Proc. Natl. Acad. Sci. USA
Vol. 78, No. 3, pp. 1828–1832, March 1981
Immunology

Isolation of lymphocyte membrane complement receptor type two (the C3d receptor) and preparation of receptor-specific antibody

(B cells/lymphoblastoid cells/complement component 3/affinity chromatography)

JOHN D. LAMBRIS, NORMAN J. DOBSON, AND GORDON D. ROSS

Division of Rheumatology–Immunology, Department of Medicine, and Department of Bacteriology–Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Communicated by Henry G. Kunkel, November 24, 1980

ABSTRACT **A glycoprotein binding complement component C3d was isolated from media used for culture of Raji human lymphoblastoid cells. Analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and gas/liquid chromatography indicated that the C3d-binding glycoprotein consisted of a single polypeptide chain with extensive intrachain disulfide bonds, a molecular weight of 72,000, and several different bound carbohydrates. Several lines of evidence indicated that this medium-derived C3d-binding protein originated from membrane complement receptor type two (CR_2, the C3d receptor), presumably shed during membrane turnover. The C3d-binding protein bound to sheep erythrocytes coated with C3d (EC3d) but not to sheep erythrocytes coated with C3b (EC3b). Antisera, prepared by immunization with the purified C3d-binding glycoprotein, inhibited lymphocyte rosette formation with EC3d but not with EC3b. Analysis by sodium dodecyl sulfate gel electrophoresis of the radiolabeled and solubilized lymphocyte antigens reactive with the anti-C3d-binding protein sera revealed a single-chain cell-surface protein of molecular weight 72,000 that was apparently identical to the isolated C3d-binding protein. Parallel assay of lymphocytes for CR_2 by direct immunofluorescence with $F(ab')_2$ anti-C3d-binding protein (anti-CR_2) and rosette formation with EC3d indicated that both assays had the same specificity and nearly the same sensitivity. With both systems CR_2 expression was limited to B cells, and was undetectable on T cells, monocytes, or neutrophils.**

Bone marrow-derived (B) lymphocytes express two distinct types of membrane receptors for the third component of complement (C3) (1), a receptor for β1H globulin (2) and surface-bound C5 (3). Complement (C) receptor type one (CR_1, the C3b receptor) has recently been isolated and shown to be a 205,000 M_r glycoprotein (4). Isolated CR_1 bound to both C4b, and C3b, and lymphocytes treated with Fab' anti-CR_1 did not form rosettes with the erythrocyte–complement complexes EC3b or EAC14b (5). Previous attempts to isolate lymphocyte C receptor type two (CR_2, the C3d receptor) have been unsuccessful (6). CR_2 differs from CR_1 in that it is expressed exclusively on lymphocytes, whereas CR_1 is also expressed on monocytes, neutrophils, erythrocytes, and kidney cells (7). Although the functions of CR_1 and CR_2 are unknown, B cells do respond to either β1H receptor triggering or the cleavage of surface C5 by releasing endogenous C3b inactivator (C3bINA) (2, *).

In the present study, media from B-type lymphoblastoid cells were shown to contain a soluble C3d-binding protein. This C3d-binding protein was purified and found to be a glycoprotein of 72,000 M_r. Antibody prepared by immunization of rabbits with the C3d-binding protein inhibited lymphocyte rosette formation with EC3d but not with EC3b, and reacted with a single cell-surface protein of 72,000 M_r.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Lymphoid Cells, Neutrophils, and Spent Culture Media. Normal human blood mononuclear cells and neutrophils were isolated on a two-step (1.08 g/ml and 1.105 g/ml) Ficoll/Hypaque density gradient (5, 8), whereas tonsil cells were isolated on 1.08 g/ml Ficoll/Hypaque (9). Monocytes were either removed with Sephadex G-10 (Pharmacia) (10) or labeled with latex (11). The human B-cell lines Raji, Daudi, and BF, and the HSB T-cell line, were maintained at 0.8–2.0 × 10^6 cells per ml by doubling the culture volume at 2-day intervals with RPMI-1640 medium containing 10% fetal bovine serum, penicillin, and streptomycin. Spent culture media was harvested by centrifugation and stored frozen at −85°C.

C Components and C-Coated Sheep Erythrocytes (EC and EAC). C components and nephritic factor (NF) were isolated and used to prepare CR_1-reactive EAC14b and EC3b as described (2, 12). EC3b was converted into EC3bi and EC3d with KSCN-inactivated serum (12) and plasmin (2). Fixation of C3 onto EC3 was monitored with [^{3}H]C3 (2, 13).

C-Receptor and Surface Ig Assays. C receptors and surface Ig were detected by rosette assay and immunofluorescence, respectively (2, 12, 14). Fluid-phase C3d-binding protein (CR_2) was detected by two different assays with EC3d containing 1.5 × 10^3 C3d molecules per EC3d complex. First, serial 25-μl dilutions of CR_2 sample were tested for inhibition of Raji cell–EC3d rosette formation by microtiter assay (6). Second, soluble CR_2 was detected by microtiter assay for inhibition of EC3d agglutination by anti-C3d. Serial 25-μl dilutions of CR_2 sample in a U-bottom plate were mixed with 25 μl of EC3d (1 × 10^8 per ml) and incubated on a shaker at 37°C for 15 min, after which time 25 μl of a 1:24 dilution of anti-C3d serum (kindly provided by Brian F. Tack, Harvard Medical School, Boston) was added to each well. After 15-min shaking at 37°C, the cells were allowed to settle for development of agglutination patterns.

