A Novel Form of Congenital Dyserythropoietic Anemia Associated With Deficiency of Erythroid CD44 and a Unique Blood Group Phenotype [In(a − b −), Co(a − b −)]

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We have used a panel of well-characterized monoclonal antibodies (MoAbs) to examine the blood cells of a patient with a novel form of congenital dyserythropoietic anaemia (CDA) characterized by intra-erythroblastic and intra-erythrocytic membranous inclusions. Twelve antibodies defining three nonoverlapping epitope groups on the extracellular domain of CD44 all failed to react with the red blood cells (RBCs) of the patient. A rabbit antibody to the cytoplasmic domain of CD44 from normal RBCs failed to react with the patient’s RBC ghosts. In contrast, the patient’s lymphocytes, granulocytes, and monocytes showed apparently normal CD44 expression. Bone marrow preparations stained with CD44 antibodies and visualized with 125I antimony Ig (F(ab)2) followed by autoradiography showed positive staining of lymphocytes and myeloid cells but not of normal orthotolidine-positive erythroblasts. The patient’s RBCs also gave weaker than normal reactions with MoAbs of anti-LW specificity while MoAbs to glycoporphins A, B, and C, Rh polypeptides, CD47, CD55, CD58, CD59, acetylcholinesterase, and Lutheran and Kell glycoproteins all gave normal reactions. Agglutination tests with human blood grouping sera demonstrated that the RBCs of

CD44 is a widely distributed cell surface glycoprotein that has been implicated in a range of biologic functions including adhesion to components of the extracellular matrix,1,2 lymphocyte homing,3 tumor metastasis,4 T-cell activation,5 and hematopoietic development.5,6 Recently, cDNA encoding the CD44 glycoprotein (CD44) has been cloned and sequenced7,9 and the existence of several different forms have been reported.10 All forms comprise a single polypeptide chain that traverses the plasma membrane once with the amino terminus in the extracellular domain. The extracellular domain comprises a distal disulfide bonded domain and a proximal domain rich in O-glycans. The multiple forms of CD44 result from inserts occurring in the O-glycan-rich domain that are presumed to result from alternative splicing of exons.10 Certain monoclonal antibodies (MoAbs) in CD44 are capable of inhibiting the development of lymphoid and myeloid precursors when added to murine bone marrow (BM) cultures in vitro.3 These observations have led to the suggestion that CD44 may play a role in the maturation of hematopoietic cells through interaction with extracellular matrix components and/or stromal cells in the marrow. In this report we describe our investigations on the hematopoietic cells of a child with a novel form of congenital dyserythropoietic anemia (CDA). The results show that the child has a gross deficiency of CD44 from her erythroid cells but not from her myeloid or lymphoid cells. It is known that the Ina and Inb blood group antigens are located on erythrocyte CD44 glycoprotein11 and consistent with this the patient’s blood type was In(a − b −). Blood grouping tests further showed that the child has the very rare Co(a−b−) blood group phenotype and is negative for the high incidence antigen AnWj.

MATERIALS AND METHODS

The clinical and hematologic features of the child with congenital anemia studied (case SF) have been reported elsewhere.12,13 Blood and BM samples. Blood samples were taken into EDTA anticoagulant. Patient SF and normal control blood samples were taken at the same time. Normal blood samples were provided by Blood Services South West (Bristol, UK). Erythrocytes from CDA type I patients SB, WM, and JB and from CDA patients of unclassified types AW and CC were obtained via the Department of Haematology (St Mary’s Hospital, London, UK) from Drs P. Saunders, J. Wallis, J.S. Lilleyman, and P.J. Darbyshire. Erythrocytes from CDA type II patient DC14 were from the frozen RBCs collection of the International Blood Group Reference Laboratory.
(IBGRL). Erythrocytes from CDA type II patient KI were kindly provided by Prof Alice Maniatis (University of Patras, Greece). Erythrocytes from CDA type III patients BL and EV were kindly provided by Drs H. Sandstrom and A. Wahlin (University Hospital, Umea, Sweden).

