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Immunology

Polymorphism of human erythrocyte C3b/C4b receptor

(complement receptors/glycoprotein/affinity chromatography)

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ABSTRACT **The human erythrocyte receptor for the major activation fragments of the third and fourth components of complement (HuE-C3bR) was isolated from individual donors. Erythrocytes were surface labeled with ^{125}I and solubilized in Nonidet P-40. HuE-C3bR was purified by using C3-Sepharose affinity chromatography and analyzed by autoradiography of $NaDodSO_4$/polyacrylamide gels. Three distinct receptor patterns were demonstrated. Type *a* had a single major band with M_r of 190,000, type *b* had a single major band with M_r of 220,000, and type *c* had two major bands of M_r 190,000 and 220,000. In all three types, a minor band accounting for <25% of the total radioactivity was usually observed at a M_r 15,000 greater than that of each major band. Identical autoradiographic patterns were obtained by affinity chromatography using methylamine-inactivated C4-Sepharose or by immunoprecipitation of solubilized membranes with a monoclonal antibody against HuE-C3bR. All three types were distinct after reduction and alkylation, although the apparent M_r uniformly increased by ≈30,000. Characterization of HuE-C3bR types in 33 unrelated individuals demonstrated that 23 had type *a*, 1 had type *b*, and 9 had type *c*. Family studies provide evidence for transmission by two codominant alleles. Thus, in the normal population two alleles appear to control expression of HuE-C3bR phenotypes and account for the polymorphism of this integral membrane glycoprotein.**

The human erythrocyte receptor[§] for the major activation fragments of the third (C3b) and fourth (C4b) components of complement (HuE-C3bR) has been previously isolated by sequential chromatography of *pooled* donor membranes. It was initially characterized on $NaDodSO_4$/polyacrylamide gels as a single glycoprotein with a M_r of ≈205,000 (2–5). Polyclonal antibodies against this glycoprotein inhibited immune adherence (2). This glycoprotein also accelerated decay of the alternative (2) and classical (4) pathway C3 convertases. Using a modified cell surface labeling procedure (6) and affinity chromatography, we previously reported the isolation of a rabbit alveolar macrophage receptor for C3b (7) and characterized its ligand binding specificity (8). Extending this method to human erythrocytes allowed us to isolate the C3b receptor from individual donors. We now report previously unrecognized polymorphism of this integral membrane glycoprotein.

MATERIALS AND METHODS

Surface Labeling and Solubilization of Human Erythrocyte Membranes. Approximately 30 ml of human blood was collected from each donor in 1.5 ml of 100 mM EDTA or in 4.5 ml of citrated dextrose (Fenwal Laboratories, Deerfield, IL). Blood was centrifuged at 800 × *g* for 8 min at 4°C, and the plasma and buffy coat were removed. The packed human erythrocytes were then washed at 4°C three times in phosphate-buffered saline (10 mM potassium phosphate/150 mM NaCl, pH 7.40). After each centrifugation, a portion was removed such that approximately one-half of the original packed cell volume was retained. Immediately before iodination, cells were resuspended in a 10% solution with phosphate-buffered saline at 22°C ($1.0–1.3 \times 10^9$ human erythrocytes per ml). The cells in 4 ml of this solution were iodinated with 0.25 mCi (1 Ci = 3.7×10^{10} Bq) of ^{125}I (New England Nuclear) by using a modified lactoperoxidase method at room temperature (6). After iodination, human erythrocytes were chilled to 4°C and lysed with 15 ml of ice-cold distilled water for 1 min followed by addition of 15 ml of 0.3 M NaCl. Both the distilled water and NaCl contained 2 mM phenylmethylsulfonyl fluoride, 3 mM ETDA, 20 mM iodoacetamide, 1 μM pepstatin, and aprotinin at 0.33 trypsin inhibitor unit/ml. The stroma were isolated by centrifugation at 33,000 × *g* for 20 min and this pellet was then solubilized with phosphate-buffered saline containing 1% Nonidet P-40 (NP-40; Sigma) containing the same protease inhibitors used in the lysis step. Approximately 5×10^9 erythrocyte stroma were solubilized in 1 ml of this buffer. Ten microliters of this solubilized membrane preparation contained typically $2–4 \times 10^5$ cpm. Samples were stored frozen at −22°C until use.

