Am. J. Hum. Genet. 41:1061–1070, 1987

Identification of a Cell-Surface Antigen Produced by a Gene on Human Chromosome 3 (cen-q22) and Not Expressed by Rh_{null} Cells

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SUMMARY

A monoclonal antibody, 1D8, which recognizes a cell-surface antigen expressed by human chromosome 3 in Chinese hamster–human somatic-cell hybrids, has been produced. Testing of hybrids containing various deletions of chromosome 3 determines that the gene encoding the antigen is regionally localized to 3q (cen-22). This regional mapping is distinct from that elsewhere reported for two other cell-surface antigens assigned to chromosome 3—namely, the human transferrin receptor and the p97 melanoma-associated antigen. In addition, biochemical characterization is different from that elsewhere reported for other chromosome 3–encoded cell-surface antigens. When tested against a panel of rare-phenotype red blood cells, the only cells that failed to react were those of the Rh_{null} phenotype. The antibody reacts only weakly with homozygous −D− and fetal red cells, in contrast with a previously described antibody, R6A, which does not react with Rh_{null} cells. Furthermore, R6A does not recognize a cell-surface antigen expressed by chromosome 3 in Chinese hamster–human somatic-cell hybrids. Thus, the monoclonal antibody 1D8 recognizes a previously undescribed cell-surface antigen encoded by human chromosome 3 and not expressed on Rh_{null} cells. The gene on chromosome 3 regulating expression of this antigen may be that defective in Rh_{null} disease or may require the normal allele at an unlinked Rh_{null} locus for expression. Linkage studies will be required to further elucidate this matter.

Received September 23, 1986; revision received May 27, 1987.

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INTRODUCTION

Five human cell-surface antigens have been assigned to chromosome 3 previously (Enns et al. 1982; Goodfellow et al. 1982; Plowman et al. 1983; Parkkinen and Schroder 1984; Rettig et al. 1984). The two antigens most completely described are the transferrin receptor and p97 melanoma-associated antigen, for which biochemical characterization and regional chromosomal assignment are available. The transferrin receptor (90,000 molecular weight [MW] reduced, 180,000 MW nonreduced) has been assigned to chromosome 3q (26-ter) (Miller et al. 1983; Rabin et al. 1984). The p97 antigen (MW 97,000) has been assigned to chromosome 3q (24-ter) (Seligman et al. 1986). A 90,000-MW antigen of unknown regional assignment has been described (Parkkinen and Schroder 1984). In spite of the similarity of its MW to that of the transferrin receptor, on the basis of its expression by various cell lines it appears to be a distinct antigen. Two other chromosome 3–encoded cell-surface antigens have been described, without regional assignment (Rettig et al. 1984). One, termed K15, is an 85,000-MW glycoprotein. The other, AJ425, does not have reported biochemical characterization.

In the present paper, we describe an IgM monoclonal antibody, 1D8, which recognizes an antigen coded for by human chromosome 3. Regional assignment to 3 (cen-q22) has been achieved. When a panel of rare-phenotype red cells was tested, by means of indirect hemagglutination, for expression of the antigen recognized by 1D8, all but Rh_{null} cells were agglutinated. Erythrocytes that lack any of the Rh blood group antigens were first described by Vos and are termed Rh_{null} cells (Vos et al. 1961). Two different genetic backgrounds can result in the Rh_{null} phenotype (Race and Sanger 1975). In the extremely rare instance, an individual is homozygous for the amorph (– – –) at the *Rh* locus, which is mapped to human chromosome 1. The less rare instance of Rh_{null} phenotype, termed regulator type, is the result of homozygosity at an unlinked locus that has not been assigned to a human chromosome.

The fact that an antigen requiring chromosome 3 for its expression is not detected in Rh_{null} cells raises the possibility that the Rh_{null} locus may map to chromosome 3. Alternatively, the antigen recognized by 1D8 may require the activity of an unlinked regulator locus for expression.

