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Immunology

Identification of an additional class of C3-binding membrane proteins of human peripheral blood leukocytes and cell lines*

(complement receptors/B cells/T cells/monocytes)

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ABSTRACT **Proteins binding the third component of complement (C3) were isolated by affinity chromatography from surface-labeled solubilized membranes of human peripheral blood cells and cell lines. The isolated molecules were subjected to NaDodSO$_4$/PAGE, and autoradiographs of these gels indicated that C3-binding proteins could be divided into three groups based on M_r: (*i*) gp200, an ≈200,000 M_r molecule previously identified as the C3b/C4b receptor or CR1; (*ii*) gp140, an ≈140,000 M_r molecule previously identified as the C3d receptor or CR2; and (*iii*) gp45–70, a heretofore unrecognized group of 45,000–70,000 M_r C3-binding molecules. The cell distribution, M_r, antigenic cross-reactivity, and specificity of gp45–70 were examined. Erythrocytes have no detectable gp45–70, but all leukocyte populations examined possess this group of molecules. On neutrophils and mononuclear phagocytes, CR1 is the predominant C3-binding glycoprotein, but gp45–70 is present on both cell populations and on macrophage and neutrophil cell lines. B plus null cells, chronic lymphocytic leukemia cells, and an Epstein–Barr virus-transformed B-cell line possess CR1, CR2, and gp45–70. On T cells and T-cell lines gp45–70 is the predominant or, in some cases, the only C3-binding protein isolated. gp45–70 is structurally characterized as a broad band or doublet with a mean M_r that is slightly different for each cell population. gp45–70 binds iC3, C3b, and C4b, but not C3d, indicating that the binding region is probably within the C3c portion of C3b. A polyclonal antibody to CR1 and monoclonal antibodies to CR1 and CR2 do not immunoprecipitate gp45–70. While gp45–70 has not been previously characterized on human cells, a C3b-binding glycoprotein of similar M_r is present on rabbit alveolar macrophages. We conclude that gp45–70 is an additional group of membrane proteins present on human leukocytes that possess ligand-binding activity for C3b.**

Several integral membrane proteins that have binding specificities for fragments of the third component of complement (3) have been isolated from human peripheral blood cells. CR1, a C3b/C4b receptor of human peripheral blood cells is an ≈200,000 M_r integral membrane glycoprotein (1, 2) and is recognized as the major C3b-binding protein on human erythrocytes (E), granulocytes (PMN, polymorphonuclear leukocytes), monocytes–macrophages, and B lymphocytes (1–4). This molecule has a number of interesting structural and regulatory features (1, 2, 5–11), including an unusual polymorphism in which there is an ≈60,000 difference in M_r among the three recognized alleles (5–7). CR2, the C3d receptor of human peripheral blood cells, is an ≈140,000 M_r integral membrane glycoprotein found on human B lymphocytes (12, 13). It is probably identical to a B-cell antigen defined by the monoclonal antibody anti-B2 (12, 14) and a C3b-binding protein purified from Raji cells (15). CR2 apparently binds to the C3d fragment within intact C3b (15).

Our laboratory has employed affinity chromatography to isolate CR1 (5, 7, 8) and CR2 (16), as well as a C3b-specific integral membrane glycoprotein from rabbit alveolar macrophages (17, 18). In our studies of CR1 and CR2 on human leukocytes, we routinely observed a third group of molecules with M_rs of 45,000 to 70,000 (gp45–70) with binding specificity for C3. This report represents our initial evaluation of these C3-binding proteins of human leukocytes, including their isolation, cell distribution, electrophoretic mobility, and specificity for C3 and its proteolytic fragments.

MATERIALS AND METHODS

Isolation of Human Peripheral Blood Cells. Normal individuals donated ≈30 ml of blood for preparation of erythrocytes (E) or up to 500 ml for preparation of leukocytes. E were washed in phosphate-buffered saline (P_i/NaCl; 10 mM potassium phosphate/150 mM NaCl, pH 7.40) and resuspended in a 10% suspension. Mononuclear and polymorphonuclear (PMN) cell populations were separated by dextran sedimentation followed by Ficoll/Hypaque centrifugation (5, 8). Cell populations enriched for T lymphocytes, B plus null lymphocytes, or monocytes were prepared and analyzed as previously described (19). Greater than 80% of the monocyte-enriched population was esterase positive. Analysis of cell surface characteristics for the enriched lymphocyte populations showed that >75% of the T-cell-enriched population formed E rosettes with only 1% possessing surface immunoglobulin, and >60% of the B plus null cells had detectable surface immunoglobulin with <5% forming E rosettes.

