Identification of Simian Immunodeficiency Virus SIV_{MAC} *env* Gene Products

FULVIA DI MARZO VERONESE,^{1*} BRIDGET JOSEPH,¹ TERRY D. COPELAND,² STEPHEN OROSZLAN,²
ROBERT C. GALLO,³ AND M. G. SARNGADHARAN¹

Bionetics Research, Inc., Rockville, Maryland 20850-4373¹; Laboratory of Molecular Virology and Carcinogenesis, National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701²; and Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892³

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A monoclonal antibody recognizing an antigenic determinant on the *env* transmembrane protein, gp32 of simian immunodeficiency virus SIV_{MAC} has been developed and designated SF8/5E11. The reactivity of this antibody was found to be type specific, since it did not cross-react with either SIV_{SMM} or SIV_{MNE} transmembrane proteins. The availability of both this antibody and the complete nucleotide sequence of SIV_{MAC} allowed us to define the organization of the *env* gene products of this virus. Radiolabel sequencing of the amino termini of both gp160 and gp32 confirmed the positions of both cleavage sites predicted by alignment of the inferred amino acid sequences of the SIV_{MAC} and human immunodeficiency virus type 1 *env* genes. The cleavage site between the signal peptide and the external *env* glycoprotein resides between the cysteine residue at position 21 and the threonine residue at position 22, starting from the first residue after the *env* gene initiator methionine. The *env* precursor polypeptide gp160 is cleaved between arginine 526 and glycine 527 to give rise to the external glycoprotein and the transmembrane of SIV_{MAC}.

The identification of human immunodeficiency virus type 1 (HIV-1) and the relevance of animal model systems to study the biology of the human T cell lymphotropic virus family have led to the characterization of a group of related simian retroviruses. Simian T cell lymphotropic virus type III now called simian immunodeficiency virus (SIV) has been isolated from various species of Old World primates, including macaques (SIV_{MAC}; 2, 4), African green monkeys (SIV_{AGM}; 13), and sooty mangabeys (SIV_{SMM}; 6, 11, 12). All macaque isolates obtained were from captive animals with immunodeficiency disease and possible exposure to either African green monkeys (4) or mangabeys (12). SIV_{MAC} induces symptoms similar to those of acquired immunodeficiency syndrome in infected macaques (4, 10), but isolates from African green monkeys and mangabeys appear to be nonpathogenic in their natural hosts. Comparison of the genome of SIV_{MAC} with those of known HIVs indicates that this virus is more closely related to HIV-2 than to HIV-1 (3, 5, 7). The biological properties of SIV_{MAC} and of HIV-1 and -2 are similar. SIV_{MAC} viral proteins show size similarities and cross-reactivity with the *gag*, *env*, and *nef* proteins of HIV-1 (9). The major viral antigens of SIV_{MAC} have been identified by radioimmunoprecipitation and sodium dodecyl sulphate-polyacrylamide gel electrophoretic (SDS-PAGE) analysis as proteins of 160, 120, 55, and 24 kilodaltons (kDa) in size (9). gp160 and gp120 were shown to be glycosylated (9), and by analogy with HIV-1, they were thought to correspond to the precursor and extracellular envelope glycoproteins of SIV_{MAC}. Alignment of the inferred amino acid sequences of the SIV_{MAC} and the human T cell lymphotropic type III_B *env* gene showed 38% homology overall and allowed prediction of the length of the signal peptide from the initiator methionine and the cleavage site between the external envelope glycoprotein and the transmembrane protein (3, 5). The predicted SIV_{MAC} transmembrane protein is 140 amino acids shorter than that of HIV-1 and should

correspond to the glycosylated 32-kDa protein recognized by infected monkey sera in immunoblots (8). In the present study we describe the development of hybridomas secreting monoclonal antibodies to this 32-kDa protein. These antibodies were instrumental in the identification of the protein as the transmembrane portion of the envelope of SIV_{MAC} and in precisely locating the cleavage site between the signal peptide and the envelope precursor gp160 and that between the external glycoprotein gp120 and the transmembrane protein gp32.