Affinity Chromatography. C3 was prepared by the method of Tack and Prahl (9) and C4 was prepared by the method of Bolotin *et al.* (10) in collaboration with P. Levine's laboratory (Department of Genetics, Washington University School of Medicine). The characterization of C3 and C4 in regards to purity and functional activity has been reported (7, 8, 11). C4 was inactivated (producing C4i) by treatment with 20 mM methylamine (Sigma) in barbital buffer (25 mM sodium barbital/150 mM NaCl, pH 8.5) for 3 hr at 37°C. Methylamine cleaves the thioester bond within the α chain of C4. This modification destroys the hemolytic activity and induces a conformational change in the molecule, making it similar to C4b (12). Sepharose beads (6B, Sigma) were then activated by cyanogen bromide and coupled to C4i, bovine serum albumin (hereafter referred to as "albumin"), or C3 for use as immunoabsorbants as described (13–15). In this system, affinity chromatography separations with C3-Sepharose or C3b-Sepharose as ligands produce identical results. It is likely that under the conditions of the coupling procedure most of the C3 no longer possesses an intact α chain thioester bond and is hemolytically inactive (so-called C3i). The

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Abbreviations: C3b and C4b, major activation fragments of the third and fourth components of complement, respectively; HuE-C3bR, human erythrocyte complement receptor for C3b and C4b; C4i, methylamine-inactivated fourth component of complement; NP-40, Nonidet P-40.

[§] In this report we have, as have others, denoted this erythrocyte glycoprotein as a receptor rather than a binding protein. Although no evidence exists that a transmembrane signal is generated upon its interaction with C3b, a similar molecule is found on human leukocytes, and on polymorphonuclear cells it mediates internalization of C3b-coated complexes (1).

conformational structure and biologic activity of this C3i molecule are similar to those of C3b (8, 16–19). Other investigators have also used C3-Sepharose to isolate HuE-C3bR (2–5).

Solubilized membranes from 1×10^{10} human erythrocytes were incubated with constant mixing for 30 min at room temperature with 0.4 ml of albumin-Sepharose and then centrifuged at $300 \times g$ for 5 min. The solubilized membranes were removed and diluted with 2 parts of distilled water before being transferred to 0.4 ml of C3-Sepharose, C4i-Sepharose, or albumin-Sepharose. After a 1-hr incubation at room temperature, samples were centrifuged at $300 \times g$ for 5 min and the supernatants were removed. The beads were transferred to 0.7×4 cm plastic columns (Bio-Rad) with 6 ml of borate buffer (65 mM sodium borate/50 mM NaCl, pH 8.0) with 1% NP-40. Columns were rinsed with 4 ml of the same buffer and eluted with four successive 1-ml aliquots of 0.1 M acetic acid/400 mM NaCl/1% NP-40. Each 1 ml of the acid eluates was collected in a tube containing 125 μl of 2 M Tris·HCl, pH 8.6/1% NP-40 for immediate neutralization. Approximately 0.05–0.10% of the total radioactivity in 1×10^{10} solubilized human erythrocytes would bind to the C3-Sepharose column and 40–60% of bound radioactivity was eluted with acid and salt. In some experiments, only 400 mM NaCl/1% NP-40 was used to elute the column. When affinity chromatography was performed at 4°C rather than room temperature, less total receptor was obtained but the pattern was identical.

For structural analysis, eluates were dialyzed against water at 4°C, lyophilized, and precipitated with acetone to remove NP-40. Before application to gels, samples were placed in 80 μl of loading buffer, consisting of 0.25 M Tris·HCl, pH 6.8/2% $NaDodSO_4$/10% (wt/vol) glycerol/0.001% bromophenol blue.

Immunoprecipitation by Monoclonal Anti-HuE-C3bR. Ascitic fluid containing a mouse monoclonal IgG1 antibody (57F) against HuE-C3bR was utilized for immunoprecipitation (20). Solubilized membranes from 3.8×10^9 human erythrocytes were incubated for 1 hr at 4°C with 50 μl (≈10 μg/ml; a 1:500 dilution of ascitic fluid) of monoclonal anti-HuE-C3bR or a control mouse monoclonal antibody in ascitic fluid of the same subclass and concentration but with no known specificity. The pellet from 0.5 ml of a 10% suspension of *Staphylococcus aureus*, Cowan I strain, was used to bind the antibody (21), and pellets were washed three times in 1 ml of phosphate-buffered saline/1% NP-40 at 4°C. The proteins bound to the pellet were removed by resuspending the pellet in 80 μl of loading buffer (see above) and heating at 80°C for 10 min. This removed approximately 40% of the total radioactivity bound to the pellet. Supernatants were collected after centrifugation and then loaded directly on gels for electrophoresis.

