CHARACTERIZATION OF A HUMAN LYMPHOCYTE SURFACE SIALOGLYCOPROTEIN THAT IS DEFECTIVE IN WISKOTT-ALDRICH SYNDROME

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The Wiskott-Aldrich syndrome is an X-linked recessive disorder characterized by reduced T lymphocyte function including decreased antibody production to carbohydrate antigens, eczema, and thrombocytopenia with platelets of reduced size and function (2, 3). Allogeneic histocompatibility-matched bone-marrow transplantation can completely correct the Wiskott-Aldrich syndrome by the engraftment of normal hematopoietic and lymphoid stem cells; partial engraftment in two cases demonstrated that the immune defects are due to a primary T lymphocyte defect (4). Splenectomy has transformed both platelet size and number to normal in Wiskott-Aldrich syndrome patients, demonstrating that these abnormalities are secondary manifestations of the disease (5).

In an earlier study, we described structural defects in surface components of both lymphocytes and platelets in the Wiskott-Aldrich syndrome (6). In lymphocytes, a previously undetected surface glycoprotein of apparent mol wt 115,000 called gpL115,1 which was present in all normal donors examined, was not detectable in three Wiskott-Aldrich patients; whereas in platelets of the one Wiskott-Aldrich syndrome patient examined, reduced amounts and restricted heterogeneity were detected of the well-characterized glycoprotein Ib (GPIb, reference 7), the Factor VIII-related von Willebrand receptor involved in platelet adhesion (8).

The deficiency of the lymphocyte surface glycoprotein gpL115 represents a...
possible link between an X-chromosome defect and a broad functional immune deficiency. Questions arose about the nature and function of gpL115.

In the current study we present substantial characterization of the gpL115 molecule from peripheral lymphocytes of normal individuals and from a lymphoblastoid cell line. Cumulatively, the characterization indicates that gpL115 is not identical to platelet GPIb, but shares with GPIb and with glycophorin of erythrocytes, the property of being a sialoglycoprotein with high content of O-linked (mucin-type acidic-type) carbohydrate moieties. gpL115 also shares characteristics with leukocyte sialoglycoprotein (LSGP), the rat and mouse thymocyte surface component responsible for peanut lectin agglutinability which consists of 60% O-linked carbohydrate residues (9, 10).

Materials and Methods

Materials. The sources of materials are as follows: galactose oxidase, soybean trypsin inhibitor, and β-galactosidase from *Escherichia coli* (Worthington Biochemical Corp., Freehold, NJ); sialidase from *Vibrio cholerae*, 20 IU per mg protein, and lactoperoxidase (Calbiochem Behring Corp., American Hoechst Corp., San Diego, CA); myosin from chick muscle (Bethesda Research Laboratories, Inc., Gaithersburg, MD); sodium dodecyl sulfate (SDS) and Nonidet P40 (NP-40) (BDH Chemicals, Ltd., Poole, England); ultrapure urea and Coomassie Brilliant Blue (Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, NJ); sodium [125I]iodide and sodium [H]borohydride (Amersham Corp., Arlington Heights, IL); L-[15]S)methionine (New England Nuclear, Boston, MA); leupeptin (acetyl-L-leucyl-L-leucyl-argininal), 8-azaguanine, polyethylene glycol (approximate mol wt of 1,000), dithiothreitol, N-acetyl-D-glucosamine, D-galactose, α-methyl-D-mannoside, phosphorylase a, albumin, carbonic anhydrase, 2-mercaptoethanol, diisopropylfluorophosphate (DFP), sodium periodate (Sigma Chemical Co., St. Louis, MO); x-ray film (X-Omat, Eastman Kodak Co., Rochester, NY); and intensifying screens (Cronex Lightning Plus; E.I. Dupont de Nemours Co., Wilmington, DE); acrylamide (Bio-Rad Laboratories, Richmond, CA); 2,5-diphenyloxazole (Fisher Scientific Co., Fair Lawn, NJ); creatine kinase (Boehringer Mannheim Biochemicals, Indianapolis, IN); pristane (2,6,10,14-tetramethylpentadecane), iodoacetamide, and cyanogen bromide (Aldrich Chemical Co., Inc., Milwaukee, WI); formaldehyde-fixed, heat-killed *Staphylococcus aureus* (The Enzyme Center, Boston, MA); Ficoll-Paque and Sepharose-6B (Pharmacia, Inc., Piscataway, NJ); rabbit antisera to mouse whole IgG (Miles Laboratories, Inc., Elkhart, IN); rabbit antisera to mouse IgG1, IgG2a, IgG2b, and IgG3 (Litton Bionetics, Charleston, SC) and acid-citrate-dextrose, NIH formula A (Fenwal Laboratories, Div. of Travenol Labs., Inc., Deerfield, IL).

Ca**+/Mg**+ free Hanks’ balanced salt solution (HBSS), Dulbecco’s Modified Eagle’s Medium (DME), penicillin, streptomycin, t-methionine, and fetal bovine serum were from M. A. Bioproducts, Walkersville, MD; fetal bovine sera were from Sterile Systems, Inc., Logan, UT; Gibco Laboratories, Grand Island, NY; and M.A. Bioproducts. All sera were heat-inactivated (56°C for 1 h). Fetal bovine serum for biosynthesis experiments was dialyzed for 24 h at 4°C against several changes of HBSS and sterilized by filtration (0.22-μm Millipore filter). Eagle’s Modified Essential Medium (Earle’s salts) lacking methionine was from Flow Laboratories, McLean, VA.

