POLYMORPHISM OF THE HUMAN C3b/C4b RECEPTOR

Identification of a Third Allele and Analysis of Receptor Phenotypes in Families and Patients with Systemic Lupus Erythematosus

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The human receptor for C3b and C4b (C3bR)[1] was initially isolated from pooled donor erythrocyte membranes (1). Although characterized as an integral membrane glycoprotein of ~200,000 daltons on SDS-polyacrylamide gels (1–4), recent studies have demonstrated that C3bR is polymorphic (5–7). In reports from our laboratory, autoradiographs of purified surface-labeled C3bR from erythrocytes (E) in 33 normal donors demonstrated three distinct patterns (5). ~70% of individuals had a single major band of ~190,000 daltons (C3bR-AA), ~3% of individuals had a single major band of ~220,000 daltons (C3bR-BB), and the remaining ~27% of individuals had both major bands (C3bR-AB). Family studies provided evidence that two codominant alleles regulated these major bands (5). Under this model, C3bR-AB individuals were heterozygous for the two alleles, A and B, and C3bR-AA or C3bR-BB individuals were homozygous for their respective alleles. Although the C3bR phenotype found on E was also present on other C3bR-bearing cells in the same individual, several cell-specific structural differences were also identified. First, on polymorphonuclear cells (PMN), the M_r of C3bR was ~5,000 daltons greater than that on E (6). Second, on E, less intense minor bands co-purified with C3bR and were found ~15,000 daltons above major bands on autoradiographs (5, 6). These minor bands, found only on E, accounted for <25% of the total C3bR. The percent represented by this minor band differed among individuals but was characteristic for a given individual. Third, among C3bR-AB individuals, vastly different (up to 30-fold) amounts of either the ~190,000- or ~220,000-dalton C3bR molecules were found on E and peripheral blood leukocytes (5, 6). The factors responsible for this variable expression among heterozygous individuals are unknown.

In a study from another laboratory, this same polymorphism was described in a larger group of 111 unrelated individuals (7). However, as our laboratory has isolated C3bR from additional normal individuals we have found several novel

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[1] *Abbreviations used in this paper:* C3b and C4b, the major activation fragments of the third and fourth component of complement; C3bR, the ~200,000-dalton receptor for C3b and C4b on human cells; C3bR-AA, -AB, -BB, -BC, -AC, -CC, C3bR phenotypes (see text); E, human erythrocytes; I, C3b inactivator; mC4, methylamine-treated C4; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PMN, polymorphonuclear cells; RIgG, rabbit immunoglobulin G; SLE, systemic lupus erythematosus.

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receptor phenotypes. These new C3bR phenotypes are regulated by a third codominant allele coding for an ~160,000-dalton C3bR. In view of the low number of C3bR found on E in many patients with systemic lupus erythematosus (SLE), we have also analyzed the frequency of C3bR polymorphic variants in this disease (3, 8). These studies demonstrate that, although the frequency of C3bR phenotypes is similar in patients with SLE and normals, certain individuals with SLE express large quantities of this ~160,000-dalton C3bR on E. Analysis of receptor phenotypes in normal and SLE families provides evidence that the amount of each C3bR allele expressed on E in heterozygous donors is genetically determined.

Materials and Methods

Purification of Human E, PMN, and Mononuclear Cells. E were obtained from 30 ml of whole blood that was collected on the day of use in 500 U of sodium heparin (Abbott Laboratories, North Chicago, IL) or in 4.5 ml of citrated dextrose (Fenwal Laboratories, Deerfield, IL) if the sample was to be stored (at 4°C) before use. The buffy coat was removed by centrifugation (800 *g* for 8 min) and the pellet was then washed four times with 0.01 M potassium phosphate, 0.15 M NaCl, pH. 7.4 (PBS) to obtain packed E from each donor. Leukocytes were obtained on the day of use from 250 ml of whole blood collected in 6% dextran (Sigma Chemical Co., St. Louis, MO), 0.15 M NaCl and 2,500 U of heparin. Mononuclear cells and PMN were then separated by the Ficoll-Hypaque method (9). The pellets typically contained >98% PMN and >90% were viable by trypan blue exclusion. The mononuclear layer contained monocytes and lymphocytes (variable ratio from each donor) that were >90% viable and contained <2% PMN.