BALB/c mice were immunized with inoculations of detergent-disrupted SIV_{MAC}. The successfully growing hybrids that resulted from fusion of the immune-mouse splenocytes with the murine myeloma line NS-1 were screened for secretion of antibody to SIV_{MAC} proteins by enzyme-linked immunosorbent assay with disrupted SIV_{MAC} as the antigen. Supernatants that scored positive in this assay were further tested by immunoblots against SIV_{MAC} viral lysates and then against lysates from both SIV_{MAC}-infected and uninfected HUT78 cells. Hybrids scoring positive on virus lysate and on virus-infected HUT78 cell lysate but negative on uninfected HUT78 lysate were cloned by limiting dilution. From all the virus-specific hybridomas, we selected the ones secreting antibodies to the diffuse 32-kDa protein recognized in electrophoretic separations, assuming that this might be an SIV envelope component. This protein was also recognized by SIV antibody-positive monkey sera. The antibody designated SF8/5E11 recognized a protein of approximately 32 kDa in size in both virus lysate and infected-cell lysate and was negative when tested against uninfected cells (Fig. 1A). In addition to binding to the 32-kDa protein, this antibody also bound to a larger 160-kDa protein in strips with infected cell lysate. These findings agree with the assumption that the 32-kDa protein represents the transmembrane component and the 160-kDa protein represents the primary *env* gene product of SIV_{MAC}. To further characterize these proteins, lysates of [³⁵S]cysteine-labeled SIV_{MAC}-infected HUT78 cells were immunoprecipitated with SF8/5E11 and

* Corresponding author.

FIG. 1. (A) Reactivity of monoclonal antibody SF8/5E11 in the immunoblot assay. The antigens on the strips were from lysates of the following: SIV_{MAC} (lane 1), HUT78 cells infected with SIV_{MAC} (lane 2), and uninfected HUT78 cells (lane 3). (B) Reactivity of monoclonal antibody SF8/5E11 in a radioimmunoassay followed by SDS-PAGE analysis. HUT78 cells infected with SIV_{MAC} were metabolically labeled with [³⁵S]methionine (100 μ Ci/ml) overnight. The cells were disrupted, and the clarified lysates were immunoprecipitated with serum from a SIV-infected monkey (lane 1) and with SF8/5E11 (lane 2).

with a SIV-positive monkey serum and the immunoprecipitates obtained were subjected to SDS-PAGE analysis. Both antibodies precipitated a similar sized protein, gp160, from the cellular extract (Fig. 1B), while the SIV-positive monkey serum also immunoprecipitated the counterpart of the HIV-1 gp120. No precipitation of the transmembrane protein was observed in either instance. This is not unusual, since precipitation of gp41, the transmembrane protein of HIV-1 from [³⁵S]cysteine-labeled infected cells cannot be demonstrated without prior enrichment of the glycoprotein fraction by lentil lectin-agarose chromatography (1, 15).

After establishing the specificity of the SF8/5E11 antibody, it was of interest whether the same epitope recognized by SF8/5E11 was also present in other SIV isolates besides SIV_{MAC}. Two other SIV isolates were tested: SIV_{SMM} and SIV_{MNe}. SIV_{SMM} was isolated from asymptomatic sooty mangabeys and rhesus macaques that developed transmissible simian acquired immunodeficiency syndrome after inoculation with tissue homogenates from infected mangabeys (12). SIV_{MNe} was isolated from a pig-tailed macaque (*Macaca nemestrina*) with immune deficiency disorders that had died in 1982 with a poorly differentiated lymphoblastic lymphoma (2). SF8/5E11 did not cross-react with the transmembrane protein of either virus in immunoblots. On the contrary, a monoclonal antibody raised against the major core protein p24 of SIV_{MAC} efficiently bound to both SIV_{SMM} and SIV_{MNe} p24s (data not shown). Moreover, no cross-reactivity was observed when HIV-1 and HIV-2 were tested in the same assay (data not shown).

Since the interpretation of serological assays currently in use requires a clear understanding of the antigens encoded by SIV_{MAC}, a definitive assignment of SIV gp160 and gp32 as the *env* precursor polypeptide and the *env* transmembrane protein, respectively, was needed. To this end, SIV_{MAC}-infected HUT78 cells were labeled with [³H]phenylalanine and [³H]valine overnight and the cellular lysates were immunoprecipitated with SF8/5E11. The precipitates obtained were subjected to preparative SDS-PAGE analysis. This antibody under these conditions efficiently precipitated gp160 and to a lesser degree gp32 and two other proteins of

FIG. 2. Preparative immunoprecipitation of extracts from [³H]phenylalanine (PHE)- and [³H]valine (VAL)-labeled HUT78 cells producing SIV_{MAC} with SF8/5E11 monoclonal antibody. SIV_{MAC}-infected HUT78 cells (5×10^7) were metabolically labeled with 10 mCi of [³H]phenylalanine or [³H]valine overnight. After being labeled, the cells were disrupted in 10 mM sodium phosphate (pH 7.2) containing 0.5% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS. The lysates were preabsorbed with protein A-Sepharose bound to rabbit antiserum to mouse κ light chain and were then clarified by centrifugation. The immunoprecipitation was performed by the addition to 7 ml of the extract of 15 μ l of SF8/5E11 ascites and 3 ml of the protein A-Sepharose rabbit antiserum. Sizes are shown in kilodaltons (K).

