Human Immunodeficiency Virus Type 1–infected Individuals Make Autoantibodies that Bind to CD43 on Normal Thymic Lymphocytes

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Summary

Sera from human immunodeficiency virus type 1 (HIV-1)-infected and -noninfected individuals were screened for antibodies that could bind to native T cell differentiation antigens. Antibodies that could immunoprecipitate CD43 (sialophorin, leukosialin) from a T cell lymphoma line were detected in sera from 27% of patients, and antibodies that could bind specifically to transfected cells expressing CD43 were detected in 47% of patients. The anti-CD43 antibodies were related to HIV-1 infection in that no patients with other chronic viral infections or systemic lupus erythematosus contained such antibodies in their sera. The anti-CD43 autoantibodies bound to a partially sialylated form of CD43 expressed by normal human thymocytes, but not by normal, circulating T lymphocytes. However, the determinant(s) recognized by the anti-CD43 autoantibodies was present on a large proportion of circulating T lymphocytes, but masked from antibody recognition by sialic acid residues. These results demonstrate that HIV-1 infection is specifically associated with the production of autoantibodies that bind to a native T cell surface antigen.

The severe immunodeficiency associated with HIV-1 infection is believed to result primarily from depletion, and to some degree, dysfunction of $CD4^+$ T lymphocytes (1). Many individuals infected by HIV-1 have serum anti-T lymphocyte antibodies (2–9), and it has been suggested that such antibodies contribute to the $CD4^+$ lymphocyte abnormalities of AIDS. Antilymphocyte antibodies that mediate complement-induced lysis of allogeneic T cells (2–4, 7, 8) or suppress mitogen-induced proliferation of allogeneic $CD4^+$ lymphocytes (8) have been detected in sera from HIV-1-infected individuals. Moreover, circulating T lymphocytes coated by autologous Ig (surface Ig^+ T cells) have been detected in a substantial proportion of infected patients (10–12). Such surface Ig^+ T cells can appear early in the course of HIV-1 infection, before decrements in the absolute $CD4^+$ lymphocytes counts (12).

Despite the potential immunosuppressive effects of antilymphocyte antibodies, their actual contribution to the immunopathogenesis of AIDS remains controversial. One problem has been the difficulty in identifying specific T cell surface antigens recognized by such antibodies. Although serum antibodies that bind to soluble, recombinant forms of CD4 (sCD4) appear specific for HIV-1 infection, (13–15), they do not bind to native CD4 on lymphocytes (13). Other lymphocyte-derived proteins that react with antibodies from $HIV\text{-}1^+$ sera (8, 16) have been described, but the identities of these proteins and their cellular location (i.e., cell surface vs. intracellular) remain uncertain.

In the present study, we show that many HIV-1-infected individuals make autoantibodies that bind specifically to the T cell surface antigen CD43. CD43, also known as sialophorin (17) or leukosialin (18), is a heavily sialylated glycoprotein expressed on the surface of virtually all thymocytes and T lymphocytes (19, 20). The anti-CD43 autoantibodies from HIV-1-infected individuals recognize a partially sialylated form of CD43 that is present on normal human thymocytes but not on mature, circulating T lymphocytes. The potential significance of these results is discussed in the context of recent insights into the role of CD43 in immune physiology.

Materials and Methods

Cells and Cell Lines. Normal human PBMC were obtained from heparinized blood samples from healthy donors and prepared by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density centrifugation. PBMC were treated with 5 μg/ml of PHA (Sigma Chemical Co., St. Louis, MO) for 3 d and then with 20 U/ml human rIl-2 (Cetus Corp., Emeryville, CA) for 5 d to obtain T lymphoblasts. Human thymocytes were obtained from infants undergoing corrective cardiac surgery at the New England Medical Center Hospital. The T cell lymphomas cell line SupT1 (21)

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was provided by Dr. James Hoxie, University of Pennsylvania, Philadelphia, PA. The T lymphoblasts and the SupT1 cell line were grown in RPMI 1640 media (containing 10% FCS [Hyclone Laboratories, Logan, UT] supplemented with glutamine [2 mM], penicillin [100 U/ml], and streptomycin [100 μg/ml]). COS cells used for transfection studies were maintained in similar media, substituting gentamicin sulfate (50 μg/ml) for penicillin and streptomycin.

Sera and mAbs. Sera from HIV-1-infected individuals was provided by the Boston City Hospital Immunodeficiency Clinic and the Fenway Community Health Center, Boston, MA; from hepatitis B antigenic ($HBsAg^+$)[1] individuals (Dr. Barbara Werner, Massachusetts State Laboratory, Boston, MA); from HTLV-1-seropositive individuals (Dr. Antonella Caputo, Dana Farber Cancer Institute, Boston, MA); and from patients with SLE (Dr. Paul Demchak, New England Center Hospital, Boston, MA). Anti-CD1 mAb (OKT6 ascites) was a gift from Dr. Judith Swack, New England Medical Center Hospital. Anti-intracellular adhesion molecule type 1 (ICAM-1) mAb (RR1/1) was provided by Dr. Timothy Springer, Center for Blood Research, Boston, MA. Anti-Leu-22 (CD43) and anti-Leu-3a (CD4) were purchased from Becton Dickinson & Co. (Mountain View, CA). PE-conjugated OKT3 (CD3) was purchased from Ortho Diagnostic Systems, Inc., Westwood, MA.

Preparation of Antibody-containing Eluates. The eluates were prepared by incubating 1 ml of human serum (diluted 1:40 in PBS, pH 7.2, containing 1% BSA and 0.2% sodium azide) with 10^9 SupT1 cells for 2 h at 4°C. The cells were washed four times with 40 ml of ice-cold PBS, and the bound antibodies were eluted using 2 ml of 0.1 M glycine, pH 2.5, for 2 min on ice. The cells were removed by centrifugation (1,000 *g*), the eluates were neutralized with 200 μl of 1 M Tris, pH 8.0, and then dialyzed extensively against PBS, pH 7.2, before use. Antibody concentration of the eluates was determined by ELISA.