Cell Lines. The Raji cell line (an Epstein–Barr-virus-negative B-lymphoid line), the GM3104, GM3161, and GM3164 cell lines (all Epstein–Barr-virus-transformed B-cell lines), and the Jurkat T-cell line were grown in T-150 flasks at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal bovine serum (Sterile Systems, Logan, UT), 4 mM glutamine, and an antibiotic/antimycotic mixture (GIBCO). HSB-2 and Molt 4, both T-cell lines, were grown

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Abbreviations: C3b and C4b, high molecular weight activation fragments of the third and fourth components of complement, respectively; iC3 and iC4, hemolytically inactive components of complement in which the internal thioester bond has been cleaved; C3bi, C3b that has undergone α-chain proteolytic cleavage by C3b inactivator; C3d, an ≈35,000 M_r α-chain fragment of C3b that results from trypsin cleavage of C3dg; CR1, a 190,000–260,000 M_r glycoprotein that is a membrane receptor for C3b/C4b; CR2, a 140,000 M_r cell surface binding protein for C3d; gp45-70, 45,000–70,000 M_r cell surface binding proteins for iC3 and C3b; H, factor H; I, C3b inactivator; E, erythrocyte(s); PMN, polymorphonuclear leukocytes; CLL, chronic lymphocytic leukemia; DAF, decay accelerating factor.

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in T-150 flasks at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal bovine serum, 25 mM Hepes buffer (GIBCO), 2 mM glutamine, and the antibiotic/antimycotic mixture. U937, a monocyte–macrophage-like cell line, was grown in T-150 flasks at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 15% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, nonessential amino acids at a concentration twice that of RPMI 1640 medium, 0.8 mM oxaloacetic acid, and the antibiotic/antimycotic mixture.

Surface Labeling and Solubilization. These procedures have been described previously (5, 7, 8, 17, 18). After iodination, cells were solubilized at 4°C in P_i/NaCl containing 1% Nonidet P-40 (NP-40, Sigma), 2 mM phenylmethylsulfonyl fluoride or 5 mM dinitrophenylfluoride, 3 mM EDTA, 1 μM pepstatin, and 20 mM iodoacetamide (1 ml of buffer per 10^8 leukocytes).

Affinity Chromatography. Affinity chromatography was performed as described (8), using either (*i*) iC3- or C3b-Sepharose beads in which the ligand was coupled to the beads by using cyanogen bromide or (*ii*) C3b- or C3d-thiol-Sepharose beads. The latter were prepared after Iida *et al.* (12) with activated thiol-Sepharose, trypsin treated with L-1-tosylamide-2-phenylethyl chloromethyl ketone, and elastase (Sigma). Elutions with cysteine followed by gel analysis were performed to confirm the structures of the bound fragments. Beads having ligands coupled by a thiol linkage contained a quantity of protein similar to those coupled by cyanogen bromide (5).

To evaluate for possible proteolytic activity not prevented by the proteolytic inhibitors listed above, experiments were performed in which unlabeled solubilized membranes of PMN, mononuclear cells, or Molt 4 cells were mixed 1:1 with labeled solubilized membranes of Raji cells, peripheral blood mononuclear cells, or E. Following a 1.5-hr incubation at 37°C, affinity chromatography was performed.

Some samples were precleared of possible factor H (H) and C3b-inactivator (I) prior to affinity chromatography by means of goat anti-human H (Atlantic Antibodies, Westbrook, MA) and goat anti-human I (Cordis, Miami, FL) immobilized on staphylococcal protein A-Sepharose beads (Pharmacia). These reagents were shown to remove I and H from serum samples. K76 monocarboxylic acid, an inhibitor of I (20, 21) and a gift from W. Miyazaki (Otsuka Pharmaceutical, Tokushima, Japan), was added to some samples (2 mg/ml) prior to affinity chromatography.

Immunoprecipitation. Immunoprecipitations were performed as described (8), using polyclonal and monoclonal (R137) antibodies to CR1 and a mouse anti-B2 (anti-CR2) monoclonal antibody (12) kindly donated by V. Nussenzweig. OKM1 (Ortho Diagnostics), a mouse monoclonal antibody that recognizes CR3 (C3bi receptor), was used in a similar manner. Briefly, the solubilized membranes were incubated with the specific antibody. Preparations in which monoclonal antibodies were used were subsequently incubated with an anti-mouse Ig. The mixtures were then incubated with *Staphylococcus aureus* Cowan strain.

