IDENTIFICATION OF THE MEMBRANE RECEPTOR FOR THE COMPLEMENT FRAGMENT C3d BY MEANS OF A MONOCLONAL ANTIBODY*

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Several cleavage products of complement component C3 interact with cellular membrane receptors and are associated with important biological activities. The larger fragment of activated C3 (C3b), which binds covalently to surface membranes or antigen-antibody complexes, is a ligand for complement receptor type 1 (CR1),[1] a glycoprotein of ~200,000 M_r found on erythrocytes, phagocytes, lymphocytes and other cells (reviewed in reference 1). CR1 has the unusual property of serving as a cofactor for the further fragmentation of bound C3b by a serum enzyme, C3b-inactivator (I) (2–4). The serum factor β1H (H) can also serve as a cofactor for the reaction between I and covalently bound C3b, but it is much less effective than CR1 on a molar basis (5).

The initial cleavage reaction of C3b releases a peptide of 3,000 M_r from its α' chain (6). The remaining fragment (iC3b) has greatest binding affinity for membrane receptor CR3 (7), which is found on phagocytes and natural killer cells. It has been suggested that CR3 consists of two polypeptides of 185,000 and 95,000 M_r (8, 9). Additional cleavages of iC3b by CR1 plus I generate C3c (140,000 M_r), which is released into the fluid phase, and C3dg (41,000 M_r) (2–4, 10). The bound C3dg fragment can be further cleaved in vitro by plasmin, elastase, and other proteases, into a fragment called C3d (~35,000 M_r) (10). Indicator particles bearing C3d can attach to and form rosettes with a subpopulation of B lymphocytes (11, 12) and with monocytes after their cultivation on glass surfaces (13). The membrane molecules that mediate this interaction are operationally defined as CR2. Some studies indicate that, in addition to C3d, CR2 also binds iC3b and C3dg, but the relative affinities of these multiple ligands for the receptor have not been measured (7). The purpose of this paper is to present evidence that CR2 is a glycoprotein of 140,000 M_r called B2 that had

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; C, complement; CR1, CR2, CR3, complement receptors for the C3 fragments C3b, C3d, and iC3b; DNP, dinitrophenyl; E, sheep erythrocytes; EAC, sheep erythrocytes coated with antibody and complement components (subscripts indicate number of hemolytic sites); EBV, Epstein-Barr virus; H, β1H; I, C3b inactivator; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; pI, isoelectric point; PMSF, phenylmethylsulfonyl fluoride; SBTI, soybean trypsin inhibitor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

been previously identified by Nadler et al. (14) and Bhan et al. (15) on the surface of B lymphocytes by means of a monoclonal antibody.

Materials and Methods

Materials. Sephadex, CM-Sephadex, Sepharose CL6B, cyanogen bromide (CNBr)-activated Sepharose 4B, Thiol-Sepharose, Ficoll-Paque, and low molecular weight markers were from Pharmacia Fine Chemicals, Piscataway, NJ. DE-52 was from Whatman Ltd., Maidstone, Kent, England. TPCK-trypsin and porcine elastase were from Worthington Biochemical Corp., Freehold, NJ. Soybean trypsin inhibitor (SBTI) was from Sigma Chemical Co., St. Louis, MO. The porcine elastase inhibitor Ac-Ala-Ala-Pro-Ala-CH_2Cl was from Enzyme Systems Products, Livermore, CA, and the neutrophyl elastase inhibitor Suc(OMe)-Ala-Ala-Pro-Val-MCA was from Peninsula Laboratories, Inc., Belmont, CA. RPMI 1640 medium and antibiotics (Pen-Strep) were from Gibco Laboratories, Grand Island, NY. Nonidet P-40 (NP-40) was from Particle Data, Inc., Elmhurst, IL. X-omat R-film XR-5 was from Eastman Kodak, Inc., Rochester, NY. Ampholines were from LKB Instruments, Inc., Gaithersburg, MD. Iodogen was from Pierce Chemical Co., Rockford, IL.

