Altered Glycosylation of Leukosialin, CD43, in HIV-1–infected Cells of the CEM Line

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Summary

CD43 (leukosialin, gpL115, sialophorin) is a major sialoglycoprotein widely expressed on hematopoietic cells that is defective in the congenital immunodeficiency Wiskott-Aldrich syndrome. It is thought to play an important role in cell–cell interactions and to be a costimulatory molecule for T lymphocyte activation. Using a metabolic $^{35}SO_4^{2-}$ radiolabeling assay or biotinylation of cell surface proteins, we describe here that CD43 are sulfated molecules the glycosylation of which is altered in human immunodeficiency virus type 1 (HIV-1)–infected leukemic T cells of the CEM line. Hyposialylation of *O*-glycans and changed substitution on *N*-acetylgalactosamine residues are observed. The glycosylation defect is associated with an impairment of CD43-mediated homotypic aggregation which can be restored by resialylation. The hyposialylation of CD43 on HIV-1$^+$ cells may explain the high prevalence of autoantibodies directed against nonsialylated CD43 that have been detected in HIV-1–infected individuals. A defect in glycosylation of important molecules such as CD43 or, as we recently described, CD45 may explain alterations of T cell functions and viability in HIV-1–infected individuals. In addition, a possible implication of hyposialylation in the HIV-1–infected cells entrapment in lymph nodes could be envisioned.

Several mechanisms have been proposed to explain AIDS pathogenesis (reviewed in reference 1). Among them, autoimmunity against lymphocytes and platelets has been observed. Autoantibodies have been described against several molecules including the following: (*a*) a 18-kD protein (2) expressed in activated lymphocytes; (*b*) MHC class II (3); (*c*) recombinant CD4 molecules (4); and (*d*) particular forms of CD43 (5). In the last case, the autoantibodies were found to react with partially sialylated CD43 expressed on thymocytes or in COS cells transfected with a CD43 cDNA (5).

The CD43 integral membrane glycoprotein is expressed on leukocytes and platelets. This molecule is heavily sialylated with 70–85 O-linked carbohydrate side chains (6). The electrophoretic mobility of CD43 on SDS-PAGE has been shown to vary from M_r of ~115,000 daltons in resting lymphocytes and CEM cells (7) to M_r of ~135,000 daltons in activated cells, due to changes in O-glycan biosynthesis (8). Moreover, neuraminidase treatment of CD43 molecules could further reduce the M_r of CD43 to 150,000 daltons (5, 7, 9). Finally, CD43 from activated cells may reach a M_r up to ~172,000 daltons after sialidase treatment (8).

CD43 glycoprotein participates in monocyte and T lymphocyte activation as an accessory signaling/activation molecule (10–13) and regulates lymphocyte adhesion (14). Various anti-CD43 mAbs have been shown to induce specific homotypic aggregation of human T lymphocytes (11) and monocytes (10).

We recently described an alteration in the sialylation status of several T cell surface glycoproteins in HIV-1–infected cells of the CEM line and identified CD45 as one of them (15). In a latently and a virus-producing HIV-1–infected T CEM cell line, up to 75% of the total tyrosine phosphatase activity displayed by CD45 molecules were precipitated by peanut lectin (*Arachis hypogaea* [PNA][1] lectin), whereas parental CEM cells did not exhibit this phenomenon. Since this lectin recognizes the terminal Galβ1 → 3GalNAc disaccharide with the restriction that it is not sialylated, a profound defect of sialic acid was then demonstrated on these CD45 molecules. In this study, nonsialylated glycoproteins with $M_r > 10^5$, other than CD45, were also detected. Here, we describe that CD43 is (*a*) a sulfated molecule in human lymphocytes; (*b*) differently substituted on *N*-acetylgalactosamine (GalNAc) residues; and (*c*) hyposialylated when it is expressed in HIV-1–infected CEM cells. As a likely consequence, HIV-1–positive CEM

[1] *Abbreviations used in this paper:* α2 → 3ST, Galβ1 → 3GalNAcα2 → 3 sialyltransferase (EC 2.4.99.4); α2 → 6ST, Galβ1 → 4G1cNAcα2 → 6 sialyltransferase (EC 2.4.99.1); GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuNAc, *N*-acetylneuraminic acid; PNA, peanut lectin (*Arachis hypogaea*).

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cells show a defect in CD43-induced homotypic aggregation. This impairment may be corrected by a resialylation procedure on intact cells. These observations may help to understand the effects of the HIV-1 virus on the immune system.

Materials and Methods

Antibodies and Reagents. The anti-CD43 mAbs used in this study were: DF-T1 (purchased from Dako A/S, Copenhagen, Denmark) and 84-3C1 (kindly provided by Dr. J. Vives, Servei d'Immunologia, Hospital Clinic i Provincial, Barcelona, Spain); anti-CD45 mAb was 9.4 (obtained from American Type Culture Collection [ATCC], Rockville, MD); and control IgG1 mAb was purchased from Dako A/S. The rabbit polyclonal antiserum raPN/NP (anti-CD43 PNA^+) was obtained as follows: hyposialylated glycoproteins from CEM_{LAI}/NP cells were partially purified by affinity chromatography on agarose-immobilized PNA lectin (E.-Y. Laboratories, Inc., San Mateo, CA) which specifically recognizes nonsialylated Galβ1 → 3GalNAc disaccharide (16). Proteins of 170–180 kD were then excised from SDS-PAGE gels and injected to New-Zealand/Californian hybrid rabbits (Lefebvre, J. C., and V. Giordanego, manuscript in preparation). FITC-PNA lectin was purchased from E.-Y. Laboratories; 2-deoxyglucose from Sigma Chemical Co. (St. Louis, MO); and cytidine-5'-monophospho-9-(3-fluoresceinylthioureido)-9-deoxy-*N*-acetylneuraminic acid from Boehringer Mannheim GmbH Biochemica (Mannheim, Germany).

