Genetic Regulation of a Structural Polymorphism of Human C3b Receptor

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ABSTRACT Two forms of the human C3b receptor (C3bR), which have relative molecular weights (M_r) of 250,000 and 260,000 and are designated F and S, respectively, have been identified in specific immunoprecipitates from erythrocytes and leukocytes by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Both forms of the receptor were visualized on gels by autoradiography of ^{125}I-labeled antigen and by silver nitrate staining. Individual donors expressed one of three possible patterns of C3bR, either the F or S form alone or both, and these patterns represented stable phenotypic characteristics of their erythrocytes and polymorphonuclear and mononuclear leukocytes. Removal of N-linked oligosaccharides by endoglycosidase-F treatment decreased the M_r of both forms but did not abolish the difference in their electrophoretic mobilities. That both forms of the receptor were functional was indicated by the capacity of all antigenic C3bR sites on erythrocytes from individuals having any of the three phenotypes to bind dimeric C3b with affinities ranging from 3 to 5 $\times 10^7$ M^{-1}.

Analyses of the occurrence of the F and S forms of C3bR in 76 individuals from 15 families revealed that this polymorphism was regulated by two alleles transmitted in an autosomal codominant manner. Of 111 normal unrelated individuals, 64.9% were homozygous for the F form (FF), 1.8% were homozygous for the S form (SS), and 33.3% were heterozygotes (FS). This distribution did not differ from that calculated by the Hardy-Weinberg equilibrium based on two codominant alleles that regulate the expression of the F and S forms and that have frequencies of 81.5 and 18.5%, respectively. The locus regulating structural polymorphism of C3bR is designated *C3BRM* (M for mobility or M_r), and is distinct from the recently described locus regulating the quantitative expression of C3bR on erythrocytes.

INTRODUCTION

The mammalian C3b receptor (C3bR),[1] which is also termed CR1, was discovered almost 30 yr ago, when human erythrocytes were observed to bind bacteria that had been treated with specific antibody and complement (1). Subsequently, the receptor was shown to be specific for C3b, the major cleavage fragment of C3 produced during proteolysis by a C3 convertase, and for C4b, the fragment produced by cleavage of C4 with C1s (2, 3). Receptors having the same specificity were found to reside also on other cell types, including neutrophils, eosinophils, monocytes, macrophages, mast cells, B and some T lymphocytes, and glomerular podocytes (4–10). Recently, the C3bR of human erythrocytes was purified to homogeneity and shown to be a glycoprotein with a relative molecular weight (M_r) of 205,000 when assessed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) (11). Monospecific rabbit antibody to this glycoprotein bound only to cell types that expressed C3bR activity, inhibited the function of C3bR on erythrocytes, neutrophils, monocytes, and lymphocytes, and immunoprecipitated from each of these populations of cells a single membrane protein having a mobility on SDS-PAGE that was identical to that of the glycoprotein that had been isolated from erythrocytes (8, 12). Thus, the C3bR of these various human cell types is a common molecular entity, a con-

[1] *Abbreviations used in this paper:* C3bR, C3b receptor; DFP, diisopropylfluorophosphate; HBSS, Hanks' balanced salt solution; PAGE, polyacrylamide gel electrophoresis.

clusion that is supported by recent studies using monoclonal antibodies (13, 14).

Specific antibodies to the C3bR and soluble forms of C3b have been used for the enumeration of cellular receptors (12, 13, 15, 16). The number of C3bR on human erythrocytes is regulated by two autosomal codominant alleles, tentatively designated $C3BRQ°H$ and $C3BRQ°L$ according to a recommended standard nomenclature (17, 18), that determine the occurrence of three phenotypes in the normal population: a high phenotype, C3BRQ H (HH), having >5,500 antigenic receptor sites (representing >600 C3b binding sites) per erythrocyte, an intermediate phenotype, C3BRQ HL (HL), having 3,000–5,499 antigenic sites (350–600 C3b binding sites) per cell, and a low phenotype, C3BRQ L (LL), having <3,000 antigenic sites (<350 C3b binding sites) per cell (15). These three phenotypes were present in 34, 54, and 12%, respectively, of normal individuals. A markedly different distribution was found for patients with systemic lupus erythematosus among whom 5, 42, and 53% had the HH, HL, and LL phenotypes, respectively (15). This mode of genetic regulation of C3bR expression was the basis of diminished numbers of erythrocyte receptors in lupus patients previously found by others (13, 19) and indicated that the abnormality in patients was inherited rather than secondarily acquired.