MATERIAL AND METHODS

Cell Lines and Culture

Chinese hamster–human somatic-cell hybrids were produced by fusion of Chinese hamster auxotrophic mutants with either human lymphocytes or human fibroblasts, according to a method described elsewhere (Jones et al. 1980). Cells were grown in either Ham's F12 or Ham's F12D supplemented with 7% dialyzed fetal calf serum (FCM 7). The following cell lines were used: A549, a human cell; HeLa, a human cell; CHO-K1, a Chinese hamster ovary cell; and the human–Chinese hamster somatic-cell hybrids 314-1, 314-2, 323-1, 371-14A, J1, 153-E9A, 724-32X-6, 567-E4E, Q72-4B, Q72-18, 706-B6A, 706-D1, 706-D6, Q21-10A, 725-18, UCH-2, UCH-2-R, UCH-12, 314-1-R, and 314-1-X.

NS-1 mouse myeloma cells were grown in RPMI 1640 12% fetal calf serum (FC 12) supplemented with 300 mg glutamine/liter (final concentration 600 mg/liter) and 2,000 mg glucose/liter (final concentration 4,000 mg/liter).

Hybridoma Isolation and Monoclonal Antibody Production

Two Balb/c mice were immunized intraperitoneally and subcutaneously with 2×10^6 A549 cells in complete Freund's adjuvant. One month later they were boosted with 0.25 ml of a 1/10 (v/v) dilution of washed human red cells and PBS, administered intravenously. Four days later the mice were killed, their spleens removed and dissociated, and, with the use of polyethylene glycol 1000, 2×10^8 spleen cells were fused with 3×10^7 NS-1 myeloma cells. Fused cells were plated in four 96-well plates in RPMI 1640 FC 12 containing 1×10^{-4} M hypoxanthine, 1.6×10^{-5} M thymidine, and 4×10^{-7} M aminopterin (HAT) and supplemented with glutamine and glucose as described in the previous section. After 10 days of growth, HAT was omitted and supernatants were tested by means of a microcytotoxicity assay: 2×10^3 314-1-R cells were incubated in 96-well plates containing 33% hybridoma supernatant and 2% normal rabbit serum as a source of complement in a total volume of 150 μl. After overnight incubation, complement-mediated cytolysis was determined by means of direct microscopic inspection. Positive wells were cloned twice by limiting dilution. Balb/c mice were primed by means of intraperitoneal injection of 0.5 ml pristane and injected with 5×10^6–1×10^7 hybridomas 5 days later. Malignant ascites were collected, centrifuged, diluted 1/10 in PBS, filtered through a 0.22-μm membrane, and frozen.

TR 3A7 is an IgG2a monoclonal antibody to the human transferrin receptor (Bies et al. 1984). R6A is an IgG_1 monoclonal antibody developed by P. A. W. Edwards (1980).

Antigen Assay

A single cell–plating cytotoxicity assay was used for antigen detection. Five hundred cells were plated in Costar 24 well plates in a volume of 0.3 ml F12 FCM 7 containing 2% normal rabbit serum as a complement source with varying concentrations of monoclonal antibody. After 6 days of growth, plates were fixed with 10% ethanol and 3.5% acetic acid and colonies were stained with crystal violet. Surviving colonies were counted, percentage survival was compared with that of a control well with no added monoclonal antibody calculated, and survival curves were generated. Killing of >90% was seen in antigen-positive cell lines, and <10% killing was seen in antigen-negative lines. This clean separation was seen because cell lines had been cloned prior to characterization and then frozen in multiple aliquots.

Indirect hemagglutination assays were performed by incubating equal volumes (10 μl) of supernatant (diluted 1/100) and a 5% suspension of red cells for 1 h at 20 C, washing the cells once, adding 30 μl of a 1/1,000 dilution of rabbit anti-mouse globulin (provided by Dr. R. W. Knowles), centrifuging for 25 s, and reading the agglutination macroscopically. For ascertaining strength of reaction, doubling dilutions of supernatant were used.