Antibodies, Complement Components, and Fragments. The monoclonal antibody anti-B2 (14) was purified from ascites by filtration on Sepharose CL6B. The first peak (IgM) contained the antibody activity. Upon sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, the only visible contaminant on the Coomassie blue-stained gels was α2-macroglobulin (<5% of the total protein). A monoclonal antibody (IgM) antidinitrophenyl (DNP) was used as a control. It was purified to homogeneity from ascites by affinity chromatography on CNBr-activated Sepharose beads bearing a DNP-bovine serum albumin conjugate (16). The antibodies were eluted in phosphate-buffered saline (PBS) containing 0.1 M DNP, filtered through a Sephadex G-25 column, and dialyzed to remove the free hapten. Four monoclonal antibodies to CR1 were purified to homogeneity (17). A polyclonal antiserum to mouse Ig was prepared by injecting rabbits with a mixture of mouse monoclonal antibodies of different isotypes. The IgG fraction of this antiserum was prepared by chromatography on DE-52, and the $F(ab')_2$ fragments obtained as described (18). Polyclonal, affinity-purified rabbit antibody to mouse IgM was isolated from this IgG fraction by filtration through CNBr-activated Sepharose 4B, coupled to a purified monoclonal antibody of the IgM isotype (anti-DNP), and elution with 3 M potassium thiocyanate. To remove KSCN, the eluate was filtered through a Sephadex G-25 column equilibrated in PBS. IgM anti-sheep erythrocytes were from Cordis Laboratories Inc., Miami, FL, and fluorescein-conjugated goat anti-mouse IgM was from Coulter Electronics, Inc., Hialeah, FL. Guinea pig Cl (19), human C2, C3, and C4, plasminogen (20), H (21), and I (22) were prepared according to published procedures. Human C2 was further purified by chromatography on CM-Sephadex C50 and DE-52 and used in the oxidized form (^{ox}C2) (23).

Sepharose Beads Coupled with Antibodies or C3 Fragments. 5 mg of each monoclonal antibody was coupled to 1 ml of CNBr-activated Sepharose 4B according to the manufacturer's instructions. Thiol-Sepharose-C3d was prepared as follows: 20 mg of C3 were incubated at 37°C for 5 min with 20 μg of TPCK trypsin in the presence of 1 g of Thiol-Sepharose beads in 0.1 M Tris buffer, pH 8.0, containing 0.1 M NaCl. 1 mM phenylmethylsulfonyl fluoride (PMSF) was added to stop the reaction. 3 ml of washed beads bearing 15 mg of C3b were then incubated twice in succession with 300 μg of porcine elastase at 37°C for 5 h. The reaction was terminated by the addition of the porcine elastase inhibitor, Ac-Ala-Ala-Pro-Ala-CH2Cl. The products of elastase digestion were examined by SDS-PAGE under nonreducing conditions. The eluate obtained from the Thiol-Sepharose-C3d beads by treatment with 0.05 M 1-cysteine in 0.01 M Tris buffer, pH 8.0, and the supernatant of elastase digestion, purified by gel filtration on Sephadex G-150, showed major bands of 35,000 and 140,000 M_r, respectively (Fig. 1).

Human Cells. Tonsil cells were suspended in RPMI 1640 containing 10 mM Hepes, 0.02% $NaHCO_3$ and 1% Pen-Strep, pH 7.4. Most of the contaminating erythrocytes and

FIGURE 1. SDS-PAGE of C3 fragments. C3 (15 μg; lane 1), C3c (15 μg; lane 2), and C3d (18 μg; lane 3) were subjected to electrophoresis in a 7.5% polyacrylamide gel under nonreducing conditions and stained with Coomassie blue.

neutrophils were removed by overlaying the tonsil cells onto Ficoll-Paque and centrifuging at 400 *g* for 30 min at room temperature. The mononuclear cells that accumulated at the interface were collected and washed several times by centrifugation in RPMI medium. Their viability was >95% as determined by trypan blue exclusion.