In the present study, a structural polymorphism of human C3b receptors is described that is detected by SDS-PAGE of the glycoprotein. Two variants of the receptor that differ by their M_r are identified and shown to be inherited in an autosomal codominant manner.

METHODS

Analysis of the C3b receptor by PAGE. Preparations of 5×10^6 purified human peripheral blood mononuclear or polymorphonuclear leukocytes (20) and of 2×10^8 erythrocytes were each suspended in 1 ml of Hanks' balanced salt solution (HBSS) (Gibco Laboratories, Grand Island, NY) and labeled with 1 mCi of $Na^{125}I$ (New England Nuclear, Boston, MA) by a lactoperoxidase catalyzed reaction (21). The labeled cells were washed five times in ice-cold HBSS containing 0.1% bovine serum albumin (HBSS-BSA) and were incubated on ice for 20 min with 2 ml of 0.5% Nonidet P-40 in phosphate-buffered saline (NP-40-PBS) and 5 mM diisopropylfluorophosphate (DFP). After removal of insoluble material by centrifugation of the detergent lysates at 44,000 g for 30 min at 4°C, the supernatants were each incubated sequentially with 50 μl of nonimmune F(ab')$_2$-Sepharose and of F(ab')$_2$ anti-C3bR-Sepharose for 1 h at 4°C. The Sepharose beads were washed once in NP-40-PBS and three times in Tris-buffered saline, pH 9.0, containing 0.25% deoxycholic acid and 0.2% SDS, before the elution of adsorbed proteins by incubation for 3 min at 100°C in 100 μl of 1% SDS. The eluted proteins were reduced and alkylated by incubation with 0.1 M dithiothreitol and 0.2 M iodoacetic acid and were analyzed by SDS-PAGE according to the method of Laemmli

(22) on slab gels of 1.5-mm thickness and containing a gradient of 3–10% acrylamide. The gels were stained with Coomassie Blue, dried, and analyzed by autoradiography.

In an alternative procedure for analysis of the C3bR not involving the use of ^{125}I, hemoglobin-free ghosts were prepared from erythrocytes by four washes in 40 vol each of 5 mM sodium phosphate pH 7.8 containing 1 mM phenylmethylsulfonylfluoride (Sigma Chemical Co., St. Louis, MO). The ghosts were used immediately or were stored at −70°C. Erythrocyte membrane proteins were extracted from the ghosts by addition of an equal volume of buffer containing 2% NP-40, 10 mM sodium phosphate, 0.3 M NaCl (pH 7.4), and 5 mM DFP (Sigma Chemical Co.) to preparations of 0.5–3.0 ml packed ghosts. After incubation at 4°C for 20 min, the insoluble material was removed by centrifugation of the detergent lysates at 44,000 g for 30 min at 4°C. The supernatants were incubated with 50 μl of F(ab')$_2$ anti-C3bR-Sepharose for 3 h at 4°C and the beads were washed and eluted according to the procedure described for the ^{125}I-labeled immunoprecipitates. The eluates containing the C3bR were reduced, alkylated, and analyzed by SDS-PAGE on gradient gels of 0.75-mm thickness. The positions of protein bands were detected by staining with silver nitrate (23). M_r markers used in SDS-PAGE included rabbit muscle myosin (200,000 M_r), phosphorylase b (93,000 M_r), BSA (69,000 M_r), ovalbumin (43,000 M_r), bovine carbonic anhydrase (30,000 M_r), soybean trypsin inhibitor (20,000 M_r), and the human erythrocyte cytoskeletal proteins, band 1 (240,000 M_r), band 2 (220,000 M_r), and actin (45,000 M_r).

Preparation of dimeric C3b, antibody to C3b receptor, and immunoabsorbents. Purified C3b, generated from native C3 by trypsin treatment, was cross-linked by incubation with a 20-fold molar excess of dimethyl suberimidate (Aldrich Chemical Co., Milwaukee, WI) for 2 h at 30°C (15). The reaction mixture was subjected to centrifugation at 4°C for 16 h at 155,000 g on a linear gradient of 7.5–30% sucrose in veronal-buffered saline, and gradient fractions were analyzed for the presence of dimeric C3b by SDS-PAGE. Dimeric C3b was radiolabeled with ^{125}I to sp act 0.3 μCi/μg with Iodo-Gen (Pierce Chemical Co., Rockford, IL) (24).