Surface-labeling and Solubilization of Cells. E (~5×10^9 cells), PMN, or mononuclear cells (~2×10^8 cells) were suspended in 4 ml of PBS and iodinated with 0.25 mCi of ^{125}I (New England Nuclear, Boston, MA) by a modified lactoperoxidase method (10). Following iodination, E were lysed at 4°C with 15 ml of distilled H_2O containing protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 3 mM EDTA, 1 μM pepstatin, and 20 mM iodoacetamide). After 1 min, 15 ml of 0.3 M NaCl with the same inhibitors was added. E stroma were then collected by centrifugation at 33,000 *g* for 20 min. E stroma (5×10^9 cell equivalents) and intact PMN and mononuclear cells (1×10^8) were then solubilized at 4°C in 1 ml PBS, 1% Nonidet P-40 (NP-40) in the presence of the same protease inhibitors used for lysis of E. After centrifugation at 33,000 *g* for 20 min, the supernatant from each cell preparation were stored at −22°C.

Isolation of C3bR by Affinity Chromatography or Immunoprecipitation. Affinity chromatography was preformed as previously described using C3, methylamine-treated C4 (mC4), bovine serum albumin, or rabbit immunoglobulin G (RIg G) as affinity ligands (5). These ligands were coupled to Sepharose (2B, Sigma Chemical Co.) by cyanogen bromide. Methylamine-treatment of C4 cleaves the thioester bond within the α chain of C4. This modification induces a conformational change in the molecule such that it is functionally similar to C4b (11). Use of C3-Sepharose or C3b-Sepharose as ligands produce identical results and C3-Sepharose has been previously used to isolate C3bR (1–3, 5). Apparently, in the process of coupling C3 to Sepharose beads the thioester bond in C3 is disrupted and a C3b-like site is exposed.

2 ml of solubilized labeled preparations from E, PMN, or mononuclear cells were incubated for 30 min at room temperature with 0.4 ml of BSA-Sepharose and then centrifuged at 300 *g* × 5 min. The solubilized preparations were then removed and diluted with 4 ml of distilled H_2O, 1% NP-40 before transfer to 0.4 ml of C3-Sepharose, mC4-Sepharose, or RIg G-Sepharose. After a 1-h incubation at room temperature, samples were then centrifuged (300 *g* for 5 min). The beads were transferred to 0.7 × 4 cm plastic columns (Bio-Rad Laboratories, Richmond, CA) with 6 ml of borate buffer (65 mM sodium borate, 50 mM NaCl, pH 8.0), 1% NP-40. Columns were rinsed with 4 ml of the

same buffer and eluted with 4 ml of 0.4 M NaCl, 1% NP-40. Eluates were then dialyzed against water at 4°C and precipitated with acetone to remove residual NP-40. Before application to gels, samples were placed in 80 μl of loading buffer that contained 0.25 M Tris-HCl, 2% SDS, 10% (wt/vol) glycerol, 0.001% bromphenol blue, pH 6.8.

For immunoprecipitation a mouse monoclonal IgG1 antibody (57F) against erythrocyte C3bR was utilized (3). This antibody was graciously provided by Victor Nussensweig, New York University Medical Center, New York. Purified membranes from *Staphylococcus aureus*, Cowan I strain (SaCI) were prepared as previously reported for use as an immunoadsorbent (12). Solubilized preparations (0.5 ml) from E, PMN, or mononuclear cells were incubated for 15 min with 0.05 ml SaCI and the SaCI was then removed by centrifugation (2,000 *g* for 5 min). The supernatant was then added to 0.02 ml of a 1:100 dilution of ascitic fluid containing 57F or an equal concentration of a control mouse monoclonal of the same subclass as 57F (provided by Joseph Davie, Washington University, St. Louis, MO). After 1 h, 0.02 ml of 1:100 dilution of a rabbit antimouse antibody was added (Dako Corp., Santa Barbara, CA). 1 h later, the preparations were transferred to 0.05 ml of SaCI. The SaCI pellets were then washed four times in 1 ml of PBS, 1% NP-40 at 4°C. The proteins bound to the SaCI were removed by resuspending the pellet in 80 μl of loading buffer (see above) and heating at 80°C for 5 min. After centrifugation, the supernatant was loaded on gels for analysis. In some experiments, a rabbit polyclonal antibody against C3bR (gift of Victor Nussensweig) was utilized rather than 57F. No second antibody was added in these experiments.

