Monoclonal antibodies against human immunodeficiency virus (HIV) type 2 core proteins: Cross-reactivity with HIV type 1 and simian immunodeficiency virus

(cross-reactive epitopes/p24 and p16 core proteins)

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ABSTRACT Four mouse monoclonal antibodies were developed after immunization with one human immunodeficiency virus (HIV) type 2 isolate and were tested for reactivity with different HIV-1, HIV-2, and simian immunodeficiency virus (SIV) isolates in an immunofluorescence assay and by immunoblot analysis. One of them, an anti-capsid (p24) antibody, called R1C7, reacted with all HIV-1, HIV-2, and SIV isolates tested, thus identifying an epitope shared by all HIV and SIV. Another anti-capsid antibody, named A4F6, reacted with three HIV-2 isolates (HIV-2NIHz, LAV-2Red, and LK001 ST9), some SIV isolates (STLV-IIIAGM, SIV-251, and SIV-309), but no HIV-1 isolates. Two anti-matrix (p16) antibodies, named R5C4 and R5F6, reacted strongly only with the HIV-2 isolates. The use of these monoclonal antibodies for rapid discrimination and identification of acquired immunodeficiency syndrome-related retroviruses is discussed.

A group of recently discovered retroviruses share many structural, biological, genetic, and antigenic properties with the human immunodeficiency virus (HIV) type 1, the etiological agent of the acquired immunodeficiency syndrome (AIDS) (1, 2). This group includes isolates from several primate species referred to as simian immunodeficiency virus (SIV) and isolates from healthy West African individuals and patients with immunodeficiency referred to as HIV-2 (3–6). Nucleic acid sequence analysis of HIV-1, HIV-2, and SIV has revealed that HIV-2 and SIV are more closely related to each other than to HIV-1 (7–9). In fact, there is only 42% overall sequence identity between the HIV-1 and HIV-2 genomes (10). Both SIV and HIV-2 display serological cross-reactivity with HIV-1 that is limited to the gag and pol viral proteins (3–6). Most importantly, there are significant differences in pathogenicity among these retroviruses. This presents a very complex epidemiological situation, particularly in Africa. To understand the mode of transmission and pathogenicity of these retroviruses, it is essential to develop specific reagents that make possible their rapid identification.

Here we report the development of four monoclonal antibodies (mAbs) directed against epitopes of capsid (CA) and matrix (MA) proteins (11) of HIV-2 and describe their reactivities with different isolates of HIV-1, HIV-2, and SIV. One of these antibodies reacts with an epitope of the CA protein of HIV-2 that is present in all HIV-1, HIV-2, and SIV isolates tested thus far. The other three antibodies react with less conserved epitopes of HIV-2 core proteins and may prove useful in discriminating among various HIV-1, HIV-2, and SIV isolates.

MATERIALS AND METHODS

Cells and Viruses. Viruses were propagated in the human T-cell line HUT-78 or in the H9 clone. The HIV-1 isolates [human T-cell leukemia/lymphoma virus (HTLV)-IIIb, HTLV-III MN, HTLV-III CC, HTLV-III RF, and HTLV-III BRIE] have been previously described (1, 12). The HIV-2 strain used for immunization was isolated from the peripheral blood lymphocytes of an AIDS patient and was designated HIV-2NIHz (13). This isolate is serologically very similar to the LAV-2Red isolate previously described (6). Fixed cells of the HUT-78 cell line infected with the LAV-2Red isolate were kindly provided by E. M. Fenyo (Karolinska Institute, Stockholm). Another HIV-2 isolate, termed LK001 ST9, from a healthy West African individual was provided by B. Hahn and G. Shaw (University of Alabama, Birmingham, AL). Slides with fixed HUT-78 cells infected with the SIV isolates 142, 157, 186, 251, and 309, recovered from rhesus macaques (Macaca mulatta), were kindly provided by M. D. Daniel and R. Desrosiers (New England Regional Primate Research Center, Harvard Medical School, Southboro, MA). The SIV isolates, simian T-cell leukemia/lymphoma virus (STLV)-IIIAGM and HT-SIV/SMM-5, from an African green monkey and a sooty mangabey (Cercocebus atys), were kindly provided by M. Essex (Harvard School of Public Health, Boston) and P. Fultz (Centers for Disease Control, Atlanta), respectively (3, 14). Equine infectious anaemia virus propagated in equine fetal kidney cells was kindly provided by R. W. Johnson (Biological Carcinogenesis Program, Frederick Cancer Research Facility, Frederick, MD). HTLV-I was grown in human cord blood T cells (C91/PL) as previously described (15).