Lentil lectin (*Lens culinaris* lectin) was purified to homogeneity from lentil beans (Peak brand; D and D Bean Co., Greeley, CO (11)) and was coupled at 2.5 mg/ml resin in 0.1 M NaHCO₃, pH 8.4, 0.1 M α-methyl-d-mannoside to Sepharose-6B activated by CNBr (12). Wheat germ lectin (*Triticum vulgare* lectin) (Bethesda Research Labs, Inc.) was coupled at 1.0 mg/ml in 0.1 M NaHCO₃, pH 8.4, 0.1 M N-acetyl-d-glucosamine to CNBr-activated Sepharose-6B and peanut lectin (*Arachis hypogaea* lectin) (Sigma Chemical Co.) was coupled at 1.0 mg/ml in 0.1 M NaHCO₃, pH 8.4, 0.1 M galactose.

Lymphocytes. Peripheral blood was collected from normal individuals and from male children clinically diagnosed as having the Wiskott-Aldrich syndrome (persistent throm-
bocytopenia with 20,000–50,000 platelets/µl, reduced platelet size, eczema, and variable immunodeficiency). Acid-citrate-dextrose was used as anticoagulant and the platelet-rich plasma was removed after centrifugation at 150 g for 8 min. Unless otherwise specified, all procedures were done at room temperature. The pelleted cells were diluted 1:1 with Ca²⁺/Mg²⁺ free Hanks' balanced salt solution (HBSS) containing 20 µg/ml of the protease inhibitor leupeptin and were separated by Ficoll-Hypaque centrifugation. The interface mononuclear cells were diluted with HBSS containing 2% fetal calf serum (FCS), pelleted at 300 g x 10 min, and incubated at 5 x 10⁵/ml in RPMI 1640 with 2% FCS for 1 h at 37°C in plastic tissue culture flasks. The nonadherent cells were pelleted and, if necessary, washed by suspending and pelleting in HBSS with 2% FCS (1–3 cycles) to reduce platelet contamination to negligible levels as judged by visual examination (opalescence) and/or microscopic examinations. The lymphocytes were washed twice in cold HBSS before radioiodination or in cold PBS before ³⁵S-labeling of surface carbohydrates. In the earliest experiments, heparin was used as anticoagulant and the platelet-depleted cell pellet was suspended in HBSS for Ficoll-Hypaque centrifugation. Adherent cells were removed by 1 h culture in 10% human serum and the nonadherent cells were then washed three times by pelleting in HBSS.

**Cell Lines.** The human T-lymphoblastoid cell lines HSB (13) and CCRF-CEM (14) were donated by Dr. H. Lazarus (Univ. of Miami Medical School) and grown in DME with 4.5 mg/ml glucose, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal calf serum and washed before use by pelleting three times in HBSS or PBS. The nonsecreting mouse myeloma cell line NS-1 used for cell fusions was donated by Dr. D. McMahon Pratt (Brigham and Women's Hospital) and was grown in the above medium.

**Radioiodination of Surface Protein Moieties.** Using a modified method (15), lymphocytes, CEM, or HSB cells were suspended at 2 x 10⁷/ml in HBSS with 10 µM NaI (200 µCi/ml for lymphocytes, 100 µCi/ml for HSB or CEM cells) and 10 µg/ml lactoperoxidase at room temperature. H₂O₂ was added in five portions (each 12 µl/ml of 0.015%) over 10 min. The reaction was terminated by adding 5–10 volumes of cold HBSS containing 0.1 mM NaI, and the cells were washed by pelleting.

**Radiolabeling of Surface Carbohydrate Moieties.** In a modified method (16) to introduce ³H-label into surface sialic acid residues, lymphocytes and CEM cells at 2-5 x 10⁷ in 1 ml were incubated in 1 mM sodium periodate in PBS at 4°C in the dark for 30 min and washed twice by pelleting in cold PBS. [³H]NaBH₄ (50 µCi in 5 µl cold 0.01 M NaOH) was added to the cells suspended in 1 ml PBS. After 20 min at room temperature the radiolabeled cells were washed by pelleting in cold HBSS. To introduce ³H-label into desialylated surface moieties (17), lymphocytes and CEM cells at 2-5 x 10⁷ in 1 ml were incubated with 0.1 IU/ml sialidase (Vibrio cholerae) and 25 µg/ml galactose oxidase (70–90 U/mg) for 30 min at room temperature and treated as described above.

**Biosynthesis of [³⁵S]Methionine-labeled Protein.** Lymphocytes or CEM cells were preincubated for 15 min in methionine-free medium, pelleted, and cultured at 1–4 x 10⁷/ml in Eagle's Minimum Essential Medium lacking nonradioactive methionine with 100 U/ml of penicillin, 100 µg/ml of streptomycin, 5% dialyzed fetal bovine serum, and 80–160 µCi/ml [³⁵S]methionine (900–1,100 Ci/mmol) for 1–2 h (CEM cells) or 18 h (lymphocytes).

To quantify [³⁵S]methionine incorporation into total cell protein, duplicate 10-µl samples were pipetted onto 2-cm squares of No. 3MM filter paper (Whatman) that were processed with trichloroacetic acid as described (18) and evaluated by scintillation counting.