$NaDodSO_4$/PAGE and Autoradiography. $NaDodSO_4$/PAGE was performed as described (8) according to the method of Laemmli (22). Autoradiography was performed with XAR-5 x-ray film (Kodak) and Cronex intensification screens (DuPont).

RESULTS

Identification of C3-Binding Proteins on Human Peripheral Blood Cells. C3-binding molecules were isolated by iC3-Sepharose affinity chromatography from the solubilized membranes of E, PMN, and Ficoll/Hypaque interface mononuclear cells and analyzed by $NaDodSO_4$/PAGE and autoradiography. Eight individuals were evaluated. The results from two representative donors are shown in Fig. 1. As expected, a C3-binding molecule with a M_r of 210,000 is isolated from E. This binding protein is identified as CR1 (1–3, 23) on the basis of its M_r, precipitation by monoclonal anti-CR1, and polymorphism (5–8). The latter is illustrated in Fig. 1, tracks 1 and 4. The CR1 phenotype observed on E is also found on other peripheral blood cells (6–8). Two C3-binding molecules are reproducibly isolated from PMN. CR1 is predominant, but a protein with an M_r of 65,000–70,000 (gp45–70) is always seen (Fig. 1, tracks 2 and 5). On mononuclear cells, three C3-binding proteins are identified (Fig. 1, tracks 3 and 6). CR1 aligns with the corresponding E molecule. The second molecule has a M_r of 140,000, and is identified as CR2 by virtue of its immunoprecipitation by anti-B2 (12, 14) (see below) and affinity for C3d (12, 13) (see below and Fig. 5). The third protein migrates in a broad band in the M_r range from 52,000–68,000.

These cell populations were also evaluated with iC4 affinity chromatography. For CR1 and gp45–70, results were similar to those for iC3-Sepharose (compare tracks 2, 3, 5, and 6 with tracks 7 and 8 in Fig. 1), with the possible exception that gp45–70 of PMN does not bind as well to iC4. However, CR2 does not bind to iC4-Sepharose, compatible with experiments indicating an inability of C4b-sensitized E to adhere to Raji cells (24).

The autoradiographs in Fig. 1 were of gels run under nonreducing conditions. Under reducing conditions, the general pattern of C3-binding molecules does *not* change.

Identification of C3-Binding Proteins on Subsets of Mononuclear Cells. To determine the distribution of these C3-binding proteins among mononuclear cells, populations enriched for B plus null cells, T cells, and monocytes were examined from five donors. Autoradiographs are shown for a repre-

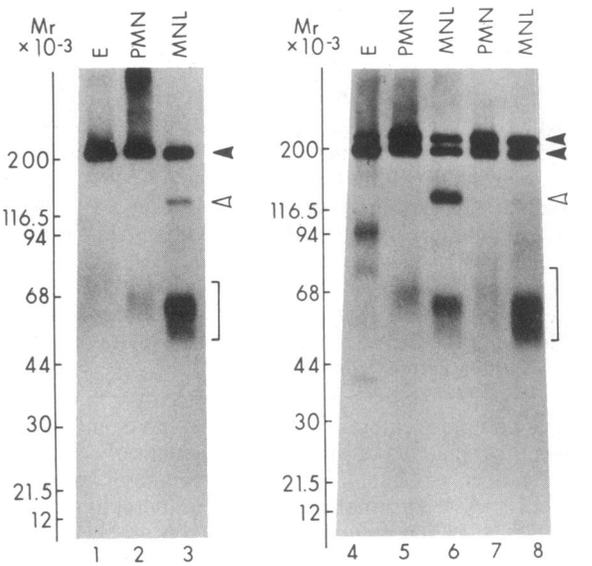

FIG. 1. Autoradiograph of C3-binding molecules isolated from human peripheral blood cell populations of two normal donors by either iC3-Sepharose (tracks 1–6) or iC4-Sepharose affinity chromatography (tracks 7 and 8). Unless otherwise noted, autoradiographs are of nonreducing $NaDodSO_4$/6–18% gradient polyacrylamide slab gels. MNL, mononuclear cells from the interface of a Ficoll/Hypaque density separation. The solid arrowheads (= CR1), open arrowheads (= CR2), and brackets (= gp45–70) are used on all autoradiographs. The band in track 4 with M_r of ≈94,000 is not routinely observed (see track 1 and refs. 5 and 7), and is also present in bovine serum albumin-Sepharose eluates. In this autoradiograph (particularly track 2) and occasionally in other autoradiographs, some tracks contain variable quantities of labeled material at the top of the gel. The nature of this material is unknown, but similar receptor patterns are obtained whether or not this material is present.