Immunization, Cell Fusion, and Establishment of Hybridoma Clones. The viruses used in the study were purified by banding twice in sucrose gradients. HIV-2NIHz virus was disrupted with 0.5% Triton X-100 in 0.6 M NaCl and was dialyzed overnight against phosphate-buffered saline (PBS). Four mice were immunized intraperitoneally with 1 ml of solubilized virus emulsified in complete Freund’s adjuvant on day 0 and the same amount of antigen in PBS on days 1, 2, 16, 17, and 18. Each mouse received the equivalent of 1 × 10⁶ virus particles each immunization. Three days after the last injection, the splenocytes of the animal with the highest serum antibody titer were fused with Sp2 myeloma cells according to a protocol described elsewhere (16). Ten to 14 days after fusion, supernatants of hybrids growing in hypoxanthine/aminopterin/thymidine medium were tested for monoclonality using limiting dilutions (17, 18).

Abbreviations: HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; mAb, monoclonal antibody; CA, capsid; MA, matrix; HTLV, human T-cell leukemia/lymphoma virus; STLV, simian T-cell leukemia/lymphoma virus.

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for specific antibody production by the immunofluorescence technique described below. Hybridomas secreting antibodies reactive against HIV-2 were subcloned by limited dilution. Cells were plated at a concentration of 0.5 cell per well in round bottom 96-well microplates. To increase plating efficiency of these cells, conditioned culture fluids harvested from Sp2 cells were included in the hypoxanthine/aminopterin/thymidine culture medium.

**Immunofluorescence and Other Immunological Assays.** The indirect immunofluorescence assay was carried out on fixed cells as described (17). Briefly, uninfected and infected HUT-78 and H9 cells were spotted on slides, air-dried, and fixed for 10 min in acetone/methanol, 1:1 (vol/vol). Supernatant (10 μl) to be tested was applied and incubated 30 min at room temperature. Fluorescein-conjugated goat anti-mouse immunoglobulin (IgA, IgM, and IgG) (Cappel Laboratories, Cochranville, PA) was the second antibody. In some cases, hybridoma supernatants were tested for specific anti-HIV-2 antibodies by using a spot-immunofluorescence technique (A.A.M., unpublished results). Class and subclass determinations of immunoglobulins secreted by hybridomas were performed by using the Ouchterlony technique with specific anti-mouse immunoglobulin typing sera (Calfchem). For detection of HIV-1 core proteins, two previously described mAbs, designated BT3 and M33, were used (18).

**Immunological Blot Assay.** Viral proteins were separated from sucrose gradient-banded HIV-1, HIV-2, and SIV by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and were blotted onto a nitrocellulose sheet according to Towbin et al. (19). The blots were reacted with each mAb as well as with HIV-1 and HIV-2 positive and negative control human sera as described elsewhere (20).

