STRUCTURAL HETEROGENEITY OF THE C3b/C4b RECEPTOR (CR1) ON HUMAN PERIPHERAL BLOOD CELLS*

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The human receptor for C3b and C4b (CR1) was initially isolated from pooled donor erythrocyte membranes and characterized as an integral membrane glycoprotein of ~205,000 daltons on SDS-polyacrylamide gels (1-3). Polyclonal antibodies against this glycoprotein immunoprecipitated a similar molecule from other CR1-bearing cells such as polymorphonuclear cells (PMN), macrophages, and B lymphocytes (1). Recently, we have demonstrated that CR1 is polymorphic in erythrocytes (E) obtained from normal donors (4). Autoradiographs of purified surface-labeled receptor demonstrated that ~70% of individuals had a single major band at 190,000 daltons, ~3% of individuals had a major band at 220,000 daltons and the remaining individuals had both major bands at 190,000 and 220,000 daltons. Family studies provided evidence for transmission of the 190,000- and 220,000-dalton major bands of CR1 by two codominant alleles. We have now extended these investigations by analyzing the structure of this receptor on human leukocytes. These studies demonstrate that although leukocytes from individual donors express CR1 polymorphism similar to that on E, specific structural differences in CR1 exist among peripheral blood cells.

Materials and Methods

Purification of Human E, Polymorphonuclear, and Mononuclear Cells. E were obtained from 30 ml of whole blood collected in 500 U sodium heparin (Abbott Laboratories, North Chicago, IL). Theuffy coat was removed after centrifugation (800 g for 8 min) and the pellet was then washed three times with 0.01 M potassium phosphate, 0.15 M NaCl, pH 7.4 (PBS) to obtain ~5 ml of packed E from each donor (4). Leukocytes were obtained at the same time as E from 250 ml of whole blood collected in 50 ml of 6% dextran (Sigma Chemical Co., St. Louis, MO), 0.15 M NaCl and 2,500 U of heparin. Polymorphonuclear (PMN) and mononuclear cells were then separated by Ficoll-Hypaque method (5). The pellets contained >96% 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 <2% PMN. In some donors mononuclear cells were further separated into monocyte-enriched or B lymphocyte-enriched populations by Dr. R. MacDermott, Washington University, as previously reported (6).

Surface Labeling, Solubilization, and Isolation of CR1. E (~5 × 10⁶ cells), PMN, or mononuclear cells (~2 × 10⁶ cells) were suspended in 4 ml PBS and iodinated with 0.25 mCi of ¹²⁵I (New England Nuclear, Boston, MA) by a modified lactoperoxidase method (4). Following iodination, E were lysed at 4°C with 15 ml of distilled H₂O containing protease inhibitors (2 mM...
phenylmethylsulfonyl fluoride, 3 mM EDTA, 1 μM pepstatin, and 20 mM iodoacetamide) for 1 min and then 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 × 10^9 cells) 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, supernatants from each cell preparation were stored at −22°C. To purify CR1, solubilized labeled preparations were thawed and immunoprecipitated as previously reported (4) with monoclonal mouse IgG1 antibody prepared against CR1 (7). Briefly, preparations were incubated with excess anti-CR1 and the antibody was then bound to purified membranes from Staphylococcus aureus, Cowan I strain (SaC1). After extensive washings, proteins bound to the pellet were removed by heating the pellet in electrophoresis buffer (0.25 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerin, and 0.001% bromophenol blue) and the buffer was then loaded directly on gels. In some donors, CR1 was also isolated by affinity chromatography with C3-sepharose (4).

SDS-Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis was performed according to the method of Laemmli (8). Reduction and alkylation of samples, molecular weight standards, and autoradiographic techniques were previously described (4). A Zeineh scanning densitometer (Biomed Instruments Inc., Fullerton, CA) was used to quantitate band intensity (4).

Results

Autoradiographs of immunoprecipitates of ^125I surface-labeled peripheral blood cells from three selected donors are shown in Figs. 1 and 2. In donor D1 (a known heterozygous individual), two major radiolabeled bands were seen on autoradiographs in all three types of peripheral blood cells (Fig. 1, tracks 1–3). In E, two predominant (major) bands were found at Mr of 190,000 and 220,000 (track 1, solid arrows to left). Less intense (minor) bands were noted ~15,000 daltons above each major band (open arrows to left of track 1). Densitometric scanning of the autoradiographs disclosed that <10% of radioactivity in the receptor was found in these minor bands. In PMN from the same donor, bands did not align with major or minor bands in E but were ~5,000 daltons greater (Mr of 195,000 and 225,000; compare track 2 with track 1). In contrast to PMN, bands derived from mononuclear cells aligned with major bands in E (track 3). The less intense minor bands that were demonstrated in E were not found in either PMN or mononuclear cells, even with prolonged exposure of autoradiographs.