Immunofluorescence. For staining SupT1 cells, sera clarified by centrifugation (10,000 *g* for 10 min) were diluted 1:50 in PBS containing 1% BSA and 0.2% sodium azide (staining buffer) and incubated for 30 min on ice with the SupT1 cells. After washing in staining buffer, the cells were incubated for 30 min on ice with FITC-conjugated goat anti-human IgG $F(ab')_2$ (Tago Inc., Burlingame, CA) diluted 1:60 in staining buffer. For staining thymocytes, 1 μg/ml anti-Leu-22 or 0.25 μg/ml of human antibody-containing eluate was used, diluted in staining buffer. Binding of anti-Leu-22 was detected by FITC-conjugated goat anti-mouse IgG $F(ab')_2$ (Tago Inc.), and binding of IgG from the eluates was detected by the goat anti-human IgG reagent described above. After staining, all cells were washed in staining buffer, fixed in 1% formalin, and analyzed on an Epics 541 flow cytometer (Coulter Electronics Inc., Hialeah, FL).

Radiolabeling. SupT1 cells (2×10^7) or 4×10^7 thymocytes or 5×10^6 COS cells were washed four times in PBS and resuspended in 1 ml of PBS containing 10 U of bovine milk lactoperoxidase (Calbiochem-Behring Corp., San Diego, CA) and 1 mCi of $Na^{125}I$ (Amersham Corp., Arlington Heights, IL). Then, 25 μl of 0.03% hydrogen peroxide was added to the cell suspension initially and at three successive times at 5-min intervals. Sodium azide (25 μl of a 20% solution) was added to the cell suspensions, the cells were washed four times in PBS, and then solubilized in lysis buffer (0.05 M Tris buffer, pH 7.2, containing 1% Triton X-100 (Sigma Chemical Co.) and 1 mM PMSF. The cell lysates were clarified by centrifugation (12,000 *g* for 30 min), and the supernatants were used for immunoprecipitations.

Immunoprecipitation and Gel Electrophoresis. For immunoprecipitation of radiolabeled cell lysates, aliquots were precleared with protein A–Sepharose 6MB beads (Pharmacia Fine Chemicals) and then mixed with antibody (1 μg of mAb or 0.25 of eluate) that was prebound to 50 μl of packed beads. The mixtures were incubated for 12–18 h at 4°C, the beads were washed six times with lysis buffer, and the immune complexes were eluted by heating for 5 min at 80°C in 2× sample buffer containing 50 mM Tris, pH 6.8, 4% SDS, 2% glycerol, 10% 2-ME and 0.02% bromphenol blue dye. The immunoprecipitates were resolved by 10% SDS-PAGE, and the dried gels were autoradiographed at −70°C for 5 d using an intensifying screen. To prepare samples for the immunoblotting experiment, 6×10^7 SupT1 cells were solubilized in lysis buffer, the lysates were clarified by centrifugation, and then precleared with protein A beads. Equal volume aliquots of the lysates were immunoprecipitated by anti-Leu-22 (1 μg), anti-Leu-3a (1 μg), or pooled eluate (0.25 μg) prebound to protein A beads (50 μl). The beads were washed six times in lysis buffer, the immune complexes were eluted by heating in 2× sample buffer, and were resolved by 10% SDS-PAGE.

Immunoblotting. Immunoprecipitates of unlabeled SupT1 cells resolved by SDS-PAGE were blotted onto nitrocellulose paper as described (22). The nitrocellulose paper was blocked in 0.05 M Tris-buffered saline, pH 7.0, containing 5% nonfat dry milk (blocking buffer), and then reacted for 18 h at 4°C with anti-Leu-22 (1 μg/ml), diluted in blocking buffer. The blot was washed extensively in blocking buffer and then reacted with alkaline phosphatase–conjugated goat anti-mouse Ig (Boehringer Mannheim Biochemicals, Indianapolis, IN) diluted 1:600 in blocking buffer for 2 h at room temperature. After further extensive washing, the blot was developed with a precipitating substrate (23).

Glycosidase Treatment of Cells and Immunoprecipitates. Neuraminidase treatment of cells was performed in RPMI 1640 with 0.1 U/ml of enzyme at 37°C for 30 min. Enzyme digestions of immunoprecipitates were performed as follows: *Vibrio cholera* neuraminidase (Calbiochem-Behring Corp.), 0.1 μ/ml for 60 min at 37°C; O-Glycanase (Genzyme, Boston, MA), 4 mU/ml for 18 h at 37°C. The enzyme reaction mixtures contained 0.17% SDS, 0.3% 2-ME, 1.25% NP-40, 5 mM calcium carbonate, 10 mM phenanthroline, and 20 mM sodium cacodylate, pH 6.5.

Plasmid Construction and Cell Transfection. The PEER-3 cDNA clone of CD43 (provided by Dr. Minoru Fukuda, La Jolla, CA [24]) was subcloned into an expression vector (CDM8) that utilizes the cytomegalovirus early promoter and contains simian virus 40 origin of replication (25). The 1.5-kb PEER-3 cDNA was isolated from Bluescript by digestion with EcoRI and low melting point agarose gel electrophoresis. The recovered cDNA was blunt ended using the Klenow fragment of DNA polymerase I, ligated to BstX1 linkers and to BstX1-digested CDM8. A DEAE-dextran method (25) was used to transfect COS cells with the CD43-CDM8 plasmid.