Cells, Cultures, and HIV-1 Strains. The parental CEM cell line (ATCC CCL 119) was maintained in RPMI 1640 medium (GIBCO BRL, Gaithersburg, MD) containing 5% FCS (Whittaker M.A. Bioproducts, Inc., Walkersville, MD), and supplemented with 2 mM glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin. The HIV-1 strains used in this study are: LAI (BRU) (17), and BCL, obtained from H. Fleury (Laboratoire de Virologie Faculté de Médicine, Bordeaux, France) (18). After a pretreatment with 2 μg/ml hexadimetrine (polybrene), (Sigma Chemical Co.) for 2 d, CEM cells were infected by suspending with virus stocks (clarified culture supernatants concentrated 100-fold by ultracentrifugation). Infected CEM cells (CEM_{LAI}, CEM_{BCL}) were found to chronically produce virus for up to 6 mo without any apparent cytopathicity. Infection was documented by the measure of reverse transcriptase activity and the evaluation of HIV-1 $p24^{gag}$ antigen with an antigen assay (HIV-1 p24; Retrovirology Coulter Co., Hialeah, FL). Thereafter, virus production decreased progressively and finally stopped. The absence of spontaneous reactivation of virus production was regularly controlled. The nonproducing HIV-1–infected CEM cells were identified by the suffix "/NP" (CEM_{LAI}/NP). Polymerase chain reaction analysis revealed the persistence of HIV-1 provirus in these cells (not shown).

Flow Cytometry Analysis. Immunofluorescence stainings were performed as previously described (15). Stained cells were analyzed on a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA).

$^{35}SO_4^{2-}$ Metabolic Radiolabeling Assay and Immunoprecipitations. Cells (4×10^6/ml) were washed and incubated for 18 h in SO_4^{2-}-free RPMI 1640 medium containing $^{35}SO_4^{2-}$ (ICN Biomedicals, Inc., Costa Mesa, CA), (1 mCi/ml) and 5% dialyzed FCS. Cells were washed twice and lysed (4×10^6 cells/ml) for 60 min in lysis buffer containing 50 mM Hepes, pH 8; 1% Nonidet P-40, 150 mM NaCl, 10 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 mg/ml aprotinin (both from Sigma Chemical Co.) and 10 mg/ml leupeptin (Boehringer-Mannheim GmbH, Biochemica). After centrifugation of the lysates (18,000 *g* for 1 h), 2 μg of various mAbs were added to 250 μl supernatant aliquots. After shaking for 1 h at 4°C, 25 μl of protein A–Sepharose 4B beads (Sigma Chemical Co.) precoated with 10 μg of rabbit anti–mouse Ig were added. For immunoprecipitations with raPN/NP, 10 μl of the rabbit serum were precoated on protein A–Sepharose 4B beads. After a 2-h agitation, precipitates were collected by brief centrifugation. The pellets were washed three times in lysis buffer and once with the same buffer containing 1 M NaCl. They were resuspended in SDS-PAGE sample buffer (2% SDS, 1.7 M 2-ME) and boiled for 3 min. Samples were resolved by SDS-PAGE (7.5% gels). Molecular mass markers (GIBCO BRL) were H chain myosin (215.5K) and phosphorylase B (105.1K). After intensification with Amplify (Amersham Corp., Arlington Heights, IL), the gels were vacuum dried and exposed to XAR5 Kodak films.

Biotinylation of Cell Surface Proteins. Cell surface proteins were biotinylated as previously described (19). Briefly, 9×10^7 cells were suspended in 2.5 ml saline solution containing 25 mM Hepes buffer, pH 8, and 1 mg/ml of sulfo-*N*-hydroxysuccinimide-biotin (Pierce Chemical Co., Rockford, IL) for 40 min at 22°C. Reaction was stopped by adding 12 μl of PBS-glycine (0.2 M, pH 7.2). Cells were then washed three times and lysed as described above. Proteins were resolved by SDS-PAGE (5% gels) in a minigel apparatus and then transferred to a Hybond-N nylon membrane (Amersham Corp). Biotinylated proteins were revealed by reaction with a streptavidin/horseradish-peroxidase complex (StreptABC Complex/HRP; purchased from Dako A/S) according to the manufacturer recommendations. The membrane was further incubated with electrochemiluminescence (ECL) Western blotting detection reagents (Amersham Corp.) and exposed to XAR5 Kodak film for 2 s to 1 min.

Glycosidase Treatments. When indicated, immune precipitates collected on Sepharose beads were treated by either sialidases and/or O-glycosidase or β-galactosidase. For desialylation, beads were resuspended with a mixture of 1 mU *Vibrio cholerae* plus 0.5 mU *Arthrobacter ureafaciens* sialidases (Boehringer Mannheim GmbH Biochemica) for 3 h at 37°C in 100 μl Tris-HCl buffer (50 mM, pH 7.8) containing 1 mM PMSF and 5 mM $CaCl_2$. When indicated, for subsequent deglycosylation, beads were washed in lysis buffer and treated with 1 mU β-galactosidase from bovine testes (Boehringer Mannheim GmbH Biochemica) in 100 μl of sodium citrate phosphate buffer (0.1 M, pH 4.3) containing 1% BSA (Sigma Chemical Co.) and 10% glycerol (vol/vol) or with 1 mU endo-α-*N*-acetyl-D-galactosaminidase (*O*-glycosidase), (EC 3.2.1.97) from *Diplococcus pneumoniae* (Boehringer Mannheim GmbH Biochemica) in 100 μl sodium cacodylate buffer (20 mM, pH 6) containing 1 mM PMSF, 1% Triton X-100, and 0.1% SDS for 3 h (or 18 h in some other experiments) at 37°C. Desialylation of cells was carried out by incubation (10^6 cells/100 μl) with the mixture of 1 mU *V. cholerae* plus 0.5 mU *A. ureafaciens* sialidases for 1 h at 37°C in PBS with gentle shaking.