BM was aspirated from SF and from a hematologically normal BM transplant donor, mixed with Hanks' balanced salt solution containing 25 U/mL preservative-free heparin and processed immediately.


**Antibodies.** Mouse MoAbs in CD44 (BRICs 35, 205, 214, 219, 222, 223, 225, 235, 238, 241, and KZ-1) were as described. Mouse monoclonal anti-LW**a** antibodies (BS46 and BSB6**a**) were generously provided by Dr H.H. Sonneborn (Biotest Serum Institute GmbH, Frankfurt am Main, Germany). Other mouse MoAbs were as previously described: antibody against glycophorin A and B (LICR/LON R1.3) and antibodies against glycophorin A LICR/LON R10, LICR/LON R18**, LICR 217, and BRIC 255 (unpublished); anti-Wr**a** (BRIC 147**a**); anti-glycophorin C (BRIC 4 and BRIC 10**a**); antibody against erythrocyte membrane anion transport protein (band 3, BRIC 169, [unpublished]); Rh blood groups related antibodies (BRIC 69**a**, and LA23-40**a**); Kell blood groups related antibodies (BRIC 18, BRIC 68, BRIC 107, and BRIC 207**a**). Lutheran blood groups related antibodies (BRIC 108**, BRIC 221, and BRIC 224 [unpublished, January 1988]); CD47 (BRIC 125 and BRIC 126**a**); CD55 (BRIC 110 and BRIC 128**a**); CD58 (BRIC 51**a**); and CD59 (BRIC 229). Rat MoAbs against glycophorin C and D (BRAC 1 and BRAC 11) were as described. Human blood group antibodies were from an in-house collection.

**Serologic techniques.** Serologic techniques were essentially as described. Indirect antiglobulin tests with mouse or rat MoAbs were performed using rabbit antirabbit Ig or rabbit antirat Ig (DAKO Ltd, High Wycombe, UK) as appropriate. Hemagglutination titration scores were calculated as described.

**Rabbit antiserum against CD44 glycoprotein.** CD44 was isolated by immuno-affinity chromatography from normal human erythrocyte membranes prepared as described and solubilized in a minimum volume of a Triton buffer (Sigma Ltd, Poole, UK) comprising 5% (wt/vol) Triton TX-100, 2 mM phenylmethylsulphonyl fluoride (PMSF), 0.5 mM L-KEDTA in phosphate-buffered saline (PBS), pH 7.3. This material was extracted using 6.6 mg CD44 antibody (BRIC 235) cross-linked to 1 mL of protein A-sepharose gel (Bioprocessing Ltd, Consett, UK) as described. The CD44 antibody column was saturated with a large volume (approximately 200 mL) of solubilized erythrocyte membranes and then washed extensively in a 1% (wt/vol) Triton buffer. CD44 was eluted with 50 mM diethylamine, 2 mM PMSF, 0.5% (wt/vol) sodium deoxycholate, pH 11.5. Fractions were adjusted to pH 8.0 with 2 mol/L glycine pH 2.0, dialyzed extensively against 10 mM L-Tris/HCl pH 8.2 and then against water. The purified CD44 was dried by centrifugal evaporation. A rabbit was immunized with a subcutaneous injection of 50 μg CD44 in Freund's incomplete adjuvant followed 3 days later with a second subcutaneous injection of 50 μg of CD44 in Freund's incomplete adjuvant. The rabbit was bled 10 days later, and serum was collected and heated at 56°C for 30 minutes. An aliquot of serum was extensively absorbed with normal, group A and group B, human erythrocytes until all agglutinating antibody was removed.

**Immune precipitation studies.** Immune precipitation was performed essentially as described. Intact erythrocytes or unsealed erythrocyte membranes were incubated with an excess of MoAb or rabbit antiserum at 0°C. Immune complexes were recovered from solubilized erythrocyte membranes on protein A-sepharose.