For antigen detection by means of indirect immunofluorescence, cells were harvested by means of a brief trypsinization, washed twice with phosphate-buffered saline, and then resuspended in a 50-μl volume of phosphate-buffered saline with 1% bovine serum albumin. Approximately 1×10^6 cells were used per assay. Fifty microliters of a 1% ascites solution of appropriate monoclonal antibody was added to each sample. From this point on, cell samples were kept at 4 C. After a 30-min incubation, cells were washed twice with cold phosphate-buffered saline, then incubated with 100 μl fluorescein-conjugated goat anti-mouse antibody (Coulter) for 30 min, washed twice, resuspended in 0.5 ml phosphate-buffered saline, and then subjected to flow cytometry in a Coulter EPICS V Model 752 cell sorter. Fluorescence data were analyzed in a Coulter EZ 88 computer system IMMUNO-PROGRAM, with subtraction of background fluorescence of cells incubated with fluoresceinated goat anti-mouse and no primary antibody.

Karyotypic and Isozyme Analysis of Hybrids

Human chromosomes were identified by means of karyotypic analysis that was performed using both trypsin banding and a modified Giemsa 11 procedure (Seabright 1971; Bobrow and Cross 1974; Alhadeff et al. 1977). Human chromosomes were also identified by means of isozyme analysis, performed as described, with the exception that aminocylase-1 was used for chromosome 3 identification, according to the method described by Voss et al. (Jones et al. 1980; Voss et al. 1980). Presence of 3q was further confirmed by expression of human transferrin receptor, as detected by monoclonal antibody TR 3A7 in a single cell–plating cytotoxicity assay.

Immunoprecipitation, Immunoblots, and Enzyme Treatment of Cells

Immunoprecipitation of antigens expressed by A549 cells was carried out according to a method described elsewhere (Jones 1980). In brief, cells were surface labeled with ^{125}I by using the lactoperoxidase technique. Cells were then solubilized in NP-40 and reacted with the appropriate monoclonal antibody. Immune complexes were immunoprecipitated using *Staphylococcus aureus*, Cowan strain 1, precoated with rabbit antibodies to mouse immunoglobulins. Samples were then eluted, subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and autoradiography.

Immunoblotting was performed essentially according to the method described by Towbin et al. (1979) as modified by Kloppel and Brown (1984). A549, 314-2, and CHO-K1 cells from 10 100-mm plates were harvested using a rubber policeman, pelleted, brought up in 0.5 ml distilled water, and freeze-thawed 10 times. Samples were then centrifuged 15 min in an Eppendorf 5412 microcentrifuge. The pellet was solubilized overnight in 1% Triton X-100 and gel buffer, (7% sodium dodecyl sulfate, 0.005% bromophenol blue, 20% glycerol, 0.1 M Tris, pH 6.8), subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred electrophoretically to nitrocellulose sheets, and reacted with a 2% solution of monoclonal antibody ascites. Nitrocellulose sheets were subsequently reacted with horseradish peroxidase–conjugated rab-

bit anti-mouse immunoglobulin. Sheets were then developed using diaminobenzidine-H_2O_2 as substrate.

Methods of enzyme treatment and 6% aminoethylisothiouronium bromide (AET) treatment of red cells have been described elsewhere (Advani et al. 1982; Anstee et al. 1984).

RESULTS

Species Specificity

The 1D8 monoclonal antibody was cytotoxic to the human cell lines A549 and Hela but not to the Chinese hamster cell CHO-K1 (fig. 1).

Chromosomal Assignment

The 1D8 monoclonal was cytotoxic to the 314-2 somatic-cell hybrid, which contains chromosome 3 as its only human genetic material (fig. 1). When tested against a clone panel of human–Chinese hamster somatic-cell hybrids containing all human chromosomes, 1D8 killed only those hybrids containing chromosome 3 (table 1); hybrids containing all other chromosomes but chromosome 3 were resistant to killing by 1D8.