SDS-Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis was performed according to the method of Laemmli (13), using a 5% or a 6–18% polyacrylamide gradient. Samples for either type of gel were loaded on a 3% stacking gel. For reduced gels, samples in loading buffer were treated in the dark at 37°C with 20 mM dithiothreitol for 30 min and then 50 mM iodoacetamide for 1 h. Autoradiographic techniques and molecular weight markers have been previously described (5). A Quick Scan Jr. densitometer (Helena Laboratories, Beaumont, TX) was used to quantitate the relatively intensity of autoradiographic bands. In some experiments, receptor was run on SDS-polyacrylamide gels and stained by silver nitrate (14).

Selection of Patients with SLE. All patients with SLE were randomly selected from clinics at Washington University School of Medicine and fulfilled at least four of the revised criteria for the diagnosis of SLE (15). Among 45 patients there were 4 males and 41 females ranging in age from 19 to 63 years (mean, 37 years). At the time of study, 37 of the 45 patients were on oral prednisone and 21 of these 37 patients had evidence of disease activity.

Statistical Methods. Potential differences in C3bR phenotype frequencies among individuals were evaluated by Chi-squared testing of contingency tables with Yate's correction (16). A Student's *t*-test for unpaired data was used for comparison of densitometric data among individuals.

Results

Identification of Novel C3bR Phenotypes. Autoradiographs of immunoprecipitated ^{125}I surface-labeled C3bR obtained from E in five selected normal donors are shown in Fig. 1. Immunoprecipitation using a mouse monoclonal antibody against C3bR (57F) demonstrates two major bands at ~190,000 and ~220,000 daltons in the first individual (track *1*). This individual is heterozygous for the A and B alleles (C3bR-AB) that determine two structurally distinct C3bR-molecules (5–7). In the adjacent track, a second individual had a single major band of ~190,000 daltons. This individual is homozygous for the A allele (C3bR-AA) determining the ~190,000-dalton C3bR (5–7). A third previously described phenotype (not shown here) consists of a single major band of ~220,000 daltons (C3bR-BB). Individuals with this phenotype are homozygous for the B allele

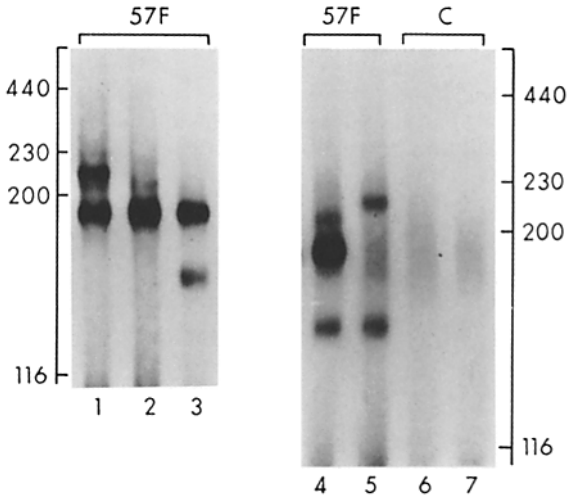

FIGURE 1. Erythrocytes from five normal individuals were surface-labeled with ^{125}I, solubilized and immunoprecipitated with monoclonal antibody (57F) against C3bR (tracks *1–5*). Control immunoprecipitations with a monoclonal antibody with no known specificity (C) were performed in parallel on labeled preparations in the individuals in tracks *4* and *5* (tracks *6* and *7*). Autoradiographs of two 5% nonreduced gels are shown.

determining the ~220,000-dalton C3bR (5–7). Faint bands seen on autoradiographs above the ~190,000 band (tracks *2* and *4*) or above the 220,000 band (track *1*) represent minor bands. These less intense or minor bands have been noted previously in normal individuals and are not inherited in a codominant fashion as are the ~190,000- and ~220,000-C3bR molecules (5, 6).