**Quantitation of erythrocyte CD44 binding sites.** CD44 binding sites were estimated using 125I-labeled Fab fragments prepared from CD44 antibodies as previously described. Erythrocytes (0.5% suspension in PBS pH 7.3 containing 1% Bovine serum albumin [PBS-A], 25 μL) were incubated with 50 μL of saturating dilution of MoAb culture supernatant for 30 minutes at room temperature, washed once in PBS-A (3.5 mL), and incubated with 1/25 dilution of fluorescein isothiocyanate (FITC) rabbit F(ab')2, antirat Ig (F313; DAKO Ltd, High Wycombe, UK) (25 μL) for 30 minutes at room temperature. PBS-A (300 μL) was added and the labeled cells analyzed using a FACStar Plus flow cytometer (Becton Dickinson, Oxford, UK). Leukocytes were analyzed after separation by dextran sedimentation and lysis of erythrocytes using 155 mM/L NH4Cl/10 mmol/L KHCO3. Washed cells resuspended in PBS-A at 1 × 107/mL (50 μL) were incubated with a saturating dilution of culture supernatant (50 μL) for 30 minutes on ice, washed once in PBS-A (3.5 mL), and incubated with 1/25 DAKO F313 (25 μL) as above for 30 minutes on ice. After one wash in PBS-A (3.5 mL) the labeled cells were resuspended in 2% formalin in PBS-A or plain PBS-A (300 μL) before fluorescence activated cell sorter (FACS) analysis. In the analysis, lymphocyte, granulocyte, and monocyte subpopulations were identified on the forward scatter/side scatter dot plot and electronic gates used to measure the FL1 (green FITC) fluorescence in each of these regions.

**Immunochemical analysis of BM samples.** The presence of CD44 on hemoglobin-containing erythrocytoblasts was investigated using cytospin preparations of BM cells from SF and a healthy subject by the procedure described by Wiener and Wickramasinghe. After fixation in a phosphate-buffered mixture of distilled water, acetone, and formaldehyde, the cells were reacted with 50 μL of KZ/1 or BRIC 235 supernatant for 30 minutes at room temperature, washed in PBS, and reincubated with 50 μL 125I-labeled antiserum Ig, F(ab')2 (0.06 μCi, 5 to 12 ng) (Amersham International, Aylesbury, UK). After further washes in PBS, the cells were fixed in methanol, stained for hemoglobin by reaction with o-tolidine, submitted to radioautography, and counterstained with Giemsa. Nucleated o-tolidine-positive (early and late polychromatic) as well as orthochromatic erythroblasts or negative cells (mainly myeloid cells and lymphocytes) were scored for radioactivity under the light microscope.

**RESULTS**

**Reactivity of SF's peripheral blood cells with MoAbs.** The erythrocytes of SF were examined in blood samples taken on May 17, 1991, September 10, 1991, and June 15, 1992. Agglutination tests with a range of MoAbs showed a gross reduction in reactivity with CD44 antibodies. The reduction was most marked in the sample taken on September 10, 1991. A total of 12 CD44 antibodies recognizing three nonoverlapping epitope groups failed to agglutinate these erythrocytes. Weak agglutination results were ob-
served with erythrocytes from the May 17, 1991 and June 15, 1992 blood samples with these antibodies. Weaker than normal reactions were also observed with monoclonal anti-LW\textsuperscript{ab} (BS 46 and BS 56). Normal reactions were obtained with MoAbs to glycophorin A, glycophorin C, Rh, Kell, Lutheran, CD55, CD58, and CD59 (data not shown).

Flow cytometric analysis of SF's erythrocytes also showed a gross reduction in CD44 reactivity (Fig 1) and weak LW\textsuperscript{ab} reactivity (not shown). CD44 antibodies gave normal reactions with SF's lymphocytes (Fig 1), granulocytes, and monocytes (data not shown). The anti-LW\textsuperscript{ab} antibodies used did not react with normal lymphocytes, granulocytes, or monocytes.