Affinity purified F(ab')$_2$ anti-C3bR was prepared as previously described (15). The purified F(ab')$_2$ anti-C3bR and preimmunization F(ab')$_2$ were radiolabeled with ^{125}I to specific activities ranging from 0.18 to 0.92 μCi/μg (24). 1 mg each of unlabeled nonimmune and immune F(ab')$_2$ were coupled to 2 ml each of CNBr-activated-Sepharose to be used as immunoabsorbents.

Assays for C3b receptors on erythrocytes. Erythrocytes from venous blood that had been anticoagulated with 10 mM ethylenediamine tetraacetate and held at 0°C were washed twice in HBSS-BSA and were counted in a model ZF Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). 2.5×10^8 erythrocytes were incubated for 60 min at 20°C in 0.2 ml HBSS-BSA with 1.15 μg of ^{125}I-nonimmune F(ab')$_2$ or ^{125}I-F(ab')$_2$ anti-C3bR; this amount of anti-C3bR yielded 97±0.9% (mean±SEM) saturation of antigenic sites on erythrocytes (15). Duplicate samples of cells in 0.075 ml were removed from each reaction mixture, layered on 0.3 ml dibutylphthalate (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, NY) in 0.5 ml polypropylene microfuge tubes, and pelleted by centrifugation at 8,000 g for 30 s. The tubes were cut and the pellets and supernatants were assessed for bound and free antibody, respectively. The amount of nonimmune F(ab')$_2$ that was bound was subtracted from the amount of anti-C3bR that was taken up to obtain the specific binding data (15).

2.5×10^8 erythrocytes were incubated for 60 min at 0°C

in 0.2 ml HBSS-BSA with incremental amounts ranging from 0.09 to 3 μg of ^{125}I-dimeric C3b alone or in the presence of 2 μg unlabeled F(ab')$_2$ anti-C3bR. Bound and free ligand were separated by the method described for anti-C3bR. The amount of ^{125}I-dimeric C3b that was bound in the presence of anti-C3bR was subtracted from that bound in the absence of antibody and specific binding was analyzed according to the method of Scatchard (15, 25).

Preparation of ^{125}I-C3bR and endoglycosidase-F treatment. C3b receptor was purified from erythrocyte membranes of selected donors by modification of a previously described method (11). The membranes were solubilized in buffer with 10 mM Tris, 150 mM KCl, 0.5% NP-40, 0.5% deoxycholate, and 2.5 mM DFP and the detergent extracts were applied to a column of Matrix Red-A (Amicon Corp., Danvers, MA) at a starting ionicity of 12 mS at 4°C. After extensive washing, the adsorbed material was eluted with buffer containing 1 M KCl. The fractions containing C3bR activity were further chromatographed on lentil lectin-Sepharose and C3b-Sepharose. The purified C3bR containing both F and S forms was dialyzed overnight at 4°C against 1 liter of borate buffered saline, pH 8.5 with 0.25% NP-40 and 2 mM DFP. The dialyzed receptor was labeled with ^{125}I by incubation for 90 min at 0°C with 250 μCi of ^{125}I-Bolton-Hunter reagent and was chromatographed on Sephacryl S-300 (Pharmacia Fine Chemicals, Piscataway, NJ) in PBS (pH 7.4) containing 0.25% NP-40 and 2 mM DFP. A fraction containing 7 μCi was further applied to a 0.7 × 50-cm column of Sephacryl S-200 (Pharmacia Fine Chemicals) equilibrated in 100 mM sodium phosphate, 50 mM EDTA, 0.1% SDS, 2 mM DFP, pH 6.1. The column fractions were screened for the presence of C3bR by analysis on SDS-PAGE and autoradiography. 2-Mercaptoethanol was added to 45 μl of a fraction containing 0.25 μCi and both F and S forms of C3bR to achieve a final concentration of 1%. The sample was heated in a boiling water bath for 5 min and was then made 1% in NP-40. 10 μl of endoglycosidase-F (26) in 50 mM EDTA and 20% glycerol was added and the samples were incubated at 37°C for 0–5 h. Small aliquots were removed at several intervals and diluted 40-fold in SDS-PAGE sample buffer to stop the reaction. Control experiments under identical conditions showed that endoglycosidase-F reduced the M_r of α_1-acid glycoprotein to that of its unglycosylated form, while having no effects on human serum albumin, a nonglycosylated protein, thus suggesting that its effect was not due to the presence of proteolytic enzymes. The endoglycosidase-F used in these experiments was a generous gift of Dr. S. Alexander of the Scripps Research Institute, La Jolla, CA.