In track *3* immunoprecipitation of C3bR in a third individual demonstrated the ~190,000-dalton C3bR and an additional band at ~160,000 daltons. This pattern has been reproducibly observed in this donor on five separate occasions over a 6-month period. Scanning densitometry of these five autoradiographs indicated that between 20 and 25% of the total radioactivity was in the ~160,000-dalton band. Identical results are also obtained when a polyclonal rabbit antibody against C3bR is utilized for immunoprecipitation (not shown). Two additional individuals with an ~160,000-dalton molecule are shown in tracks *4* and *5*. The individuals in tracks *3* and *4* have similar receptor phenotypes. The individual in track *5*, however, demonstrates that the 160,000-dalton molecule may also be found with the ~220,000-dalton C3bR. Of note is that in this donor the ~160,000- and ~220,000-dalton bands are of equal intensity. (By scanning densitometry, ~50% of the radioactivity is present in the ~160,000 band). This healthy individual is the daughter of a patient with SLE, and patients with SLE as well as some family members have relatively large amounts of the ~160,000-dalton molecule on E (see below). 10 individuals (5 males and 5 females) with this ~160,000-dalton band on E have been identified.

Immunoprecipitations were also performed in parallel with a control mouse monoclonal antibody on individuals in tracks *4* and *5*. This nonspecific antibody has the same subclass as the antibody against C3bR and was used at the same concentration. The ~160,000-, ~190,000-, or ~220,000-dalton molecules are

not precipitated by this nonspecific antibody (tracks *6* and *7*). To evaluate the possibility that the ~160,000-dalton molecule was a proteolytic breakdown product of the larger ~190,000- or ~220,000-dalton molecules, mixing experiments were performed. In these experiments, labeled E from donors with C3bR-AA or C3bR-BB were mixed with unlabeled E from a donor with the ~160,000-dalton molecule. In both cases, no ~160,000-dalton molecule was isolated by immunoprecipitation from the C3bR-AA or C3bR-BB donors (not shown).

In Fig. 2, C3bR was immunoprecipitated from a previously identified C3bR-AB normal individual (track *1*) and three selected patients with SLE (tracks *2–4*). The first patient (track *2*) has a faint major band at ~220,000 daltons and a predominant major band at ~160,000 daltons. The second patient has a typical C3bR-AA phenotype (track *3*) and the last patient has a major band at ~190,000 daltons that aligns with the AA phenotype in track *3* and a predominant major band at ~160,000 daltons. In the two patients with SLE, scanning densitometry demonstrates the ~160,000-dalton band contains ~85% of the total radioactivity in the receptor. Of interest is the band below the ~190,000-dalton major band in track *4* (arrow head). We have only found this band in donors with the ~160,000-dalton molecule. A similar M_r band is also faintly visualized in the patient in track *2*. Since it co-purifies with C3bR by immunoprecipitation, this band probably represents a minor band associated with the ~160,000-dalton major band.

In previous reports, phenotypes expressed on E were also found on other C3bR bearing peripheral blood leukocytes (6, 7). To assess this finding in individuals with the ~160,000-dalton molecule, C3bR was purified by affinity chromatography from E and Ficoll-Hypaque–purified PMN and mononuclear cells in two normal individuals with the ~160,000-dalton molecule. Results in both individuals were similar and a representative autoradiograph of eluates obtained by C3-Sepharose from one of these individuals is shown in Fig. 3. In track *1*, eluates obtained from E demonstrate a single major band at ~190,000 daltons, a major band at ~160,000 daltons, and a faint minor band above the

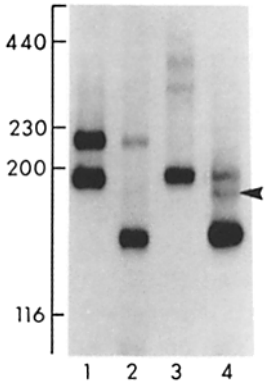

FIGURE 2. Erythrocytes from one normal individual (track *1*) and three individuals with SLE (tracks *2–4*) were surface-labeled with ^{125}I and solubilized, and C3bR was purified by immunoprecipitation. A single 5% nonreduced gel was subjected to autoradiography. The faint high M_r bands seen above 260,000 daltons in track *3* do not appear regularly on immunoprecipitations and their identity is unknown.

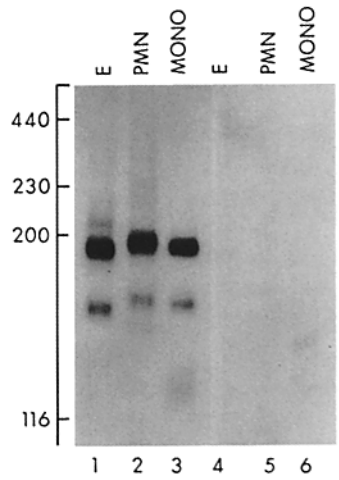

FIGURE 3. Peripheral blood cells from a single donor were surface-labeled with ^{125}I, solubilized, and subjected to affinity chromatography with C3-Sepharose (tracks *1–3*) or RIg G-Sepharose (tracks *4–6*). An autoradiograph of a 5% nonreduced gel is shown for E in tracks *1* and *4*, for PMN in tracks *2* and *5* and for unseparated mononuclear cells in tracks *3* and *6*.