**Preparation of Monoclonal Antibodies.** Two BALB/cByJ female mice (The Jackson Laboratory, Bar Harbor, ME) received intravenous injections of 2 x 10⁶ CEM cells in HBSS followed after 3 wk by 0.5 x 10⁶ CEM cells and after further 5 wk by 2 x 10⁶ CEM cells. 5 d later, the mice were sacrificed and their spleen cells (1.4 and 2.2 x 10⁶ cells) mixed at a 10:1 ratio with NS-1 myeloma cells and 35% polyethylene glycol and hybridized as described (19). After 15 d, culture supernatants from the 53 largest colonies chosen from the 960 microtiter wells were assayed by immunoprecipitation (described below) with NP-40 extracts of ¹²⁵I-labeled CEM cells as antigen, followed by SDS-electrophoresis and autoradiography to identify the precipitated antigens. One colony, which secreted
antibody that immunoprecipitates $^{125}$I-gpL115$_{CEM}$ was cloned twice at limiting dilution to yield the cell line L10. Antibody isotypes were determined by double diffusion in Ouchterlony plates with isotype-specific antisera. L10, an IgG1, has also been grown as ascites in pristane-treated BALB/cByJ mice. Other cell lines from the same fusions are L2, which secretes an anti-gpL115 antibody, and L1, L8, L4, and L9, which secrete antibodies recognizing other CEM surface proteins. As negative controls in immunoprecipitation, we used L4 antibody, an IgG1 that immunoprecipitates an unidentified CEM-surface protein of ~160,000 daltons and M3, a mouse monoclonal IgG1 generated against guinea pig macrophage surface antigens. (E. Remold-O'Donnell et al., to be published) which does not immunoprecipitate $^{125}$I-labeled human lymphocyte or CEM antigens.

Lectin Affinity Chromatography. Lymphocytes or CEM were extracted with 0.5% NP-40, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM diisopropylfluorophosphate, 3 mM iodoacetamide (1 ml extract per 1–8 × 10$^7$ lymphocytes or 1–2 × 10$^7$ CEM cells) for 3 min at room temperature and 8 min at 4°C. Insoluble material was removed at 12,000 g$_{max}$ × 15 min. The “NP-40 extracts” (0.8–2 ml) were applied to 1-ml columns of lentil lectin-Sepharose, wheat germ lectin-Sepharose or peanut lectin-Sepharose, which were washed with 0.3% NP-40, 10 mM Tris-HCl pH 7.4, 150 mM NaCl (0.3% N.T.S.). For each column, two fractions were collected, the “nonadherent” fraction and the “adherent fraction,” the latter being the fraction eluted with 0.1 M α-methylmannoside in the case of lentil lectin, 0.4 M N-acetylglucosamine in the case of wheat germ lectin, and 0.4 M galactose in the case of peanut lectin in 0.3% N.T.S.

Sialidase Treatment. $^{125}$I-labeled lymphocytes or CEM cells at 1–2 × 10$^7$/ml in HBSS or NP-40 extracts of $^{125}$I-labeled cells were incubated for 30 min at room temperature with 2 mM diisopropylfluorophosphate without or with 0.02 IU/ml Vibrio cholerae sialidase.

Immunoprecipitation. Washed formaldehyde-fixed S. aureus were incubated for 30 min at room temperature with rabbit antiserum to mouse IgG (20 μl antiserum/6 mg of bacteria) and the resulting bacteria-antibody complexes washed as described (20) by pelleting once in Buffer B (10 mM Tris-HCl, pH 8.6, 0.1% SDS, 0.05% NP-40, 300 mM NaCl) and once in Buffer A (12 mM sodium phosphate, pH 7.4, 200 mM NaCl). The complexes were incubated with hybridoma culture supernatant (200 μl/6 mg bacteria) for 1 1/2 h at room temperature and the washing procedure was repeated. The ternary complexes were incubated for 1–3 h with 50–200 μl $^{125}$I-labeled or $^{35}$Smethionine-labeled cell fractions. The resulting bacteria-second antibody-antibody-antigen complexes were washed twice and extracted for 2 min at 100°C with 2% SDS in 60 mM Tris-glycine buffer, pH 6.8, with or without 2% 2-mercaptoethanol. Fixed bacteria were removed by centrifugation.

In a simpler variation used to screen hybridoma colonies, 75 μl of hybridoma culture supernatant were incubated with 75 μl of NP-40 extract of $^{125}$I-labeled CEM for 2 h at room temperature and then combined with 6 mg fixed S. aureus containing bound rabbit antibodies to mouse IgG. Incubation, washing, and extraction were as above.

Two-dimensional Electrophoresis. Two-dimensional isoelectrofocusing-SDS-electrophoresis analysis was performed according to a modified procedure (21, 22). The first dimension isoelectrofocusing tube gel was composed of 3.3% polyacrylamide, 9.2 M urea, and 3.2% Triton X-100 with 0.4% ampholytes (Ampholine, LKB) of range pH 2.5–4, 2% of range pH 3.5–5, 2% of range pH 5–7, and 0.6% of pH 3.5–10. The sample dissolved in 9.5 M urea, 0.2% NP-40, and 60 mM dithiothreitol was applied to the basic end and focused for 16 h at 400 V and 2 h at 1,000 V. The pH values are averages measured after elution of 5 mm slices of duplicate gels with water.