RESULTS

Peripheral blood erythrocytes and mononuclear and polymorphonuclear leukocytes from three representative normal donors were surface-labeled with ^{125}I and the membrane proteins that were immunoprecipitated by nonimmune F(ab')$_2$-Sepharose or F(ab')$_2$ anti-C3bR-Sepharose were analyzed by SDS-PAGE and autoradiography (Fig. 1). No radiolabeled material was observed in the control precipitates obtained with nonimmune F(ab')$_2$. Immune precipitates from each of the three cell types of donor A yielded a major band of radioactivity having an M_r of 250,000, whereas the corresponding immune precipitates from donor C
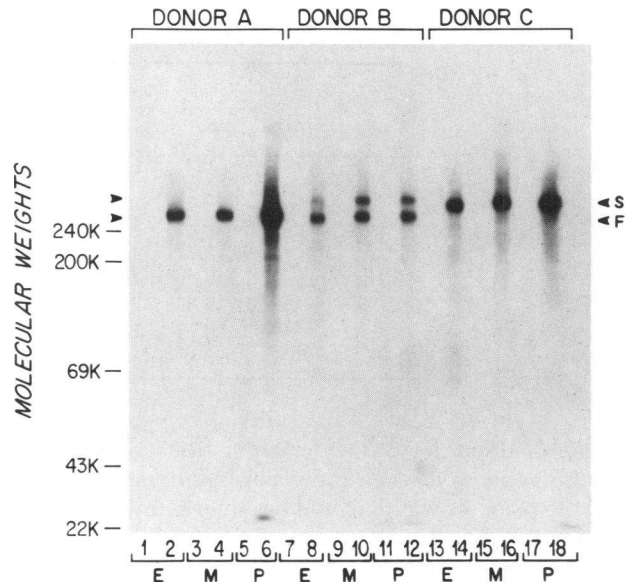

FIGURE 1 Autoradiographs of SDS-PAGE of ^{125}I-labeled membrane proteins immunoprecipitated from erythrocytes (*E*), mononuclear leukocytes (*M*), and polymorphonuclear leukocytes (*P*) of three representative individuals. Even-numbered lanes represent immunoprecipitates obtained with anti-C3bR and odd-numbered lanes represent the nonimmune controls.

yielded a major band of radioactivity having a higher M_r of 260,000. Immune precipitates from donor B were composed of two radiolabeled bands of M_r 250,000 and 260,000, which corresponded to the bands of donors A and C, respectively. Reexamination of each donor over periods of at least 8 mo yielded identical results. Thus, the C3bR can exist in two forms, which are distinguished by their differential mobilities on SDS-PAGE, fast (F) and slow (S), and individuals can be classified as having either one or both of these forms.

The cells taken from donor B (Fig. 1) appeared to express more of the radiolabeled F than S form of the C3bR. To quantitate the relative expression of the S and F forms of the C3bR among individuals having both forms, erythrocytes and neutrophils from donor B and five other individuals were surface labeled with ^{125}I and labeled receptor was obtained by immunoprecipitation with F(ab')$_2$ anti-C3bR-Sepharose. After SDS-PAGE and autoradiography, the regions of the gels corresponding to the positions of the F and S forms were excised and assessed for the presence of ^{125}I. Erythrocytes from donors B and J.C. had less radiolabeled S than F; these cells from M.M. and J.W. had equal amounts of the labeled forms of C3bR, and cells from A.H. and J.N. expressed more S than F (Table I). Comparable relative expression of the S and F forms

TABLE I
Relative Amounts of the S and F Forms of C3bR in Donors Expressing Both Forms

Donor	Erythrocytes			Neutrophils		
	S	F	S/F ratio	S	F	S/F ratio
	cpm			cpm		
B (Fig. 1)	4,800	7,850	0.61	3,825	5,358	0.71
J.C.	3,875	4,507	0.86	4,094	4,920	0.83
A.H.	1,782	1,292	1.38	7,073	8,267	0.86
M.M.	2,743	2,685	1.02	4,786	5,326	0.90
J.N.	3,208	2,643	1.21	3,485	4,493	0.78
J.W.	1,926	1,822	1.06	5,245	4,459	1.18

of the C3bR on erythrocytes from these individuals was also observed when the experiment was repeated and the receptor was detected by silver nitrate staining of SDS-PAGE gels. The neutrophils of all six individuals also expressed both the F and S forms of C3bR. There was less variability of the ratio of S to F expression on neutrophils than on erythrocytes among the individuals, and in five of six instances, the amount of F was slightly greater than that of S (Table I).