approximately 75 and 300 kDa in size (Fig. 2). These proteins are not yet characterized. The radioactive gp160 band was excised from the gel, eluted in water containing 1mM phenylmethylsulfonyl fluoride and 10 nmole of apomyoglobin, and subjected to radiosequencing by Edman degradation. Valine was unambiguously assigned to positions 4, 6, and 10, and phenylalanine was at position 7 for 20 cycles analyzed (Fig. 3A and B). The sequence determined was found to be a perfect match with the amino acid sequence predicted from the nucleotide sequence of the amino-terminal region of the SIV_{MAC} *env*. Thus, as predicted, the gp160 precipitated by SF8/5E11 corresponds to the SIV *env* precursor polypeptide. These results allowed us to precisely locate the cleavage site for the signal peptide and the external *env* glycoprotein between the cysteine residue at position 21 and the threonine residue at position 22 starting from the first residue after the *env* gene initiator methionine.

Following the same approach outlined above, the radioactive gp32 band was excised from the gel, eluted, and subjected to radiosequencing by Edman degradation. Valine was unambiguously assigned to positions 2 and 4 for 20 cycles analyzed (Fig. 3C). The determined position of valine corresponded exactly to the amino acid sequence predicted from the nucleotide sequence of the carboxy-terminal region of the SIV *env* gene. These results allowed us to precisely identify gp32 as the transmembrane protein of SIV arising by cleavage of the precursor polypeptide between the arginine residue at position 526 and the glycine residue at position 527 (Fig. 4). The cleavage is at a position analogous to the cleavage site in HIV-1, following conserved lysine-arginine residues (15).

In conclusion, we described the development of a hybridoma secreting monoclonal antibody to the *env* transmembrane protein of SIV_{MAC}. The availability of both this antibody and the complete nucleotide sequence of SIV_{MAC} (8, 9) allowed us to define the organization of the *env* gene of

FIG. 3. Amino-terminal sequence analysis of SIV gp160 labeled with [^3H]valine (A) or [^3H]phenylalanine (B) and of SIV gp32 labeled with [^3H]valine (C). The radioactive gp160 and gp32 bands were excised from the gel and eluted in water containing 1 mM phenylmethylsulfonyl fluoride and 10 nmol of apomyoglobin. The eluted radiolabeled proteins were dialyzed at 4°C versus 5% acetic acid. The proteins were then subjected to radiolabeled sequencing (14) in a Beckman updated 890B spinning-cup Sequenator. Ten percent of cycles 2 and 11 were converted to the phenylthiohydantoin derivative of the amino acid and analyzed to ensure that the apomyoglobin protein was sequenced correctly. The remainder of the Sequenator fractions were transferred to scintillation vials, dried, dissolved in 10 ml of Aquassure (New England Nuclear Corp.), and counted for 10 min. The recovery of radioactivity in each cycle is given in the figure. Positive identifications are indicated by the open circles.

this virus. Besides gp32 and gp160, this antibody also immunoprecipitated two additional proteins of 75 and 300 kDa in apparent size, but their physical and chemical natures remain to be determined. In every instance, the monoclonal antibody recognized only a 32-kDa transmembrane protein and never the higher-molecular-weight transmembrane species that would result from translational readthrough of the in-frame termination codon occurring directly after the transmembrane protein (3, 5, 7). This is consistent with the results obtained using sera from infected macaques. In immunoblots with lysates from chronically SIV_{MAC}-infected cells, these sera always recognized a band of approximately 32 kDa in size representing what we confirmed to be the transmembrane protein of SIV_{MAC} (8).

The SF8/5E11 monoclonal antibody recognized an epitope in the SIV_{MAC} transmembrane protein not shared with

FIG. 4. Diagram indicating the cleavage between signal peptide and gp160 and between gp120 and gp32. Arrows indicate the identified cleavage sites. Amino acid residues identified by asterisks are those determined by radiolabel sequence analysis. Numbers above the amino acids denote the portions in the deduced amino acid sequence for the primary gene product.

HIV-1 and HIV-2, defining its reactivity as type specific. Moreover, SF8/5E11 was not cross-reactive with SIV_{SMM} and SIV_{MNe}. The nucleotide and amino acid sequences of SIV_{SMM} are not available at this time. SIV_{MNe} gag proteins have been partially sequenced, and their amino acid sequences were found to be 92% homologous to the translated nucleotide sequences of SIV_{MAC} (6a). Furthermore, SF8/5E11 cross-reacted with a number of molecular clones from individual macaque isolates other than the original SIV_{MAC} but failed to cross-react with some others (T. Kodama and R. Desrosiers, personal communication). Thus, it appears that the epitope recognized by this antibody is situated in a hypervariable region of gp32.

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