sentative donor and demonstrate that each of the enriched populations has a distinct pattern of C3-binding molecules (Fig. 2, tracks 5–7). The B plus null fraction has prominent CR1 and CR2, compatible with previous studies of this population (3, 4). In addition, gp45–70 appears as a doublet with mean M_rs of 47,000 and 55,000. In the monocyte-enriched population, CR1 is the major C3-binding molecule. gp45–70 is a broad band with a mean M_r of 59,000. CR2 is not present.

The predominant C3-binding protein of the T cells is represented by two distinct bands of approximately equal intensity with mean M_rs of 52,000 and 57,000. Bands corresponding to CR1 and CR2 are also present. These could represent contaminating B plus null cells in the T-cell-enriched population; however, CR1 has been shown to be present on a subset of T cells (23) and, as will be shown below, CR1 and CR2 are present on T-cell lines. Additional examples of enriched T-cell populations are in Fig. 4 (track 1) and Fig. 5 *Left* (tracks 1–3).

Variables Affecting Affinity Chromatography. In addition to employing a variety of affinity chromatography ligands to address the specificity of these C3-binding proteins (see below), we performed experiments for each cell type to determine if differences in autoradiographic patterns were due to variable binding to, or elution from, the affinity column (5, 7, 8). These studies indicated that >85% of the labeled material in CR1, CR2, and gp45–70 bound to the affinity column and that >80% of this material was eluted by 400 mM NaCl. The first point was established by incubating the solubilized preparation with iC3-Sepharose a second time or with an antiserum to CR1 or CR2. For the second point, elution of the column with 6 M guanidine after an initial high-salt elution led to the recovery of 5–20% as much material as was eluted originally.

An important factor was the use of a low ionic strength (50 mM NaCl) buffer during the incubation of the solubilized cell preparation with the iC3-Sepharose. This led to the recovery of ≈3 times as much specifically bound material for each of the three labeled molecules as compared to a 150 mM NaCl buffer. Others have noted that CR1 has higher affinity for C3b at low ionic strength (25). Incubation temperature (4°, 22°, and 37°C) and freezing and thawing of the solubilized preparations did not affect the results.

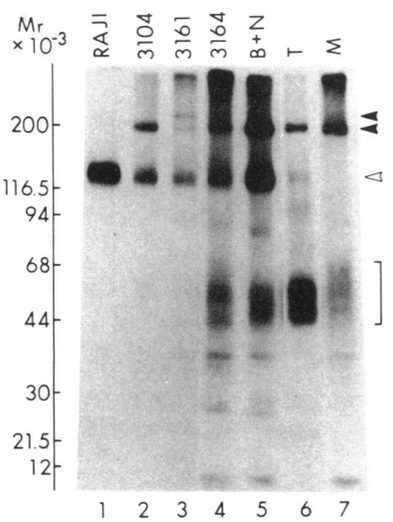

FIG. 2. Autoradiograph of C3-binding molecules isolated from B-cell lines (tracks 1–4) and from enriched peripheral blood mononuclear cell populations (tracks 5–7). The faint bands (<2% of total radioactivity in this intentionally overexposed gel) with M_rs of 40,000 or less are nonspecific, as they were also seen in eluates of bovine serum albumin- and IgG-Sepharose that were run in parallel.

We considered the possibility that H, I, or both were present in the solubilized membranes and converting the iC3-Sepharose to C3bi- or C3d-Sepharose. However, neither clearance of possible H and I from samples nor addition of K76 monocarboxylic acid affected the results. Also, analysis of the C3b eluted from the thiol-Sepharose after affinity chromatography demonstrated only the intact α and β chains of C3. In addition, OKM1, a mouse monoclonal antibody that recognizes the C3bi receptor (CR3) (26) does not precipitate gp45–70 (not shown).