~190,000-dalton major band. In PMN from this same donor, two bands are also found. However, these bands are ~5,000 daltons greater than the ~190,000- or ~160,000-dalton major bands on E (track *2*). On unseparated mononuclear cells two bands are also found (track *3*). These bands more closely align with the ~160,000- and ~190,000-dalton major bands on E. Similar results are obtained when C3bR is purified from peripheral blood cells by immunoprecipitation or by affinity chromatography with mC4-Sepharose (not shown).

Affinity chromatography was performed with RIgG-Sepharose as a control ligand in parallel on the preparations shown in tracks *1–3*. No molecules corresponding to C3bR were isolated. C3bR autoradiographic patterns on 6–18% gradient gels (not shown) are similar to those on 5% gels; however, the small M_r difference between C3bR on PMN and C3bR on mononuclear or E is not as readily apparent on gradient gels. If samples are reduced with dithiothreitol and alkylated with iodoacetamide before electrophoresis, there is an ~30,000-dalton increase in the apparent M_r of the molecules but no change in the band pattern. This effect of reducing conditions on the apparent M_r of C3bR has been noted previously (5).

Population Studies in Normal Individuals and Patients with SLE. To evaluate the frequency of the ~160,000-dalton molecule, C3bR phenotypes in 104 *unrelated* normal individuals were analyzed. As shown in Table I, homozygous C3bR-AA individuals were most common with 72 of 104 (69.2%) possessing this phenotype. The heterozygous C3bR-AB phenotype was found in 25 of the 104 individuals (24.0%). There were four (3.8%) homozygous C3bR-BB individuals and three (2.9%) individuals with both the ~190,000- and ~160,000-dalton molecules (C3bR-AC). Although a single normal individual with the ~220,000- and ~160,000-dalton molecules (C3bR-BC) is shown in Fig. 1, this individual, found in family studies (detailed below), is not included in this table of unrelated donors. No individual with only the ~160,000-dalton molecule (C3bR-CC) has

been found.

Since the ~160,000-dalton molecule (*a*) co-purifies with other C3bR molecules by affinity chromatography or immunoprecipitation, (*b*) is found on E with either the ~190,000- or ~220,000-dalton C3bR, and (*c*) is present on other C3bR bearing peripheral blood cells, it seemed likely that the ~160,000-dalton molecule represented a third codominant C3bR allele. Under this model, the expected frequencies for C3bR phenotypes were calculated based upon Hardy-Weinberg equilibria for three codominant alleles at a single locus (Table I). As can be seen, the observed phenotype frequencies closely agree with the expected frequencies ($\chi^2 = 0.06$, $p > 0.9$). Of note is that individuals with the C3bR-BC or -CC phenotypes would be so uncommon (1 of 333 or 1 of 10,000, respectively) that screening of a much larger number of individuals would be necessary to find these phenotypes in unrelated donors. The calculated gene frequencies for the three alleles in the 104 individuals are shown in Table I. Gene frequencies were also evaluated among races in the 104 normal individuals. For 37 blacks, the gene frequencies were A = 0.78, B = 0.20 and C = 0.01. For 67 Caucasians the gene frequencies were A = 0.86, B = 0.13, and C = 0.01. The three remaining patients were Oriental and were all C3bR-AA. The differences among blacks and Caucasians were not significantly different ($\chi^2 = 2.21$, $p > 0.3$).

In 45 patients with SLE, the frequency of different receptor phenotypes on E was not significantly different from that in normal individuals ($\chi^2 = 0.41$, $p > 0.9$, Table II). However, in the three patients with SLE who expressed the ~160,000-dalton C3bR on E, autoradiographs of C3bR demonstrated a much greater quantity of the ~160,000-dalton molecule than that found in normal

TABLE I

Frequency of C3bR Phenotypes in 104 Unrelated Normal Individuals

C3bR-phenotype	$M_r \times 10^{-3}$	Observed	Expected	
		No. (%)	Genotype	%
AA	190	72 (69.2)	AA	68.9
AB	190/220	25 (24.0)	AB	26.6
BB	220	4 (3.8)	BB	2.6
AC	190/160	3 (2.9)	AC	1.7
BC	220/160	0 (0)	BC	0.3
CC	160	0 (0)	CC	0.01

Gene frequency: A = 0.83, B = 0.16, and C = 0.01.