The same radiolabeled solubilized preparations from E, PMN, and mononuclear cells of donor D1 were also immunoprecipitated with a control mouse monoclonal antibody with no known specific activity (Fig. 1, tracks 4–6, respectively). This monoclonal had the same subclass (IgG1) and was used at the same concentration as anti-CR1. Radiolabeled bands corresponding to the receptor bands were not precipitated by nonspecific monoclonal antibody. With either the nonspecific or specific monoclonal antibody, bands were occasionally noted at ~94,000 daltons, particularly in E preparations (tracks 1 and 4). As reported previously, this 94,000-dalton band is not found on autoradiographs of CR1 isolated by affinity chromatography with C3-Sepharose (4).

Experiments similar to those shown in Fig. 1 were performed with two selected homozygous donors, D2 and D3 (Fig. 2). In donor D2, a single major band was found at 190,000 daltons on E (track 2, solid arrow) that aligned with the lower major band in donor D1 (track 1). In donor D3, a single major band was found at 220,000 daltons on E (track 3, solid arrow) that aligned with the upper major band in donor D1 (track 1). Less intense minor bands were noted ~15,000 daltons greater than the major bands in donors D2 and D3 (open arrows). Densitometric scanning of autoradiographs
Fig. 1. Purified peripheral blood cells were labeled with \(^{125}\text{I}\), solubilized, and immunoprecipitated with monoclonal anti-CR1 (tracks 1-3) or control antibody with no known specificity (tracks 4-6). Autoradiographs of all samples run on the same nonreduced 5% gel are shown for erythrocytes (RBC) in tracks 1 and 4, for PMN in tracks 2 and 5, and for mononuclear cells (MONO) in tracks 3 and 6.

in these donors demonstrated that \(\sim 12\%\) of the radioactivity in the receptor was found in minor bands in both donors.

 Autoradiographs of immunoprecipitations of solubilized labeled PMN and mononuclear cells in homozygous donors D2 and D3 gave single band patterns (tracks 4-7). In both donors, bands immunoprecipitated from PMN did not align with major or minor bands in E and were \(\sim 5,000\) daltons greater than the radiolabeled major band found on their respective E (compare track 4 with track 2, and track 5 with track 3). In mononuclear cells, single bands in both donors (tracks 6 and 7) aligned with major bands in their respective E (tracks 2 and 3) and were lower than those in PMN (tracks 4 and 5). As in donor D1 in Fig. 1, minor bands were not apparent in leukocytes from donors D2 or D3.

 Differences between CR1 on PMN and E or mononuclear cells were also apparent on reduced and alkylated gels. In addition, patterns found with gradient gels (6-18%) were similar to those on 5% nongradient gels. When CR1 was purified by affinity chromatography with C3-Sepharose, autoradiography demonstrated similar patterns among these and other donors. Minor bands on E and differences in CR1 between PMN and E or mononuclear cells were found whether preparations were isolated by affinity chromatography or immunoprecipitation.

 In some donors CR1 was immunoprecipitated from monocyte-enriched (\(\sim 81\%\) monocytes, 19% B lymphocytes) or B lymphocyte-enriched populations (\(\sim 90\%\) B lymphocytes, 8% null, and 2% T cells). Both monocyte and B lymphocyte-enriched populations had similar \(M_r\) receptors on autoradiographs as found in nonseparated mononuclear preparations. In cells obtained from human eosinophil cultures from normal donors (>98% eosinophils, gift of Drs. Salomon Asmar and Jeff Herzig, Washington University) immunoprecipitation of CR1 demonstrated a \(M_r\) receptor similar to that found on PMN.

 Mixing experiments were performed to determine whether alterations in CR1 occurred during solubilization and purification. In these experiments, labeled cells were mixed after surface labeling with equal numbers of unlabeled cells of a different type. Combined preparations were then solubilized and CR1 purified by immuno-
Peripheral blood cells from three selected donors (D1, D2, and D3) were surface labeled, immunoprecipitated by anti-CR1, and analyzed as in Fig. 1. Autoradiographs of a single 5% nonreduced gel are shown for erythrocytes (RBC) in tracks 1-3, for PMN in tracks 4 and 5, and for mononuclear cells (MONO) in tracks 6 and 7.

