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Antibodies to the HIV-1 p17 Protein Cross-React with Human Superoxide Dismutase-2

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Antibodies reacting with the *gag* protein p17 of the human immunodeficiency virus type 1 (HIV-1) can occasionally be found in the serum of non-HIV-1-infected individuals. Conversely, anti-p17 antibodies can also react with human tissues from non-infected individuals. Here we report on the isolation from human liver of a molecule that is immunoreactive with anti-p17 antibodies. This molecule was purified to homogeneity and identified as superoxide dismutase-2 (manganese type SOD). Both human SOD-2 and HIV-1 p17 contain the LQPALK hexapeptide which may serve as a common antigenic determinant. This study indicates that human SOD-2 is a target for anti-p17 antibodies and suggests that HIV-1-negative individuals may possess SOD-2 auto-antibodies that cross-react with HIV-1 p17.

Antibodies reacting with the *gag* proteins p17 (a matrix protein underneath the viral envelope) or p24 (a major capsid potein) of the human immunodeficiency virus type 1 (HIV-1) have sometimes been detected in uninfected individuals during systematic blood screening (1-8). The HIV-1-negative status of these individuals was confirmed by a variety of tests, including immunoassays for HIV-1 antigens, viral culture for HIV-1, polymerase chain reaction (PCR) for HIV-1 DNA, and absence of seroconversion over time (5-8). Infection with HIV-2 or HTLV-1 was also ruled out (5-8). The occurrence of antibodies reacting with HIV-1 p17 and/or p24 in HIV-1-negative individuals increases in frequency in patients with autoimmune diseases (9). Conversely, immunoreactivity with anti-HIV-1p17 or p24 has been reported in normal human tissues from individuals with no HIV-1 infection (10-13). The cause of such immunoreactivity remains unknown, although some raised the possibility of infection with other retroviruses (5,6,8,14), such as bovine immunodeficiency virus (15), or the possibility of expression of retroviral proto-oncogene proteins (10) or intrinsic substance(s) with antigenic determinant(s) shared with HIV-1 (11-13). Thus, it is not known whether the anti-HIV-1 antibodies recognize "viral" or "human" antigens. The present study aimed at identifying and characterizing the antigen(s) in human tissues that is recognized by antibodies to HIV-1 p17.

METHODS

The supernatants from brain, heart, liver, kidney and skeletal muscle homogenates were examined. These tissues came from three uninfected autopsy patients. Two anti-HIV-1 p17 monoclonal antibodies were used: Cellular Products Inc. # 0801005, and Chemicon MAB-822.

We employed the following procedures.

1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. Homogenized tissues with Tris-buffered saline (50 mM Tris-HCl and 150 mM NaCl, pH 7.6, containing 50,000 U/l aprotinin, 5 mM EDTA and 1 mg/l leupeptin) were centrifuged at 100,000 × *g* for 1 h. The supernatant was separated by SDS-PAGE on 15% polyacrylamide gels and electroblotted onto a nitrocellulose membrane. The membrane was incubated for 3 h with an anti-HIV-1 p17 antibody (1:2000 dilution), followed by incubation with a biotinylated anti-mouse IgG (1:1000; Amersham). After reaction with streptavidin-labelled alkaline phosphatase (1:3000; Amersham), color was developed with nitro-blue tetrazolium (Sigma) and 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

2. Immunohistochemistry. Formalin-fixed tissue sections were incubated overnight with an anti-HIV-1 p17 antibody (1:1000), followed by incubation with biotinylated anti-mouse IgG (1:200; Vector). After reaction with avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector), labelling was visualized with 0.05% diaminobenzidine and 0.024% hydrogen peroxide.

3. Gel filtration chromatography. 2 ml of sample was applied to a HiLoad 16/60 Sephacryl S-100 HR column (Pharmacia) (buffer: 150 mM NaCl, flow rate: 0.25 ml/min) using the FPLC system (Pharmacia).

4. Ion-exchange chromatography. One ml of sample was applied to a Mono Q HR 5/5 column (Pharmacia) and eluted with a linear gradient (0 to 0.5 M NaCl in 20 mM Tris-HCl, pH 8.3) for 24 min using the FPLC system (Pharmacia).