The possibility that one of the forms of C3bR was generated from the other during the solubilization and immunoprecipitation of labeled cells was assessed in mixing experiments. ^{125}I-labeled erythrocytes from donors A and C (Fig. 1) were mixed with unlabeled erythrocytes from the same or the other donor and were subjected to the standard solubilization and immunoprecipitation procedures. The mixing of labeled erythrocytes from donor A with unlabeled cells from donor C did not alter the F mobility of ^{125}I-C3bR; similarly, the S electrophoretic mobility of ^{125}I-C3bR of donor C was not altered by the presence of erythrocytes from donor A.

A preparation of ^{125}I-labeled C3bR containing both forms was treated with endoglycosidase-F for 0, 2, and 5 h at 37°C and then analyzed by SDS-PAGE. Addition of endoglycosidase-F without incubation did not alter the M_r of the F and S forms (Fig. 2), whereas incubation with the enzyme for 2 and 5 h decreased the M_r of both forms without altering the difference in their M_r. Thus, N-linked carbohydrates do not appear to account for the structural difference between the two forms of C3bR.

The least complex genetic basis that can be proposed for this polymorphism of the C3bR assumes that the F and S forms are the products of two alleles at a single locus, which is tentatively designated C3BRM (for regulation of electrophoretic mobility or M_r). According to this proposal, donors A and C (Fig. 1) would be homozygous for these alleles, having the C3BRM F

(FF) and C3BRM S (SS) phenotypes, respectively, and donor B would be a heterozygote, having the phenotype C3BRM FS (FS).

The mode of inheritance of these phenotypes was assessed by examining the C3bR of erythrocytes from 116 unrelated normal individuals and 76 related individuals from 15 families. In these analyses, the C3bR was identified on polyacrylamide gels by staining with silver nitrate to avoid frequent radioiodination of cells. All three C3bR phenotypes could be recognized by the silver nitrate staining method (Fig. 3). Although con-
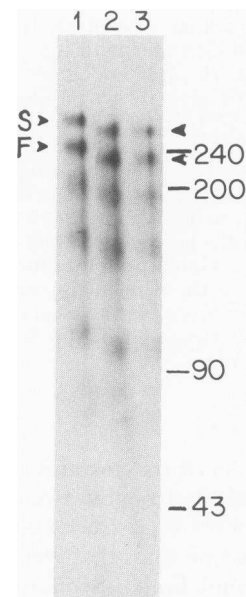

FIGURE 2 Autoradiograph of SDS-PAGE of endoglycosidase-F-treated ^{125}I-C3bR. The C3bR was purified from erythrocytes of individuals expressing the F and S forms. Lanes 1, 2, and 3 represent ^{125}I-C3bR after 0-, 2-, or 5-h incubations with endoglycosidase-F, respectively. The positions of C3bR are designated by arrow heads.
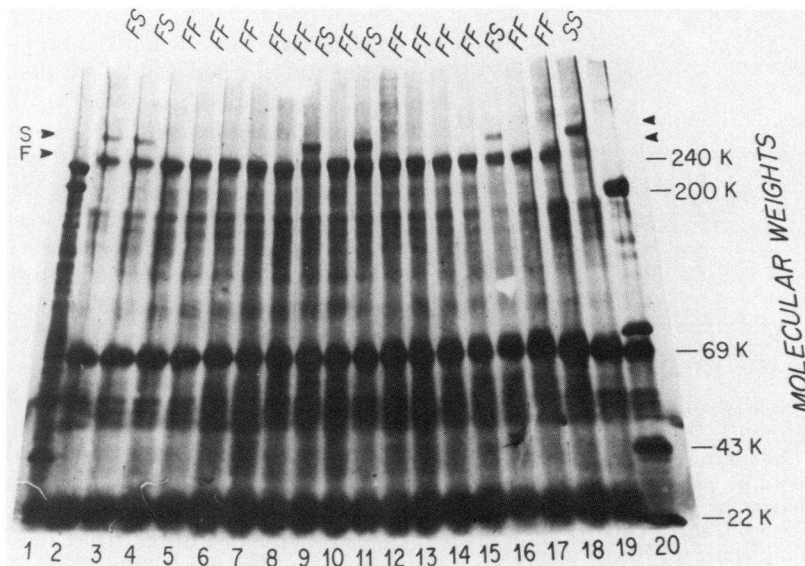
FIGURE 3 SDS-PAGE of anti-C3bR immunoprecipitates from erythrocytes of 18 normal individuals. Proteins were detected by staining with silver nitrate. Lane *1* contains proteins from erythrocyte membranes and lane *20* contains the M_r standards myosin, phosphorylase *b*, BSA, ovalbumin, and carbonic anhydrase. The C3bR phenotype of each individual as determined by this method is designated by the symbols *FF*, *FS*, and *SS* above the corresponding lanes. The positions of C3bR are designated by arrow heads.