Flow cytometry also revealed that SF blood samples contained a minor population of erythrocytes with normal expression of CD44. The sample taken on May 17, 1991, 51 days after blood transfusion, contained a population of erythrocytes with normal expression of CD44 amounting to 28% of the total. The blood sample taken on September 10, 1991, 167 days after blood transfusion, contained only 3% CD44-normal erythrocytes and the sample taken on June 15, 1992, 84 days after transfusion, contained 8% normal erythrocytes (data not shown). It is likely that the weak agglutination reactions obtained with CD44 antibodies on the samples taken on May 17, 1991 and June 15, 1992 were caused by the presence of these CD44-normal cells and that these cells were transfused erythrocytes with normal CD44 reactivity. Erythrocytes from both the parents of SF and her unaffected sister gave normal reactions with all antibodies.

Reactivity of CD44 antibodies with BM cells. When normal and SF BM preparations were reacted with BRIC 235 or KZ-1 CD44 antibodies and subsequently with \textsuperscript{125}I-antimouse Ig [F(ab')\textsubscript{2}], considerable radioactive labeling of myeloid cells and lymphocytes was found in normal as well as in SF BMs (>90%). By contrast, with both CD44 antibodies the percentage of radioactively labeled o-tolidine-positive erythroblasts was greatly decreased in SF, being 5% to 8% compared with 85% to 96% in the normal control.

Reactivity of erythrocytes from SF and her family with blood group antibodies. SF RBCs typed as group B, CcDeEe, MNs, P\textsubscript{1}, Le(a−b+) Lu(a−b+), Jk(a+b+). SF RBCs were negative in the direct antiglobulin test. The blood sample taken on September 10, 1991 typed as In(a−b−), Co(a−b−). The high frequency antigen AnW\textsubscript{j} was also absent from the September 10, 1991 sample and weak reactions were obtained with anti-LW\textsuperscript{ab} antibodies. Weak (in some cases mixed field) agglutination reactions were noted with anti-In\textsuperscript{a}, anti-AnW\textsubscript{j}, and anti-S on the samples taken on May 17, 1991 and June 15, 1992. As discussed above, these reactivities are likely to reflect the presence of residual transfused erythrocytes. Normal-strength reactions were obtained with antisera to the following antigens: Cr\textsuperscript{a}, Te\textsuperscript{a}, Dr\textsuperscript{a}, At\textsuperscript{a}, Hy, Gy\textsuperscript{a}, Jf\textsuperscript{a}, MER-2, Ge2, Di\textsuperscript{b}, Sc1, Lan, Rh29, Kp\textsuperscript{b}, Js\textsuperscript{a}, Kn, Jk3, U, Er\textsuperscript{a}, Wr\textsuperscript{b}, Kn\textsuperscript{a}, Yt\textsuperscript{a}, Cs\textsuperscript{a}, Ch, Rg, JMH, H, P, I, i, Vel, Fy3. The parents and sister of SF had
CD44 deficiency in a novel form of CDA

Fig 3. Immunoblotting of normal and SF erythrocyte membranes with monoclonal anti-CD44, anti-LW<sup>ab</sup>, and anti-Lutheran. Erythrocyte membrane proteins were separated on 8% (wt/vol) acrylamide gels under nonreducing conditions, transferred to PVDF membrane, and stained using MoAbs as described in Materials and Methods. Fifty micrograms of erythrocyte membrane protein was applied to each sample slot. The position of standard molecular weight marker proteins (Mr × 1,000) is indicated on the figure. Normal control (C) erythrocyte membranes (a, c, e, g, i, k) and SF erythrocyte membranes (b, d, f, h, j, l through n). SF membranes (SF or SF2, b, d, f, h, j, m) from blood taken on September 10, 1991. (SF1, i) from sample taken on May 17, 1991 and (SF3, n) from sample taken on June 15, 1992. (a, b) Stained with CD44 antibody BRIC 35; (c, d) stained with CD44 antibody BRIC 235; (e, f) stained with CD44 antibody KZ-1; (g, h) stained with anti-LW<sup>ab</sup> BS56; (i, j) stained with anti-Lutheran BRIC 221; (k through n) stained with a mixture of three CD44 antibodies against three nonoverlapping epitope groups BRIC 222, BRIC 235, and KZ-1.

common In and Co phenotypes and gave normal reactions with anti-LW and anti-AnW<sub>j</sub> sera (Fig 2). Lacey et al<sup>35</sup> describe an antibody made by a Co(a-b-) individual that gave weak reactions with other Co(a-b-) RBCs; this antibody gave normal-strength reactions with SF RBCs.