R6A was not cytotoxic to either the A549 cell or the 314-2 somatic-cell hybrid. In addition, fluorescence analysis by means of flow cytometry demon-

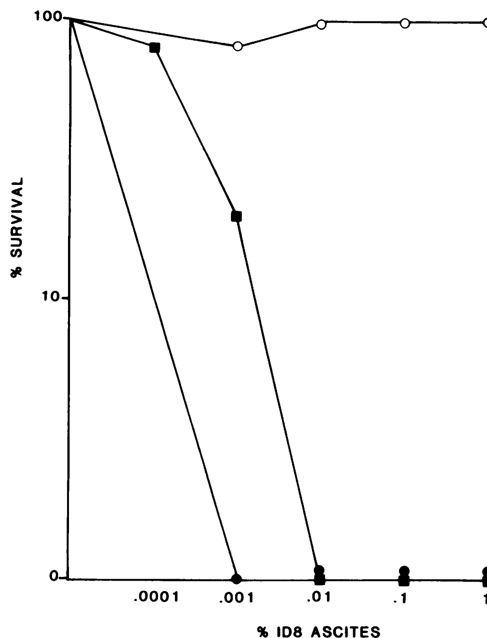

FIG. 1.—Single cell–plating cytotoxicity curves for CHO-K1 (–○–), A549 (–●–), and the 314-2 (–■–) somatic-cell hybrid, demonstrating that 1D8 does recognize a cell-surface antigen expressed in human cells and in a human–Chinese hamster somatic-cell hybrid containing chromosome 3 as its only human genetic material but does not do so in Chinese hamster cells.

TABLE 1

Human Chromosome Constitution of 15 Hybrid Clones and Result of Killing by 1D8 Monoclonal Antibody

Human Chromosome	Hybrid Clone															% Concordant
	314-1	314-2	323-1	371-14A	JI	153-E9A	725-32X-6	567-E4A	Q826-26A	Q72-18	Q21-10A	706-B6A	706-D1	725-18	706-D6	
1	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	60
2	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	53
3	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	100
4	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	67
5	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	53
6	−	−	−	+	−	−	−	−	+	+	+	−	+	−	+	53
7	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	60
8	−	−	−	+	−	−	−	−	−	+	−	+	−	−	+	53
9	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	60
10	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	60
11	−	−	−	+	+	−	−	−	+	+	+	−	−	−	−	60
12	−	−	+	−	−	−	−	+	+	+	−	−	+	−	−	47
13	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	60
14	−	−	+	+	−	−	−	−	−	+	+	+	−	+	+	60
15	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−	73
16	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	53
17	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	60
18	−	−	−	+	−	−	−	−	−	−	−	−	+	−	+	60
19	−	−	−	+	−	−	−	−	+	+	+	−	−	−	−	67
20	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	60
21	−	−	−	+	−	+	+	−	+	+	+	−	−	+	+	40
22	−	−	−	+	−	−	−	−	−	+	−	−	+	−	+	53
X	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	60
1D8 Killing	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	

strated that 314-2 did not bind R6A (0% cells positive), whereas this hybrid was positive for 1D8 (36% cells positive), as well as for TR 3A7 (90% cells positive). It is of interest that the cytotoxicity assay is apparently more sensitive than flow cytometry for detecting the antigen recognized by 1D8, a difference likely due to the efficiency of IgM isotype antibodies in fixing complement.

Regional Mapping

Three independent hybrids containing 3q, 314-1-X, UCH-12, and UCH-2 were killed by 1D8. All were also killed by the TR 3A7 anti–human transferrin receptor monoclonal (table 2). Segregants resistant to anti–transferrin receptor antibodies have been derived from 314-1 and UCH-2 and are designated 314-1-R and UCH-2-R, respectively. 314-1-R Has suffered a deletion 3q (24-ter) and UCH-2-R has suffered a deletion 3q (22-ter), as described elsewhere (Miller et al. 1983; Seligman et al. 1986). Both hybrids are susceptible to killing by the 1D8 monoclonal antibody but are not killed by the TR 3A7 monoclonal antibody (table 2). Thus, 3 (cen-q22) is necessary for expression of the cell-surface antigen recognized by 1D8.

