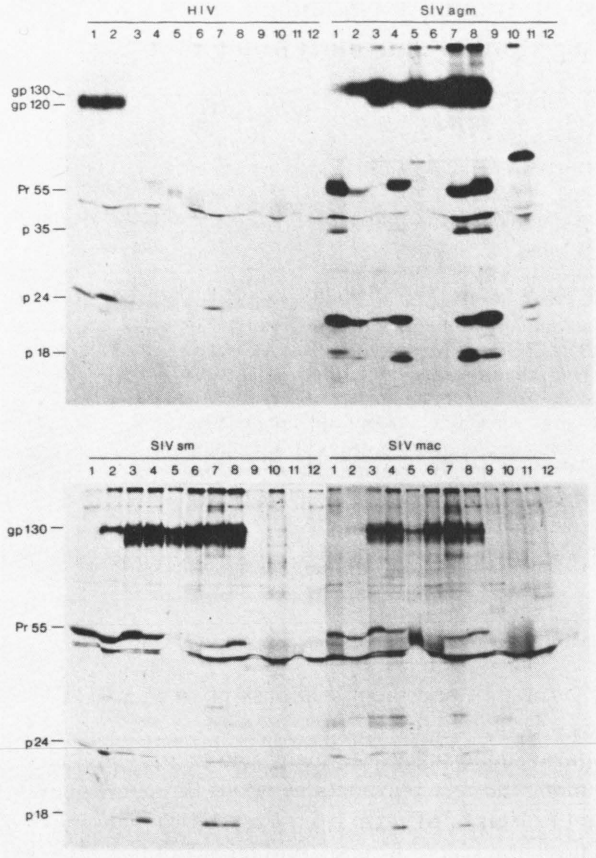


Fig. 1. Serologic cross-reactions between primate lentiviruses. Cells were labelled with [^{35}S]cysteine. Immunoprecipitations were performed with extracts of labelled cells and sera of an AIDS patient (lane 1), an HIV-infected haemophiliac (lane 2), two SIVmac-infected rhesus monkeys from which SIVmac was isolated [4] (lanes 3 and 4), two SIVagm-infected green monkeys kept in Germany (lanes 5 and 6) and two SIVsm-infected sooty mangabeys, one of which was the source of SIVsm (lanes 7 and 8). Negative control sera of man (lane 9), green monkey (lane 10), rhesus monkey (lane 11), and sooty mangabey (lane 12) were included. Radioactive polypeptides reacting with the sera were analyzed by PAGE and detected by fluorography.

were regularly recognized by antibodies to HIV-1 and the three SIVs. The p18 of SIVmac, SIVagm-a and SIVsm contain cross-reactive epitopes. The cross-reactivity of HIV-1 p18 could not be tested in this assay, since it is not even recognized by homologous sera in the extract of H9 cells. The two SIVagm antisera were exceptional because they failed to recognize homologous and heterologous core polypeptides. Also, five additional sera of African green monkeys lacked antibodies to homologous and heterologous core polypeptides (data not shown).

The control serum from an African green monkey obviously contained antibodies to HTLV-I, since it reacted with gp68 and other polypeptides of MT-2 cells (Fig. 1, lane 10).

The intensities of immunoprecipitated polypeptide bands varied between virus producing cells, reflecting different efficiencies of virus production. To exclude artifacts which could result from these variations in sensitivity, the immunoprecipitations were repeated with iodinated polypeptides of purified viruses and one serum of each species. All reactions were confirmed, including the non-reactivity of the green monkey sera with viral core polypeptides (data not shown). Previous studies have shown that antisera to SIVmac [6, 17], SIVagm [5] and SIVsm [7] cross-reacted to various degrees with the core but not with envelope polypeptides of HIV-1, while some sera of AIDS patients weakly recognized the glycoproteins gp130 of SIVmac [9, 17]. Our independent isolates of SIVagm and SIVsm reacted similarly.