Epstein-Barr virus (EBV)-transformed B lymphoblastoid lines (Laz 388, Lazγ471, and Lazγ475), Burkitt's lymphoma lines (Daudi, Ramos, and Raji), and chronic myelocytic leukemia line NALM-1 were kindly provided by Dr. Herbert Lazarus, Dana-Farber Cancer Institute; Burkitt's lines Namalva and Wilson were provided by Dr. Ian McGrath, National Cancer Institute.

Surface Labeling of Lymphocytes and Preparation of Extracts. 4×10^7 tonsil lymphocytes or Raji cells were washed three times in PBS, resuspended in 100 μl PBS, and incubated with 1 mCi of ^{125}I in Iodogen-coated tubes for 15 min at 0°C. Free iodine was removed from the cells by centrifugation through fetal calf serum. After additional washings by centrifugation in PBS, the cells were incubated in 100 μl of 0.5% NP-40 in PBS containing 1 mM PMSF and 50 μg/ml of the synthetic elastase inhibitor, Suc(OMe)-Ala-Ala-Pro-Val-MCA. After incubation for 30 min at room temperature, 400 μl of PBS containing the same protease inhibitors was added and the mixture was centrifuged to remove insoluble materials. The supernatant was dialyzed against PBS, further diluted to 1 ml with distilled water, and incubated with 0.5 ml of packed Sepharose CL6B for 30 min at room temperature, to remove materials that adhere nonspecifically to the beads. The supernatant contained $\sim 2 \times 10^7$ cpm, >95% of which could be precipitated in 10% trichloroacetic acid.

Affinity Chromatography of Membrane Proteins

Chromatography on Sepharose C3. This was performed as described (24). Briefly, the NP-40 extracts were incubated for 1 h at room temperature with 0.5 ml of Sepharose C3. The beads were kept in suspension by rotation of the tube. Then the beads were packed in a small column, washed with 40 ml of 0.065 M borate buffer, pH 8.0, containing 0.05 M NaCl, 0.1% NP-40, and eluted with 3×300 μl of 0.01 M acetate buffer, pH 4.1, containing 0.5 M NaCl, 0.1% NP-40, and 1 mM PMSF. The eluates containing radioactive materials were pooled, supplemented with 0.2 mg/ml of bovine serum albumin (BSA), and dialyzed against PBS with 0.1% NP-40.

Chromatography on Sepharose-Anti-B2. This was performed as described above, except that the packed beads were washed four times with 2 ml of 0.1 M Tris, pH 8.0, containing 0.3 M NaCl, 0.1% NP-40, and 1 mg/ml BSA, and that the elution was performed with 3 M KSCN. The eluate was then passed through a small column of Sephadex G25 equilibrated in PBS, 0.1% NP-40, and 0.2 mg/ml BSA to remove KSCN, and then dialyzed against PBS-0.1% NP-40.

Rosette Formation. Sheep erythrocytes (E) were sensitized with rabbit IgM anti-E (A) and reacted sequentially with purified complement components C1, C4, and ^{oxy}C2 at concentrations sufficient to give 300 hemolytic sites of each component per erythrocyte. EAC14^{oxy}2 were then incubated with various amounts of C3 in order to generate the EAC14^{oxy}23b intermediates bearing either 1.5 or 10 C3 sites. The EAC14^{oxy}23b cells

were converted to EAC14^{oxy}23bi by incubation for 1 h at 37°C with purified H and I at final concentrations of 5 and 1 μg/ml, respectively. EAC14^{oxy}23bi cells were incubated with 5 μg/ml of plasmin for 30 min at 37°C to generate EAC14^{oxy}23d. Less than 0.1 hemolytic sites per cell remained on this intermediate. For rosette formation, lymphocytes (10^6/ml were mixed with EAC14^{oxy}23d (5×10^7/ml) in RPMI medium containing 10% fetal calf serum and 1 mg/ml SBTI. The tubes were rotated for 30 min at 37°C. In some experiments lymphocytes were first incubated with antibody to cell surface components for 30 min at 37°C before the addition of the erythrocyte intermediates. Rosettes were counted under the microscope as described (25).