**Immunoblotting analysis of erythrocyte membranes from SF and her family.** When the electrophoretically separated components of erythrocyte membranes from SF were examined by immunoblotting with CD44 and LW<sup>ab</sup> antibodies the reduced reactions observed in agglutination tests were confirmed (Fig 3, membranes prepared from the September 10, 1991 blood sample). Normal staining patterns were observed with Lutheran antibodies (Fig 3) and antibodies to erythrocyte band 3, CD58, and glycoporphins A, B, C, and D (data not shown). Improved staining of CD44 in normal erythrocyte membranes was obtained by using a mixture containing equal volumes of antibodies against each of three CD44 epitopes. Weaker-than-normal staining was observed in SF erythrocyte membranes prepared from the May 17, 1991 (SF1) and June 15, 1992 (SF3) blood samples and very weak staining was observed with membranes prepared from the September 10, 1991 (SF2) blood sample (Fig 3). Antibodies to band 3 showed a slightly increased electrophoretic mobility when compared with control samples (data not shown). This increased mobility was also apparent on Coomassie stained gels (Mr 107,000 for leading edge of SF band 3 compared with Mr 113,000 for control samples [data not shown]).

A rabbit antiserum was prepared to purified normal human erythrocyte CD44 and an aliquot was rendered specific for the cytoplasmic and/or transmembrane domain of the molecule by exhaustive absorption with intact normal erythrocytes. The rabbit antisera were used in immune precipitation experiments with normal, intact human erythrocytes and with unsealed erythrocyte membranes. The precipitated components were examined by immunoblotting with CD44 antibody KZ-1. The results are shown in Fig 4. Whole rabbit serum precipitated CD44 from intact erythrocytes (ie) and from unsealed membranes (um). Absorbed rabbit serum precipitated CD44 from unsealed erythrocyte membranes, where the cytoplasmic domain of CD44 was available for antibody binding, but not from intact human erythrocytes. Control serum, taken from the same rabbit before immunization, did not precipitate CD44.

In immunoblotting experiments markedly reduced binding of rabbit anti-CD44 and of absorbed rabbit anti-CD44
Immune precipitation

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<th>whole</th>
<th>abs.</th>
<th>control</th>
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<td>ie um</td>
<td>ie um</td>
<td>ie um</td>
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Fig 4. Immunoprecipitation and immunoblotting of normal and SF membranes with rabbit anti-CD44. (a through f) Immune precipitation from intact erythrocytes or from unsealed erythrocyte membranes using rabbit anti-CD44, absorbed anti-CD44 and preimmune serum. The precipitated proteins were separated on an 8% (wt/vol) acrylamide gel, transferred to PVDF, and stained by immunoblotting with monoclonal CD44 antibody KZ-1. The position of standard molecular weight marker proteins (Mr x 1,000) is indicated on the figure. (a, c, e) Precipitates from intact erythrocytes (ie). (b, d, f) Precipitates from unsealed erythrocyte membranes (um). (a, b) Rabbit anti-CD44 (whole serum); (c, d) rabbit anti-CD44 extensively absorbed with intact erythrocytes (abs serum); (e, f) preimmune rabbit serum (control serum). (g through l) Erythrocyte membrane proteins were separated on 8% (wt/vol) acrylamide gels under nonreducing conditions, transferred to PVDF membrane, and stained using rabbit anti-CD44, absorbed anti-CD44, and preimmune, control serum. Fifty micrograms of erythrocyte membrane protein was applied to each sample slot. (g, i, k) Normal erythrocyte membranes (C). (h, j, l) SF membranes (sample bled on September 10, 1991). (g, h) Stained with rabbit anti-CD44. (i, j) Stained with absorbed rabbit anti-CD44. (k, l) Stained with preimmune serum. A component (marked with an asterisk) of Mr 70,000 was observed in SF membranes but not in normal membranes. This component was not found in skeletons prepared from SF RBCs or in membranes prepared from the erythrocytes of the parents of SF (data not shown). The nature of this component is unknown.