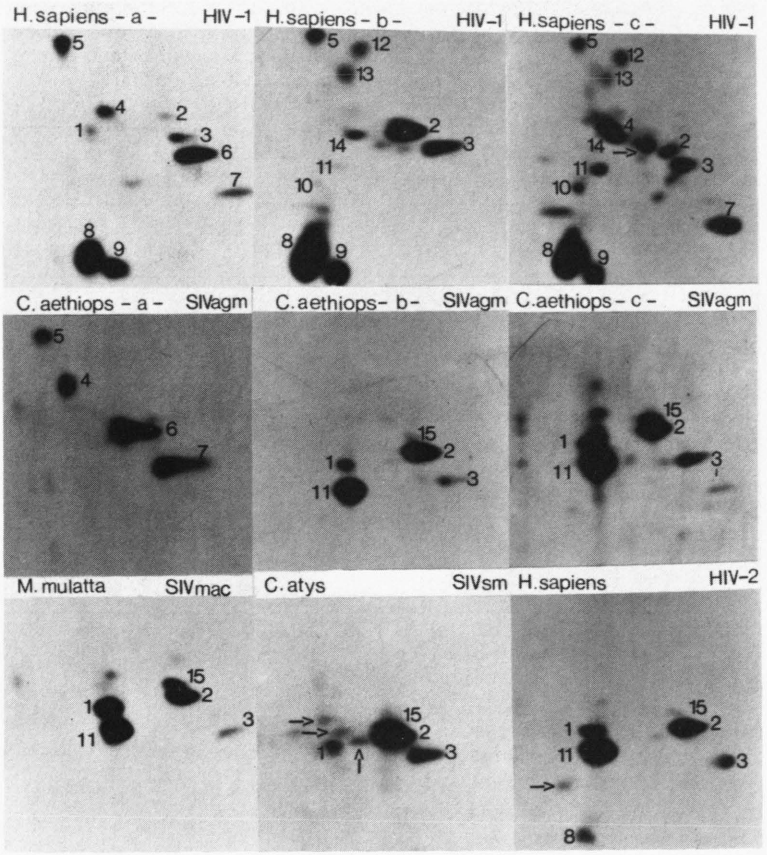
In contrast to these observations and the expected high variability of the *env* gene, immunogenic epitopes seemed to be conserved among the envelope glycoproteins of SIVs from different simian species. Thus our SIV isolates represent a serological subgroup within the immunodeficiency viruses of primates.

The lack of antibodies against the core polypeptides, that we have observed in African green monkeys, could be due to the low immunogenicity of these polypeptides in green monkeys, since sera of individual virus carriers rarely reacted with the core polypeptides of their own virus [5].

Because differences between the core polypeptides could not be detected by immunoprecipitation, we chose the more sensitive technique of two-dimensional peptide mapping. This technique is fast and detects minor differences in size, charge and hydrophilicity of tryptic fragments. The American HIV-1a was harvested from Jurkat cells infected by HTLV-IIIB [18]. The German isolates HIV-1b and HIV-1c were propagated in MT-2 cells (H. Schmitz *et al.*, in preparation). The three independent SIVagms were isolated from individual green monkeys kept in Japanese research facilities. One of the animals originated from parents imported from Kenya (SIVagm-b), the other two were imported from Kenya

(SIVagm-a) and Ethiopia (SIVagm-c). For peptide mapping, SIVagms were produced in Molt-4 cells [19]. HIV-2 was produced in MT-2 cells co-cultivated with LAV-II/CEM cells [10]. SIVmac was obtained from HUT-78/SIVmac [4], and SIVsm from H9/SIVsm [14]. Iodinated virus was incubated with hyperimmune sera prepared in rabbits against the HTLV-IIIB core polypeptides p18 and p24, respectively [20]. These sera precipitated the core polypeptides p18 and p24 of HIV-1 exclusively and cross-reacted with respective polypeptides of HIV-2 and the SIVs. The precipitates were separated on polyacrylamide gels, excised, digested with TPCK-treated trypsin and eluted from the gel. Two-dimensional analyses of the labelled fragments were performed on cellulose-coated thin-layer plates [21]. To confirm the identity of the tryptic fragments of two proteins migrating to similar positions on a peptide map, mixtures of peptides from the respective proteins were analyzed (not shown).