SDS-PAGE. This was performed according to Laemmli (26). Human C4, a mouse monoclonal antibody of the IgG1 isotype, and BSA were used as molecular weight markers when the electrophoresis was performed under nonreducing conditions. After electrophoresis, the gels were stained with Coomassie blue. Radioautography was carried out at −70°C using Kodak XR-5 film.

Two-dimensional Electrophoresis. This was carried out by use of a modified O'Farrell technique (27). Electrofocusing was performed in the first dimension. A mixture of ampholines of pH 3–10, 6–8, and 9–11 was used to obtain the desired pH range. After the first dimension run, one strip of the slab gel was cut into 0.5-cm segments, and these were eluted with 2 ml degassed, distilled water overnight at room temperature with continuous agitation. The eluate was counted in a gamma counter and its pH measured. Other strips were run in the second dimension (SDS-PAGE), and the slab gels were subjected to radioautography.

Cell Surface Phenotype Determination by Indirect Immunofluorescence. $1–2 \times 10^6$ cells were treated with 0.1 ml of a 1:100 dilution of anti-B2 or a 1:100 dilution of an isotype-identical nonreactive monoclonal antibody, incubated at 4°C for 30 min, and washed three times by centrifugation. These cells were then reacted with 0.1 ml of a 1:60 dilution of fluorescein-conjugated goat anti-mouse IgM, incubated at 4°C for 30 min, washed three times, and analyzed on an Epics V cell sorter (Coulter Electronic Inc.). For each sample, a quantitative assessment of the number of positive cells was made, i.e., the number of cells reactive with anti-B2 minus the number of cells reactive with the control antibody, divided by the 10,000 cells tested.

Results

Isolation from the Lymphocyte Membrane of Molecules with Binding Affinity for C3 as well as for Anti-B2. Extracts from ^{125}I-labeled membranes of Raji cells and tonsil lymphocytes were subjected to affinity chromatography on Sepharose C3 and analyzed by SDS-PAGE and radioautography. Both eluates contained a band of 120,000 M_r under nonreducing conditions (Fig. 2) and 140,000 M_r under reducing conditions (not shown). An additional band of 200,000 M_r was seen only in the eluate from tonsil lymphocytes. The band of 200,000 M_r was identified as CR1 since it was specifically removed by treatment of the extracts with Sepharose beads coupled to monoclonal antibodies to CR1. It appears therefore that a C3b-like site, with binding affinity for CR1, was exposed on C3 after its coupling to Sepharose beads.

When the same labeled membrane extracts were subjected to affinity purification on Sepharose-anti-B2, the SDS-PAGE radioautography of the eluates showed a single band with 120,000 M_r under nonreducing conditions (Fig. 2) and 140,000 under reducing conditions.

The radiolabeled molecules of 140,000 M_r eluted from either Sepharose C3 or Sepharose-anti-B2 were further analyzed by two-dimensional gel electrophoresis. The results shown in Fig. 3 demonstrate that all molecules of 140,000 M_r isolated from tonsil lymphocytes or from Raji cells have the same isoelectric point

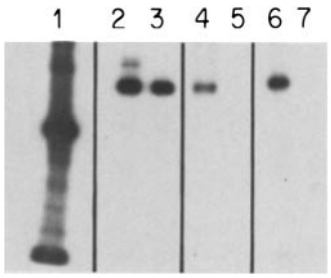

FIGURE 2. SDS-PAGE and radioautography of polypeptides isolated from surface-labeled lymphocyte extracts by affinity chromatography. Tonsil lymphocytes were surface labeled, solubilized, and the extracts pretreated with Sepharose CL6B as described in Materials and Methods. The extracts (lane 1) were subjected to affinity chromatography on Sepharose C3. The eluate (lane 2) containing the polypeptides of 200,000 and 140,000 M_r was absorbed with Sepharose-anti-CR1 (lane 3). The extracts were also incubated with 20 μl of either Sepharose-anti-B2 or with Sepharose-anti-DNP for 1 h at room temperature with continuous agitation, in 300 μl of PBS containing 0.1% NP-40 and 0.2 mg/ml BSA, pH 7.4. The anti-B2 and anti-DNP beads were then extensively washed, eluted with SDS-PAGE sample buffer, and the eluates subjected to electrophoresis in 5–10% gradient gels, under reducing (lanes 6, 7) or nonreducing conditions (lanes 4, 5). Lanes 4 and 6 contain the eluates from Sepharose-anti-B2 and lanes 5 and 7 the control eluates from Sepharose-anti-DNP. The SDS-PAGE of lanes 1–3 was performed in 5–10% gradient gels under nonreducing conditions.