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Electrophoresis in slab gels was performed according to the method of Laemmli, using a straight 5% or a 6–18% polyacrylamide gradient. Samples for both gels were loaded into a 3% stacking gel (22). For reduced gels, samples in loading buffer were treated with 20 mM dithiothreitol for 30 min at 37°C and then 50 mM iodoacetamide for 1 hr in the dark or with 5% (vol/vol) 2-mercaptoethanol at 80°C for 10 min. Autoradiographs of dried gels were exposed with a Cronex Lightning Plus screen (Dupont) and X-Omat AR film (XAR-5, Kodak) at −70°C. Autoradiographs were scanned with a Zeineh densitometer (Biomed) to estimate intensity of the autoradiographic bands. Calibration of molecular weights was based upon fibronectin (M_r 440,000 nonreduced, 220,000 reduced), catalase (M_r 230,000 nonreduced), myosin (M_r 200,000), β-galactosidase (M_r 116,000), and phosphorylase *b* (M_r 94,000). Fibronectin was a gift from Jay McDonald (Washington University), and other markers were from standard kits (Bio-Rad; Pharmacia).

RESULTS

Purification of HuE-C3bR by Affinity Chromatography. Aliquots of solubilized labeled human erythrocyte membranes (1×10^{10} cells) from three selected individuals were subjected to affinity chromatography with C3-Sepharose and autoradiography (Fig. 1). An autoradiograph of the eluate from individual D1 showed a major band of M_r 190,000 (Fig. 1, track 1). A less intense band with $M_r \approx 15{,}000$ greater was seen above the major band. In a second individual (D2), a major band was found with a M_r of 220,000 (track 3). As in donor D1, a less intense band was observed that had $M_r \approx 15{,}000$ larger than that of the major band. In a third individual (D3), two radiolabeled bands were found with M_r values of 190,000 and 220,000 (track 5) that aligned with major bands found in individuals D1 (190,000) and D2 (220,000). With labeled, solubilized membranes from the same donors, identical radiolabeled bands were seen on autoradiographs of eluates from C4i-Sepharose (not shown). No corresponding bands were isolated when albumin (Fig. 1, tracks 7, 8, and 9) or rabbit immunoglobulin (not shown) was used as the affinity reagent.

Experiments were performed to determine if differences in autoradiographic patterns were due to elution methods or variable binding to the affinity column. First, radiolabeled solubilized membranes were immunoprecipitated after affinity chromatography with C3-Sepharose. Autoradiographs demonstrated that >95% of the total receptor among donors of all three patterns had been removed by C3-Sepharose. Second, 6 M guanidine/1% NP-40 was used to strip the affinity column after salt and acid elution. Comparing autoradiographs of both eluates indicated that at least 80% of the receptor had been removed by the initial salt or salt and acid elution. Furthermore, autoradiographic patterns of the guanidine eluates were identical to initial salt and acid eluates, indicating that certain bands were not preferentially removed by salt and acid. Third, separate C3-Sepharose columns were eluted by 400 mM NaCl/1% NP-40 or 400 mM NaCl/0.1 M acetic acid/1% NP-40. Both methods gave similar yields and autoradiographic patterns in the same donor. Finally, a separate method of purifying the receptor gave identical results (see below).

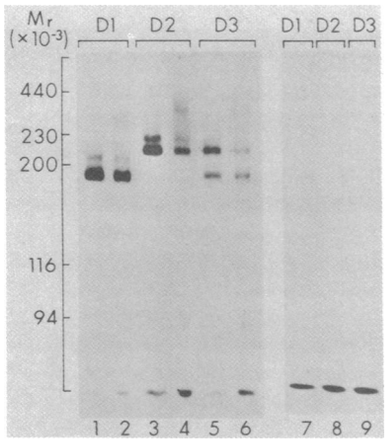

FIG. 1. Autoradiograph of HuE-C3bR isolated by affinity chromatography or immunoprecipitation. Solubilized ^{125}I-labeled erythrocyte membranes from three donors (D1, D2, and D3) were used for affinity chromatography on C3-Sepharose (tracks 1, 3, and 5) or albumin-Sepharose (tracks 7, 8, and 9). From the same donor (tracks 2, 4, and 6) receptor obtained by immunoprecipitation of solubilized erythrocyte membranes with a monoclonal antibody was run in parallel. Unless noted otherwise, autoradiographs are obtained from $NaDodSO_4$/5% polyacrylamide gels after electrophoresis under nonreducing conditions.