Resialylation. CD43-coated beads were treated by 0.3 mU Galβ1 → 3GalNAc α2 → 3Sialyltransferase (EC 2.4.99.4), (α2 → 3ST), (Boehringer Mannheim GmbH Biochemica) in 100 μl Mes-NaOH buffer (50 mM, pH 6.1) containing 100 mM cytidine-5'-monophospho-*N*-acetylneuraminic acid (CMP-NeuNAc) (Boehringer Mannheim GmbH Biochemica), 0.1% BSA, 1 mM PMSF, and 0.5% Triton X-100 for 3 h at 37°C. For resialylation of intact cells, 2×10^6 cells were suspended in 100 μl PBS containing 20 mM cytidine-5'-monophospho-9-(3-fluoresceinylthioureido)-9-deoxy-*N*-acetylneuraminic acid and 0.25 mU of α2 → 3ST (Boehringer Mannheim GmbH Biochemica) and gently shaken at 37°C for 15 min. Cells were then tested for specific mAb aggregation. For resialylation controls, after four washes with PBS, cells were fixed

in 1 ml of 0.4% formalin in PBS and analyzed on a FACScan®.

Cell Aggregation. Cells (5×10^5/ml) were suspended in RPMI 1640 medium with 5% FCS containing 1 μg/ml various mAbs or Ig controls and incubated for 20 min at 37°C before plating in flat-bottomed microtiter plates (100 μl/well). Cells were then incubated at 37°C in 7% CO_2 in a humidified incubator for 4 h. Thereafter, cells were gently shaken by micropipetting. Cell layers were photographed after additional 1-h incubation period.

Results

Lymphocyte CD43 Molecules Are Sulfated and Modified in HIV-1–infected CEM Cells. To study possible modifications of CD43 molecules in HIV-1–infected CEM cells, we used different labeling methods. A sulforadiolabeling technique appeared to be more sensitive, since a 5-h exposure of the gel gave similar results as a several day exposure in the case of [^{35}S]methionine labeling (not shown). After $^{35}SO_4^{2-}$ metabolic radiolabeling, immunoprecipitations were carried out with the anti-CD43 mAb DF-T1. As shown on Fig. 1, *a* and *b*, CD43 molecules appeared as a 115–120-kD band in parental CEM cells (Fig. 1 *a*, lane *3*) and in PHA-activated PBL, (Fig. 1 *b*, lane *3*); while those from CEM_{LAI}/NP as a heterogeneous 160–170-kD band (Fig. 1 *a*, lane 7). CD43 from CEM_{BCL} that are productively HIV-1–infected showed two bands, one at 115–120 kD and the other broad band at 135–160 kD (Fig. 1 *a*, lane *6*). Another anti-CD43 mAb 84-3C1 (9) gave the same results (Fig. 1 *a*, lanes *2*, *4*, and *5*).

Since the differences in CD43 mobility that we observed in CEM_{LAI}/NP with M_r of 160,000–170,000 daltons were close to those described after neuraminidase treatment of CD43 from activated lymphocytes (M_r of ~172,000 daltons), (8), we hypothesized that HIV-1–infected CEM cells expressed hyposialylated CD43 molecules.

Because CD43 from CEM_{LAI}/NP (Fig. 1 *a*, lanes *5* and 7) were revealed to a lesser degree than those immunoprecipitated from parental CEM (Fig. 1 *a*, lanes *2* and *3*) or from CEM_{BCL} (Fig. 1 *a*, lanes *4* and *6*), we developed a rabbit polyclonal antiserum against these hypothetical hyposialylated CD43. A hybrid New Zealand/Californian rabbit was immunized with SDS-PAGE semipurified 170–180-kD glycoproteins previously precipitated by immobilized PNA lectin from CEM_{LAI}/NP lysates. By the sulforadiolabeling assay, the antiserum (raPN/NP) could precipitate the upper and lower CD43 bands with much more efficiency than anti-CD43 mAbs (Fig. 1 *a*, lanes *9*, *11*, and *12*). The reactivity of raPN/NP toward CD43 was confirmed by Western blotting assay: CD43 previously immunoprecipitated from parental CEM and CEM_{LAI}/NP, by the raPN/NP antiserum, or by anti-CD43 mAb DF-T1 and transferred onto nylon membrane were equally detected by DF-T1 (not shown). It has to be pointed out that the raPN/NP serum recognizes 115–120-kD CD43 forms from parental CEM cells while immunization was performed with 170–180-kD glycoproteins from CEM_{LAI}/NP cells. Thus, this antiserum recognizes different forms of the same CD43 glycoprotein that are differentially sialylated as we demonstrate below. The raPN/NP antiserum also recognized 180–190-kD CD45 molecules in the Western blotting assay (not shown). CD45 appeared also as a characteristic 180–190-kD doublet immunoprecipitated by raPN/NP in the sulforadiolabeling assay from CEM cells (Fig. 1 *a*, lane *9*) and from PBL (Fig. 1 *b*, lane *2*), indicating that CD45 molecules from these two cell species are also sulfated (Lefebvre, J.-C., unpublished observations).

Modified CD43 Molecules from HIV-1–infected CEM Cells Are Partially Sialylated. As shown by others (7, 9, 20, 21), treatment of CD43 molecules from parental CEM cells with sialidases induced a dramatic reduction in their electrophoretic mobility (Fig. 2 *a*, lanes *3* and *4*). CD43 from CEM_{LAI}/NP showed also an additional reduction in their mobility upon