Quantitation of CD44 on erythrocytes from SF and her family. Purified radio-iodinated Fab fragments from murine monoclonal CD44 antibodies were used in quantitative binding assays to determine the level of CD44 expression on the erythrocytes of SF and her family. The results (Table 1) show a gross reduction in CD44 levels on RBCs from SF but apparently normal levels of expression on both parents and the sister of SF. Fab fragments from antibodies to glycophorin A and glycophorin C were used as controls and these gave normal levels of binding to SF cells (Table 1).

CD44 expression and Colton blood types of erythrocytes from patients with CDA I, CDA II, CDA III, and unclassified CDA. Erythrocytes from patients with CDA type I (SB, WM, and JB), CDA type II (DC and KI), CDA type III (BL and EV), and from patients with unclassified CDA (AW and CC) were tested by titration with MoAbs. No reduction in hemagglutination titration score was found with antibodies in CD44 (BRIC 35, BRIC 235, and KZ-1), with Lutheran blood groups related antibody (BRIC 221), or with antibody against glycoporphins A and B (LICR/LON R1.3, results not shown). Flow cytometric analyses with CD44 antibodies and quantitation using 125I-labeled CD44 antibodies were
also normal with these cells. In tests with anti-LW MoAbs some reduction in titration scores was observed with erythrocytes from patients SB (CDA I), DC (CDA II), and CC (CDA unclassified). Tests with human blood group antibodies gave normal positive reactions with anti-In\textsuperscript{b}, anti-An\textsubscript{Wj}, and anti-Co\textsuperscript{a}.

**DISCUSSION**

Case SF\textsuperscript{12,13} was born in 1982 to haematologically normal, unrelated Danish parents. She had severe anemia, marked erythroblastosis and hyperbilirubinemia at birth, and required regular blood transfusions during the first year of life. She now receives transfusions only when her hemoglobin, which is usually around 8.5 g/dL, decreases in association with common infections. The anemia is normochromic and normocytic and is caused by a combination of ineffective erythropoiesis and peripheral hemolysis. Hematologic features include a reticulocyte count of 2% to 15%, an HbF level of 40% (ie, higher than in other CDAs), a negative acidified serum lysis test (Ham's test), normoblastic erythroid hyperplasia, and nonspecific dysplastic features in several late erythroblasts—mainly basophilic stippling of the cytoplasm and marked irregularities in nuclear outline. Electron microscopy showed large distinctive intracytoplasmic inclusions consisting of compact masses of tubules and sacules within late erythroblasts, reticulocytes, and RBCs. The characteristic ultrastructural findings in CDA types I, II, and III which are, respectively, a spongy appearance of abnormally electron-dense heterochromatin in a high proportion of erythroblasts, a double membrane running parallel to and 40 to 60 nm away from the erythroid cell membrane, and numerous giant mononucleate and multinucleate erythroblasts, were not observed. \(\xi\)- and \(\epsilon\)-globin chains were present in some circulating RBCs. Studies of the globin genes showed that the patient had a normal \(\xi\text{aa}/\xi\text{aa}\)-globin genotype and that her elevated HbF level was unlikely to be caused by mutations in the \(\gamma\)-\(\beta\)-globin gene cluster. The features of the CDA affecting case SF do not resemble those of CDA types I, II, or III and this patient suffers from a novel type of CDA.