TABLE II

Analysis of C3bR Phenotypes in Patients with SLE

C3bR phenotype	SLE	Normal
	No. (%)	*No. (%)*
AA	28 (62)	72 (69)
AB	12 (27)	25 (24)
BB	2 (4.4)	4 (3.8)
AC	2 (4.4)	3 (2.9)
BC	1 (2.2)	0 (0)
Total	45 (100)	104 (100)

individuals. By scanning densitometry, the ~160,000-dalton molecule in the three SLE patients contained 48, 85, and 85% of the total radioactivity in the receptor (see Fig. 2). In contrast, in the three unrelated C3bR-AC normal individuals the ~160,000 band possessed 15, 20, and 22% of the total radioactivity in the receptor. In the 25 unrelated normal C3bR-AB individuals, the mean intensity of the ~190,000 band was 57.7 ± 4.0% (mean ± SEM). This was not significantly different than the mean intensity of the ~190,000 band in the 12 C3bR-AB patients with SLE (63.3 ± 5.8%, $t = 0.81$, $p > 0.4$).

To further evaluate the inheritance of C3bR phenotypes, children from 16 normal families and 2 SLE families were studied (Table III). These studies demonstrate that the three C3bR molecules were inherited in a codominant fashion at a single locus. Homozygous parents for the A allele (AA × AA) have only homozygous children (AA), and parents that are each homozygous for one of the A or B alleles (AA × BB) have only heterozygous offspring (AB). If either parent is heterozygous for different alleles (AA × AB or AA × AC) the expected equal number of homozygous (AA) and heterozygous children (AB or AC, respectively) are found. Finally, in parental pairings in which all three alleles are present (AB × AC, AB × BC) only offspring with the expected genotypes are found. In no case did any individual express more than two of the three alleles.

Variable Amount of Different C3bR Molecules on Autoradiographs in Heterozygous Siblings. Unrelated C3bR-AB normal individuals may express markedly different amounts of the 190,000 and 220,000 molecules on E (5–7). Among unrelated C3bR-AB normal individuals and patients with SLE, the quantity of radioactivity in the ~190,000-dalton major band on E as determined by densitometry of the autoradiograph varied from 17 to 90% of the total radioactivity in both bands. In five unrelated C3bR-AC normal individuals and patients with SLE, the amount of radioactivity in the ~190,000-dalton major band on E also varied extensively from 15 to 85%. Although there is variability among heterozygous donors, repeat isolation of C3bR in the same heterozygous donor gives a similar percent of radioactivity in each band (6). Furthermore, silver staining of unlabeled C3bR from selected heterozygous individuals on gels also demonstrates a similar proportion of major bands as those found on autoradiographs of surface-labeled C3bR (not shown). To evaluate whether variable expression of C3bR molecules in heterozygous individuals is inherited, densitometric scanning of autoradiographs of C3bR on E in *related* heterozygous siblings in 10 families was performed (Table IV). In contrast to the considerable variability among all

TABLE III

Inheritance of C3bR Phenotypes in 18 Families

Phenotypes of parents	Phenotypes of children					
	AA	BB	CC	AB	AC	BC
AA × AA	21	0	0	0	0	0
AA × BB	0	0	0	7	0	0
AA × AB	19	0	0	18	0	0
AA × AC	2	0	0	0	2	0
AB × AC	0	0	0	0	2	1
AB × BC	0	1	0	1	2	0

TABLE IV
Phenotypic Expression of C3bR Among Heterozygous Siblings

Family	C3bR phenotype	C3bR in M_r ~190,000 band*
		%
1	AB	73, 72, 70, 68, 61
2	AB	63, 61, 60
3	AB	90, 86
4	AB	62, 61
5	AB	80, 71
6	AB	60, 56
7	AB	79, 76, 70
8	AC	22, 14
9	AC	82, 81
10	AC	85, 78

* Percent determined by densitometric scanning of autoradiographs in C3bR-AB or C3bR-AC siblings.

unrelated heterozygous donors, heterozygous siblings have similar proportions of the two C3bR molecules. There is <15% difference in the amount of the ~190,000-dalton C3bR among heterozygous siblings in any given kindred. Of interest is that of the two siblings who possess large amounts of the ~160,000-dalton molecule (kindred 8), a female has SLE (shown in Fig. 2, track *4*) and her brother is healthy at the age of 58.