**RESULTS**

**Development and Characterization of mAbs.** Out of 118 individual hybridomas that grew in hypoxanthine/aminopterin/thymidine medium, 48 secreted antibodies reactive with HIV-2NIHZ-infected HUT-78 cells. However, 44 of these also reacted with uninfected HUT-78 cells, which clearly demonstrated that the antigen used for immunization contained material originating from the cells that were used for propagation of the virus. Four hybridomas reacted with HIV-2NIHZ-infected HUT-78 cells but lacked reactivity with uninfected HUT-78 cells and were, therefore, considered virus-specific. These hybridomas were cloned twice and were designated R1C7, A4F6, R5C4, and R5F6. They continue to secrete specific antibodies 3 months after fusion. All four are of the IgG1 subclass as determined by the Ouchterlony technique by using class- and subclass-specific anti-mouse immunoglobulin typing antisera.

**Reactivity of mAbs with HIV-1, HIV-2, and SIV Isolates.** The four anti-HIV-2NIHZ mAbs were tested for reactivity with cells infected with different HIV-1, HIV-2, and SIV isolates by using an indirect immunofluorescence assay. Negative control cell populations included uninfected HUT-78 and H9 cells, HTLV-I-infected cord blood T cells (C91/PL), and equine infectious anemia virus-infected equine fetal kidney cells. For detection of HIV-1-infected cells, two previously characterized mAbs directed against the CA protein (BT3) and MA protein (M33) of HIV-1 were included in this study. An example of immunofluorescence reactivity of the most broadly reactive mAb raised against HIV-2NIHZ, designated R1C7, is shown in Fig. 1. This antibody exhibited strong positivity with HUT-78 cells infected with HIV-2NIHZ or STLV-IIIAGM and also with H9 cells infected with HTLV-IIIPL, but not with uninfected HUT-78 cells (Fig. 1). The data from an extensive survey of immunofluorescence reactivities with various isolates are summarized in Table 1. The antibody R1C7 reacted with all HIV-1, HIV-2, and SIV isolates tested thus far. The A4F6 mAb reacted with members of the HIV-2 group (HIV-2NIHZ, LK001 ST9, and LAV-2Rod) and also with some SIV isolates (STLV-IIIAGM, SIV-251, and SIV-309), but not with HIV-1 isolates. The R5C4 and R5F6 mAbs were strongly reactive with the HIV-2NIHZ, LK001 ST9, and LAV-2Rod isolates. None of these four mAbs reacted with uninfected H9 or HUT-78 cells or with HTLV-1 or equine infectious anemia.

**Fig. 1.** Immunofluorescence reactivity of the R1C7 mAb with uninfected HUT-78 cells (A), HIV-2NIHZ-infected HUT-78 cells (B), HTLV-IIIag-infected H9 cells (C), and STLV-IIIAGM-infected HUT-78 cells (D).
TABLE 1. Immunofluorescence reactivity of mAbs with different HIV-1, HIV-2, and SIV isolates

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>Immunofluorescence reactivity</th>
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<tbody>
<tr>
<td></td>
<td>R1C7(CA)</td>
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<tr>
<td>HIV-1</td>
<td></td>
</tr>
<tr>
<td>HTLV-III_B</td>
<td>+</td>
</tr>
<tr>
<td>HTLV-III_MN</td>
<td>+</td>
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<tr>
<td>HTLV-III_RF</td>
<td>+</td>
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<tr>
<td>HTLV-III_CC</td>
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<tr>
<td>HTLV-III_Rutz</td>
<td>+</td>
</tr>
<tr>
<td>HIV-2</td>
<td></td>
</tr>
<tr>
<td>HIV-2_NIH-Z</td>
<td>+</td>
</tr>
<tr>
<td>LK001 ST9</td>
<td>+</td>
</tr>
<tr>
<td>LAV-2_Rod</td>
<td>+</td>
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<tr>
<td>SIV</td>
<td></td>
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<tr>
<td>STLV-III_AGM</td>
<td>+</td>
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<tr>
<td>SIV-251</td>
<td>+</td>
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<tr>
<td>SIV-309</td>
<td>+</td>
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<tr>
<td>SIV-142</td>
<td>+</td>
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<td>SIV-157</td>
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<tr>
<td>SIV-186</td>
<td>+</td>
</tr>
<tr>
<td>SMM-5</td>
<td>+</td>
</tr>
<tr>
<td>HTLV-1 C91/PL</td>
<td>-</td>
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<tr>
<td>EIAV EFK</td>
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The M33 and BT3 mAbs are directed against HIV-1 core proteins; the remaining mAbs are anti-HIV-2\_NIH-Z antibodies.