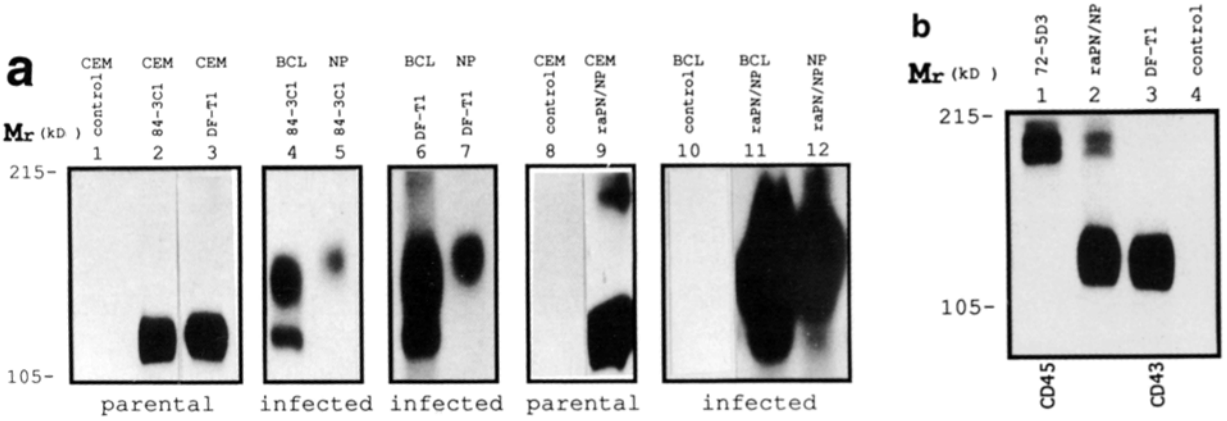

Figure 1. Variations in electrophoretic mobility of $^{35}SO_4^{2-}$-labeled CD43 molecules. Immunoprecipitations were carried out from parental CEM cells (lanes *1–3*, *8–9*), virus-producing (CEM_{BCL}; lanes *4*, *6*, *10*, and *11*) or latently HIV-1–infected (CEM_{LAI}/NP, lanes *5*, *7*, and *12*) CEM cells, with anti-CD43 mAbs DF-T1 (lanes *3*, *6* and *7*), 84-3C1 (lanes *2*, *4*, and *5*), or with the polyclonal rabbit serum raPN/NP (lanes *9*, *11*, and *12*). A control irrelevant IgG_1 mAb was used in lane *1* and a preimmune rabbit serum in lanes *8* and *10*. (*b*) Variations in electrophoretic mobility of $^{35}SO_4^{2-}$-labeled CD43 molecules. Immunoprecipitations were carried out from normal PHA-activated PBLs. The rabbit polyclonal raPN/NP serum (lane *2*) recognized CD43 and the 180–190-kD doublet CD45. The controls were: anti-CD45 mAb 9.4 (lane *1*), anti-CD43 mAb DF-T1 (lane *3*), and preimmune rabbit serum (lane *4*).

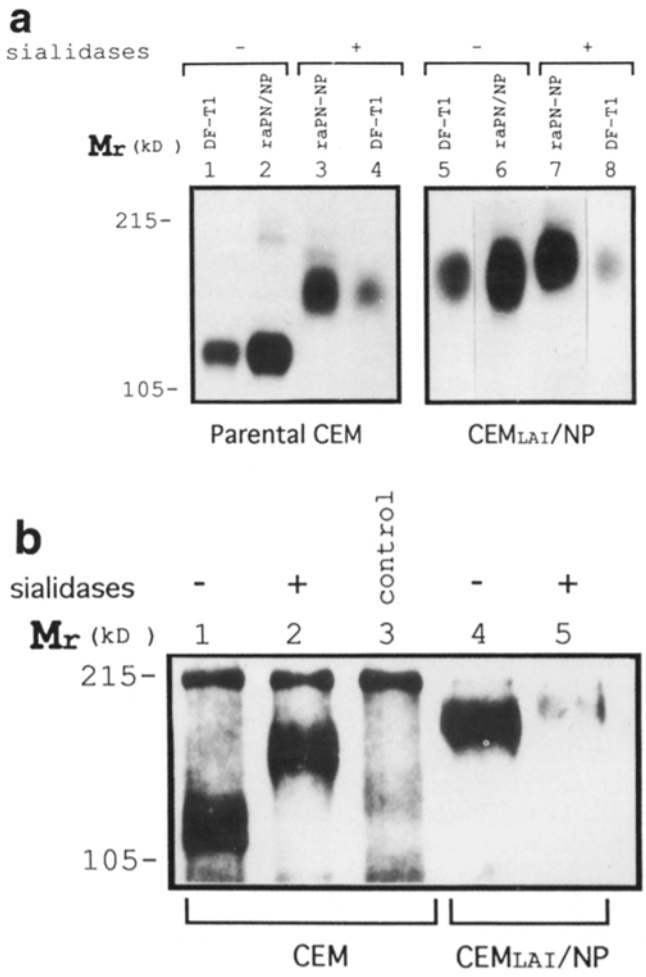

Figure 2. (*a*) Desialylation of CD43 molecules. After metabolic sulforadiolabeling, CD43 was immunoprecipitated from lysates of parental CEM cells (lanes *1–4*) and CEM_{LAI}/NP (lanes *5–8*) by DF-T1 mAb (lanes *1*, *4*, *5*, and *8*) or by the polyclonal rabbit raPN/NP serum (lanes *2*, *3*, *6*, and *7*) and treated by sialidases (lanes *3*, *4*, *7*, and *8*) or left untreated (lanes *1*, *2*, *5*, and *6*). (*b*) Immunoprecipitation and desialylation of cell surface biotin-labeled CD43 molecules. CD43 were immunoprecipitated from CEM cells (lanes *1–3*), CEM_{LAI}/NP (lanes *4* and *5*) by DF-T1 mAb. Control was an irrelevant IgG1 mAb. Coated beads were treated by a mix of sialidases (lanes *2* and *5*) or left untreated (lanes *1* and *4*).

sialidases treatment (Fig. 2 *a*, lanes 7 and *8*). The desialylated CD43 molecules treated by neuraminidases were more efficiently precipitated by raPN/NP (Fig. 2 *a*, lanes *3* and *7*) than by the DF-T1 mAb (Fig. 2 *a*, lanes *4* and *8*). To further demonstrate that CD43 molecules from HIV-1–infected CEM cells were only partially sialylated we used a resialylation procedure. Treatment of CD43 from CEM_{LAI}/NP and CEM_{BCL} with $\alpha 2 \rightarrow$ 3ST (EC 2.4.99.4), (22) gave a partial restoration of their electrophoretic mobility (Fig. 3, lanes *3* and *5*), while CD43 from parental CEM cells were not modified by this treatment (Fig. 3, lane *1*).