Polyacrylamide Gel Electrophoresis and Autoradiography. NP-40 extracts and purified cell fractions were solubilized by heating with an equal volume of 2% SDS in 60 mM Tris-glycine buffer, pH 6.8, with or without 2% 2-mercaptoethanol for 2 min at 100°C. SDS electrophoresis (23) and autoradiography conditions were as described (24) with gels of 7% polyacrylamide or of 7.5–14% exponential polyacrylamide gradients. The standard proteins myosin, β-galactosidase, phosphorylase a, albumin, creatine kinase, carbonic anhydrase, and soybean trypsin inhibitor indicated mol wt of 200,000, 130,000, 94,000,
Results

Lentil Lectin Chromatography of gpL115 from Lymphocytes and Lymphoblastoid Cell Lines. To characterize its carbohydrate moieties, gpL115 was analyzed by affinity chromatography on columns of lentil lectin-Sepharose. Lentil lectin shows specificity for mannose (26), a core component of asparagine-linked (N-linked) carbohydrate units. \(^{125}\text{I}\)-labeled-gpL115 of normal lymphocytes passed unretarded through lentil lectin-Sepharose columns (Fig. 1, left), indicating that gpL115 contains little or no exposed mannose residues and suggesting the absence of N-linked carbohydrate.

Two long-term lymphoblastoid cell lines HSB (14) and CEM (13) were surface radioiodinated. Both contain \(^{125}\text{I}\)-labeled components that co-migrate on SDS-electrophoresis with gpL115 of normal peripheral lymphocytes (not shown). The component of apparent mol wt 115,000 in CEM cells is the most prominent \(^{125}\text{I}\)-labeled cell surface protein. On chromatography of extracts of \(^{125}\text{I}\)-labeled CEM cells, the major radiolabeled 115,000 mol wt component was found to be lentil lectin-nonadherent (Fig. 1, right) suggesting its identity with gpL115 of normal lymphocytes.

Wheat Germ Lectin Chromatography of gpL115. When chromatographed on wheat germ lectin-Sepharose, \(^{125}\text{I}\)-labeled gpL115 of normal lymphocytes was found totally in the adherent fraction (Fig. 2A, left). Wheat germ lectin has two

![Figure 1](image.png)
FIGURE 2. (A) Chromatography on wheat germ lectin-Sepharose of ^125^I-labeled surface polypeptides of normal lymphocytes without (Native) and with (Asialo) sialidase treatment of the starting fraction, an NP-40 extract. Shown is an autoradiograph of an SDS-electrophoresis gel (nonreducing conditions). The left three lanes show chromatography of a native extract, with the starting fraction (Start), the nonadherent fraction (Non Ad.), and the adherent fraction (Ad.; \( N\)-acetylglucosamine eluate) indicated. The three lanes on the right show chromatography of a sialidase-treated extract. Positions of mol wt marker proteins and gpL115 are indicated on the left. The shift in electrophoretic mobility of gpL115 after sialidase treatment can be noted by comparing Native and Asialo starting fractions. Asialo-gpL115, designated by the arrows on the right, migrates with apparent mol wt 150,000; its identity as a species of gpL115 was verified by immunoprecipitation (shown below).\(^2\) Note that native gpL115 from lymphocytes is wheat germ lectin-adherent and asialo-gpL115 is nonadherent. (B) Chromatography on wheat germ lectin-Sepharose of ^125^I-labeled surface polypeptides of CEM cells. Details and findings are as in A.
specificities, binding both to N-acetylgalactosamine (27, 28) and to clustered sialic acid residues (29, 30). To determine which of these specificities mediate the binding of gpL115, the starting fraction, an NP-40 extract of 125I-labeled lymphocytes, was treated with sialidase to remove sialic acid. Sialidase treatment was found, first of all, to reduce the electrophoretic mobility of gpL115 from apparent mol wt 115,000 to apparent mol wt 150,000 (Fig. 2A, comparison of “Native” and “Asialo” starting fractions). This large reduction in electrophoretic mobility suggests that gpL115 has significant sialic acid content. On chromatography, asialo-gpL115 was found in the wheat germ lectin–nonadherent fraction (Fig. 2A, right), indicating that native gpL115 adheres to wheat germ lectin via sialic acid residues and does not adhere via N-acetylgalactosamine residues.

The native 115,000 mol wt 125I-labeled molecule from CEM cells, like lymphocyte gpL115, was found to be wheat germ lectin–adherent (Fig. 2B, left). Sialidase treatment shifted this molecule to apparent mol wt 150,000 and converted it to a wheat germ lectin–nonadherent form (Fig. 2B, right), strongly suggesting that this CEM surface molecule is identical to gpL115 of lymphocytes.

Radiolabeling of the Carbohydrate Moieties of gpL115. Surface carbohydrate moieties were labeled by treating lymphocytes with periodate followed by [3H]-NaBH₄, which converts terminal 9-carbon sialic acid residues to 3H-labeled 7-carbon 5-acetamido-3,5-dideoxy-L-arabino-2-heptulosonic acid residues (31). 3H-label was found in several lymphocyte surface glycoproteins, including a component of apparent mol wt 115,000, presumably gpL115 (Fig. 3, left). Lymphocytes were also treated with sialidase-galactose oxidase followed by [3H]NaBH₄, which yields 3H-labeled asialo-glycoproteins. The 3H-labeled asialo-glycoproteins of lymphocytes migrate with apparent mol wt ≥150,000 (Fig. 3). After similar treatments of CEM cells, the major 3H-labeled native surface glycoprotein has apparent mol wt 115,000 and the major 3H-labeled asialo-glycoprotein has apparent mol wt 150,000 (Fig. 3).