Isolation of HuE-C3bR by Immunoprecipitation. Immunoprecipitation of radiolabeled solubilized membranes by monoclonal anti-HuE-C3bR produced bands on autoradiographs similar to those obtained by affinity chromatography (Fig. 1, tracks 2, 4, and 6). Immunoprecipitation of HuE-C3bR immediately after solubilization (no freezing), or in the absence of protease inhibitors did not alter the band pattern (not shown). Patterns obtained from human erythrocytes that were stored in citrated dextrose up to 5 days at 4°C were identical to those obtained from fresh human erythrocytes collected in EDTA or heparin (not shown). Bands at M_r 260,000–320,000 did not bind to C3-Sepharose (Fig. 1) and have not routinely appeared on immunoprecipitations (for example, see Fig. 2). Electrophoresis and autoradiography of material isolated by repeat immunoprecipitation of radiolabeled solubilized membranes, after initial immunoprecipitation, showed no bands on autoradiographs (not shown). Patterns obtained on $NaDodSO_4$/6–18% polyacrylamide gradient slab gels were similar (not shown) to those on straight 5% slab gels, although better separation of radiolabeled bands was obtained with 5% gels. Lower M_r bands (<80,000) were not seen on autoradiographs of the gradient gels.

Autoradiographs of immunoprecipitates of three individuals from a separate experiment are shown in Fig. 2. In this experiment, two different donors (D4 and D5) and the same donor (D2) that was used in Fig. 1 were evaluated. A major radiolabeled band was observed in donor D5 at M_r 190,000 (track 2) and in donor D2 again at M_r 220,000 (track 3). As is apparent, donor D5 had a pattern similar to donor D1 in Fig. 1. Less intense bands with M_r values ≈15,000 greater than those of the major bands were observed in both donors. Radiolabeled bands in donor D4 (track 1) were seen with M_r values of 190,000 and 220,000 and aligned with the major bands in donors D2 and D5. Thus, donor D4 had a two-band pattern like donor D3 in Fig. 1; however, the M_r 190,000 band in donor D4 appeared to contain a greater proportion of the total radioactivity than the same band in donor D3. As additional individuals with this "doublet" pattern were studied, quantitation of the ratio of the radioactivity in the M_r 220,000–190,000 bands was found to be relatively constant for each donor but to vary from individual to individual (see below). On most gels of donors with patterns like D4, faint bands corresponding to less intense bands with M_r values ≈15,000 greater than those of the major bands were also observed (Figs. 2 and 4).

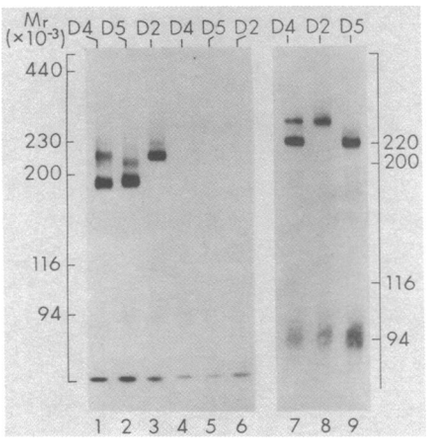

FIG. 2. Effect of reduction and alkylation on M_r of HuE-C3bR. Autoradiograph of HuE-C3bR isolated by immunoprecipitation from three donors (D4, D5, and D2). Receptors are shown without (tracks 1–3) and with reduction and alkylation with dithiothreitol and iodoacetamide (tracks 7–9). Although reduced and nonreduced samples were run in different gels in this figure, similar results in other experiments were obtained when samples were run in the same gel. Control immunoprecipitations (tracks 4–6) were performed with a mouse monoclonal antibody of no known specificity.

When immunoprecipitated samples from the same donors (D2, D4, and D5) were reduced with 2-mercaptoethanol or alkylated with iodoacetamide after reduction with dithiothreitol, apparent M_r uniformly increased ≈30,000 (Fig. 2, tracks 7, 8, and 9). The gel patterns of the major species when reduced and alkylated were identical to the pattern when the species were not reduced. Minor (less intense) bands were more difficult to resolve under reducing conditions, although faintly seen above major bands (Fig. 2).