(pI) of 8.2 under denaturing conditions, and give superimposable spots on two-dimensional gels.

Next, we studied the interaction between the molecule of 140,000 M_r and C3 fragments. Extracts from ^{125}I-surface-labeled lymphocytes were prepared, absorbed with Sepharose-anti-CR1 to remove the polypeptide of 200,000 M_r, and subjected to affinity chromatography on Sepharose C3. Samples of the eluate were incubated for 1 h at room temperature with 20 μl of Sepharose beads bearing C3c, C3d, anti-B2, or anti-DNP. The beads were washed extensively and counted in a gamma counter. The results, summarized in Table I, show that the polypeptide of 140,000 M_r has binding affinity for C3d and for anti-B2, since 46% of the counts bound to Thiol-Sepharose-C3d and 34% to Sepharose-anti-B2. The binding to Sepharose C3c and to the control beads bearing Sepharose anti-DNP was not significantly different (15–17%). As expected, the binding to Sepharose-anti-B2 was reduced from 34 to 16% in the presence of antibodies to B2 in the fluid phase. It should be pointed out, however, that the fluid-phase anti-B2 antibodies had little effect on the interaction between the molecules of 140,000 M_r and Thiol-Sepharose-C3d, suggesting that the epitope reacting with anti-B2 is not the binding site for C3d. The specificity of interaction between Thiol-Sepharose-C3d and the 140,000 M_r molecules was further demonstrated by incubating the washed beads with 1 ml of 0.05 M cysteine in 0.1 M Tris pH 8.0. 46% of the counts associated with the beads were released. The eluate was concentrated and analyzed by SDS-PAGE and radioautography. Only two bands, C3d (35,000 M_r) and carrier BSA, were apparent in the stained gel. The radioautography showed a single labeled band of 140,000 M_r (not shown).

Correlation Between the Expression of B2 Antigen and C3d Receptor. Several cell lines were analyzed simultaneously, both for expression of C3d receptor as assessed by rosette formation, and for the presence of B2 antigen, as determined