Hyposialylated CD43 Molecules Are Efficiently Transported to the Cell Surface. Cell surface proteins were biotinylated on intact cells and immunoprecipitated from cell lysates by raPN/NP. This biotinylation assay, which is not restricted to sulfated molecules, showed that electrophoretic mobility

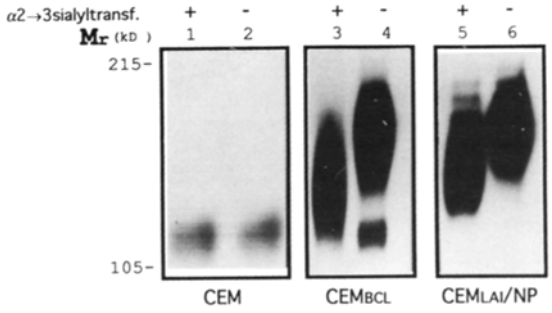

Figure 3. Resialylation of CD43 by $\alpha 2 \rightarrow$ 3ST. CD43 molecules were immunoprecipitated by the rabbit raPN/NP serum after metabolic sulforadiolabeling from parental CEM (lanes *1* and *2*), HIV-1–infected virus producing CEM_{BCL} (lanes *3* and *4*) and from virus nonproducing CEM_{LAI}/NP (lanes *5* and *6*). Before gel resolution, coated beads were treated by $\alpha 2 \rightarrow$ 3ST (lanes *1*, *3*, and *5*) or left untreated (lanes *2*, *4*, and *6*).

of CD43 from CEM_{LAI}/NP (*a*) was reduced (Fig. 2 *b*, lane *4*) as compared with CD43 from parental CEM cells CD43 (Fig. 2 *b*, lane *1*); (*b*) can be further reduced by sialidase treatment (Fig. 2 *b*, lane *5*), demonstrating at least some degree of sialylation; (*c*) was also reduced as compared with in vitro–desialylated CD43 from parental CEM cells (Fig. 2 *b*, lane *2*), indicating that hyposialylation is not sufficient to explain the significant difference in gel migration between parental CEM and CEM_{LAI}/NP cell CD43, the glycosylation of which is likely more complex.

Hyposialylated CD43 Molecules from HIV-1–infected Cells Bear More Complex O-glycans. We next tried to deglycosylate by endo-α-*N*-acetyl-D-galactosaminidase (O-glycosidase) treatment, (EC 3.2.1.97), CD43 molecules. This enzyme is known to hydrolyze the α-linkage between disaccharide Gal–GalNAc from its serine or threonine moiety with the restriction that it is not substituted by any sialic acid or fucose or *N*-acetylglucosamine (GlcNAc) residue (23). As shown on Fig. 4,

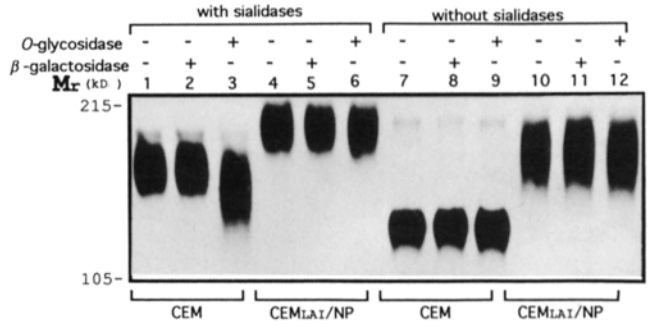

Figure 4. Deglycosylation of CD43. After metabolic sulforadiolabeling, combined enzymatic treatment (sialidases, β-galactosidase, and O-glycosidase) was performed on beads coated with CD43 molecules immunoprecipitated by the rabbit polyclonal raPN/NP serum from lysates of parental CEM cells (lanes *1–3*, *7–9*) and HIV-1–infected virus nonproducing CEM_{LAI}/NP (*4–6*, *10–12*). Before deglycosylation, coated beads were previously treated with a mix of sialidases (lanes *1–6*) or nontreated (lanes *7–12*). Thereafter, beads were washed in lysis buffer and directly denaturated for resolution by SDS-PAGE 7.5% (lanes *1*, *4*, *7*, and *10*) or treated by β-galactosidase (lanes *2*, *5*, *8*, and *11*) or by O-glycosidase (lanes *3*, *6*, *9*, and *12*).

deglycosylation by O-glycosidase was effective on CD43 from parental CEM, only after a pretreatment with sialidases (Fig. 4, lane *3*). In contrast, deglycosylation of CD43 molecules from CEM_{LAI}/NP was not possible even after a preincubation with sialidases (Fig. 4, lane *6*). The variation in electrophoretic mobility of sialidase- and O-glycosidase–treated CD43 from parental CEM cells appears to be very weak, however it is significant and comparable with that described by Ardman et al. (5). Moreover, the absence of variation of CEM_{LAI}/NP cell CD43 treated under the same conditions suggests that these molecules have a more complex carbohydrate structure than parental CEM cell CD43. As a control, hydrolysis of galactoses by β-galactosidase from bovine testes, which splits terminal galactoses from Galβ1 → GalNAc, Galβ1 → 4GlcNAc, and Galβ1 → 3GlcNAc, was not observed through an electrophoretic mobility shift, even after desialylation (Fig. 4, lanes *2*, *5*, *8*, and *11*). After labeling of galactose residues by the galactose oxidase/tritiated sodium borohydride reduction method (24), the enzymatic activity of β-galactosidase was verified by a significant release of $[^3H]NaBH_4$ galactose residues (not shown). Therefore the observation that removal of galactose residues by β-galactosidase treatment did not affect CD43 electrophoretic mobility highlights (*a*) the slight variation induced by O-glycosidase treatment of CEM cell CD43; and (*b*) the absence in variation of CEM_{LAI}/NP cell CD43 treated under the same conditions.