A diffuse band at a M_r of ≈94,000, most prominent in the reduced gel of Fig. 2, was occasionally seen. It was nonspecific because it was not present in autoradiographs of receptor protein produced by affinity chromatography (Fig. 1) and because immunoprecipitates with a control mouse monoclonal antibody also showed a heterogeneous band at the same M_r (tracks 4, 5, and 6). No labeled material in the M_r range of HuE-C3bR was immunoprecipitated by control mouse monoclonal antibody in any donor.

Proposed Classification and Prevalence of HuE-C3bR Types. HuE-C3bR was characterized in 33 unrelated individuals. Patterns on autoradiographs were like those described in Figs. 1 and 2. Therefore, tentative HuE-C3bR types were assigned according to the model proposed in Fig. 3. In this model, type *a* has a major radiolabeled band at M_r of 190,000 and type *b* has a major radiolabeled band with a M_r of 220,000. In both types minor bands with M_r values ≈15,000 larger than major bands are present. In type *c*, the major and minor bands align with those in types *a* and *b*. Type *a* was most common and was found in 23 individuals (70%). Type *b* was found in one individual (3%) and type *c* was found in 9 individuals (27%).

Family Studies. The HuE-C3bR types of four kindreds are shown in Fig. 4. In kindreds I and II both sets of parents in tracks 1 and 2 are type *a* and all eight children from both families are type *a*. Track 6 adjacent to kindred I shows an unrelated type *c* donor for comparison. Variability among children in the intensity of the minor band is apparent. For kindred III, a study of three generations is included. Tracks 1 and 2 in the upper gel are parents (type *c* and *a*, respectively) of the four children in tracks 3–6, two of which are type *a* and two type *c*. The type *c* individual shown in track 5 of the upper gel is also shown in track 1 of the lower gel. He married a type *a* woman (track 2) and he fathered two type *a* children and one type *c* child shown in tracks 3–5. Thus, in these studies of children from individuals with type *a* or *c*, type *c* is observed in the child only when one of the parents has this pattern and is not seen when both parents have

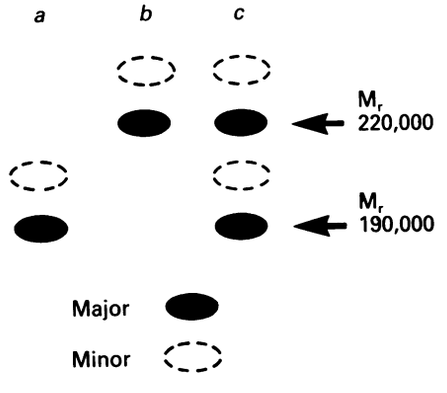

FIG. 3. Proposed classification of HuE-C3bR types (see text).

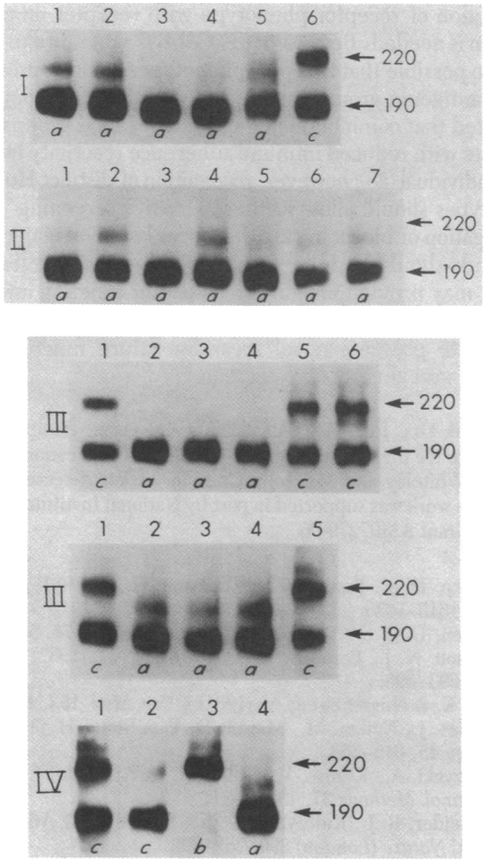

FIG. 4. Family studies of HuE-C3bR types. HuE-C3bR was isolated from four kindreds by immunoprecipitation. Kindred I, tracks 1 and 2, parents of children in tracks 3–5; track 6, unrelated type *c* individual shown for comparison. Kindred II, tracks 1 and 2, parents of children in tracks 3–7. Kindred III (upper row), tracks 1 and 2, parents of children in tracks 3–6. Kindred III (lower row), tracks 1 and 2, parents of children in tracks 3–5 (the parent in track 1 of this row is the same individual as the child in track 5 of the row above). Kindred IV, tracks 3 and 4, parents of child in track 2; track 1, unrelated type *c* individual for comparison.

the *a* type. The M_r 190,000 and 220,000 bands appear to represent two codominant alleles.