taminating proteins were observed on these gels that were not detected by autoradiography (Fig. 3), these represented BSA, which was present in the storage buffer for F(ab')$_2$ anti-C3bR-Sepharose, and heavy and light chains of the F(ab')$_2$ anti-C3bR that were released from the immunoadsorbent during elution by SDS. These contaminants and the unidentified minor bands of M_r of 100,000 to 180,000 did not interfere with C3bR identification. Repeat analysis by this procedure of 23, 13, and three individuals initially assigned the FF, FS, and SS phenotypes, respectively, confirmed these assignments.

The occurrence of the C3bR phenotypes in 116 unrelated normal individuals, of whom four were Asian and one was black, was studied by SDS-PAGE. The frequencies in men and women of FF, FS, and SS phenotypes were similar; one black individual had the SS phenotype and the four Asian persons had the FF phenotype. In the 111 white individuals, the calculated frequencies of the genes *C3BRM*°*F* and *C3BRM*°*S* were 81.5 and 18.5%, respectively, and the observed frequencies of C3bR types and those expected from the Hardy-Weinberg equilibrium based on two codominant alleles did not differ (x^2 = 1.258; 0.4 > P > 0.3; Table II). In the family studies, four of the six possible combinations of parental phenotypes were available for analysis (Table III). The pattern of in-

heritance of the C3bR phenotypes was consistent with an autosomal codominant mode of transmission.

The relationship of this structural polymorphism of C3bR to the number of C3bR expressed on erythrocytes was examined (Fig. 4). All three phenotypes of receptor number, HH, HL, and LL, were found among individuals having the FF, FS, and SS structural phenotypes, respectively, suggesting that the structural polymorphism of C3bR has no major effects on quantitative expression. However, the number of antigenic sites per cell of individuals with the FF phenotype was

TABLE II
Observed and Expected° C3b Receptor Phenotypes in Unrelated Caucasian Individuals

	FF	FS	SS
Male (n = 65)	42	22	1
Female (n = 46)	30	15	1
Total (n = 111)	72	37	2
Observed frequency (%)	64.9	33.3	1.8
Expected frequency° (%)	66.4	30.2	3.4

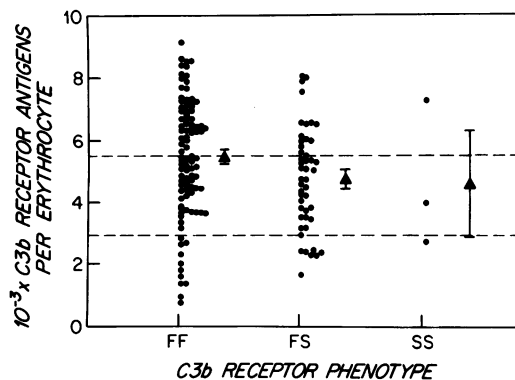° Based on the Hardy-Weinberg equilibrium $(p + q)^2$, where p and q represent observed gene frequencies.

FIGURE 4 Number of C3bR antigenic sites on erythrocytes from individuals having the FF, FS, and SS structural phenotypes. Bars represent the mean±SEM in each group. The dashed lines divide the three phenotypes of quantitative C3bR expression: HH, having >5,500 antigenic sites/erythrocyte; HL, having 3,000–5,499 antigenic sites/erythrocyte; and LL, having <3,000 antigenic sites/erythrocyte.