Discussion

Previous studies demonstrated that two codominant alleles regulated polymorphism of C3bR on human peripheral blood cells (5–7). In this report, normal individuals and patients with SLE were found to have an ~160,000-dalton molecule on E that co-purified with two previously reported C3bR molecules. Several lines of evidence indicate that this ~160,000-dalton molecule is C3bR. First, the molecule was immunoprecipitated by monoclonal or polyclonal antibodies against C3bR. Second, the ~160,000-dalton molecule was isolated by C3- or mC4-Sepharose affinity chromatography. Third, in individuals with the ~160,000-dalton molecule on E, a similar receptor phenotype was also expressed on other C3bR-bearing leukocytes. Indeed, donors with the ~160,000-dalton molecule on E demonstrated an ~5,000-dalton increase in the M_r of this molecule on PMN (Fig. 2). This M_r increase is identical to that reported for other C3bR molecules on PMN (7).

Two previous studies failed to identify this ~160,000-dalton C3bR molecule. The first study by our laboratory evaluated 33 unrelated normal individuals and was probably too small a sample to identify individuals with the uncommon ~160,000-dalton C3bR (5). However, a second report by another laboratory in a larger group of 111 unrelated normal individuals also failed to detect this molecule (7). In this study silver nitrate staining of SDS-polyacrylamide gels was used to screen most donors. Examination of published gels using this methodology demonstrate heavy background staining in the ~160,000-dalton range, which could have prevented identification of the ~160,000 C3bR. Furthermore, the amount of the ~160,000 C3bR on E in normal heterozygous individuals is small.

All three of the unrelated normal individuals with the ~160,000-dalton C3bR have less than ~25% of the total C3bR in this band on autoradiographs. Only patients with SLE or their relatives have a large amount of the ~160,000-dalton C3bR and these individuals were not examined in the second study. The ~160,000-dalton molecule does not represent a proteolytic fragment of the larger ~190,000- or ~220,000-dalton C3bR molecules. It is reproducibly found in only certain individuals, the quantity of the molecule is constant in a given individual, and it is found on all cell types that possess C3bR. Mixing experiments failed to disclose production of the ~160,000-dalton molecule from donors with the other two alleles. Finally, the ~160,000-dalton C3bR is inherited.

Family studies demonstrate that three codominant alleles at a single locus are responsible for C3bR phenotypes. None of the 225 individuals in this study expressed more than two of the three C3bR molecules. The frequencies of C3bR phenotypes observed in 104 unrelated normal individuals are also compatible with Hardy-Weinberg equilibria for this model. We have chosen to designate the three alleles as A, B, C in order of decreasing gene frequency. In normal individuals, the most common allele (A) determines the ~190,000-dalton C3bR molecule and has a gene frequency of 0.83. The second allele (B) determines the ~220,000-dalton C3bR molecule and has a gene frequency of 0.16. The third allele (C) determines the ~160,000-dalton C3bR molecule and has a gene frequency of 0.01. There were no major differences in gene frequencies for these three alleles between blacks and Caucasians or between patients with SLE and normals. However, since the gene frequencies for each population were not identical, it is possible that small differences in gene frequencies would be demonstrated if a larger number of individuals had been surveyed (i.e., a higher frequency of the B allele in the black population).

In individuals who are heterozygous (C3bR-AB, C3bR-AC), scanning densitometry of the autoradiograph demonstrated a large difference in the quantity of radioactivity in each C3bR band. It is quite unlikely that this difference reflects variable surface-labeling of C3bR molecules. This would require similar molecules on different donors to label with greatly different efficiency. Furthermore, silver staining of gels with receptor purified from selected heterozygous donors demonstrates that the amount of different C3bR molecules visualized by silver staining is similar to that seen on autoradiographs of ^{125}I surface-label receptors (reference 7, this paper). Therefore, among unrelated heterozygous donors any one of the three C3bR molecules may be preferentially expressed. In C3bR-AB individuals, the quantity of the ~190,000-dalton molecule on E was similar in patients with SLE and normals. However, in C3bR-AC individuals a much greater amount of the ~160,000-dalton molecule was found in patients with SLE compared with that in normal individuals. It is unlikely this finding is acquired since the healthy brother of a patient with SLE also had a large amount of the ~160,000-dalton C3bR on E. Furthermore, heterozygous siblings (C3bR-AB or C3bR-AC) in nine other families had a similar proportion of different C3bR molecules on E. These findings strongly suggest that an inherited factor controls the expression of these different C3bR molecules. Except for the greater expression of the ~160,000-dalton C3bR in a small number of patients with SLE (3 of 45), no major differences in C3bR phenotypes were found among normal