Isoelectrofocusing of gpL115. On two-dimensional analysis (isoelectrofocusing-SDS electrophoresis) of partially purified fractions, 125I-labeled gpL115 from CEM cells was found as a broad band or “streak,” primarily in the extreme acidic region at pH 4.1 (Fig. 4, top). On the other hand, sialidase-treated gpL115 focuses as a defined spot at pH ~5.0 (Fig. 4, bottom). Identical behavior on isoelectrofocusing was found for native and sialidase-treated 125I-labeled gpL115 from lymphocytes (not shown). The large difference in the behavior of native and sialidase-treated gpL115 on isoelectrofocusing indicates a prominent role for multiple sialic acid residues in determining the physiochemical characteristics of gpL115 (see Discussion).

Peanut Lectin Chromatography of gpL115. Peanut lectin shows specificity for the disaccharide Galβ1-3GalNAc (32), a sequence found in mucin-type O-linked saccharides (33). When extracts of 125I-labeled lymphocytes were chromatographed on peanut lectin–Sepharose, gpL115 was found to be peanut lectin–nonadherent (Fig. 5A, left). Sialidase-treated gpL115 from lymphocytes, however, was found to be peanut lectin–adherent (Fig. 5A, right). 125I-labeled native gpL115 from CEM cells was also found to be peanut lectin–nonadherent, and the sialidase-treated form to be peanut lectin–adherent (Fig. 5B), which provides further evidence that the gpL115 molecules from CEM cells and lymphocytes
FIGURE 3. \(^{3}\)H-labeled surface glycopeptides of normal peripheral lymphocytes (two left lanes) and CEM cells (two right lanes). Intact cells were reacted with \(^{3}\)HNaBH\(_4\) after periodate treatment to generate reactive carbonyl groups in terminal sialic acid residues (Native) or after treatment with sialidase and galactose oxidase (Asialo). Shown is a fluorograph of NP-40 cell extracts fractionated by SDS-electrophoresis under nonreducing conditions with mol wt \((\times 10^{-6})\) indicated on the right. Arrow on the left indicates the position of native lymphocyte gpL115 and the major co-migrating \(^{3}\)H-labeled native glycopeptide of CEM. Arrow on the right indicates the position of the major CEM \(^{3}\)H-labeled asialo-glycopeptide which migrates with apparent mol wt 150,000.

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Generation of Anti-gpL115 Monoclonal Antibody. Hybridoma technique was used to generate a cell line secreting monoclonal antibody to gpL115. The cell line, L10, was generated using CEM cells as the immunizing agent. L10 antibody is an IgG1 molecule that immunoprecipitates \(^{125}\)I-labeled native and sialidase-treated gpL115 (Fig. 6). Both gpL115 from lymphocytes and CEM cells are immunoprecipitated by L10 antibody (Fig. 6), further demonstrating their identity.

Synthesis of gpL115 by Lymphocytes. Since detection of gpL115 relied exclusively on surface labeling techniques, we sought to determine whether gpL115 is a biosynthesis product of lymphocytes and CEM cells or an acquired surface component. Lymphocytes from five normal donors cultured with \[^{35}\]S\)methionine at 2–4 \(\times\) 10\(^7\)/ml were found to incorporate 21% ± 3% of added radioactivity into trichloroacetic acid–precipitable protein in 18 h. CEM cells, in contrast,
FIGURE 4. Two-dimensional analysis of native and sialidase-treated $^{125}$I-labeled gpL115 from CEM cells. Shown is an autoradiograph of two-dimensional gels with isoelectrofocusing on the horizontal axis (pH gradient is indicated) and SDS-electrophoresis under reducing conditions on the vertical axis (mol wt marker positions indicated on the right). NP-40 extracts of $^{131}$I-labeled CEM cells were incubated without (Native) or with (Asialo) sialidase. The former fraction was then purified by wheat germ lectin chromatography and the latter by peanut lectin chromatography. Note that sialidase treatment shifts gpL115 to an isoelectric point that is less acidic and more sharply defined. Identical results were obtained when $^{125}$I-labeled gpL115 was first purified (by lentil lectin and wheat germ lectin chromatography) and then incubated without (Native) or with (Asialo) sialidase (not shown).

incorporated >60% within 2 h. On immunoprecipitation, L10 antibody precipitates a $^{[35S]}$methionine-labeled component of apparent mol wt 115,000 from lymphocytes and CEM cells that was not found in immunoprecipitates of two control monoclonal IgG1 antibodies (Fig. 7). The $^{[35S]}$methionine-labeled component precipitated by L10 antibody co-migrates with $^{125}$I-gpL115 under reducing and nonreducing conditions (not shown), thus establishing that lymphocytes and CEM cells synthesize gpL115.