Another consideration was that, despite multiple protease inhibitors in the solubilizing buffer, enzymes in the leukocyte preparations were cleaving CR1 or CR2, resulting in a lower M_r molecule possessing a C3-binding site. To address this question, a variety of unlabeled solubilized cell preparations, including PMN (with CR1 and gp45–70), mononuclear cells (with CR1, CR2, and gp45–70), and Molt 4 cells (with CR2 and gp45–70) (see below) were incubated with the labeled solubilized membranes of Raji cells (with CR2 only), leukocytes, or E (with CR1 only). Subsequent affinity chromatography revealed no alterations in the band patterns (not shown), thus demonstrating an apparent lack of proteolytic activity in the unlabeled preparations and thus no evidence for cleavage of labeled CR1 or CR2 to form gp45–70.

Identification of C3-Binding Proteins of Chronic Lymphocytic Leukemia (CLL) Cells and Human T-, B-, and Monocyte Cell Lines. The previous mononuclear populations were only enriched for the given cell type. Therefore, the less intense bands could be derived from contaminating cell populations. To further establish the pattern for each cell population, C3-binding proteins were isolated from patients with CLL and from four B- and three T-cell lines.

C3-binding proteins were isolated from peripheral blood mononuclear cells of three patients with CLL. All three patients' cells possessed a similar pattern. Fig. 3 is an autoradiograph comparing one patient's peripheral blood and bone marrow C3-binding proteins with those of a normal donor's enriched B- plus null-cell population. These results indicate that CR1, CR2, and gp45–70 are present on these monoclonal B cells. Moreover, each aligns with its counterpart from normal human peripheral blood B plus null cells.

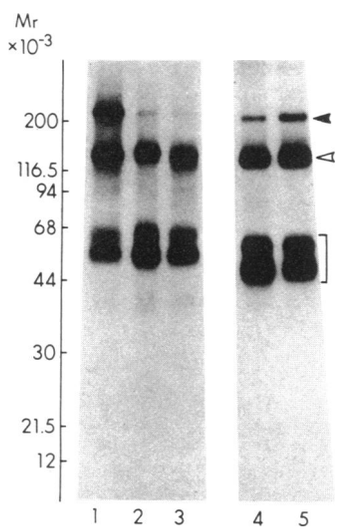

FIG. 3. Autoradiograph of C3-binding molecules isolated from the lymphocytes of a patient with chronic lymphocytic leukemia. The cell populations from which the C3b-binding molecules were isolated contained >95% B lymphocytes as determined by morphology and Ig staining. Samples from peripheral blood (tracks 2 and 4) and bone marrow (tracks 3 and 5) were run under reducing (tracks 2 and 3) and nonreducing (tracks 4 and 5) conditions. For comparison, C3-binding molecules were isolated from B plus null cells of a normal individual (track 1).

As previously reported by Barel *et al.* (15), Raji cells have a single C3-binding protein, CR2 (Fig. 2, track 1). The GM3104 and GM3161 B lines possess CR1 and CR2 but lack gp45–70 (Fig. 2, tracks 2 and 3). In the GM3164 B line, all three species of C3-binding proteins are present (Fig. 2, track 4), and these cells therefore resemble normal peripheral blood B plus null cells (track 5).

A T-cell population from a normal individual, three T-cell lines, and a monocyte-like cell line (U937) are compared in Fig. 4. gp45–70 is the predominant C3-binding protein isolated from these cell populations. There is heterogeneity of M_r among gp45–70. Molt 4 possesses a distinct doublet with mean M_rs of 48,000 and 62,000. On HSB-2 cells, gp45–70 migrates as a single but diffuse band with a M_r of 62,000 that aligns with the upper band of Molt 4. The Jurkat line possesses a C3-binding protein with a mean M_r of 55,000. CR2 is present on two of the three T-cell lines. It is similar in M_r to CR2 of Raji and B cells (compare to Figs. 2 and 3) and is immunoprecipitated by anti-B2 (not shown) (12). U937 resembles peripheral blood mononuclear phagocytes in that it possesses CR1 and gp45–70 but no CR2. On the U937 line there is more gp45–70 relative to CR1 (compare Fig. 4, track 5, with Fig. 2, track 7).