5,403±184 (mean±SEM) and was slightly higher (0.05 > P > 0.03) than the mean of 4,739±248 sites/cell of persons with the FS phenotype. The infrequent occurrence of the SS phenotype did not allow reasonable determination of the mean number of erythrocyte receptors in this group.

The relative capacities of the F and S forms of C3bR to bind C3b were determined by comparing the uptake by erythrocytes of labeled F(ab')$_2$ anti-C3bR and dimeric C3b. Erythrocytes from 10 individuals having the FF receptor phenotype bound at saturation an average of 625 C3b molecules and 5,762 F(ab')$_2$ anti-C3bR molecules per cell, yielding a ratio of 9.2 antigenic sites per functional receptor. The average association constant of the receptor for dimeric C3b was 3.13±0.17 × 10^7 M^{-1} (mean±SEM). Erythrocytes from a single SS individual bound 509 C3b molecules and 4,632 F(ab')$_2$ anti-C3bR molecules per cell at saturation, yielding a ratio of 9.1 antigenic sites per functional receptor. This individual's erythrocyte receptors bound dimeric C3b with an association constant of 4.96 × 10^7 M^{-1}. Erythrocytes from five FS individuals bound 470 C3b molecules and 4,001 F(ab')$_2$ anti-C3bR molecules per cell, yielding a ratio of 8.5 antigenic sites per functional receptor, and receptors on these cells bound dimeric C3b with an association constant of 4.01±0.35 × 10^7 M^{-1}. Thus, there are no marked differences in the capacities of the F and S forms of the C3bR to bind soluble dimeric C3b.

DISCUSSION

The C3bR of peripheral blood erythrocytes and leukocytes exists in two forms, F and S, that have M_r of

250,000 and 260,000, respectively, when assessed by SDS-PAGE (Figs. 1 and 3). Previous studies (11, 13, 27) of the purified C3bR had demonstrated only one band, having a M_r of 205,000, that probably corresponded to the F form because of the more frequent occurrence of individuals expressing only this form of C3bR. The higher M_r of both forms of the C3bR in this study compared with the results of previous investigations from this laboratory is accounted for by differences in the analytical systems used and by the presence previously of NP-40 in samples that causes a more rapid migration of the C3bR on SDS-PAGE (data not shown).

The pattern of inheritance of the F and S forms of the receptor is consistent with autosomal codominant expression of two alleles (Table III). The observed frequencies of the FF, FS, and SS phenotypes in a population of 111 unrelated normal individuals selected for race did not differ from those predicted by the Hardy-Weinberg equilibrium based on gene frequencies of 0.81 and 0.19 for C3BRM°F and C3BRM°S, respectively (Table II). These gene frequencies were calculated for the purpose of the Hardy-Weinberg analysis; actual gene frequencies in a larger population may vary slightly. When gels were overloaded, a small amount of immunoprecipitable material having the M_r of the F form may be apparent in an SS individual (Fig. 1, lane 18, and Fig. 3, lane 19). This finding would not invalidate a genetic basis for the polymorphism, but would suggest the presence of two closely linked loci as for human C4, with phenotypes being determined by varying expression of one or the other locus.

Although the structural polymorphism of C3bR appears to be genetically regulated, based on the family and population studies, another explanation for the appearance of two forms of the C3bR is that they have a precursor-product relationship. According to this model, individuals having only the S or F form rep-

TABLE III
Inheritance of F and S Forms of C3b Receptor

Parental phenotypes	Number of families	Offspring phenotypes		
		FF	FS	SS
FF × FF	4	17 (17)*	0 (0)	0 (0)
FF × FS	8	9 (10.5)	12 (10.5)	0 (0)
FF × SS	2	0 (0)	4 (4)	0 (0)
FS × SS	1	0 (0)	0 (2)	4 (2)

* Numbers in parentheses represent the expected phenotypes based on a two codominant allelic mode of inheritance.

resent extremes of a continuum of conversion of F to S or of S to F and the variable ratios of the two forms among FS individuals represent varying completeness of conversion. However, this model would not readily explain either the inheritance of the F and S forms (Table III) or the close approximation between the observed frequencies of FF, FS, and SS individuals and those calculated by the Hardy-Weinberg equilibrium. In addition, this putative mechanism for post-synthetic conversion of one form of the receptor to the other would have to yield the same apparent phenotype in three separate cell types for each person (Fig. 1, Table I). Thus, a nongenetic mechanism accounting for the occurrence of two forms of the C3bR seems unlikely, but cannot be excluded without pulse-chase experiments of biosynthetically labeled C3bR.