Defect in gpL115 in Lymphocytes of Wiskott-Aldrich Syndrome Patients

Lymphocytes of patients with the Wiskott-Aldrich syndrome and normal individuals were radioiodinated and their surface components examined by SDS electrophoresis and autoradiography. In all, lymphocytes from 8 patients were
examined in 12 experiments. $^{125}$I-labeled gpL115 was found to be deficient in lymphocytes of all patients. However, the levels of $^{125}$I-gpL115 varied, being nondetectable in patient 1 (Fig. 1 of reference 6) patients 2, 3, and 5 (Fig. 8) and detectable, but decreased relative to levels in normal lymphocytes, in patients 4, 6, 7, 8 (Fig. 8).
FIGURE 6. Immunoprecipitation of native and sialidase-treated $^{125}$I-labeled gpL115 by L10 monoclonal antibody. NP40 extracts of normal lymphocytes and CEM cells were incubated without (Native) or with (Asialo) sialidase and immunoprecipitated with hybridoma culture supernatant of L10 cells (lanes 1, 3, 5, 7) or M3 cells (lanes 2, 4, 6, 8). Shown is an autoradiograph of an SDS electrophoresis gel (reducing conditions) with mol wt $\times 10^{-3}$ of marker proteins indicated on the left.

FIGURE 7. Immunoprecipitation of gpL115 labeled biosynthetically with $[^{35}]$S]methionine. Normal lymphocytes and CEM cells were cultured with $[^{35}]$S]methionine. gpL115-containing fractions were prepurified by NP-40 extraction and wheat germ lectin chromatography and immunoprecipitated with L10, (lanes 1 and 4) or L4 (lanes 2 and 5) or M3 (lanes 3 and 6). Shown is a fluorograph of an SDS electrophoresis gel (reducing conditions) with the position indicated of $^{125}$I-labeled gpL115 which was electrophoresed in parallel.
**FIGURE 8.** T-labeled surface proteins of lymphocytes of six normal individuals (N2-N7) and eight Wiskott-Aldrich syndrome patients (P1-P8). Shown is a composite of autoradiographs of SDS electrophoretic separations of NP-40 extracts of 125I-labeled lymphocytes from six experiments. Electrophoretic separations were of nonreduced extracts in four experiments (eight lanes starting from the left) and reduced extracts in two experiments (six lanes on the right). Within each of the six experiments, normal and patient were matched for cell number, radiiodination conditions, electrophoresis, and autoradiography conditions. Arrows on the left indicate gpL115. The patients indicated by P1, P2, and P3 have been previously described (6). P1,T indicates patient 1 examined 26 mo after bone marrow transplant; prior to transplant his lymphocytes had nondetectable levels of 125I-labeled gpL115 (6). Note that 125I-gpL115 levels are reduced or nondetectable in all patients. Note that some patients (P3, P6, and possibly P2) have an 125I-labeled component of electrophoretic mobility slower than gpL115 (indicated by dots on the left) which is not found in normal individuals.
FIGURE 9. $^{125}$I-labeled surface proteins of normal lymphocytes and CEM cells after treatment with varying concentrations of sialidase. Normal lymphocytes and CEM cells were treated with A, E, no additive; B, F, 0.002; C, G, 0.007, and D, H, 0.02 IU/ml sialidase (Vibrio cholerae), washed, radiiodinated, and extracted with NP-40. Shown is an autoradiograph of an SDS electrophoresis gel (nonreducing conditions) with the position of marker proteins and native gpL115 indicated by the large arrows on the left. Note that sialidase treatment generates partially desialylated species of gpL115 (small arrows) that migrate as discrete bands of decreasing mobility. Higher sialidase concentrations did not further alter the electrophoretic mobility of gpL115 (not shown). A pattern identical to the right lane was found when CEM cells were treated with sialidase from Clostridium perfringens, (0.32 U/ml; Bethesda Research Laboratories; not shown).

In addition, an $^{125}$I-labeled polypeptide of apparent mol wt 135,000 (indicated in Fig. 8 by dots on the left) was found in lymphocytes of two patients (patient 3 and patient 6) and on one of two analyses of the cells of patient 2. This $^{125}$I-labeled 135,000 mol wt component was not detected in lymphocytes of normal individuals and did not co-migrate with asialo-gpL115 (apparent mol wt 150,000; not shown).

Thus, lymphocytes of patients with the Wiskott-Aldrich syndrome are characterized by the absence or the deficiency of gpL115 molecules that are accessible to surface radioiodination. In addition, lymphocytes of some Wiskott-Aldrich syndrome patients contain a surface polypeptide of apparent mol wt 135,000 not found in lymphocytes of normal individuals.

Treatment of normal lymphocytes or CEM cells with limiting concentrations of sialidase was found to generate species of gpL115 of decreasing electrophoretic mobility (Fig. 9). The partially desialylated species of gpL115 display varying, but discrete, apparent electrophoretic mobilities intermediate between those of the native and the “asialo”-gpL115 molecules (Fig. 9).2 This finding introduces

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2 The sialidase-treated gpL115 species of apparent mol wt 150,000 has been referred to as “asialo” since higher sialidase concentrations do not further alter its mobility. We cannot, at this stage, rule out the presence of residual resistant sialyl moieties.
the possibility that the abnormal 135,000 mol wt component found in lymphocytes of some Wiskott-Aldrich syndrome patients is a partially desialylated variant(s) of gpL115.