Results

Detection of Anti-T Lymphocyte Antibodies in $HIV\text{-}1^+$ Sera. To determine if antibodies to native T lymphocyte surface antigens are produced by HIV-1-infected individuals, we screened sera by immunofluorescent flow cytometry to identify those containing antibodies that could bind to a T cell

[1] *Abbreviations used in this paper:* $HBsAg^+$, hepatitis B antigenemic; HTLV-1, human T lymphocyte virus type 1; ICAM-1, intracellular adhesion molecule type 1; WA, Wiskott-Aldrich.

lymphoma line (termed SupT1). This cell line was chosen because it expresses multiple T cell antigens including high levels of CD4 (26). Sera from several HIV-1-seropositive individuals contained antibodies that stained the SupT1 cell line (Fig. 1). In contrast, no sera from hepatitis B-antigenemic individuals ($HBsAg^+$) and few sera from individuals seropositive for human T lymphocyte virus type 1 (HTLV-1) infection or with the autoimmune disease SLE demonstrated staining of SupT1 cells greater than sera from healthy, HIV-1-seronegative control subjects (Fig. 1).

Identification of Cell Surface Protein Recognized by Anti-T Lymphocyte Antibodies. To identify the SupT1 surface antigen(s) recognized by the serum antibodies, sera were absorbed to SupT1 cells, and the eluted antibodies (termed eluates) were used to immunoprecipitate detergent-solubilized lysates from surface ^{125}I-labeled SupT1 cells. Of 18 eluates prepared from different $HIV\text{-}1^+$ sera (sera also positive for SupT1 staining), eight immunoprecipitated a single SupT1 surface protein with a M_r of 120 kD on SDS-PAGE (Fig. 2 *a*). The 120-kD protein was not immunoprecipitated by eluates prepared from the two $HTLV\text{-}1^+$ sera and the one SLE serum that did stain the SupT1 cells. Thus, antibodies that immunoprecipitated the 120-kD SupT1 protein were detected only in $HIV\text{-}1^+$ sera.

Biochemical Characterization of 120-kD SupT1 Protein. To assess the contribution of carbohydrate to the 120-kD SupT1 protein, immunoprecipitates were subjected to glycosidase digestion. Neuraminidase digestion of the immunoprecipitated 120-kD SupT1 protein shifted its M_r to ~150 kD (Fig. 2 *b*, lane *N*), a result consistent with removal of negatively charged sialic acid residues (18, 19). Subsequent *O*-Glycanase digestion of the neuraminidase-treated immunoprecipitate resulted in a M_r of ~110 kD, indicating the presence of *O*-linked oligosaccharides (Fig. 2 *b*, lane *N/O*). Treatment of the immunoprecipitated 120-kD protein with *N*-Glycanase did not result in a discernable shift in relative molecular mass of the protein (data not shown), suggesting that little or no *N*-linked oligosaccharides were present. These biochemical characteristics suggested that the 120-kD SupT1 protein was similar to CD43 (sialophorin, leukosialin), a cell surface sialoglycoprotein predominantly expressed by cells of hematopoietic origin (19, 20).

The 120-kD SupT1 Protein Is Identical to CD43. The results of two experiments established that the 120-kD protein immunoprecipitated by the eluates is CD43. First, an anti-CD43 mAb (anti-Leu-22; clone L60) (27) bound to the 120-kD SupT1 protein that was immunoprecipitated by pooled eluates, and then electroblotted onto nitrocellulose paper (Fig. 2 *c*). Second, the eluates immunoprecipitated a 130-kD protein from COS cells transfected with a cDNA clone encoding CD43 (Fig. 2 *d*, lane *A*) but not from control COS cells transfected with a cDNA clone of ICAM-1 (Fig. 2 *d*, lane *B*). anti-Leu-22 specifically immunoprecipitated a protein with a M_r of 120 kD from the COS cells expressing CD43 (Fig. 2 *d*, lane *C*), suggesting that in transfected COS cells, anti-Leu-22 recognizes a more sialylated form of CD43 than that recognized by the eluates.

Anti-CD43 Autoantibodies Bind Only to CD43 Expressed by Thymocytes. To determine if the eluates could bind to normal human cells of T cell lineage known to express CD43, two eluates (from two different HIV-1-infected subjects) containing anti-CD43 antibodies were tested for binding to thymocytes, fresh peripheral blood T lymphocytes, and PHA-activated T lymphocytes that had been maintained in IL-2. The eluates bound only to thymocytes, whereas the anti-CD43 mAb (anti-Leu-22) bound to all three cell types (Fig. 3 *a*). However, the eluates bound to all cell types if the cells were first treated with neuraminidase, a treatment that eliminated the epitope recognized by anti-Leu-22 (Fig. 3 *b*). These results suggested that the eluates recognize a non–sialic acid epitope of CD43 present on both thymocytes and mature T lymphocytes, but accessible to autoantibody binding only on thymocytes.

The eluates were also tested for binding to freshly obtained peripheral blood T lymphocytes and T cell lines from HIV-