The unequal expression of the two forms of C3bR on cells from FS individuals (Fig. 1, Table I) may indicate different rates of biosynthesis or catabolism of the F and S forms. The number of C3bR molecules expressed on erythrocytes has been shown to be an inherited characteristic suggesting some mechanism for genetic regulation of biosynthesis. However, products of regulatory genes are usually not limited to a cis-effect. Moreover the variability in the relative amounts of S and F expressed by neutrophils of heterozygous individuals was less marked than that of erythrocytes. The latter cell type persists in the circulation for prolonged periods of time and its C3bR may be more subject to alterations by catabolism than are receptors on the relatively short-lived neutrophils. The absence of a consistent diminution on erythrocytes of one form of the receptor relative to the other suggests that differential rates of catabolism may not be the only basis for the variable S-to-F ratios on this cell type.

The structural polymorphism of the C3bR is unusual, because it can be detected by SDS-PAGE, whereas the demonstration of the polymorphic forms of the human serum complement proteins, C4, C2, C3, C6, C8, and factor B require procedures that separate proteins according to net charge rather than size (28–33). Two forms of human C4 differing in their M_r have been described, but they represent products of two separate loci, C4A and C4B (34). Similar to the findings relating to the C3bR are the allotypic α-chain variants of murine C4 that can be distinguished by SDS-PAGE; these have been shown to differ in the extent of their glycosylation (35–37). Although we have shown that N-linked carbohydrates do not account for the difference in M_r of the F and S forms of the C3bR (Fig. 2), we have not determined whether there are differences in their primary sequence, O-linked carbohydrates, or both. However, the apparent

codominant expression and cis-regulation of the C3bR forms do suggest that C3bRM is the locus of the C3bR structural gene.

Antigenically detectable C3bR on erythrocytes from individuals having the FF, FS, and SS phenotypes, respectively, bound dimeric C3b with a similar affinity, suggesting that the two forms of the receptor may not differ in function. However, other activities of the receptor, such as serving as cofactor for cleavage of C3b by factor I (11), facilitating phagocytosis (2), and mediating adsorptive endocytosis by polymorphonuclear leukocytes and monocytes (38) must be assessed before one can conclude that the two forms are functionally equivalent. Relevant to the possibility of variable function of these receptor allotypes differing by M_r is the recent finding that the allotypic form of murine C4 having the lower M_r α-chain exhibits a 60–80% reduction in hemolytic activity (35).

The relationship between structural polymorphism and quantitative expression of the C3bR on human erythrocytes was examined to determine whether these two inherited characteristics were regulated by separate loci. The number of receptors as determined by binding of monospecific antibody to cells has been shown in two previous studies to correlate with a high degree of significance with the number of functional receptors (15, 39). All three structural phenotypes were found among individuals with high, intermediate, or low numbers of erythrocyte C3bR antigenic sites (Fig. 4), indicating that the gene, C3BRM, regulating structural polymorphism is distinct from that determining numbers of the C3bR. However, individuals with the FS phenotype had a slightly lower mean number of receptors than did persons with the FF phenotype, a finding that may be explained by the diminished expression of the S form of the receptor relative to that of the F form in some heterozygous individuals (Figs. 1 and 3). Linkage of the loci regulating receptor structure and quantitative expression could not be reliably determined in this study, because only families in which one parent is homozygous and the other is heterozygous at both loci would be informative. In the single family having the necessary parental phenotypes, C3BRQ HL; C3BRM FS and C3BRQ H; C3BRM F, independent segregation of the alleles at these two loci was observed among the offspring.

The LL quantitative phenotype of erythrocyte C3bR expression is more prevalent among lupus patients than among normal individuals (13, 15, 19). In addition, the receptor was absent from glomerular podocytes in lupus patients having severe proliferative nephritis in contrast to the apparently normal expression of glomerular receptors in other forms of lupus nephritis and nonlupus endocapillary nephritides (10).

Since diminished quantitative expression of the C3bR appears to be related to the development of systemic lupus erythematosus, it will be essential to compare the two structural variants of the receptor for their relative functional capabilities and occurrence among patients with immune complex diseases.

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