Discussion

gpL115, the lymphocyte surface glycoprotein that is deficient in Wiskott-Aldrich syndrome, is a sialoglycoprotein that can be radiolabeled in protein moieties by \(^{125}\text{I}\)lactoperoxidase and in carbohydrate moieties by \(^{3}\text{H}\)NaBH4. gpL115 can be labeled by culturing lymphocytes with \(^{35}\text{S}\)methionine, indicating that it is synthesized by lymphocytes.

Comparison of radioiodinated surface proteins of lymphocytes and the lymphoblastoid cell line CEM by electrophoresis, isoelectrofocusing, sialidase treatment, lectin affinity chromatography, and immunoprecipitation established that gpL115 is also present on the surface of the CEM cells, a finding used to advantage in the generation of a monoclonal antibody to gpL115. The designation of CEM cells as a suitable source establishes the opportunity for isolation and chemical analysis of gpL115. Through the use of surface marker antigens, CEM has been shown to be a cell line of early thymocyte origin (34), thus implying that thymocytes, which were not examined in this study, also express surface gpL115.  

gpL115 was identified through its deficiency in Wiskott-Aldrich syndrome patients (6) and has also been found defective in lymphocytes of two non-Wiskott immunodeficient patients. \(^{125}\text{I}\)-labeled gpL115 was not immunoprecipitated by monoclonal antibodies directed against the surface antigen CALLA (references 35, 36; common acute lymphoblastic leukemia antigen; antibodies supplied by Dr. J. Ritz, Dana Farber Cancer Institute), thus establishing the nonidentity of gpL115 and CALLA. Likewise, gpL115 was not immunoprecipitated by monoclonal anti-T12 antibody (reference 37; supplied by Dr. S. Schlossman, Dana Farber Cancer Institute), demonstrating the nonidentity of the surface antigens T12 and gpL115.

The quantity of sialic acid on gpL115 is not known, but appears to be large since desialylation dramatically alters the physical behavior of the molecule. On SDS electrophoresis, desialylation shifts gpL115 from a mobility corresponding to mol wt 115,000 to a mobility corresponding to mol wt 150,000. Good correlation of mol wt with electrophoretic mobility in the presence of SDS has been established for a wide range of polypeptides (38) and the behavior of gpL115 is, thus, atypical. Anomalous behavior on SDS electrophoresis is a feature of glycoproteins with high content of carbohydrate and is caused by the inability of carbohydrate to bind SDS (39, 40).

On isoelectrofocusing, sialidase treatment shifts gpL115 from a very broad band or "streak," primarily in the extreme acidic region (pH 4.1) to a well-defined "spot" at pH 5. We do not know why the native molecule is found as a broad streak. Heterogeneity at the level of sialylation is a possibility, but would not explain the finding that the streak of native gpL115 extends to pH regions

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\(^{5}\) Using fluorescent techniques, LI0 anti-gpL115 monoclonal antibody stains 99% of normal lymphocytes and thymocytes (R. Parkman et al., to be published).

\(^{6}\) R. Parkman et al., manuscript in preparation.

\(^{7}\) We have at present no estimate of the true molecular weight of gpL115.
less acidic than the spot of sialidase-treated gpL115 (observed in five of five experiments). We favor the explanation that native gpL115 is incompletely focused, i.e., that the rate of focusing of native gpL115 is atypically slow due to its elongated conformation enforced by its high sialic acid content.

Lymphocytes of patients with the Wiskott-Aldrich syndrome are characterized by a defect in gpL115. $^{125}$I-labeled gpL115 was deficient in eight patients; the extent of the deficiency varied from nondetectable levels (two patients) to levels that are decreased relative to normal individuals (four patients). In addition, an $^{125}$I-labeled surface protein of apparent mol wt 135,000, which was not found in normal individuals, was detected in three of eight Wiskott-Aldrich patients. We speculate that this abnormal component is a variant of gpL115.$^6$ gpL115 of normal individuals can be converted to apparent mol wt 135,000 by treating intact lymphocytes with limiting sialidase concentrations (Fig. 9).

The thesis that a single defective gene in Wiskott-Aldrich syndrome codes for both the defective lymphocyte and platelet surface components gpL115 and GPIb is unlikely since the molecules are not identical, i.e., they do not co-migrate on electrophoresis (not shown). We also did not detect partial identity, in that GPIb ($^{125}$I-labeled and $[^3H]$NaBH$_4$-labeled) was not immunoprecipitated by L10 anti-gpL115 monoclonal antibody and $^{125}$I-gpL115 was not immunoprecipitated by a polyclonal antibody to GPIb/glycocalcin (not shown; rabbit antisera supplied by Dr. G. A. Jamieson, The American National Red Cross, Bethesda, MD).

A more likely hypothesis is that the concomitant defect of gpL115 and GPIb in Wiskott-Aldrich syndrome results from a single defective processing element, possibly a synthesis enzyme such as a glycosyl transferase, or a degradation enzyme. The current study indicates that gpL115, like GPIb, is one of a small group of glycoproteins with high content of O-linked carbohydrate units (see below); and it is, thus, likely that they share some biosynthesis and/or degradation steps.