The results presented here provide clear evidence that the erythrocytes of SF have a gross deficiency of CD44 but that other peripheral blood cells (lymphocytes, granulocytes, monocytes) have normal expression of this cell surface glycoprotein. CD44 deficiency is also apparent in erythroid cells in SF's BM. Immunoblotting with a rabbit antisera directed at the cytoplasmic (and transmembrane) domain of CD44 indicates that these domains are also deficient in SF erythrocytes. This result makes it unlikely that the lack of reactivity with MoAbs to the extracellular domain is caused by abnormal glycosylation rather than deficiency of the whole protein. The \(\text{In}^a\) and \(\text{In}^b\) blood group antigens are carried on erythrocyte membrane CD44.\textsuperscript{13} Consistent with CD44 deficiency, SF RBCs typed as \(\text{In}\,(a\,-b\,-)\). It is well known that erythrocytes from \(\text{Lu}(a\,-b\,-)\) individuals of the dominant \(\text{In}(Lu)\) form of inheritance have an apparent deficiency of the Lutheran glycoproteins and CD44\textsuperscript{11} and that these cells also lack the high incidence antigen \(\text{AnWj}\) as well as having weakened expression of other blood group antigens.\textsuperscript{35} Therefore, \(\text{In}(Lu)\) type erythrocytes also type as \(\text{In}(a\,-b\,-)\). It was shown that SF has normal expression of erythrocyte Lutheran glycoproteins and therefore is not of the \(\text{In}(Lu)\) type. This is, to our knowledge, the first report of an individual with a selective, gross deficiency of erythrocyte CD44. Analysis of the erythrocytes of the parents and the unaffected sister of SF showed normal levels of CD44 and so we were unable to unequivocally establish inheritance; however, SF has been affected since birth and the defect would, therefore, appear to be recessively inherited. The unique nature of this case is further supported by our observations that CD44 deficiency is not a feature of RBCs obtained from several other patients with CDA that we have examined. In contrast, reduced expression of LW antigens is not uncommon in CDA patients and has also been seen, transiently, in autoimmune anemias.\textsuperscript{37} SF RBCs were negative in the direct antiglobulin test, suggesting an absence of autoantibody.

The only other type of CDA which has so far yielded to analysis at the molecular level is CDA II (HEMPAS). The primary defect in different patients with CDA II appears to

### Table 1. Quantitation of CD44 on the Erythrocytes of SF and Family Using Radiiodinated Fab Fragments of MoAbs

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<thead>
<tr>
<th>Antibody</th>
<th>SF</th>
<th>Father</th>
<th>Mother</th>
<th>Sister</th>
<th>Normal Erythrocytes</th>
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<tr>
<td>CD44</td>
<td></td>
<td></td>
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<tr>
<td>BRIC 222</td>
<td>1.491 ± 398 (3)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>11,484 (2)</td>
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<td>BRIC 205</td>
<td>1.798 ± 484 (3)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7,605 ± 1,158 (11)</td>
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<tr>
<td>KZ-1</td>
<td>2.202 ± 333 (3)</td>
<td>9.539 (1)</td>
<td>10,073 (1)</td>
<td>10,915 (1)</td>
<td>8,434 ± 1,396 (12)</td>
</tr>
<tr>
<td>GPA</td>
<td></td>
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<td>BRIC 256</td>
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<td>BRIC 217</td>
<td>255,206 (1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>GPC</td>
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<td>BRIC 10</td>
<td>160,276 (1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>145,219 (1)</td>
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Determinations were performed on SF erythrocytes from the blood sample taken on September 10, 1991. Figures in parentheses represent the number of determinations performed. Where three or more determinations were performed the result is expressed as mean ± SD. Background binding was estimated as 1,274 sites per cell using BRIC 205 Fab fragments and trypsin treated normal erythrocytes in a single determination.

Abbreviations: ND, not determined; GPA, glycophorin A; GPC, glycophorin C.
occurred in one or other of the genes encoding enzymes involved in the N-glycosylation pathway and these defects result in plasma membrane abnormalities.34 In contrast, the primary abnormality in CDA I and CDA III is more likely to affect nuclear membranes or other nuclear constituents and gross abnormalities of the plasma membrane have not been recorded.38,39 In CDA II the abnormality in N-glycosylation of RBC membrane proteins is most readily detected by observing the increased electrophoretic mobility on SDS-PAGE of the major RBC glycoprotein band 3.14,40 In the case of patient SF, a small shift in Mr of band 3 was observed but this was not of the same order as that for CDA II (unpublished observations, October 1991).