Hyposialylated Lymphocytes Are Not Found in PBLs of HIV^+ Individuals. Using FITC-PNA lectin, we have screened resting and PHA-activated PBL from fifteen HIV^+ individuals. This lectin is specific for Galβ1 → 3GalNAc disaccharide, branched on O-glycan with the restriction that it is not sialylated (16). As shown on Fig. 5, we have described a strong reactivity of this lectin against CEM_{LAI}/NP cells (Figs. 5 *a*, histogram *12*; 5 *b*, histogram *2*), (15) by contrast to CEM cells which are almost unreactive (Figs. 5 *a*, histogram *4*; 5 *b*, histogram *1*). In accordance with an earlier report, we failed to find any hyposialylated circulating lymphocytes in HIV^+ individuals (Fig. 5 *b*, histogram *4*) as in noninfected individuals (Fig. 5 *b*, histogram *3*), (5).

Hyposialylated CD43 Molecules Are Recognized by Neuraminidase Sensitive mAbs at the Surface of HIV-1–infected CEM Cells. As shown above, partially sialylated CD43 may be immunoprecipitated from HIV-1–infected CEM cells by the mAbs DF-T1 and 84-3C1, and these molecules are efficiently transported to the cell surface. Using immunofluorescence staining, we confirmed that the mAbs were able to recognize intact parental CEM (Fig. 5 *a*, histograms *2* and *3*), CEM_{LAI}/NP (Fig. 5 *a*, histograms *10* and *11*), and CEM_{BCL} cells (Fig. 5 *a*, histograms *18* and *19*). The binding of anti-CD43 DF-T1 and 84-3C1 mAbs appeared to be slightly lower in HIV-1–infected CEM cells. As shown on Fig. 5 *a*, removal of sialic acids by treatment of the cells with sialidases completely abolished the binding of DF-T1 and 84-3C1 mAbs to parental CEM, CEM_{LAI}/NP, and CEM_{BCL} (Fig. 5 *a*, histograms *6*, *7*, *14*, *15*, *22*, and *23*), as compared with flow cytometry profiles obtained with an irrelevant IgG_1 mAb (Fig. 5 *a*, histograms *1*, *9*, and *17*). These results are in agreement with the previously reported sensitivity of the anti-CD43 mAbs to neuraminidase treatment (9, 25). Our results showed hyposialylated CD43 from CEM_{LAI}/NP or CEM_{BCL} are nevertheless significantly recognized by DF-T1 and 84-3C1 mAbs.

Impairment of CD43-mediated Homotypic Aggregation of HIV-1–infected CEM Cells. The CD43 DF-T1 mAb was able to induce in parental CEM cells an homotypic aggregation (Fig. 6, panel *2*). It is known that this phenomenon is not due to a simple cross-linking of cells by antibodies but an active process (26, 27). We then verified that CD43-mediated aggregation we observed was inhibited by metabolic poisons. Addition of 0.2% azide plus 5 mM 2-deoxyglucose completely abolished cell–cell adhesion. Moreover, irrelevant IgG1 or anti CD45 9.4 mAb did not induce CEM cells aggregation (not shown). This specific homotypic cell adhesion was not observed with HIV-1–infected CEM_{BCL} or CEM_{LAI}/NP cells (Fig. 6, panel *5*). Intact CEM_{LAI}/NP and CEM_{BCL} cells were then resialylated with fluorescent precursor NeuNAc by α2 → 3ST. As shown in Fig. 5 *c* (histograms *1* and *2*), the fluorescence associated with neuraminic acid was detected at the surface of treated cells, thus demonstrating that resialylation was efficient. Resialylation was able to correct the defect in homoaggregates formation induced by CD43 mAb (Fig. 6, panel *6*). Similar results were obtained with the 84-3C1 mAb (not shown).

Discussion

In this paper, we describe a reduction in electrophoretic mobility of CD43 molecules in HIV-1–infected CEM cells. This modification of CD43 can not be due to alternative exon splicing since the molecule is known to be the product of a single coding exon (28) of a single gene (29). Earlier reports have shown that a change in O-glycan biosynthesis leading to a hexasaccharide substitution on CD43 from activated lymphocytes, instead of a tetrasaccharide as in CD43 from resting T cells leads to a shift of CD43 M_r from ~115,000 to 135,000 daltons by SDS-PAGE (8). Moreover, neuraminidase treatment of immunoprecipitated CD43 induced an even more dramatic change (5, 7, 9) to a M_r of ~150,000 daltons (7). The modifications we observed on CD43 molecules (M_r of ~160,000–170,000 daltons) immunoprecipitated from virus producing or latently HIV-1–infected CEM cells are reminiscent of those described after in vitro desialylation of CD43 molecules from activated lymphocytes (8). In the last case, changes in O-glycan biosynthesis together with sialidase treatment gave CD43 molecules with M_r of 172,000 (8). Moreover, CD43 glycoproteins from CEM_{LAI}/NP cells could be further desialylated in vitro, thus demonstrating some degree of sialylation.