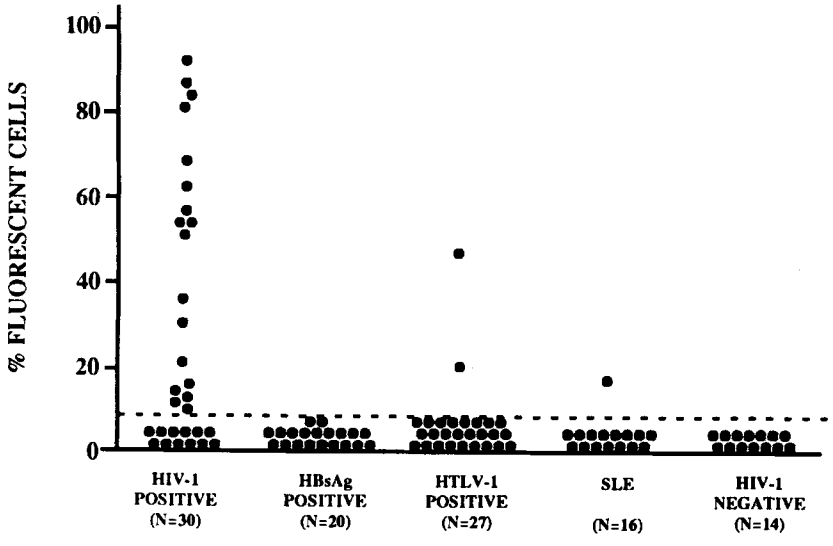

Figure 1. Immunofluorescent screening of sera for antibodies that could bind to the SupT1 T cell lymphoma line. Percent fluorescent cells indicates the percentage of cells stained by each serum (diluted 1:50) greater than stained by buffer alone. Each dot represents the percentage of cells stained by an individual serum. The horizontal dotted line indicates 3 SD above the mean percentage of cells stained by sera from 14 healthy, individually tested, HIV-1 seronegative laboratory personnel.