5. Protein sequencing. A CBB (Coomassie Brilliant Blue)-stained band on PVDF (polyvinylidene difluoride) membrane was cut out

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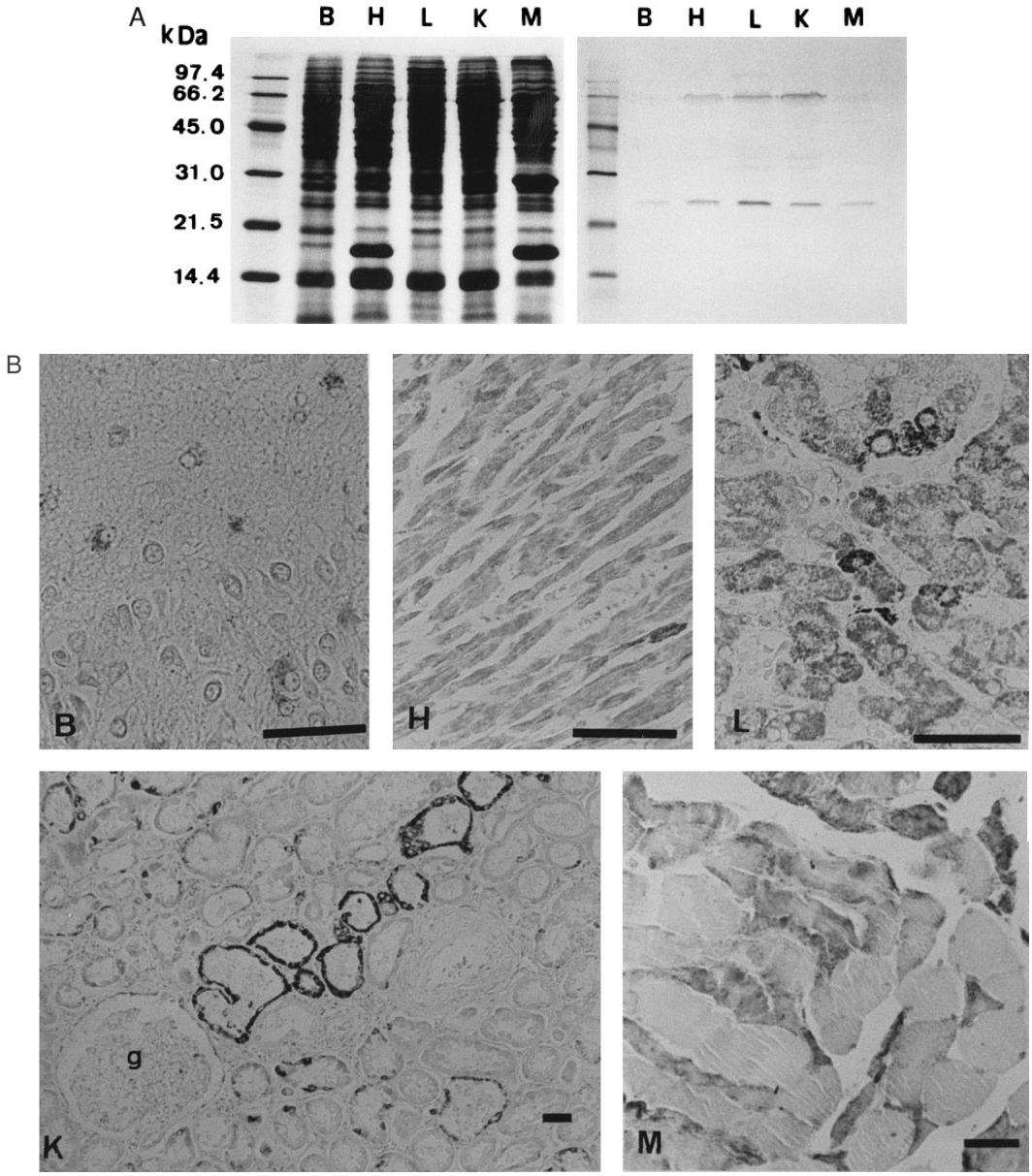

FIG. 1. (A, Left) SDS-PAGE of the supernatants from brain (B), heart (H), liver (L), kidney (K) and skeletal muscle (M) homogenates. Coomassie Brilliant Blue stain. (Right) Western blotting using an antibody to HIV-1 p17. (B) Immunohistochemistry of human tissues using an antibody to HIV-1 p17. Intense immunoreactivity is seen in the cytoplasm of some astrocytes (B), virtually all cardiac muscles (H), many hepatocytes (L), some renal tubular epithelial cells (K) and about half of the skeletal muscle fibers (M). g, glomerulus. Counterstained with hematoxylin. All scale bars = 50 μm.

and applied to the Hewlett-Packard G 1005 A protein sequencing system.