Significant resialylation of CD43 was obtained with α2 → 3ST, which links a NeuNAc moiety to Galβ1 → 3GalNAc disaccharide attached to Ser/Thr amino acids in O-linkage. Similar observations were made on CD45 from CEM_{LAI}/NP (15). Resialylation of these CD45 molecules was obtained by α2 → 3ST and Galβ1 → 4GlcNAc 6 sialyltransferase (EC 2.4.99.1) (α2 → 6ST) demonstrating that several glycopro-

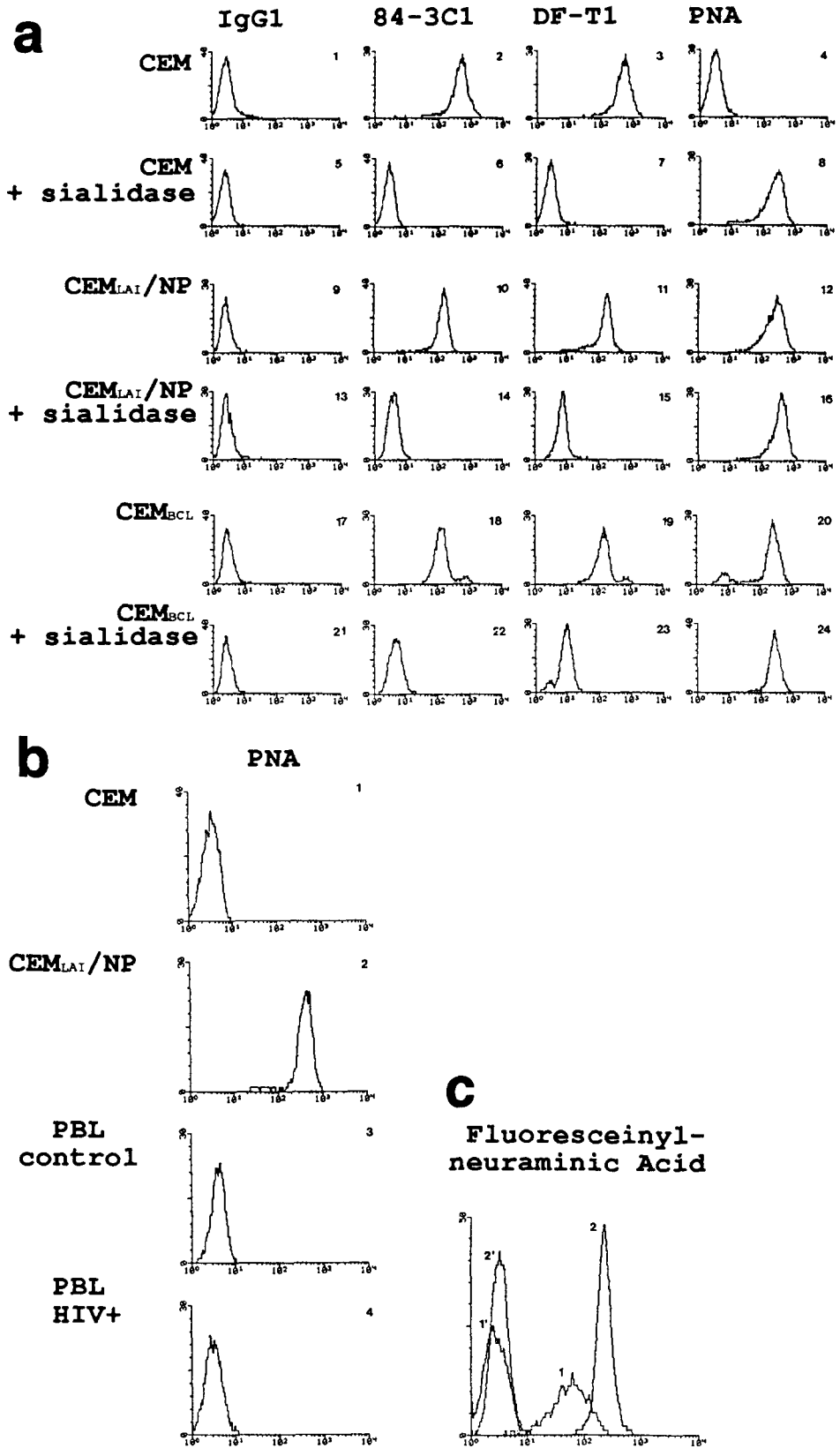

Figure 5. Flow cytometry analysis of membrane proteins. (*a*) The following cells were tested: parental CEM (histograms *1–8*), nonproducing HIV-1–infected CEM cells (CEM_{LAI}/NP or histograms *9–16*) and virus-producing HIV-1–infected CEM cells (CEM_{BCL} or histograms *17–24*), with or without previous sialidase treatment as indicated. Cells were stained with 2 μg anti-CD43 84-3C1 (histograms *2*, *6*, *10*, *14*, *18*, and *22*), or DF-T1 (histograms *3*, *7*, *11*, *15*, *19*, and *23*) mAbs or FITC-PNA lectin (1 μg PNA/ml), (histograms *4*, *8*, *12*, *16*, *20*, and *24*), control was an irrelevant IgG1 mAb (histograms *1*, *5*, *9*, *13*, *17*, and *21*). (*b*) The reactivity of FITC-PNA lectin (1 μg PNA/ml) was tested towards parental CEM (histogram *1*) or CEM_{LAI}/NP cells (histogram *2*) or PBLs from an HIV^+ individual (histogram *4*). Control was PBLs from a noninfected individual (histogram *3*). (*c*) CEM_{LAI}/NP (histogram *1*) and CEM_{BCL} cells (histogram *2*) were resialylated by fluoresceinyl-sialic acid attached by α2→3ST. Controls were nonstained nonresialylated CEM_{LAI}/NP (histogram *1'*) and CEM_{BCL} (histogram *2'*). The fluorescence intensity of 10^5 cells was analyzed on a FACScan®.