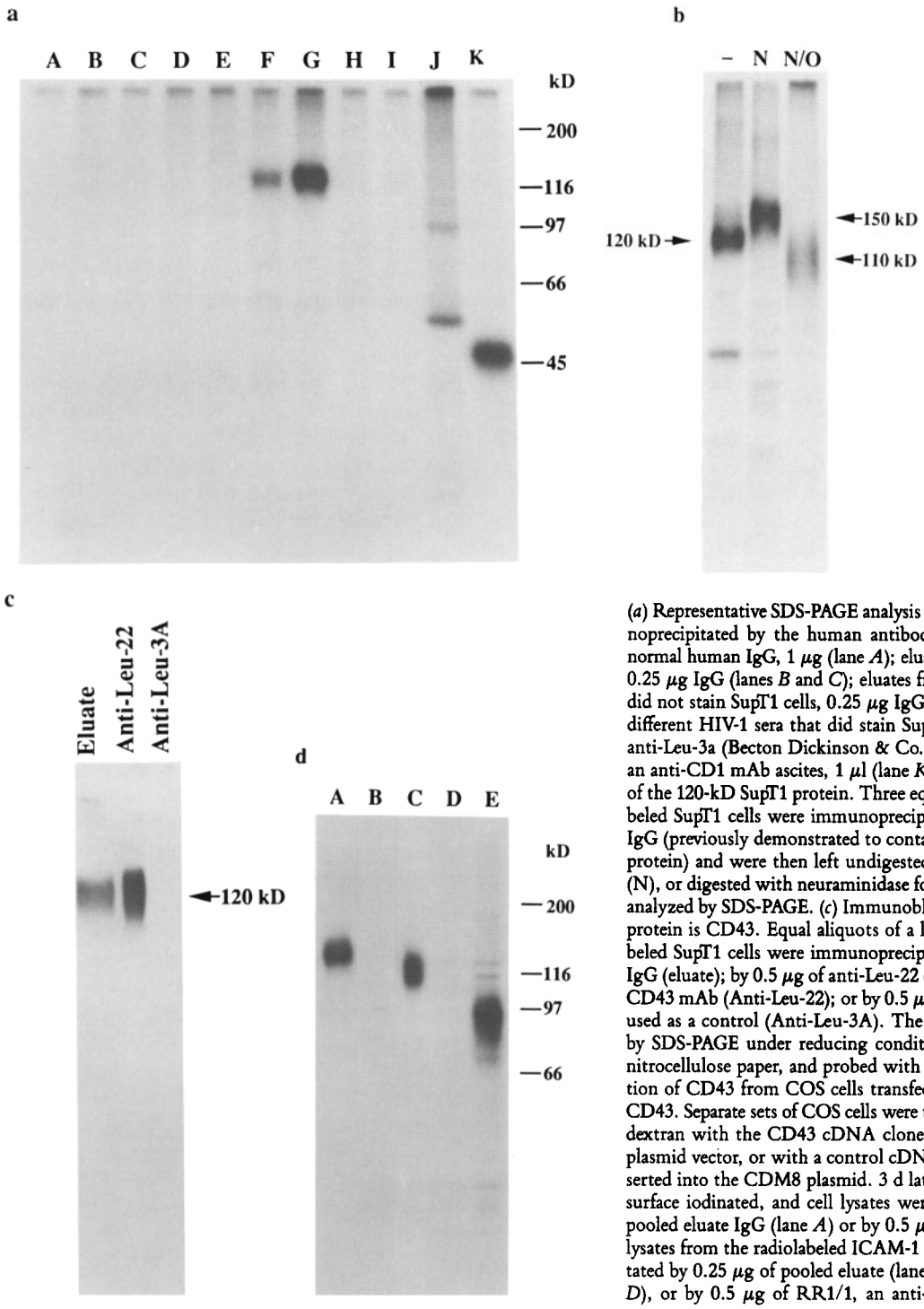

Figure 2. Identification and characterization of the SupT1 cell surface protein recognized by the human antibody–containing eluates. (*a*) Representative SDS-PAGE analysis of the SupT1 surface protein immunoprecipitated by the human antibody–containing eluates. Antibodies: normal human IgG, 1 μg (lane *A*); eluates from two different HIV-1$^-$ sera, 0.25 μg IgG (lanes *B* and *C*); eluates from two different HIV-1$^+$ sera that did not stain SupT1 cells, 0.25 μg IgG (lanes *D* and *E*); eluates from four different HIV-1 sera that did stain SupT1 cells, 0.25 μg IgG (lanes *F–I*); anti-Leu-3a (Becton Dickinson & Co.) an anti-CD4 mAb, 1 μg (lane *J*); an anti-CD1 mAb ascites, 1 μl (lane *K*). (*b*) Biochemical characterization of the 120-kD SupT1 protein. Three equal aliquots of lysates from radiolabeled SupT1 cells were immunoprecipitated by 0.25 μg of pooled eluate IgG (previously demonstrated to contain antibodies to the 120-kD SupT1 protein) and were then left undigested (–), digested with neuraminidase (N), or digested with neuraminidase followed by O-Glycanase (N/O) and analyzed by SDS-PAGE. (*c*) Immunoblot evidence that the 120-kD SupT1 protein is CD43. Equal aliquots of a lysate prepared from 6×10^7 unlabeled SupT1 cells were immunoprecipitated by 0.25 μg of pooled eluate IgG (eluate); by 0.5 μg of anti-Leu-22 (Becton Dickinson & Co.), an anti-CD43 mAb (Anti-Leu-22); or by 0.5 μg of anti-Leu-3a, an anti-CD4 mAb used as a control (Anti-Leu-3A). The immunoprecipitates were resolved by SDS-PAGE under reducing conditions, electroblotted onto 0.45-μm nitrocellulose paper, and probed with anti-Leu-22. (*d*) Immunoprecipitation of CD43 from COS cells transfected with a cDNA clone encoding CD43. Separate sets of COS cells were transiently transfected using DEAE-dextran with the CD43 cDNA clone PEER-3 inserted into the CDM8 plasmid vector, or with a control cDNA encoding ICAM-1 (43), also inserted into the CDM8 plasmid. 3 d later, each set of transfected cells was surface iodinated, and cell lysates were immunoprecipitated by 0.25 μg pooled eluate IgG (lane *A*) or by 0.5 μg of anti-Leu-22 (lane *C*). Control lysates from the radiolabeled ICAM-1 transfectants were immunoprecipitated by 0.25 μg of pooled eluate (lane *B*), by 0.5 μg of anti-Leu-22 (lane *D*), or by 0.5 μg of RR1/1, an anti-ICAM-1 mAb (lane *E*).

1-seropositive individuals (n = 3). In all cases, the eluates bound only to neuraminidase-treated cells (data not shown). These results suggest that peripheral blood T lymphocytes from HIV-1-infected individuals either do not express partially sialylated forms of CD43, or if they do, are targeted for rapid removal from the circulation.

Immunoprecipitation of cell lysates from radiolabeled thymocytes confirmed that the anti-CD43 autoantibodies recognize a partially sialylated form of CD43. The pooled eluates immunoprecipitated a single protein with a M_r of ~130 kD, whereas anti-Leu-22 immunoprecipitated a protein with a M_r of ~120 kD (Fig. 3 *c*, lanes *A* and *B*). However, after digestion of each immunoprecipitate with neuraminidase, the proteins immunoprecipitated by the pooled

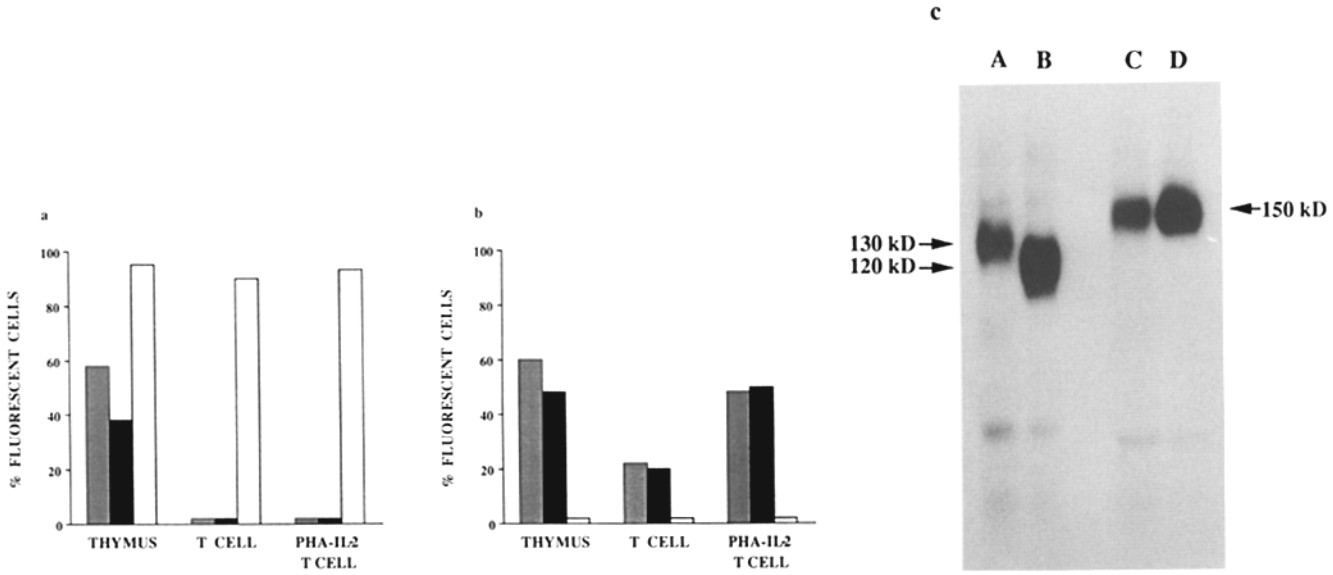

Figure 3. The eluates identify a partially sialylated CD43 form expressed by normal thymocytes. (*a*) The eluates bind to thymocytes but not T lymphocytes. Eluates from patients M-1 (*shaded bars*) and E-1 (*black bars*), and anti-Leu-22 (*white bars*) were tested by indirect immunofluorescence for binding to thymocytes (thymus), peripheral blood T lymphocytes (T cells), and T lymphocytes stimulated with PHA for 3 d and maintained in IL-2 for 7 d (PHA-IL-2 T cells). The results are representative of three separate experiments in which thymocytes and T lymphocytes from other donors were tested. (*b*) The eluates bind to all cell types after cells are treated with neuraminidase (0.1 U/ml in RPMI 1640 for 30 min at 37°C), but reactivity of anti-Leu-22 is lost. (*c*) Immunoprecipitation of CD43 from normal human thymocytes by the pooled eluates (lanes *A* and *C*) and anti-Leu-22 (lanes *B* and *D*). The immunoprecipitates in lanes *C* and *D* were treated with neuraminidase (0.1 U/ml) before electrophoresis. 0.5 μg of pooled eluates or 0.5 μg of anti-Leu-22 were used to immunoprecipitate equal aliquots of ^{125}I-labeled cell lysate from 4 × 10^7 normal thymocytes.