Biochemical Characterization of Antigen Recognized by 1D8

Immunoprecipitation studies with the A549 cell line did not reveal a specific band precipitated by 1D8. A control immunoprecipitation with the TR 3A7 monoclonal did reveal a specific band at MW 180,000, which reduced to 90,000 and is consistent with the human transferrin receptor. Immunoblot analysis of a membrane preparation of A549 did not reveal a specific band recognized by 1D8, under conditions in which TR 3A7 recognized a band corresponding to the human transferrin receptor.

Treatment of red cells with the enzymes papain, ficin, trypsin, alpha-chymotypsin, pronase, neuraminidase, or the thiol reagent AET failed to abolish antibody reactivity.

Hemagglutination of Rare-Phenotype Red Cells

1D8 Agglutinated red cells both directly and indirectly when used with anti-mouse globulin. When 1D8 was tested against a panel of red cells of rare phenotype, the only cells that failed to react with it were two unrelated Rh_{null}

TABLE 2

REGIONAL ASSIGNMENT OF GENE EXPRESSING 1D8 ANTIGEN

Hybrid	Karyotype	Killing by TR3A7	Killing by 1D8
314-2	3	+	+
314-1	3	+	+
314-1-X	3q	+	+
UCH-2	3q,21q	+	+
UCH-12	3q	+	+
314-1-R	3q del (24-ter)	−	+
UCH2-R	3q del (22-ter),21q	−	+

samples, one of the regulator type and one of unknown genetic background. Homozygous −D− red cells and red cells obtained from umbilical cord samples reacted only weakly with the antibody. 1D8 Reacted with cells of the following rare phenotypes, which lack high-frequency antigens: O_h, U−, En(a−), p, P_2^k, LKE−; LW(a+b−); LW(a−b−); Lu(a+b−); dominant Lu(a−b−); X-linked Lu(a−b−); Lu:−13; K+k−; Kp(a+b−c−); Js(a+b−); K_o, McLeod; Fy(a−b−); Jk(a−b−); Di(a+b−); Yt(a−b+); Co(a−b+); Co(a−b−); AnWj−; Ch−; Rg−; Cr(a−); Cs(a−); Yk(a−); Emm−; Er(a−); Ge:−2,−3; Ge:−2,3; Gy−Hy−; Gy+Hy−; adult i; JMH−; Jr(a−); Kn(a−); McC(a−); Lan−; Ok(a−); Sd(a−); and Vel−.

DISCUSSION

We describe a new chromosome 3–encoded cell-surface antigen. With use of the IgM monoclonal 1D8 and a cytotoxicity assay, the antigen recognized by 1D8 has been demonstrated to be expressed on human–Chinese hamster somatic-cell hybrids containing human chromosome 3 but not on hybrids lacking chromosome 3. Regional assignment of the gene responsible for expression of the antigen to 3 (cen-q22) has been achieved by using deletion mutants.

Biochemical characterization of the antigen by means of immunoprecipitation or immunoblotting has been unusuccessful. The antigen may either be a member of a class of compounds not immunoprecipitated—e.g., glycolipids—or be present in a concentration below the limits of sensitivity of our assay. The fact that treatment of red cells with a variety of proteolytic enzymes did not abolish antibody reactivity suggests that the antigen recognized by 1D8 may not be a protein accessible to digestion by soluble proteases. Failure of neuraminidase to abolish reactivity suggests that sialic acid residues are not involved in the antigen's recognition by antibody.

The transferrin receptor and the p97 melanoma-associated antigen have been assigned to human chromosome 3 previously. The antigen recognized by 1D8 appears to be distinct from both of these, in that its regional localization is different. A 90,000-MW cell-surface antigen has been assigned to chromosome 3 (Parkkinen and Schroder 1984). This antigen has a molecular weight similar to that of the reduced transferrin receptor, and biochemical characterization of this antigen in nonreduced form has not been published. Expression on various cells suggests that this antigen may be distinct from the transferrin receptor. Regional localization data are not available. On the basis of biochemical characteristics, the antigen recognized by 1D8 appears distinct from this antigen. Two additional cell-surface antigens have been assigned to chromosome 3, without regional mapping data yet being available (Rettig et al. 1984). One of these, defined by the monoclonal antibody K15, is an 85,000-MW glycoprotein and appears to be biochemically distinct from the antigen that we here describe. Another, AJ425, does not have its biochemical characterization described. The possible relationship of AJ425 to the antigen recognized by 1D8, in terms of biochemistry or regional localization, cannot be commented on at this time.