individuals and patients with SLE. Thus, C3bR polymorphism is unlikely to be important in the majority of patients with SLE. Nevertheless, these results identify a novel C3bR phenotype that appears to be associated with SLE.

The large difference in the M_r among the polymorphic forms of C3bR raises important questions regarding the molecular basis for this polymorphism. Although glycoproteins may exhibit anomalous migration when M_r is characterized on SDS-polyacrylamide gels, a 60,000-dalton difference in M_r among alleles is a unique observation. Most polymorphic variants of serum proteins are found by isoelectric focusing and represent charge differences (17). However, previously characterized allelic variants of murine C4 demonstrate an ~5,000 difference in M_r by SDS-polyacrylamide gels (18, 19). In this case, the uncommon allele was approximately one-third as hemolytically efficient as the common allele (19, 20). To date, the C3bR-AA and C3bR-BB phenotypes have been found to bind both C3 and C4 (5) and to be equally efficient in the C3b inactivator-mediated cleavage of C3b (21). Evaluation of the functional capacities of the AC phenotype is in progress. In the allelic variants of murine C4, a variation in carbohydrate was found to account for the M_r difference on gels (19). Although evidence indicates that N-linked carbohydrates do not account for the M_r difference between the ~190,000- and ~220,000-dalton C3bR (7), the contribution of O-linked carbohydrates of C3bR M_r is unknown. Recently, O-linked carbohydrates have been demonstrated to account for ~40,000 daltons of the apparent M_r of the LDL receptor[2] and HSV1 glycoprotein (22). Further carbohydrate as well as peptide studies are needed to ascertain the structural basis for the apparent M_r differences in these polymorphic forms of C3bR.

In summary, further analysis of the C3b/C4b receptor in normal individuals and patients with SLE has proven to be instructive. Some of the interesting features of this integral membrane glycoprotein include: (*a*) three alleles coding for polymorphic variants with relatively large M_r differences, (*b*) several cell-specific structural variations, (*c*) variable but genetically regulated gene product expression among heterozygotes, and (*d*) an apparent association between a certain C3bR phenotype and SLE. Studies to elucidate the molecular basis and functional consequences of these observations are in progress.

Summary

We have isolated C3bR from surface-labeled erythrocytes of 180 normal individuals and 45 patients with SLE. These studies have identified a previously unrecognized C3bR molecule on E with a M_r of ~160,000 daltons on nonreduced SDS-polyacrylamide gels. A similar receptor phenotype is also found on other C3bR-bearing peripheral blood leukocytes. Family studies demonstrate that this ~160,000-dalton molecule represents a third allele that is inherited in a codominant fashion at the same locus as the two previously described C3bR alleles. In unrelated normal donors a common allele (A) determines an ~190,000-dalton C3bR (gene frequency 0.83), a second allele (B) determines an ~220,000-dalton C3bR (gene frequency = 0.16), and a third rare allele (C) determines an

[2] Cummings, R., S. Kornfeld,, W. Schneider, K. Hobgood, H. Tolleshauq, M. Brown, and J. Goldstein. Biosynthesis of the N- and O-linked oligosaccharides of the low density lipoprotein receptor. Manuscript submitted.

~160,000-dalton C3bR (gene frequency = 0.01). There were no major differences in gene frequencies among Caucasians and blacks or normal individuals and patients with SLE. However, compared with normal individuals, heterozygous C3bR-AC patients with SLE express large amounts of the ~160,000-dalton C3bR on E. Expression of C3bR molecules among heterozygous siblings is similar, suggesting that an inherited factor controls expression of the two molecules in heterozygous donors. These observations constitute an instructive example of a structural polymorphism of an integral membrane glycoprotein and provide a structural and genetic basis for further molecular and functional analyses of C3bR in normal and patient populations.

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