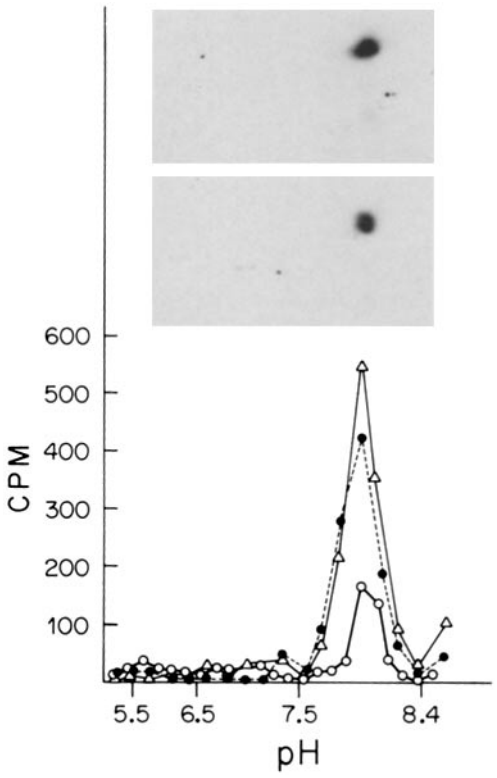

FIGURE 3. Comparison by two-dimensional electrophoresis of the 140,000 M_r molecules with binding affinity for C3 or for anti-B2. Extracts of surface-labeled Raji cells and tonsil lymphocytes were affinity purified either on Sepharose C3 or Sepharose-anti-B2 and subjected to electrofocusing under reducing conditions in slab gels. After focusing, strips of the gel containing each preparation were cut into segments and extracted with water. The graph shows results of the measurement of radioactivity and pH of the eluates obtained from strips containing Raji cell extracts purified on Sepharose C3 (△); Raji cells purified on Sepharose-anti-B2 (●); and tonsil lymphocyte extracts purified on Sepharose C3 (○). The tonsil extracts had been preabsorbed with Sepharose-anti-CR1 to remove the contaminating CR1. Separate strips containing the focused materials were subjected to SDS-PAGE and radioautography. The radioautographs of the Sepharose C3 and Sepharose-anti-B2 affinity-purified Raji cell extracts are shown in the *top* and *middle* panels, respectively. The results show that the membrane B2 antigen and the membrane protein with binding affinity for C3 have the same M_r and pI.

by indirect immunofluorescence. The results shown in Table II indicate that the proportion of cells bearing these two phenotypic markers in the different cell lines is very similar.

Effect of Anti-B2 on Rosette Formation Between Lymphocytes and EAC14^{oxy}23d. Anti-B2, but not anti-CR1, inhibited rosette formation between tonsil lymphocytes and EAC14^{oxy}23d in a dose-dependent fashion (Table III). Although relatively high antibody concentrations were used, the inhibition was not complete. The effect, however, appears to be specific, since only anti-CR1, but not anti-B2, inhibited rosette formation between the lymphocytes and EAC14^{oxy}23b.

The results of the treatment of Raji cells with anti-B2 alone were variable. Sometimes a small but significant inhibition of rosette formation with

TABLE I

Binding of the 140,000 M_r Polypeptide to Immobilized C3 Fragments or Anti-B2

Beads*	Antibody in the fluid phase‡	140,000 M_r protein	
		Input	Bound§
		cpm	*cpm* (%)
S-anti-B2	None	4,000	1,371 (34.3)
S-anti-B2	Anti-B2	3,900	619 (15.8)
S-anti-DNP	None	4,100	639 (15.5)
S-C3	None	3,000	1,005 (33.5)
S-C3c	None	3,000	510 (17.0)
T-C3d	None	3,600	1,658 (46.0)
T-C3d	Anti-B2	3,500	1,387 (39.6)
T	None	3,700	294 (7.9)

* S, CNBr-activated Sepharose beads; T, Thiol-Sepharose beads.

‡ Final concentration of 330 μg/ml.

§ Sepharose C3, Sepharose C3c, and Thiol-Sepharose-C3d had 1.5×10^{16} molecules bound per ml of packed beads; Sepharose-anti-B2 and Sepharose-anti-DNP had 3×10^{15} molecules of IgM bound per ml of packed beads. 40 μl of 50% suspensions of beads pretreated with 5 mM of diisopropylfluorophosphate were centrifuged in a Beckman microfuge tube. The Sepharose C3–affinity-purified, anti-CRI–absorbed, ^{125}I-labeled tonsil lymphocyte extracts were diluted and added to the packed beads. The incubation with continuous agitation was performed at room temperature in 300 μl of phosphate buffer containing 0.07 M NaCl, 0.1% NP-40, 1 mg/ml SBTI, 100 μg/ml of the elastase inhibitor, Ac-Ala-Ala-Pro-Ala-CH_2Cl, and 0.2 mg/ml of BSA at pH 7.4.

TABLE II

Correlation Between the Expression of B2 Antigen and Rosette Formation with EAC14^{oxy}23d

Cell lines	Origin of cell lines	B2 antigen*	Rosette formation with EAC14^{oxy}23d$_{10}$
		%	
Raji	Burkitt lymphoma	78	86
Daudi	Burkitt lymphoma	50	59
Laz 388	EBV-transformed	85	77
Laz 471	EBV-transformed	15	20
Laz 475	EBV-transformed	0	1
Namalwa	Burkitt lymphoma	7	10
Ramos	Burkitt lymphoma	5	0
Wilson	Burkitt lymphoma	0	0
Nalm 1	Chronic myelocytic leukemia	0	0

* As determined by indirect immunofluorescence.