1D8 Resembles the monoclonal antibody R6A, described by Anstee and Edwards (1982) and used by Ridgwell et al. (1983) to immunoprecipitate a 34,000-MW protein, by failing to react with Rh_{null} cells and by reacting weakly with homozygous −D− cells (Anstee and Edwards 1982; Ridgwell et al. 1983). However, unlike R6A (G. L. Daniels, unpublished observations), 1D8 reacts only weakly with cord red cells. Furthermore, the antigen recognized by R6A is not detectable by either cytotoxicity or indirect immunofluorescence on the 314-2 somatic-cell hybrid. Thus, 1D8 and R6A appear to define distinct antigens not expressed by Rh_{null} cells. In addition to Rh antigens, Rh_{null} cells do not express the LW blood group antigens LW^a, LW^b, or LW^{ab}. 1D8 Does agglutinate LW(a−b−) cells, demonstrating that it does not recognize an LW antigen. Although Rh_{null} cells demonstrate a number of membrane abnormalities—e.g., increased osmotic fragility, abnormal shape, abnormal phosphatidyl ethanolamine distribution, and enhanced transbilayer mobility of phosphatidylcholine—they do not appear to have a generalized resistance to agglutination by monoclonal antibodies (Kuypers et al. 1984). Thus, it is likely that, in concert with the LW and Rh antigens, the antigen defined by 1D8 is not expressed on Rh_{null} cells, owing to a common shared defect in biosynthesis. A gene required for expression of the 1D8 antigen has been localized to chromosome 3 (cen-q22). The data that we have developed do not allow one to determine whether this locus is identical to that for the regulator-type Rh_{null} defect. Linkage studies to determine whether the regulator-type Rh_{null} defect cosegregates with chromosome 3q markers would further elucidate this matter.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grants CA34831 and CA18734 awarded by the National Cancer Institute, by the U.S. Department of Health and Human Services, and by a grant from the Veteran's Administration. The expert technical assistance of Betty Kao is gratefully acknowledged, as is the assistance of Roxie Kight in manuscript preparation. Dr. P. A. W. Edwards and B. Gorick kindly supplied monoclonal antibody R6A ascites and supernatant. The assistance of Dr. P. A. Bunn and Philip Jewett in flow cytometry is gratefully acknowledged.

REFERENCES

Advani, H., J. Zamor, W. J. Judd, C. L. Johnson, and W. L. Marsh. 1982. Inactivation of Kell blood group antigens by 2-aminoethylisothioruonium bromide. Br. J. Haematol. **51**:107–115.

Alhadeff, B., M. Velivasakis, and M. Siniscalco. 1977. Simultaneous identification of chromatid replication and of human chromosomes in metaphases of man-mouse somatic cell hybrids. Cytogenet. Cell Genet. **19**:236–239.

Anstee, D. J., and P. A. W. Edwards. 1982. Monoclonal antibodies to human erythrocytes. Eur. J. Immunol. **12**:228–232.

Anstee, D. J., K. Ridgwell, M. J. A. Tanner, G. L. Daniels, and S. F. Parsons. 1984. Individuals lacking the Gerbich blood-group antigen have alterations in the human erythrocyte membrane sialoglycoproteins beta and gamma. Biochem. J. **221**:97–104.

Bies, R., Y. E. Miller, P. Seligman, C. Chitambar, and C. Jones. 1984. Biochemical characterization of a mutant human transferrin receptor. Clin. Res. **32**:29A.

Bobrow, M., and J. Cross. 1974. Differential staining of human and mouse chromosomes in interspecific cell hybrids. Nature **251**:77–79.

Edwards, P. A. W. 1980. Monoclonal antibodies that bind to the human erythrocyte-membrane glycoproteins glycophorin A and band 3. Biochem. Soc. Trans. **8**:334–335.