eluates, and anti-Leu-22 migrated identically with a M_r of 150 kD (Fig. 3 *c*, lanes *C* and *D*). These results are consistent with those from the COS cell transfection experiments where two forms of CD43 were identified (Fig. 2 *d*), differing only in their degrees of sialylation. Taken together with the immunofluorescence results, the data confirm that a partially sialylated CD43 form is normally expressed on a large subpopulation of thymocytes but not on mature T lymphocytes (19).

Discussion

These studies demonstrate that anti-CD43 autoantibodies can be detected in sera from HIV-1 infected individuals and that these autoantibodies bind to a form of CD43 expressed by normal thymocytes. The results also indicate that the autoantibodies recognize a CD43 epitope(s) on circulating T lymphocytes that is masked by sialic acid residues. These characteristics distinguish the anti-CD43 autoantibodies from antilymphocyte antibodies described previously (2–9) that bind circulating lymphocytes. The absence of partially sialylated CD43 forms on circulating lymphocytes (19) suggests that the anti-CD43 autoantibodies we detected could not contribute to depletion of circulating $CD4^+/CD43^+$ lymphocytes. Rather, the thymocyte specificity of these antibodies suggests that they may interfere with replenishment of the circulating lymphocyte pool. Because anti-CD43 autoantibodies were found only in HIV-1-infected individuals, it is possible that they are involved in the immunopathogenesis of AIDS.

It has been demonstrated that binding of mAbs to CD43 can induce biochemical and functional changes in T cells in vitro. These antibody-mediated effects include induction of phosphoinositide hydrolysis with resultant second messenger formation (28); homotypic adhesion of lymphocytes (29) and monocytes (30); and activation of thymocytes (31) and T lymphocytes (29, 32) by a mechanism independent of TCR/CD3 complex–mediated signaling (28). It is plausible that the effects mediated by anti-CD43 mAbs in vitro would be mirrored by human anti-CD43 autoantibodies in vivo, resulting in inappropriate activation of thymocytes during the process of maturation. Antibodies that can inappropriately activate or cause adhesion of thymocytes may heighten thymocyte susceptibility to HIV-1 infection (33) or facilitate intercellular virus transmission. Moreover, thymocyte-specific anti-CD43 antibodies could target thymocytes for destruction by complement-mediated lysis and thus contribute to the severe thymic atrophy in AIDS (34, 35). If the thymus is required for normal replenishment of $CD4^+$ lymphocytes in adult humans as it is in adult mice (36, 37), then thymic dysfunction or destruction would be expected to prevent replenishment of mature $CD4^+$ cells killed by HIV-1.

We observed that 8 of the 30 HIV-1-seropositive subjects tested (27%) had serum anti-CD43 antibodies that could immunoprecipitate CD43 from the SupT1 cells. Yet, 18 of the 30 HIV-1-seropositive subjects (60%) had serum antibodies that stained the SupT1 cell line (see Fig. 1). Because some

patients may have had anti-CD43 antibodies that could not immunoprecipitate CD43, we tested sera by immunofluorescence for the presence of antibodies that could bind specifically to transfected COS cells that express CD43. By this method, sera from 14 of the 30 HIV-1-seropositive subjects (47%) specifically stained the CD43-expressing COS cells (mean fluorescence intensity of each positive sera $\geq$ 3 SD above the mean fluorescence intensity obtained using pooled normal human sera). No sera from HIV-1-seronegative homosexual men (n = 14) specifically stained the CD43-expressing cells. These data suggest that anti-CD43 autoantibodies are a common feature of HIV-1 infection and provide further evidence that such antibodies are restricted to individuals infected by HIV-1.

The most provocative data suggesting that anti-CD43 autoantibodies may contribute to the immunodeficiency of AIDS come from the study of children with the Wiskott-Aldrich (WA) syndrome, an X chromosome–linked, severe immunodeficiency syndrome. Lymphocytes from children with the WA syndrome express diminished amounts or unstable forms of CD43 (17, 38). The clinical course of the WA syndrome includes progressive T cell depletion, susceptibility to opportunistic and pyogenic infections, inability to produce antibodies against polysaccharide antigens, thrombocytopenia, and severe eczema (39). Several of these clinical features, particularly T cell depletion and susceptibility to opportunistic infections, also characterize HIV-1 infection. If normal CD43 expression is important for T cell development and if anti-CD43 autoantibodies can interfere with this process, then there may be a link between the immunopathogenesis of AIDS and the WA syndrome.

Why are anti-CD43 autoantibodies produced by HIV-1-infected individuals? One possibility is that in noninfected individuals, immunogenic epitopes of CD43 on circulating lymphocytes evade immunologic recognition because they are masked by sialic acid residues. However, in conditions where increased lymphocyte destruction is thought to occur (e.g., HIV-1-induced lymphocyte cytopathicity), autoimmunogenic CD43 epitopes may be exposed. The exposure of such epitopes could result in the induction of autoantibodies, before $CD4^+$ lymphocyte depletion and the corresponding defect in humoral immune responses occur.