To find additional type *b* individuals, parents of two type *c* individuals were screened (≈1/6 should be type *b* by Hardy–Weinberg equilibrium if type *c* represents a heterozygote of two codominant alleles). An example of the family from one type *c* individual is shown in Fig. 4 (kindred IV). This type *c* individual (track 2) is unusual because of the low ratio of radioactivity in the M_r 220,000 band to that in the M_r 190,000 band (as shown below, all other type *c* individuals studied had ratios from 0.4 to 1.4 by densitometric scanning; the individual shown in track 1 is an unrelated type *c* individual shown for comparison). Repeat examination in this unusual type *c* individual (track 2) disclosed that low amounts of radioactivity were reproducibly found in her M_r 220,000 band. Nevertheless, her father (track 4) had a type *a* pattern and her mother (track 3) was type *b*. Therefore, this kindred provides evidence for one allele from each parent determining HuE-C3bR types because the individual in track 2 expresses both major bands of her parents. The only member of the family of donor D2 in Fig. 1 (type *b*) available for study is her 8-year-old child. He is type *b*, suggesting that his father was type *b* or *c*.

Quantitative Measurement and Reproducibility of Band Intensity for HuE-C3bR Types. Band intensity was quantitated by densitometric scanning of autoradiographs of nonreduced gels in 41 individuals. Determination of band density by densitometric scanning gave results similar to those obtained by directly measuring radioactivity in labeled bands cut from dried gels. In eight individuals with type *c*, either the M_r 190,000 or the M_r 220,000 major band could predominate. The ratio of radioactivity in the M_r 220,000 to the M_r 190,000 band was 0.68 ± 0.16 (mean ± SEM) with a range of 0.1 to 1.4. Repeat examinations in five of the type *c* individuals gave ratios that agreed within 0.1 of the initial result. Minor bands in the type *c* pattern were sometimes difficult to quantitate but always accounted for <20% of the total radioactivity. The minor bands in 30 individuals with type *a* accounted for 8.1 ± 1.2% (mean ± SEM) of the total radioactivity in the receptor, with a range from no detectable minor band to 22%. Type *b* was found in three individuals and the minor band accounted for 11.3% of the total radioactivity in the receptor bands in these individuals (6%, 14%, and 14%).

To analyze the reproducibility of the minor band patterns, repeat examinations were performed on selected donors, some up to 12 months apart. In three individuals with no detectable minor bands (one type *a* and two type *c*), minor bands were not found upon repeat examination. For six individuals with 6–18% of the total radioactivity in minor bands (three type *a* and three type *c*), repeat evaluations were within this same range. Moreover, for each individual the repeat values were within ±3% of the initial result. Thus, although among individuals variability in intensity was observed for the major and minor band patterns, repeat studies in the same individual produced similar results.

DISCUSSION

Multiple lines of evidence support the identification of the radiolabeled macromolecules isolated in this study as the human erythrocyte receptor for C3b and C4b. First, all variants were bound to and eluted from C3-Sepharose during affinity chromatography but were not isolated by use of nonspecific columns such as albumin- or IgG-Sepharose. Second, identical patterns were obtained with C4i as an affinity ligand [C4i has the same conformational structure as C4b (12)]. In previous reports, HuE-C3bR has been shown to mediate binding of human erythrocytes to indicator particles coated with C4b or C3b and polyclonal antibody against HuE-C3bR was found to block binding (23). Third, immunoprecipitation by a monoclonal antibody against HuE-C3bR gave autoradiographic receptor patterns identical to those obtained by affinity chromatography. Fourth, the M_r values of the isolated receptor molecules were similar to those reported by others (2–5).