The identification of HIV-2 proteins reacting with mAbs is described in Fig. 2. Positive (+) and negative (−) refer to reactivity in an indirect immunofluorescence assay. The reactivity of cells infected with different HIV-1, HIV-2, and SIV isolates was in the range of 10–90%. EIAV, equine infectious anemia virus; EFK, equine fetal kidney cells.

virus-infected cells. Similarly, the mAbs BT3 and M33 directed against HIV-1 core proteins reacted only with cells infected with HIV-1 isolates. It should be mentioned that both the R1C7 and A4F6 mAbs (directed against the HIV-2 CA protein) reacted with fixed cells but not with live cells, whereas R5C4 and R5F6 (directed against the HIV-2 MA protein) reacted with fixed as well as live cells.

Identification of Viral Proteins Reacting with mAbs. The viral proteins reacting with the different mAbs were identified by immunological blot analysis (Fig. 2). All four mAbs reacted with the gag proteins of HIV-2. Whereas antibodies R5C4 and R5F6 reacted with the MA (p16) protein of HIV-2 (Fig. 2A, lanes 1 and 2), A4F6 and R1C7 reacted specifically with the CA protein (p24) of HIV-2 (Fig. 2A, lanes 3 and 4). All four mAbs reacted with a gag precursor protein p55 of HIV-2, which is sometimes packaged in the virion. The cross-reactivity found with immunofluorescence was confirmed by immunological blot analysis. Antibody R1C7, which is specific for CA of HIV-2, also reacted with the CA proteins of HIV-1 and SIV (Fig. 2B and C, lane 4). A4F6 reacted with p24 of HIV-2 and SIV (STLV-III\_AGM), whereas R5C4 and R5F6, which are specific for the MA protein of HIV-2, did not cross-react with HIV-1 or SIV. Again, all the mAbs recognizing the gag proteins reacted with the precursor p55.

**DISCUSSION**

Four mouse mAbs were developed after immunization with the HIV-2\_NIH-Z isolate and were tested for reactivity with different HIV-1, HIV-2, and SIV isolates in an indirect immunofluorescence assay. One of them, R1C7, consistently reacted with all HIV-1, HIV-2, and SIV isolates tested so far, indicating that this particular epitope is highly conserved. By using immunological blot analysis, the epitope reacting with the R1C7 mAb was assigned to the CA protein of these immunodeficiency retroviruses. This is in line with previous observations showing serological cross-reactivity between HIV-1, HIV-2, and SIV core proteins (3–6).

Given its broad reactivity, the R1C7 mAb can be used for detection of members of the AIDS-related retrovirus family in tissues, body fluids, or cultured cells. Also, it may prove

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**Fig. 2.** Immunological blot analysis of mAbs and human sera. Electrophoretically separated HIV-2\_NIH-Z (A), HTLV-III\_B (B), and STLV-III\_AGM (C) proteins were blotted onto nitrocellulose and were reacted with R5C4 (lane 1), R5F6 (lane 2), A4F6 (lane 3), R1C7 (lane 4), HIV-1-positive serum (lane 5), negative control serum (lane 6), or HIV-2-positive serum (lane 7).
useful for assessing the level of p24 antigemia, which has been shown to be an early sign of HIV infection (21, 22). On the other hand, identification of an epitope shared by all members of the family of immunodeficiency retroviruses may have practical implications. For example, it has been shown that cross-reactivity between HIV-1 and HIV-2 does not consistently allow detection of HIV-2 seropositivity with HIV-1-specific assays (23, 24). This indicates a need for HIV-2-specific assays to screen blood donations and to perform HIV-2 seroepidemiological surveys. However, the availability of the RIC7 mAb may help to develop an HIV-1/HIV-2-specific assay (for instance, by using a synthetic peptide that reacts with the RIC7 mAb), which should be simpler, faster, and more economic.