Specificity of the C3-Binding Proteins. With each purification, bovine serum albumin- or IgG-Sepharose columns were run in parallel with iC3-Sepharose. Molecules corresponding in M_r to CR1, CR2, and gp45–70 were not isolated. C3b-Sepharose, iC3-Sepharose, and C3b-thiol-Sepharose columns gave identical results for each cell type examined (E, PMN, mononuclear cells, B plus null cells, T cells, U937, and HSB-2). As noted, iC4 affinity columns also produced similar results compared to iC3- or C3b-Sepharose for CR1 and gp45–70 (Fig. 1) but did not isolate CR2. These data suggest that iC3 and C3b are functionally similar as ligands and that identical results are obtained whether C3b is coupled to Sepharose by cyanogen bromide activation or by a thiol group. The specificity of gp45–70 was further addressed by comparing the binding of these molecules to iC3-Sepharose and C3d-thiol-Sepharose affinity columns (Fig. 5). In none of the cell types examined did gp45–70 bind to the C3d affinity column, indicating that the binding site is probably in the C3c portion of C3b.

For each cell population, immunoprecipitation with monoclonal or polyclonal anti-CR1 and monoclonal anti-CR2 (anti-B2) was performed and compared to affinity column-derived material. In all cases the corresponding molecules obtained by either isolation technique aligned by gel analysis. The anti-CR1 and anti-CR2 antibodies did not recognize gp45–70 (not shown). These immunoprecipitations were performed on both the complete solubilized membrane preparations and on C3-binding proteins previously isolated by affinity chromatography.

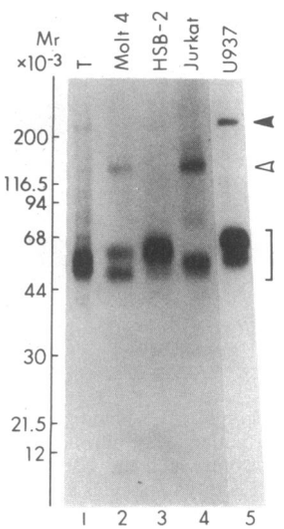

FIG. 4. Autoradiograph of C3-binding molecules isolated from an enriched T-cell population from a normal donor (track 1), three T-cell lines (tracks 2–4), and a monocyte-like cell line (track 5).

DISCUSSION

These studies were initiated because of the difference in M_r between the 64,000 that we reported for the rabbit alveolar macrophage C3b-binding protein (17, 18) and the 205,000 reported for the human C3b/C4b receptor (1–4). We first evaluated human E with the same experimental protocol that we had employed to isolate the rabbit macrophage molecule. In so doing, we confirmed the M_r of E CR1 as reported by others (2, 10, 27), but we also described three polymorphic variants and several additional interesting structural and regulatory features (5, 7, 8). However, in employing affinity chromatography to isolate C3-binding proteins from leukocytes, molecules with M_r of ≈140,000 and 45,000–70,000 were observed in addition to CR1.

As our work was in progress (16), Iida *et al.* (12) reported that the 140,000 M_r molecule was in all likelihood CR2. They identified a previously described B-cell-specific antigen of ≈140,000 M_r (14), recognized by a monoclonal antibody (anti-B2), as a receptor for C3d. Relative affinities of this molecule for C3b and C3d have not been reported, but the distribution of this molecule on peripheral blood cells and other data support the assertion that the ligand for the 140,000 M_r molecule is the C3d fragment of C3b. We (this report) and others (13) have confirmed their result. In addition, we have provided evidence that CR2, although present in small quantities, can be isolated reproducibly from enriched T-cell populations. Although this result may be secondary to contaminating B cells, the molecule is found as well on two (Molt 4 and Jurkat) of three T-cell lines evaluated. Taken together, these data suggest that some T cells express CR2.

The major observation in this report relates to the identification on human leukocytes of C3-binding proteins with M_rs