The blood group antigen is characteristically elevated in CDA II but other blood group antigens are not known to be markedly affected. There is a report of one patient having CDA II in association with the relatively rare Kell blood group phenotype Kp(a-b-),41 but this blood group phenotype appeared to be unrelated to the disease.33 All the other CDA samples that we have examined have normal Ina/Inb and Coa/Cob phenotypes and normal expression of AnWj. The absence of Ina/Inb antigens from SF RBCs represents a unique phenotype that can be readily explained by deficiency of CD44 (discussed above). The AnWj antigen (formerly Ant 0) is known to be reduced on RBCs of Lu(a-b-) individuals of the dominant In(Lu) type and to be inherited independently of the Lutheran Blood Group System.42-44 The absence of the AnWj antigen from SF's RBCs raises the possibility that this determinant may be closely associated with erythrocyte CD44. The remarkable occurrence of a third very rare phenotype [Co(a-b-)] in SF RBCs seems unlikely to be a coincidence but is not easy to interpret because the biochemical nature of the membrane component expressing the Coa/Cob antigens is unknown. Nevertheless, the possibility that SF has inherited a silent Co allele from each parent and that this is independent from the CD44 deficiency cannot be formally ruled out (Fig 2). The expression of the Colton antigens is unaffected by inheritance of the In(Lu) gene. The Co(a-b-) phenotype has previously been described in four families.35 One of these Co(a-b-) individuals55 has been tested and found to have normal expression of the Inb antigen35 (and unpublished observations, August 1992). These results show that Ina/Inb expression is independent of Coa/Cob expression in Lu(a-b-) RBCs of the (InLu) type and in at least one of the previously described individuals with the Co(a-b-) phenotype. The Co(a-b-) phenotype has also been described in several Finnish patients who had monosomy 7.45,46 Evidence locating the locus for Colton blood groups to chromosome 7p has been presented recently.47 The CD44 gene is located on chromosome 11p13.48 Reciprocal translocations involving the short arms of chromosomes 7 and 11 have been described in myeloid leukemia.49 However, no evidence of chromosomal abnormalities was found on examination of BM aspirates from SF (N.I., unpublished, December 1987). Nevertheless, further studies of the expression of CD44 and the Colton blood group antigens on the erythrocytes of patients with chromatid abnormalities and/or chromosome 11 abnormalities might be rewarding.

The complete absence of CD44 from RBCs and most orthotolidine-positive erythroblasts, but not from myeloid or lymphoid cells, indicates a specific defect in either the erythroid CD44 gene or in control of its expression in erythroid cells. The concomitant occurrence of the Coa(a-b-) phenotype suggests that the latter is more likely and such a conclusion would be consistent with the abnormal expression of hemoglobin genes in this patient reported by Tang et al.13 Erythroid CD44 cDNA has recently been cloned and sequenced from reticulocytes.50 The derived amino acid sequence was identical to that reported for the hematopoietic isoform of CD44 from other cells. Harn et al51 discuss the possibility that alternative processing of 3' untranslated regions of CD44 mRNA may regulate gene expression by modulating RNA stability or translational efficiency. Whether or not abnormalities at this level are responsible for the erythroid specific deficiency of CD44 in SF must await further study.

Clearly, the identification and characterization of the erythrocyte membrane component responsible for Colton blood groups would greatly facilitate attempts to establish the linkage, if any, between CD44 deficiency and the Coa(a-b-) phenotype in this patient. The erythrocytes of SF also have novel intra-erythroblastic and intra-erythrocytic membrane inclusion52 in addition to the two abnormalities in the erythrocyte plasma membrane described here. Implying that there is a fundamental defect in membrane assembly of erythroid cells from this patient. An understanding of the defect(s) in erythropoiesis in patient SF may provide a useful approach to understanding the mechanism of membrane assembly and the regulation of differentiation in normal erythrocytic cells.

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A novel form of congenital dyserythropoietic anemia associated with deficiency of erythroid CD44 and a unique blood group phenotype [In(a-b-), Co(a-b-)]

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