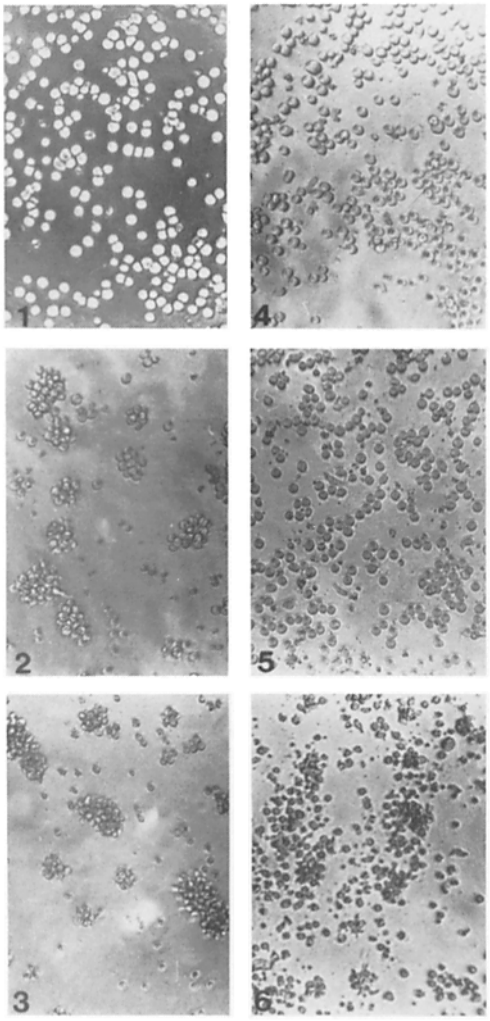

Figure 6. Homotypic aggregation of CEM cells. Parental CEM cells (panels *1*, *2*, and *3*) and latently HIV-1–infected CEM_{LAI}/NP (panels *4*, *5*, and *6*) were incubated with control irrelevant IgG_1 mAb (panels *1* and *4*) or anti-CD43 DF-T1 (panels *2* and *5*) or previously resialylated with α2→3ST (panels *3* and *6*) before incubating with DF-T1 mAb.

teins from HIV-1–infected CEM cells were hyposialylated on O- and N-linked carbohydrate side chains. Since all the known sialyltransferases are located in the *trans*-Golgi, the partial sialylation of CD43 molecules could not be due to a defective trafficking of the molecules inside the cell.

As described for murine CD43 (30), we also showed that lymphocyte CD43 molecules are sulfated in human CEM cells and activated human PBL. CD43 sulfation might be of importance for ligand–receptor interactions since SO_4^{2-} ions have been shown to be involved in various recognition phenomena (31–34) and required for the binding of GlyCAM-1 to L-selectin (35).

The hyposialylation of CD43 in HIV^+ cells is reminiscent of the defect affecting CD43 in the congenital immunodeficiency Wiskott-Aldrich syndrome. In this syndrome, CD43 molecules are either defective or have a reduced migration in SDS-PAGE. It has been proposed that they are affected by a desialylation process (7, 36, 37) or by a more complex modification of O-glycans induced by an increased activity of β1 → 6-*N*-acetylglucosaminyltransferase which links a GlcNAcβ1 → 6- (with subsequent elongation by a NeuNAcα2 → 3Galβ1 → 4-) instead of a NeuNAcα2 → 6- to GalNAc attached to Ser/Thr of CD43 in normal lymphocytes (38, 39).

We have demonstrated that CD43 molecules from HIV-1–infected CEM cells were not only partially sialylated but also differently substituted on their Galβ1 → 3GalNAc disaccharides since deglycosylation of CD43 from these cells by O-glycosidase was without effect. Moreover, since resialylation was possible on terminal Gal with an α2 → 3 linkage (Fig. 3, lanes *3* and *5*), the inactivity of O-glycosidase on CD43 from CEM_{LAI}/NP is certainly due to a substitution on penultimate GalNAc moieties rather than on terminal Gal residues.

Using restoration of CD43 expression in negative cells, Manjunath et al. (14) have demonstrated that CD43 interferes with T lymphocyte adhesion. CD43-mediated homotypic aggregation has been extensively described (10, 11, 14, 26, 27, 40).

Our observations suggest that HIV-1 may alter CD43 functions through modification of the sialylation status of the molecule. Sialic acid residues are thought to play a major role in cell–cell interactions. In particular, sialic acid requirement has been shown for the recognition of their counter structures by selectins (41–47), or CD22 ligand–receptor interactions (48–50).

There is an apparent discordance between the sialic acid requirement of CD43-mediated homotypic aggregate formation and the enhanced adhesion demonstrated in CD43-deficient CEM cells (14) where it was proposed that electronegative charges of numerous sialic acid residues attached to CD43 serve to repulse $CD43^+$ cells, and thus act to limit cell–cell adhesion (51). However, we favor the idea that autoaggregability of modified CD43-deficient CEM cells is a phenomenon entirely different from homotypic aggregation induced by anti-CD43 activation which requires integrity of CD43 molecules.

Autoimmunity against hyposialylated CD43 has been demonstrated with a high prevalence in HIV-1–infected individuals (5). Modified CD43 molecules responsible for this reactivity were found on normal thymocytes but not on peripheral blood lymphocytes of normal donors (52) or HIV-1–seropositive individuals (5). The authors suggested that hyposialylated CD43 molecules were provided by an increased destruction of lymphocytes in HIV^+ individuals. Our data suggest that hyposialylated CD43 molecules in HIV^+ cells may be responsible for the generation of autoantibodies. Similar to Ardman et al. (5), we did not find partially sialylated CD43 on peripheral blood lymphocytes of HIV^+ individuals. This could be due to the low percentage of hyposialylated lymphocytes in the peripheral blood of HIV^+ individuals and/or their trapping in lymph nodes. (Indeed, as a preliminary report, we have observed that ~2–5% of lymphocytes from lymph nodes from two HIV^+ individuals were reactive with the PNA lectin specific for Gal–GalNAc disaccharides.) Using neuraminidase treatment of rat lymphocytes, it has been shown that sialylated molecules play an important role in the

trafficking of the cells between the different lymphoid sites. The desialylated lymphocytes are removed from the circulation to be initially trapped in the rat liver and subsequently emigrate to the lymph nodes where they concentrate (53, 54). Together with CD45, CD43 represents 38% of the total T lymphocyte surface glycoproteins (55). The altered sialylation of these two glycoproteins in HIV-1–infected cells may contribute to the observed phenomenon of entrapment of HIV^+ cells in lymph nodes (56). The low percentage of cells bearing hyposialylated CD43 may be compensated by the strong immunogenicity of carbohydrate antigens (57) to explain the generation of autoimmunity.

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