Core polypeptides p24 and p18 of the nine viruses had distinct peptide maps (Fig. 2a and 2b). Two patterns of p18 core polypeptides could be distinguished (Table I): the first comprises HIV-1a, HIV-1b and HIV-1c, the second is represented by SIVagm-b and SIVagm-c, SIVmac, SIVsm, and HIV-2. Both groups shared fragments 1 to 3. The second group lacked fragments 4 to 10 with the exception of spot 8 in HIV-2. They all contained fragment 15 which is not found in HIV-1 isolates. p18s of the second type were more conserved than those of the first type. SIVagm-a p18 belongs to the first type, since it shares fragments with HIV-1 but not with other isolates.



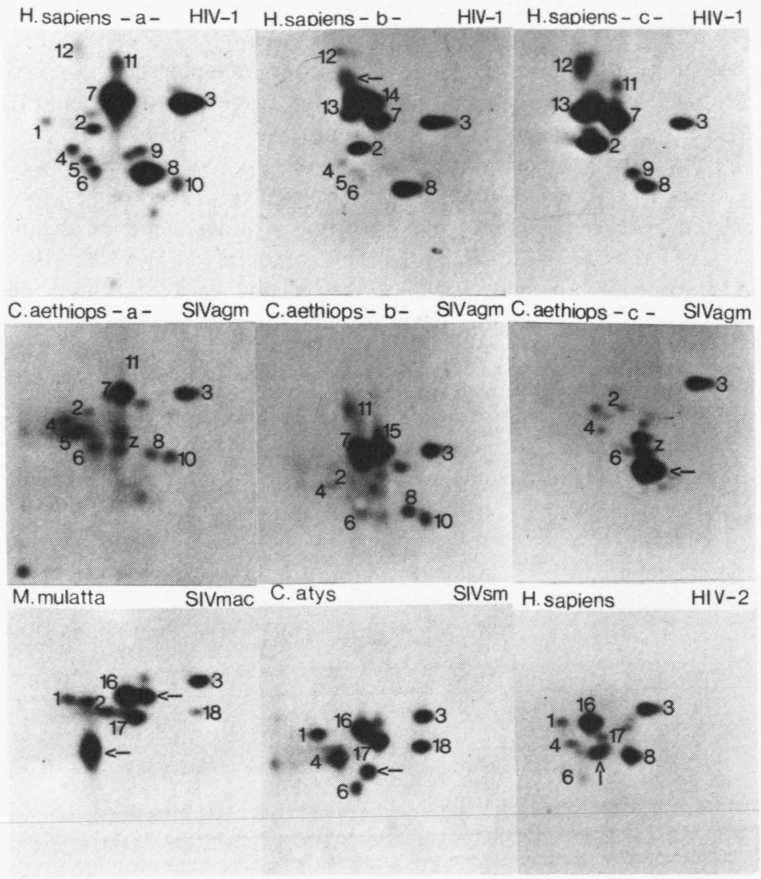


Fig. 2. Peptide maps of core polypeptides HIV-1 related viruses. ^{125}I-labelled polypeptides were digested with TPCK-trypsin and subjected to peptide mapping. Samples were applied in the lower left corner. Electrophoresis from left to right was followed by chromatography from bottom to top. (a) Peptide maps from polypeptide p18. Arrows indicate fragments found only in a single isolate. Z is unique to SIVagm-a and SIVagm-c. (b) Peptide maps from polypeptide p24.