Enns, C., H. Suomalainen, J. Gebhardt, J. Schroder, and H. Sussman. 1982. Human transferrin receptor: expression of the receptor is assigned to chromosome 3. Proc. Natl. Acad. Sci. USA **79**:3241–3245.

Goodfellow, P., G. Banting, R. Sutherland, M. Greaves, E. Solomon, and S. Povey. 1982. Expression of the human transferrin receptor is controlled by a gene on chromosome 3: assignment using species specificity of a monoclonal antibody. Somatic Cell Genet. **8**:197–206.

Jones, C., F. T. Kao, and R. T. Taylor. 1980. Chromosome assignment of the gene for folypolyglutamate synthetase to human chromosome 9. Cytogenet. Cell Genet. **28**: 181–194.

Jones, P. 1980. Pp. 398–425 *in* B. Mishell and S. M. Shiigi, eds. Selected methods in cellular immunology. W. H. Freeman, San Francisco.

Kloppel, T. M., and W. R. Brown. 1984. Rat liver membrane secretory component is larger than free secretory component in bile: evidence for proteolytic conversion of the membrane form to free form. J. Cell. Biochem. **24**:307–317.

Kuypers, F., M. Linde-Sibenius-Trip, B. Roelofsen, M. J. A. Tanner, D. J. Anstee, and J. A. F. Op den Kamp. 1984. Rh_{null} human erythrocytes have an abnormal membrane phospholipid organization. Biochem. J. **221**:931–934.

Miller, Y. E., C. Jones, C. Scoggin, H. Morse, and P. A. Seligman. 1983. Chromosome 3q (22-ter) encodes the human transferrin receptor. Am. J. Hum. Genet. **35**:573–583.

Parkkinen, E., and J. Schroder. 1984. Assignment of a gene coding for a cell surface antigen with a molecular weight of 90,000 to human chromosome 3. Heriditas **101**:217–222.

Plowman, G. D., J. P. Brown, C. A. Enns, J. Schroder, B. Nikinmaa, H. H. Sussman, K. E. Hellstrom, and I. Hellstrom. 1983. Assignment of the gene for human melanoma-associated antigen to chromosome 3. Nature **303**:70–72.

Rabin, M., A. McClelland, L. Kuhn, and F. Ruddle. 1985. Regional localization of the human transferrin receptor gene to 3q 26.2-q ter. Am. J. Hum. Genet. **37**:1112–1116.

Race, R. R., and R. Sanger. 1975. Blood groups in man. 6th ed. Blackwell Scientific, Oxford.

Rettig, W. J., N. C. Dracopoli, T. A. Goetzger, B. A. Spengler, J. L. Biedler, H. F. Oettgen, and L. J. Old. 1984. Somatic cell genetic analysis of human cell surface antigens: chromosomal assignments and regulation of expression in rodent-human hybrid cells. Proc. Natl. Acad. Sci. USA **81**:6437–6441.

Ridgwell, K., S. J. Roberts, M. J. A. Tanner, and D. J. Anstee. 1983. Absence of two membrane proteins containing extracellular thiol groups in Rh_{null} human erythrocytes. Biochem J. **213**:267–269.

Seabright, M. 1971. A rapid banding technique for banding human chromosomes. Lancet **2**:971–972.

Seligman, P. A., C. D. Butler, E. J. Massie, J. Kaur, J. P. Brown, G. Plowman, Y. Miller, and C. Jones. 1986. The p97 antigen is mapped to the q24-qter region of chromosome 3; the same region as the transferrin receptor. Am. J. Hum. Genet. **38**:540–548.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.

Vos, G. H., D. Vos, R. L. Kirk, and R. Sanger. 1961. A sample of blood with no detectable Rh antigens. Lancet **1**:14–15.

Voss, R., I. Lerer, S. Povey, E. Solomon, and M. Bobrow. 1980. Confirmation and further regional assignment of aminoacylase-1 on human chromosome 3 using a simplified detection method. Ann. Hum. Genet. **44**:1–9.