Glycoprotein carbohydrate moieties are of two types. The N-linked “complex” and “high mannose” units consist typically of 8–14 monosaccharide residues (sialic acid, galactose, fucose, mannose, and N-acetylglucosamine) linked to asparagine via N-glycosidic bonds. gpL115 is lentil lectin-nonadherent, suggesting the absence of N-linked carbohydrate. The second type, the “O-linked” units, also called “mucin-type” or “acidic type” because of the high sialic acid content, are linked to serine or threonine via O-glycosidic bonds. These units are generally small, consisting of three, four, or six residues. For example, in glycophorin O-linked units consist of galactose, N-acetylgalactosamine, and two terminal sialic acid residues (41) and in platelet GPIb, they also contain N-acetylglucosamine (42). Typically, mannose is not found.

A few glycoproteins consist of >50% O-linked carbohydrate; these include erythrocyte glycophorin (43), platelet GPIb (7), “masking” glycoproteins on certain tumor cells (44, 45), and LSGP (leukocyte sialoglycoprotein; 9), a rat T lymphocyte and thymocyte surface component. LSGP may be the counterpart in the rat of gpL115. LSGP displays the same unusual behavior as gpL115 on lentil lectin (46) and peanut lectin chromatography (10); its asialo-form is the

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$^6$ When lymphocytes from a suitable patient become available, this hypothesis will be tested by immunoprecipitation with L10 antibody.
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major peanut lectin-adherent surface glycoprotein of rat thymocytes and T lymphocytes (10).

gpL115 adheres to wheat germ lectin, but asialo-gpL115 does not, indicating that wheat germ lectin-adherence is due to clustered sialic acid residues on the native molecule. Clustered sialic acid is a feature of glycoproteins with high content of O-linked sugar. gpL115 does not adhere to peanut lectin, but asialo-gpL115 does—a very important finding since peanut lectin shows specificity for the disaccharide galactoseβ(1-3)-N-acetylgalactosamine. These findings indicate that native gpL115 contains the sequence sialic acid-galactoseβ(1-3)-N-acetylgalactosamine, a sequence particular to O-linked, mucin-type carbohydrate units.

This study establishes similarity of the carbohydrate portions of the lymphocyte and platelet components altered in Wiskott-Aldrich syndrome. Like gpL115, GPIb and its soluble form glycocalcin (8) adhere to wheat germ lectin (7) and asialo-GPIb adheres to peanut lectin (8). GPIb (glycocalcin) consists to 60% of carbohydrate that appears to be exclusively mucin-type units linked to serine and threonine (7, 42).

Due to sialic acid content, glycoproteins with high O-linked carbohydrate content are thought to adopt extended conformation and to confer negative charge characteristics required in cell-cell interactions (9). In certain mouse adenocarcinomas a surface sialoglycoprotein with >50% O-linked carbohydrate, referred to as a “masking” glycoprotein, is thought to confer on the cells the capacity to invade allogeneic and xenogeneic hosts (44, 45). Similarly, sialidase treatment of mouse lymphoma cells exposes cryptic tumor antigens on glycolipid molecules that are, themselves, devoid of sialic acid (47). Finally, removal of sialic acid from lymphocytes has been shown to induce their loss from the circulation (48).

The cumulative findings suggest that the defect(s) in the sialoglycoprotein gpL115 in the Wiskott-Aldrich syndrome adversely affect(s) the ability of the lymphocytes to interact with other cells and/or to survive in circulation.

Summary

gpL115 is a lymphocyte surface component that is deficient in patients with the X-chromosome-linked immune deficiency Wiskott-Aldrich syndrome (6). The glycoprotein nature of gpL115 is demonstrated through labeling in carbohydrate moieties by [3H]NaBH₄ and its synthesis by lymphocytes through labeling with [35S]methionine. Native gpL115 adheres to wheat germ lectin-Sepharose and sialidase-treated gpL115 does not adhere, indicating that native gpL115 adheres via clusters of sialic acid residues. When tested on peanut lectin, which shows specificity for the disaccharide Galβ1-3GalNAc, gpL115 is nonadherent and sialidase-treated gpL115 is adherent, indicating the presence of the sequence sialic acid-Galβ1-3GalNAc, which is characteristic for O-linked (mucin-type, acidic-type) carbohydrates. A surface glycoprotein with all the above characteristics was found on the lymphoblastoid cell line CEM. CEM cells were used as immunogen to generate the monoclonal antibody L10, an IgG₁, which binds native and sialidase-treated gpL115. Sialidase-treatment of gpL115 significantly alters its physical properties, reducing its electrophoretic mobility and changing its behavior on isoelectrofocusing. Cumulatively, these findings indicate that gpL115, like glycophorin of erythrocytes and GPIb of platelets, is a sialoglyco-
protein with significant quantities of O-linked carbohydrate.

On treatment with limiting sialidase concentrations, gpL115 of normal lymphocytes is transformed into a series of partially desialylated species of decreasing electrophoretic mobility. This finding resembles the situation with lymphocytes of some Wiskott-Aldrich syndrome patients. Lymphocytes of eight Wiskott-Aldrich syndrome patients were found to be deficient in 125I-labeled gpL115. Lymphocytes from three of these patients displayed an abnormal 125I-component of apparent mol wt 135,000.

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Note added in proof: After submission of the manuscript, we became aware of a report describing F10-44-2, an LSGP-like glycoprotein of mol wt 105,000 on human blood mononuclear cells (Dalchau, R., J. Kirkley, and J. w. Fabre. 1980. Monoclonal antibody to a human brain-granulocyte-T lymphocyte antigen probably homologous to the W 3/13 antigen of the rat. Eur. J. Immunol. 10:745). This molecule may be identical to gpL115.

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