Table I. Structural relationship of polypeptides p18 and p24 of HIV and SIV.

p18		Spot no.														Non-HIV-1	Extra
Virus	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	spots
HIV-1a	*H. sapiens*-a	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
HIV-1b	*H. sapiens*-b	−	+	+	−	+	−	−	+	+	+	+	+	+	+	−	−
HIV-1c	*H. sapiens*-c	−	+	+	+	+	−	+	+	+	+	+	+	+	+	−	*[1]
SIVagm-a	*C. aethiops*-a	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
SIVagm-b	*C. aethiops*-b	+	+	+	−	−	−	−	−	−	−	+	−	−	−	+	−
SIVagm-c	*C. aethiops*-c	+	+	+	−	−	−	−	−	−	−	+	−	−	−	+	−
SIVmac	*M. mulatta*	+	+	+	−	−	−	−	−	−	−	+	−	−	−	+	−
SIVsm	*C. atys*	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	*
HIV-2	*H. sapiens*	+	+	+	−	−	−	−	+	−	−	+	−	−	−	+	*

p24		Spot no.														Non-HIV-1				Extra
Virus	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	spots
HIV-1a	*H. sapiens*-a	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
HIV-1b	*H. sapiens*-b	−	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−	−	−	*
HIV-1c	*H. sapiens*-c	−	+	+	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−
SIVagm-a	*C. aethiops*-a	−	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	z[2]
SIVagm-b	*C. aethiops*-b	−	+	+	+	−	+	+	+	−	+	+	−	−	−	+	−	−	−	−
SIVagm-c	*C. aethiops*-c	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	*, z
SIVmac	*M. mulatta*	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	*
SIVsm	*C. atys*	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	+	+	+	*
HIV-2	*H. sapiens*	+	−	+	+	−	+	−	+	−	−	−	−	−	−	−	+	+	−	*

[1] Only found in a single virus.
[2] Only found in SIVagm-a and SIVagm-c.

The p24s also could be classified into two groups. HIV-1a, -1b, and -1c formed one group. Among the SIVagm isolates, the similarity to HIV-1 decreased from SIVagm-a to SIVagm-c. A major new spot was detected in SIVagm-c (Fig. 2b, arrow) but p24 of this virus was still more similar to HIV-1 than to the second group (SIVmac, SIVsm and HIV-2). The second group contained spots 16, 17, and 18 (except fragment 18 in HIV-2) and lacked spots 5, 7, and 9 to 14.

All p24 maps presented here differ from each other as do those of p18s. The structural relationship of the core polypeptides examined is closer within the group of SIVmac/SIVsm/HIV-2 than among the three independent HIV-1 isolates. This relatedness of HIV-2 to SIVmac/SIVsm and its lack of similarity to HIV-1 agrees with results of nucleotide sequence comparison of HIV-2 and HIV-1, restriction endonuclease mapping, nucleic acid hybridization and serological assays [9–11, 22–24].

Our SIVagm isolates -a and -b are more closely related to HIV-1 than to the other SIVs. Contamination with cellular or other viral proteins appears unlikely since HIV-1b, -c and HIV-2 yielded greatly different peptide maps even though they were all produced in MT-2 cells. Furthermore, peptide maps of one virus grown in either Jurkat or H9 cells revealed only a slight variation in the intensity of various spots (not shown). We have analyzed uncloned virus isolates. Thus, the peptide maps represent the potential variants contained in our tissue cultures.

These data clearly demonstrate the heterogeneity of the core polypeptides among independent isolates of HIV-1 as well as SIVagms. However, in contrast to previously described SIVagm isolates ours originated from East Africa where HIV-1 is endemic. They were more distinct from each other than our three HIV-1 isolates.

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Note added in proof:

It has been shown by Kestler *et al.* (Nature **331,** 619–621, 1988), that the initial SIVagm [5] and HTLV-IV [9] isolates have been derived from SIVmac infected cell cultures.

SIVagm-a, -b and -c described here are now called SIV[AGM-1], SIV[AGM-5] and SIV[AGM-7], respectively [19].

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