EAC14^{oxy}23d was detected. More frequently, rosette formation was enhanced, and the aggregation of erythrocytes onto one pole of the lymphocyte (capping) was observed. When, however, anti-B2–treated Raji cells were incubated with F(ab′)$_2$ fragments of rabbit IgG or anti-mouse IgM to cross-link the monoclonal

TABLE III

Inhibition by Monoclonal Anti-B2 Antibody of Rosette Formation Between Tonsil Lymphocytes and EAC14^{oxy}23d

Treatment of lymphocytes	Concentration of monoclonal antibody	Indicator cells	Rosette formation
	μg/ml		%
Medium	—	EAC14^{oxy}23d$_{10}$	51.0
Anti-B2	30		14.0
Anti-B2	10		18.9
Anti-B2	3		25.0
Anti-CR1	25		58.6
Medium	—	EAC14^{oxy}23b$_{1.5}$	51.3
Anti-B2	30		47.7
Anti-CR1	5		1.6

TABLE IV

Inhibition of Rosette Formation Between Raji Cells and EAC14^{oxy}23d by Incubation of Lymphocytes with Monoclonal Antibodies to B2 Followed by a Second Antibody to Mouse Ig

Raji cells incubated with:			Rosette formation with EAC14^{oxy}23d$_{1.5}$
First step		Second step	
Monoclonal anti-B2	Monoclonal anti-DNP	Rabbit anti-mouse IgM	
	μg/ml		%
0.5	—	0.5	0
0.5	—	—	21.2
0.05	—	0.5	2.5
0.05	—	—	21.3
0.005	—	0.5	21.5
—	5	0.5	28.0
—	5	—	29.0
—	—	0.5	22.5
—	—	—	23.4

antibodies and, presumably, B2, rosette formation with EAC14^{oxy}23d was totally abolished (Table IV). The degree of inhibition was dependent on the dose of anti-B2. For example, treatment of lymphocytes with 0.5 or 0.05 μg/ml of anti-B2 followed by anti-mouse IgM completely inhibited the binding of EAC14^{oxy}23d, but at concentrations of 0.005 μg/ml, anti-B2 was ineffective. Lymphocytes incubated in 50 μg/ml of a nonrelevant monoclonal antibody (IgM anti-DNP), followed by incubation in 0.5 μg/ml anti-mouse IgM, were not impaired in their ability to bind EAC14^{oxy}23d.

Discussion

The possible identity between the B2 antigen and CR2 was initially suspected because both markers are associated only with B cells, and because they are

similarly distributed among established cell lines (Table II) and B cell malignancies (28, 29). Indeed, B2 and CR2 are expressed in most chronic B lymphocytic leukemias and poorly differentiated lymphomas but not in large cell lymphomas and myelomas, which correspond phenotypically to transformed B lymphoblasts and plasma cells (30).

Our working hypothesis was confirmed by isolating a C3d-binding polypeptide from the membrane of Raji cells and tonsil lymphocytes, and showing that it not only had the same M_r and pI as B2 but also that it was recognized by the monoclonal antibody to B2 (Fig. 3, Table I). Since it could be argued that other membrane molecules with binding affinity for C3d were involved in the previously described phenomenon of rosette formation between B lymphocytes and C3d-bearing particles, we studied the effect of the treatment of lymphocytes with anti-B2 on their subsequent interaction with $EAC14^{oxy}23d$. As shown in Table III, when tonsil lymphocytes were treated with the monoclonal antibody, rosette formation was inhibited. In the case of Raji cells, strong inhibition was observed only when the lymphocytes were further treated with a second antibody directed against mouse Ig (Table IV).

These observations demonstrate that CR2 is the B2 antigen and suggest also that the inhibitory effect of the monoclonal antibody on rosette formation is probably the result of the cross-linking of CR2 on the lymphocyte membrane, rather than of a direct competition with the ligand for the receptor-binding site. Indeed, the epitope recognized by anti-B2 and the binding site for C3d on the 140,000 M_r polypeptide must be topographically distinct, since the interaction between purified B2 and Thiol-Sepharose-C3d is not prevented by the monoclonal antibody (Table I).