Depletion of $CD4^+$ lymphocytes has been noted in many HIV-1-seropositive, healthy individuals (40) when the proportion of virus-infected lymphocytes is estimated to be small ($\sim$1:50,000) (41) and plasma viremia is low or undetectable (41, 42). Such observations support the notion that in addition to direct virus cytopathicity, other mechanisms may contribute to $CD4^+$ lymphocyte depletion early in the course of HIV-1 infection. For example, cytolysis of infected $CD4^+$ cells in asymptomatic individuals by CTL combined with defective T cell replenishment could manifest as a selective depletion of the $CD4^+$ lymphocyte pool. Understanding the effects of anti-CD43 autoantibodies on T cell maturation may provide further insight into the immunopathogenesis of $CD4^+$ cell depletion in AIDS.

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References

1. Bowen, D.L., H.C. Lane, and A.S. Fauci. 1985. Immunopathogenesis of the acquired immunodeficiency syndrome. *Ann. Intern. Med.* 103:704.
2. Pollack, M.S., C. Callaway, D. LeBlanc, H.M. Robinson, F. Mastrota, B. Dupont, and B. Koziner. 1983. Lymphocytotoxic antibodies to non-HLA antigens in the sera of patients with acquired immunodeficiency syndrome (AIDS). *Prog. Clin. Biol. Res.* 133:209.
3. Klostar, B., R. Tomar, and T. Spira. 1984. Lymphocytotoxic antibodies in the acquired immunodeficiency syndrome (AIDS). *Clin. Immunol. Immunopathol.* 30:330.
4. Pruzanski, W., J. Jacobs, P. Lorne, and M.D. Laing. 1984. Lymphocytotoxic antibodies against peripheral blood B and T lymphocytes in homosexuals with AIDS and ARC. *AIDS Res.* 1:211.
5. Kiprov, D.D., R.E. Anderson, P.R. Morand, D.M. Simpson, J.C. Shermann, J.A. Levy, and A.R. Moss. 1985. Antilymphocyte antibodies and seropositivity for retroviruses in groups at high risk for AIDS. *N. Engl. J. Med.* 312:1517.
6. Dorsett, B., W. Cronin, V. Chuma, and H.L. Ioachim. 1985. Anti-lymphocyte antibodies in patients with the acquired immune deficiency syndrome. *Am. J. Med.* 78:621.
7. Tomar, R.H., P.A. John, A.K. Hennig, and B. Kloster. 1985. Cellular targets of antilymphocyte antibodies in AIDS. *Clin.*