The identification and characterization of the RIC7 epitope may also be useful for vaccine development. Recently, several groups have underscored the importance of the immune response to gag proteins because clinical progression to AIDS is associated with reduction in antibodies to p24, while patients with AIDS can die with high level of antibodies to env proteins (25–27). The highly conserved RIC7 epitope should therefore be considered as a potential candidate that might be included together with other conserved env and gag epitopes in a recombinant vaccine expected to be effective against all AIDS-related retroviruses.

Another anti-p24 mAb, called A4F6, reacted with all three HIV-2 isolates (HIV-2_0.7, LAV-2_Rd, and LK001_ST9) and some SIV isolates (STLV-III AIM, SIV-251, and SIV-309) but with none of the HIV-1 isolates. Therefore, this antibody identifies a relatively conserved epitope that is shared by HIV-2 and some SIV isolates. This is in agreement with data showing that HIV-2 is closer to SIV than to HIV-1 (5, 6), but it demonstrates a heterogeneity within the SIV group. Quite interestingly, restriction endonuclease maps and nucleotide sequence analysis have shown that STLV-III AIM, SIV-251, and SIV-309, which are A4F6 positive, are much closer to each other than to the other SIV isolates that are A4F6 negative. In fact, it has been proven that STLV-III AIM was derived from cell cultures infected with SIV-251 (28). The heterogeneity within the SIV group raises the following question. To what extent are some SIV isolates close to HIV-2, or is there an overlap between the HIV-2 and SIV groups?

The RSC4 and RSF6 mAbs reacted in immunological blots with the MA protein (p16) of HIV-2NLV, but they did not react with HIV-1 (HTLV-III NL) or SIV (STLV-III AIM). By using immunofluorescence with fixed cells, these mAbs reacted strongly only with HIV-2NLV_LAV-2_Rd and LK001_ST9. Quite interestingly, these mAbs also reacted with live HIV-2-infected cells. This finding suggests that there is expression of the MA protein on the cell surface. Obviously this is an important observation, which warrants a detailed analysis. Because RSC4 and RSF6 have identical patterns of reactivity and competition studies using fluorescein-labeled RSF6 and found that unlabeled RSC4 completely blocks the reaction of labeled RSF6 (data not shown).

The existence of multiple immunodeficiency retroviruses presents a complex epidemiological situation, particularly considering their different pathogenic propensities. HIV-1 is a cause of a fatal disease (AIDS) recognized as an epidemic of global dimensions, with a high prevalence in Central Africa (29). In West Africa, the number of AIDS cases is relatively low in spite of the fact that a considerable number of healthy HIV-2 seropositive individuals has been identified (23, 30). In the case of SIV, there are very limited data available regarding the pathogenicity of these viruses in their natural hosts. However, it is well established that some SIV isolates cause immunodeficiency in rhesus macaques and sooty mangabeys infected in captivity (3–5, 14). Further-

more, an extensive molecular analysis of different viral isolates clearly demonstrated that a considerable heterogeneity exists within the SIV as well as the HIV-2 groups of viruses (6–9, 13, 28). Thus, it is conceivable that different strains or subgroups of these immunodeficiency viruses may differ in their pathogenicity. Therefore, it is of ultimate importance to develop a systematic classification of all available immunodeficiency retroviruses that might prove indicative of the pathogenic potential of any additional isolate. The extent of the relationship between viruses is usually determined by molecular genetic methods, which are, however, expensive and time-consuming. As demonstrated in this work, mAbs can be used as rapid and simple tools for discrimination between different isolates. From this point of view, we consider our mAbs as a step toward the development of a serotypic classification of the entire HIV-related retrovirus family.

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