Other investigators have used methods similar to those described here to identify membrane molecules with binding affinity for immobilized and denatured C3 or C3 fragments in lymphoid cells and macrophages. Barel et al. (31) isolated a glycoprotein of 140,000 M_r from Raji cells and characterized it as a C3b receptor. Cole et al. (32) isolated molecules of 140,000 and 63,000 M_r from the membrane of human mononuclear cells and suggested that the smaller polypeptide was derived from the larger one. Lambris et al. (33) purified a C3d-binding glycoprotein (73,000 M_r) from the supernatant of cultures of Raji cells and identified it as CR2, but other C3d-binding polypeptides with higher M_r were observed during the intermediate steps of purification. In light of the present studies, we speculate that the protein of 140,000 M_r described by the above-mentioned investigators may be identical to B2, and that the smaller polypeptides represent degradation products. Further studies are necessary to clarify this point.

Previous studies of the distribution of B2 antigen provide significant insight into the cellular expression of the C3d receptor. Within the hematopoietic system, the B2 antigen is found in the majority of B cells from peripheral blood and lymphoid organs but not on mature or progenitor cells of myeloid, erythroid, or T lymphoid lineage (14, 34).

Several lines of evidence suggest that the expression of B2 is limited to discrete stages of B cell differentiation. B2 is detected simultaneously with or after the appearance of IgM in the cytoplasm of pre-B cells, and is lost from the cell

surface when B cells develop lymphoblastoid morphology and lose cell surface IgD (30). B2 is more strongly expressed on cells from lymphoid organs than from peripheral blood (14), but after stimulation with pokeweed mitogen, the amount of B2 on peripheral blood B cells increases. After 4–5 d in culture, when stimulated B cells develop presecretory cytoplasmic IgM and lose surface IgD, the B2 antigen disappears (34). In lymphoid organs, B2 is weakly expressed on cells of primary follicles or in the mantle zone of secondary follicles, but very strongly in germinal centers. Interestingly, in the latter location, B2 antigen also appears to be present between cells, suggesting that it may be shed (15).

The function of CR2 is not known. Many reports in the literature (reviewed in reference 35) suggest that cleavage products of C3 play a role in immunoregulation, but it has been very difficult to clarify the nature of the C3 fragments and C3 receptors involved. Particularly intriguing are the observations that the retention of antigen in lymphoid follicles, as well as the development of B memory cells and germinal centers, are antibody- and C3-dependent processes (36–38). These studies have been interpreted as meaning that the generation of B memory cells is triggered by antigen-antibody-C3 fragments trapped in follicles by complement receptors (39). Since the fragments that remain associated with immune complexes after the cleavage of bound C3b by CR1 + I are most likely C3dg (40), CR2 could be involved in this process.

Summary

The B2 antigen characterized by means of a monoclonal antibody (14) is a 140,000 M_r protein expressed only in certain stages of the differentiation of lymphocytes of the B lineage. Here we examine the relationship between B2 and the membrane complement receptor type 2 (CR2) for the complement fragment C3d (11, 12), which is also associated only with B cells. Both phenotypic markers are distributed in a similar manner among B cell malignancies and, as shown here, among established cell lines.

A polypeptide with binding affinity for C3d was isolated from the membrane of B2-positive cells, i.e., tonsil lymphocytes and Raji cells. We found that this C3d-binding protein not only had the same M_r and isoelectric point (pI) as the B2 antigen, but that it was recognized by the monoclonal antibody to B2. However, anti-B2 does not mask the ligand-binding site of CR2 since it does not prevent the interaction of the purified 140,000 M_r polypeptide with immobilized C3d.

Rosette formation between tonsil lymphocytes and erythrocyte intermediates bearing C3d was specifically inhibited by anti-B2. In the case of Raji cells, rosette formation was strongly inhibited only when the lymphocytes were sequentially treated with anti-B2 and with a polyclonal antibody against mouse Ig.

In short, B2 and CR2 have a similar distribution among normal and malignant cells, have the same M_r and pI under denaturing conditions, and react with a single monoclonal antibody. We conclude that B2 is identical to CR2.

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