HuE-C3bR was initially characterized as a single-chain glycoprotein that under nonreducing or reducing conditions had a M_r of 195,000–205,000 (2–5). More recently, the M_r of HuE-C3bR after reduction and alkylation was characterized as 220,000–250,000 but no comparison was made to nonreduced samples (24–26). Our study demonstrates that reduction and alkylation is associated with an apparent increase in M_r of ≈30,000 for all HuE-C3bR types. Addition of alkylated groups to the protein backbone could not account for these increases, because reduction with mercaptoethanol alone produced similar findings. Decreases in migration more likely reflect unfolding of the HuE-C3bR structure due to cleavage of internal disulfide bonds under reducing conditions. This uniform shift of M_r under reducing conditions is additional evidence suggesting that the three receptor patterns (both major and minor bands) are due to closely related molecules. Several explanations may account for the failure of other groups to observe the M_r patterns found in this study. First, because a single receptor type (type *a*) is found in 70% of

normal individuals, less frequent types may not have been apparent when 2–20 liters of pooled blood were used to isolate HuE-C3bR (2–5). Second, the $NaDodSO_4$/polyacrylamide techniques employed by others may not have been adequate to separate the different HuE-C3bR types.

HuE-C3bR patterns were reproducible on repeat examination. Over a 12-month period all individuals tested with one (types *a* and *b*) or two major bands (type *c*) always had the same pattern. Less intense bands, seen at $M_r \approx 15{,}000$ greater than major bands, were termed minor for several reasons. By densitometric scanning, the minor band in all individuals always contained small amounts (<25%) of the total radioactivity in the receptor. Of course, major and minor bands may not label with equal efficiency and other labeling methods will be necessary to establish that minor bands make up a small amount of total HuE-C3bR on the membrane surface. Second, by employing methods identical to those described herein for erythrocytes, minor bands have not been found on human peripheral blood granulocytes or mononuclear cells purified by Ficoll/Hypaque methods (unpublished data). However, major band patterns on leukocytes are similar to those on erythrocytes—i.e., individuals with type *a* and *b* have single bands differing in M_r by 30,000 and those with type *c* have both of these bands. These observations in C3b receptor-bearing cells suggest that each HuE-C3bR type is genetically determined among normal individuals.

This study provides preliminary evidence that expression of HuE-C3bR types is governed by two codominant alleles. Because individuals with type *c* express bands of *a* and *b* types, type *c* probably represents a heterozygote phenotype. Type *a* individuals would represent the homozygous phenotype of one common allele and type *b* individuals the homozygous phenotype of a second, less-common, allele. Although small numbers of individuals have been characterized, frequencies of phenotypes found in this study are consistent with Hardy–Weinberg equilibrium for this model. Furthermore, the patterns of HuE-C3bR in families are consistent with this mode of inheritance. Parents who were homozygous for the same allele (type *a*) had children of the same type and parents who were homozygous for different alleles (types *a* and *b*) had a child that was heterozygous (type *c*); parents who were homozygous (type *a*) and heterozygous (type *c*) had children that were either homozygous or heterozygous (types *a* and *c*). Although two alleles imply that separate transcriptional proteins account for major bands, alleles could determine differences in enzymes involved in posttranslational modification. In this case, M_r differences among major bands could reflect posttranslational modifications of a common precursor molecule. However, the magnitude of the difference in M_r and the presence of variation in both bands among heterozygotes provides evidence against this hypothesis. Taken together, the data suggest that a difference in transcriptional protein structure is more likely to account for the major HuE-C3bR bands, but additional structural studies will be required to differentiate these possibilities.

A number of other membrane receptors have been isolated and characterized, but we are unaware of other examples of polymorphism in membrane receptors. Structural variation in HuE-C3bR may reflect beneficial or nonadvantageous mutations. Clearly all major and minor bands bound to C3- or C4i-Sepharose, but these relatively crude functional observations may not reflect *in vivo* receptor function before detergent solubilization. Recently a wide variation in HuE-C3bR number has been documented in normal individuals and patients (20, 26, 27). Correlation of receptor phenotype with receptor number and function is needed. Because HuE-C3bR is a membrane protein, it is also possible that receptor phenotypes could represent different antigenic groups among individuals. A previous study suggested that common major and minor blood groups did not correlate with reduced immune adherence reactivity in one unusual individual (28); however, recognition of distinct HuE-C3bR phenotypes should allow further extensive screening. Finally, investigation of biochemical differences between major and minor bands should be informative. Differences in cellular processing may provide insight into receptor function and evolution. Identification of distinct receptor phenotypes in the human erythrocyte presents novel areas for future functional, biochemical, and genetic investigation.

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