Immunol. Immunopathol. 37:37.
8. Strickler, R.B., T.M. McHugh, D.J. Moody, W.J.W. Morrow, D.P. Stites, M.A. Shuman, and J.A. Levy. 1987. An AIDS-related cytotoxic autoantibody reacts with a specific antigen on stimulated CD4+ T cells. *Nature (Lond.).* 327:710.
9. Ozturk, G.E., P.F. Kohler, C.R. Horsburch, Jr., and C.H. Kirkpatrick. 1987. The significance of antilymphocyte antibodies in patients with acquired immune deficiency syndrome (AIDS) and their sexual partners. *J. Clin. Immunol.* 7:130.
10. Stites, D.P., C.H. Casavant, T.M. McHugh, A.R. Moss, S.L. Beal, J.L. Zeigler, A.M. Saunders, and N.L. Warner. 1986. Flow cytometric analysis of lymphocyte phenotypes in AIDS using monoclonal antibodies and simultaneous dual immunofluorescence. *Clin. Immunol. Immunopathol.* 38:161.
11. Daniel, V., K. Schimpf, and G. Opelz. 1989. Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients. *Clin. Exp. Immunol.* 74:178.
12. Ardman, B., K. Mayer, J. Bristol, M. Ryan, M. Settles, and E. Levy. 1990. Surface immunoglobulin-positive T lymphocytes in HIV-1 infection: Relationship to CD4+ lymphocyte depletion. *Clin. Immunol. Immunopathol.* 56:249.
13. Chams, V., T. Jouault, E. Fenouillet, J.C. Gluckman, and D. Klatzmann. 1988. Detection of anti-CD4 autoantibodies in the sera of HIV-infected patients using recombinant soluble CD4 molecules. *AIDS (Lond).* 2:353.
14. Thiriart, C., J. Goudsmit, P. Schellekens, F. Barin, D. Zagury, M. DeWilde, and C. Bruck. 1988. Antibodies to soluble CD4 in HIV-1 infected individuals. *AIDS (Lond.).* 2:345.
15. Kowalski, M., B. Ardman, L. Basiripour, Y. Lu, D. Blohm, W. Haseltine, and J. Sodroski. 1989. Antibodies to CD4 in individuals infected with human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA.* 86:3346.
16. Warren, R.Q., E.A. Johnson, R.P. Donnelly, M.F. Lavia, and K.Y. Tsang. 1988. Specificity of anti-lymphocyte antibodies in sera from patients with AIDS-related Complex (ARC) and healthy homosexuals. *Clin. Exp. Immunol.* 73:168.
17. Remold-O'Donnell, E., D.M. Kenney, R. Parkman, L. Cairns, B. Savage, and F.S. Rosen. 1984. Characterization of a human lymphocyte surface sialoglycoprotein that is defective in Wiskott-Aldrich syndrome. *J. Exp. Med.* 159:1705.
18. Carlsson, S.R., and M. Fukuda. 1986. Isolation and characterization of leukosialin, a major sialoglycoprotein on human leukocytes. *J. Biol. Chem.* 261:12779.
19. Remold-O'Donnell, E., C. Zimmerman, D. Kenney, and F.S. Rosen. 1987. Expression on blood cells of sialophorin, the surface glycoprotein that is defective in Wiskott-Aldrich syndrome. *Blood.* 70:104.
20. Borche, L., F. Lozasno, R. Vilella, and J. Vives. 1987. CD43 monoclonal antibodies recognize the large sialoglycoprotein of human leukocytes. *Eur. J. Immunol.* 17:1523.
21. Smith, S.D., M. Shatsky, P.S. Cohen, R. Warnke, M.P. Link, and B.E. Glader. 1984. Monoclonal antibody and enzymatic profiles of human malignant T-lymphoid cells and derived cell lines. *Cancer Res.* 44:5657.
22. Towbin, H., T. Stahelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 81:573.
23. Blake, M.S., K.H. Johnston, G.J. Russell-Jones, and E.C. Gotschlich. 1984. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal. Biochem.* 136:175.
24. Pallant, A., A. Eskenazi, M.G. Mattei, R.E.K. Fournier, S.R. Carlsson, M. Fukuda, and J.G. Frelinger. 1989. Characterization of cDNAs encoding human leukosialin and localization of the leukosialin gene to chromosome 16. *Proc. Natl. Acad. Sci. USA.* 846:1328.
25. Seed., B., and A. Aruffo. 1987. Molecular cloning of the CD2 antigen, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. *Proc. Natl. Acad. Sci. USA.* 84:3365.
26. Hoxie, J.A., J.D. Alpers, J.L. Rackowski, K. Huebner, B.S. Haggarty, A.J. Cedarbaum, and J.C. Reed. 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. *Science (Wash. DC).* 234:1123.
27. Stross, W.P., R.A. Warnke, D.J. Flavell, S.U. Flavell, D. Simmons, K.C. Gatter, and D.Y. Mason. 1989. Molecule detected in formalin fixed tissue by antibodies MT1, DF-T1, and L60 (Leu-22) corresponds to CD43 antigen. *J. Clin. Pathol.* 42:953.
28. Silvermann, L.B., R.C.K. Wong, E. Remold-O'Donnell, D. Vercelli, J. Sancho, C. Terhorst, F. Rosen, R. Geha, and T. Chatila. 1989. Mechanism of mononuclear cell activation by an anti-CD43 (sialophorin) agonistic antibody. *J. Immunol.* 142:4194.
29. Axelsson, B., R. Youseffi-Etmad, S. Hammarstrom, and P. Perlmann. 1988. Induction of aggregation and enhancement of proliferation and IL-2 secretion in human T cells by antibodies to CD43. *J. Immunol.* 141:2912.
30. Nong, Y.H., E. Remold-O'Donnell, T.W. LeBien, and H.G. Remold. 1989. A monoclonal antibody to sialophorin (CD43) induces homotypic adhesion and activation of human monocytes. *J. Exp. Med.* 170:259.
31. Bierer, B.E., A. Peterson, J. Park, E. Remold-O'Donnell, F.S. Rosen, B. Seed, and S.J. Burakoff. 1988. T-cell activation: the T-cell erythrocyte receptor (CD2) and sialophorin (CD43). *Immunology and Allergy Clinics of North America.* 8:51.
32. Mentzer, S.J., E. Remold-O'Donnell, M.A.V. Crimmins, B.E. Bierer, F.S. Rosen, and S.J. Burakoff. 1987. Sialophorin, a surface sialoglycoprotein defective in the Wiskott-Aldrich syndrome, is involved in human T lymphocyte proliferation. *J. Exp. Med.* 165:1383.
33. Gowda, S.D., B.S. Stein, N. Mohagheghpour, C.J. Benike, and E.G. Engelman. 1989. Evidence that T cell activation is required for HIV-1 entry in CD4+ lymphocytes. *J. Immunol.* 142:773.
34. Reichert, C.M., T.J. O'Leary, D.L. Levens, C.R. Simrell, and A.M. Macher. 1983. Autopsy pathology in the acquired immune deficiency syndrome. *Am. J. Pathol.* 112:357.
35. Davis, A.E., Jr. 1984. The histopathological changes in the thymus gland in the acquired immune deficiency syndrome. *Ann. NY Acad. Sci.* 437:493.
36. Hirsch, R., M. Eckhaus, H. Auchincloss Jr., D.H. Sachs, and J.A. Bluestone. 1988. Effects of in vivo administration of anti-T3 monoclonal antibody on T cell function in mice. I. Immunosuppression of Transplantation Responses. *J. Immunol.* 140:3766.
37. Ghobrial, R.R.M., M. Boublik, H.J. Winn, and H. Auchincloss, Jr. 1989. *In vivo* use of monoclonal antibodies against murine T cell antigens. *Clin. Immunol. Immunopathol.* 52:486.
38. Reisinger, D., and R. Parkman. 1987. Molecular heterogeneity of a lymphocyte glycoprotein in immunodeficient patients. *J. Clin. Invest.* 79:595.
39. Rosen, F.S., M.D. Cooper, and R.J. Wedgewood. 1984. The primary immunodeficiencies. *N. Engl. J. Med.* 311:300.
40. Volberding, P.A., S.W. Lagakos, M.A. Kock, C. Pettinelli, et. al., and the AIDS Clinical Trials Group of the National Insti-

tute of Allergy and Infectious Diseases. 1990. Zidovudine in asymptomatic human immunodeficiency virus infection. A controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. *N. Engl. J. Med.* 322:941.

41. Ho, D.D., T. Moudgil, and M. Alam. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* 321:1621.

42. Coombs, R.W., A.C. Collier, J.-P. Allain, B. Nikora, M. Leuther, G.F. Gjerset, and L. Corey. 1989. Plasma viremia in human immunodeficiency virus infection. *N. Engl. J. Med.* 321:1626.

43. Staunton, D.E., M.L. Dustin, and T.A. Springer. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature (Lond.).* 339:61.