RESULTS

Western blot analysis of different human tissues showed a distinct immunoreactive band at ~23 kDa and a minor positive band at ~66 kDa in all organs examined (Fig. 1A). Immunostaining of tissue sections with these antibodies showed granular immunoreactivity in the cytoplasm of all above organs: some brain astrocytes, virtually all cardiac muscles, many hepatocytes, some renal tubular epithelial cells and about half of the skeletal muscle fibers (Fig. 1B). We selected the liver for the isolation of the antigen because of the availability of comparatively large amounts of starting material. The 80-100% ammonium sulfate cut from liver homogenates was subjected to gel filtration and

ion-exchange chromatography (Fig. 2, left). At each purification step, the immunoreactive fractions were identified by Western blotting (Fig. 2, right). The products of ion-exchange chromatography contained two bands: a major immunoreactive band and a minor non-immunoreactive band (Fig. 2). The immunoreactive band was electroblotted onto a PVDF membrane for N-terminal amino acid sequence determination. The N-terminal sequence was identical with that of human superoxide dismutase-2 (SOD-2: manganese type SOD)(16,17) (Fig. 3A). An homology search revealed that human SOD-2 and HIV-1 p17 both contained the identical LQPALK hexapeptide (Fig. 3B)(16,18).

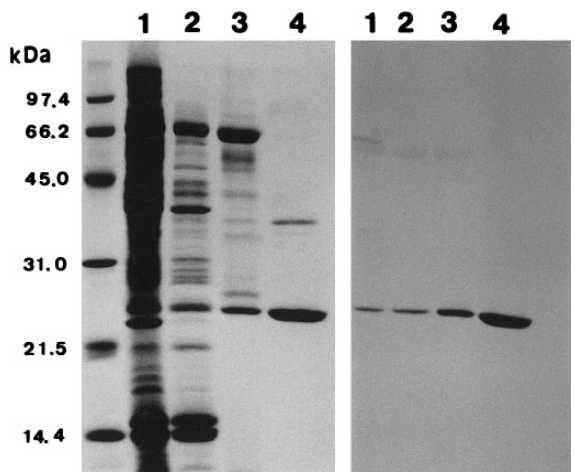

FIG. 2. Purification of anti-HIV-1 p17 reactive materials. (Left) Coomassie Brilliant Blue stain. (Right) Western blotting using an anti-p17 antibody. Lane 1, crude supernatant from liver homogenate; lane 2, 80-100% ammonium sulfate precipitate; lane 3, gel filtration chromatography; lane 4, ion-exchange chromatography.

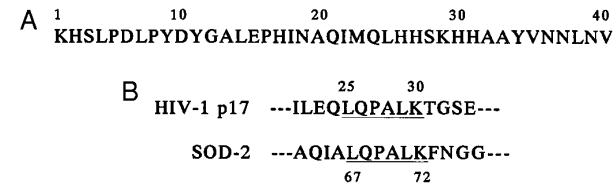

FIG. 3. (A) Amino acid sequence of the immunoreactive band from ion-exchange chromatography. (B) Amino acid sequence common to HIV-1 p17 and SOD-2.

DISCUSSION

The present study indicates that human SOD-2 is recognized by anti-HIV-1 p17 antibodies. This observation is consistent with the previous localization of p17-like immunoreactivity to mitochondria (13), where SOD-2 is found. We suggest that the LQPALK hexapeptide is the common antigenic determinant between the two proteins Furthermore, our results suggest that reactivity of serum from non-infected individuals with HIV-1 p17 protein is due to an anti-SOD-2 autoantibody. Production of this autoantibody seems to increase in patients with autoimmune diseases (9). Although SOD-2, a scavenger for superoxides, is localized in mitochondria, a small amount of SOD-2 has been reported to exist in the serum of normal individuals (19-21). Serum levels increase under conditions of cell destruction such as myocardial infarction (20). This serum SOD-2 might stimulate antibody production, although local immune response to SOD-2 is also possible.

The present study has two implications. First, detection of anti-HIV-1 p17 immunoreactivity in tissue sections from AIDS patients does not necessarily imply HIV-1 infection. The signal may simply be due to endogenous SOD-2. Second, in certain asymptomatic patients with HIV-1 infection, a high anti-p17 titer has been observed and this antibody has been reported to inhibit the proliferation of HIV-1 (22-24). In addition to this beneficial effect of the anti-p17 antibody, the present study suggests a potential adverse effect: a persistent high titer of anti-p17 over a long period of time may cause a general and gradual cellular dysfunction through inhibition of SOD-2 activity.

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