Discussion

In a previous study we demonstrated polymorphism of E CR1 in normal individuals (4). In the present report, this polymorphism was shown to extend to other peripheral blood cells that possess this integral membrane glycoprotein. A phenotype was expressed on PMN, eosinophils, and mononuclear cells (B lymphocytes and monocytes) that was similar to that on E. Donors with a single major band on E had a single band on leukocytes; donors with both major bands on E had two bands on leukocytes. Since a similar phenotype was expressed on all peripheral blood leukocytes in the same donor, differences in posttranscriptional modification of CR1 molecules...
could account for the small $M_r$ differences observed in leukocytes (~5,000-dalton increase in PMN or eosinophils). Further structural studies are needed to determine whether carbohydrate or peptide differences account for $M_r$ increases in PMN or eosinophil CR1. Since CR1 on PMN has been shown to mediate absorptive endocytosis (9), it will be of considerable interest to determine whether the 5,000-dalton difference is related to this function.

Two other differences were noted between CR1 structure on E compared with PMN or mononuclear cells. First, minor bands were not found on leukocytes. In E, although minor bands are variable from individual to individual (none to 25% of the radioactivity incorporated into the receptor) they are reproducible in the same individual (4). One possibility is that they represent an acquired change in CR1 structure during the 120-d lifetime of E. However, employing differential centrifugation to separate young and old E (10-fold enhancement of reticulocytes in young cell population), no differences in minor band intensity were found on young vs. old E (unpublished data). This suggests that minor bands are not acquired but are constant once the E has entered the circulation. We favor the possibility that these minor bands represent incompletely processed precursor molecules that bind specifically to C3-Sepharose (4).

Another observation of interest is the variable autoradiographic intensity of polymorphic CR1 receptors among different cell types in unrelated heterozygous individuals (donors D1 and D4 through D7 in Table I). As noted previously, heterozygous individuals express variable ratios of the 220,000 and 190,000 bands on E but the ratio is characteristic for each individual (4). In the present study, when CR1 was examined on leukocytes of heterozygous donors, 220,000 (mononuclear)- or 225,000 (PMN)-dalton bands were more intense on autoradiographs than on E. Differences in band intensity on E among individuals and between E and leukocytes in the same individuals could be accounted for by variable surface-labeling or turnover of receptor molecules. Neither of these possibilities seems likely, however, since there would have to be large differences (up to 30-fold) in labeling efficiency or turnover of similar $M_r$ receptor molecules on E among individuals or on E compared with leukocytes in the same individuals. We feel our data are most compatible with unequal expression of the two receptor molecules among heterozygous individuals. Other examples of unequal expression in heterozygous individuals have been reported among peripheral blood cells. In individuals who are heterozygous for peptidase A (two alleles at a single locus), different quantities of each allelic product are found on leukocytes than on E.

### Table I

| Donor | E* | PMN† | MONO* |
|-------|----|------|-------|
| D1    | 0.4| 1.7  | 1.5   |
| D4    | 0.1| 1.5  | 1.2   |
| D5    | 0.4| 1.2  | 0.8   |
| D6    | 0.7| 1.6  | 1.7   |
| D7    | 3.1| ND   | 5.6   |

* Ratio of radioactivity found in 220,000-dalton divided by 190,000-dalton band. Values were determined by densitometric scanning as described in Materials and Methods for E and mononuclear cells (MONO).

† Similar ratio of 225,000-dalton divided by 195,000-dalton band in PMN.

ND, not done.
This mechanism would provide an explanation not only for the differences among cell types but also for differences on E among heterozygous donors. Although a number of other membrane receptors have been characterized, examples of structural heterogeneity among donors and cell types such as that reported for CR1 in this study have not been noted. If there are functional differences between these receptor molecules, expression of different CR1 molecules on certain cells could have important biologic consequences. Further characterization of the structural differences in the human CR1 receptor among cell types and individuals are in progress.

Summary

In these studies CR1 polymorphism previously demonstrated on erythrocytes (E) was also found on CR1-bearing peripheral blood leukocytes including polymorphonuclear (PMN), eosinophils, monocytes, and B lymphocytes. However several cell-specific differences in CR1 were found: (a) an ~5,000-dalton increase in CR1 on PMN and eosinophils, (b) unequal band intensity among heterozygotes suggests that there is preferential expression of 220,000- or 225,000-dalton receptors on leukocytes compared to E, and (c) “minor” bands, ~15,000 daltons larger than the major receptor molecule, were found on E but not on leukocytes. These observations constitute a unique example of heterogeneity of an integral membrane receptor.

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