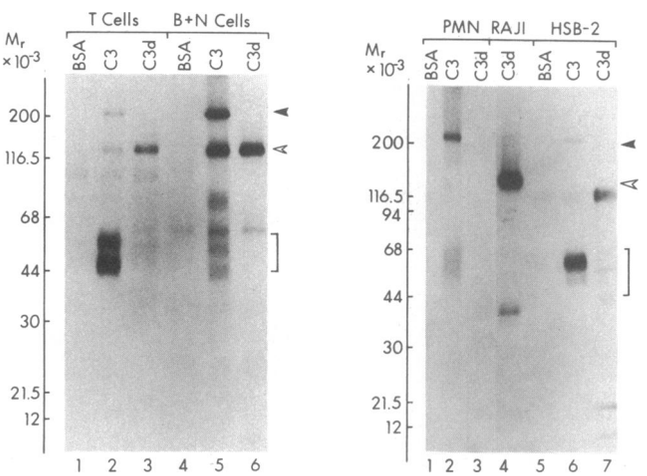

FIG. 5. Autoradiographs of C3-binding molecules isolated by bovine serum albumin- and C3-Sepharose and C3d-thiol-Sepharose. The few bands present that do not align with CR1, CR2, or gp45–70 for the most part bind to albumin- as well as iC3-Sepharose or C3d-thiol-Sepharose. The bands with a M_r of 110,000 (*Left*, tracks 1–4, and *Right*, tracks 5–7) and 62,000 (*Left*, tracks 4–6) are examples. The band with a M_r of 70,000 in *Left* (track 5) and the band with a M_r of 40,000 in *Right* (track 4) are variable (see Fig. 2, track 1, for comparison).

of 45,000 to 70,000. The gp45–70 molecules bind iC3 and C3b but not C3d. Though gp45–70 is structurally heterogeneous among cell types, the pattern and M_r of the molecules isolated from each cell type is reproducible. On nonreduced gels gp45–70 is present on PMN (a broad band with a mean M_r of 68,000), monocytes (a broad band with a mean M_r of 59,000), B lymphocytes (a doublet with M_rs of 47,000 and 55,000), and T lymphocytes (a doublet with M_rs of 52,000 and 57,000). In addition, there is heterogeneity of the molecule among various T cell lines (Fig. 4).

Other than similar but not identical M_rs and isolation based on C3b- but not C3d-binding activity, we have no definitive evidence that these molecules are related. However, there is precedence for heterogeneity of M_r among related receptors. For example, Fcγ receptors of different human cell populations (28), CR1 on human peripheral blood cells (5–8), and the receptor for the T-cell growth factor of peripheral blood T cells and the HUT-102B2 T-cell line (29) all demonstrate such heterogeneity. In addition, the specificity of our affinity chromatography system for C3-binding proteins is supported by the fact that two of the three molecules isolated are recognized receptor proteins for C3b (1–4) and C3d (12–15). Finally, a C3b-binding protein on rabbit alveolar macrophages has a M_r similar to that of these molecules (17, 18).

Except for the T cells and T-cell lines, gp45–70 is not the major C3-binding protein of these cell populations. Most T cells have a limited ability to form rosettes with C3b-coated particles and therefore have been considered to be C3b receptor negative. Further, since polyclonal antibodies to CR1 inhibit rosette formation between C3b-coated E, PMN, monocytes and B-lymphocytes (2–4), our demonstration of an "additional" C3b-binding protein on these cell populations requires comment. First, we have purposely identified these molecules as C3-binding proteins, since we have provided no evidence that these molecules function as receptors on the cell surfaces of leukocytes. We have shown, though, that surface-labeled molecules with M_rs of 45,000 to 70,000 can be specifically and reproducibly isolated from the solubilized membranes of these cell populations. Second, the binding of complement-coated particles, such as rosette formation with C3b-coated erythrocytes, may require a specific receptor spatial relationship, density, or mobility, while the binding of soluble ligands may not be so restricted. Third, since the evidence suggests that this molecule is not responsible for rosette formation, gp45–70 may be more analogous to decay accelerating factor (DAF). DAF is an integral membrane glycoprotein that is thought to modulate complement activation by inhibiting the classical (C4bC2a) and alternative pathway (C3bBb) convertases (30). DAF has greater inhibitory activity for C4bC2a than for C3bBb, while gp45–70 binds C3b more than C4b. These two proteins may be widely distributed on human cells where they could serve to control complement activation. Fourth, its electrophoretic mobility, lack of antigenic cross-reactivity, ligand specificity for C3b, and cell distribution indicate that gp45–70 is not a proteolytic fragment of CR1, CR2, or CR3. Moreover, it is not DAF, since gp45–70 is not present on E or immunoprecipitated by anti-DAF (unpublished data). In addition, DAF is not isolated by C3b or C4b affinity chromatography. Taken together, the evidence strongly points to gp45–70 being an additional C3b-binding protein of human cells.

Further studies are required to more definitively address both the function of this molecule and the heterogeneity of its M_r. As demonstrated in this report, though, gp45–70 can be surfaced labeled and specifically isolated by C3 affinity chromatography, and thus additional functional and structural studies can be performed.

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