Binding of ^{3}H-Labeled Lymphoid Components to EC3 and EAC14b. One-hundred microliters of ^{3}H-labeled Raji culture media (2) was mixed with 100 μl of E, EAC14b, EC3b, or EC3d (2 × 10^8 per ml) in Veronal-buffered (3.5 mM, pH 7.2) 1% bovine serum albumin/3.2% dextrose/0.2% NaN_3/30 mM NaCl,

Abbreviations: B lymphocytes or B cells, bone marrow-derived lymphocytes; C, complement; CR_1, complement receptor type one (C3b–C4b receptor); CR_2, complement receptor type two (C3bi–C3d receptor); CR_3, complement receptor type three (C3bi receptor); C3bINA, C3b-inactivator; E, erythrocyte; EAC, erythrocyte–antibody–complement complex; EC, erythrocyte–complement complex, NF, nephritic factor; NP-40, Nonidet P40; T lymphocytes or T cells, thymus-derived lymphocytes.

* Ross, G. D., Lambris, J. D. & Dobson, N. J. (1980) *Abstracts of the 4th International Congress on Immunology*, July 1980, Paris, abstr. 15.3.22.

and incubated on a rotator for 15 min at 37°C. After three washes with 1% bovine serum albumin in phosphate-buffered saline, each cell type was solubilized with 1 ml of NCS solution (Amersham) and radioactivity was measured in 10 ml of OCS scintillation fluid (Amersham).

Molecular Weight and Carbohydrate Analysis. Proteins were treated with 2% $NaDodSO_4$, either with or without 0.1 M dithiothreitol, at 100°C for 5 min and then electrophoresed in 7% polyacrylamide gels (15). Molecular weight was determined from mobility by comparison to known markers. Coomassie blue-stained bands were cut out of the gel slab and analyzed for carbohydrates by gas/liquid chromatography after protein elution with $NaDodSO_4$ and precipitation with trichloroacetic acid (16).

Evaluation of Antibody Specificity for Lymphoid Cell Components. Lymphoid cells were either labeled with ^{125}I and lactoperoxidase (17) or labeled endogenously by culture in [^{3}H]leucine (2). $F(ab')_2$ antibodies, prepared with pepsin and Sephadex G-150 (Pharmacia) (11), were conjugated to Sepharose CL-4B (Pharmacia) with cyanogen bromide (18) at a ratio of 10 mg of protein per ml of gel. Radiolabeled cells (1×10^8) were solubilized with 1% Nonident P40 (NP-40; Particle Data Laboratories, Elmhurst, IL) in 50 mM Tris·HCl, pH 7.5, containing 2 mM phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, at 50 μg/ml, 25 mM benzamidine, 10 mM EDTA, 50 mM ε-aminocaproic acid, and 10 mM KCl, and then insoluble debris was removed by centrifugation at 40,000 × g for 16 hr. The solubilized cells were cycled two times through a column containing 2 ml of $F(ab')_2$ antibody-Sepharose CL-4B in phosphate-buffered saline and then, after a wash with phosphate-buffered 0.3 M NaCl/1% Triton X-100 (Sigma)/0.5% deoxycholate, the bound antigens were eluted with 4 M guanidine. The eluate was dialyzed versus 62.5 mM Tris·HCl, pH 6.8, concentrated to 1 ml with polyethylene glycol powder, treated with 2% $NaDodSO_4$ either with or without 0.1 M dithiothreitol for 5 min at 100°C, and electrophoresed in 7% polyacrylamide gels (15). After electrophoresis, the gel tracks were sliced into 2-mm segments whose radioactivities were measured either directly (^{125}I) or in OCS after solubilization overnight in 30% (wt/wt) H_2O_2 at 56°C (^{3}H).

Preparation of C3d-Sepharose for Affinity Chromatography. Five milliliters of trypsin-Sepharose, prepared with 3 mg of trypsin per ml of gel (18), was mixed with 20 mg of C3 for 2 min at 37°C and then washed three times by centrifugation. A NF-stabilized C3-convertase was then assembled on the trypsin-generated Sepharose-bound C3b by addition of 16 mg of factor B, 16 μg of factor D, and 11.5 mg of NF. After 10 min at 37°C, 100 mg of C3 was added and incubation was continued for 60 min at 37°C. The gel was washed two times, resuspended in 35 ml of fresh serum, and incubated 60 min at 37°C. C3b fixation, monitored with ^{3}H-labeled C3, was 2.8 mg per ml of gel, of which most was presumably converted to C3bi by the serum C3bINA. Bound C3bi was then degraded to C3d with plasmin (2) at 60 μg/ml for 30 min at 37°C.

RESULTS

Evidence for a Lymphocyte-Derived C3d-Binding Protein in Culture Media from B-Type Lymphoblastoid Cell Lines. Three findings indicated that B-type lymphoblastoid cells synthesized and released a C3d-binding protein resembling CR_2. First, 40-fold concentrated (PM-10 membrane, Amicon, Lexington, MA) spent culture media from both Raji and Daudi cells inhibited lymphocyte-EC3bi and -EC3d rosette formation to a titer of 32–64, but did not inhibit formation of EC3b or EAC14b rosettes. By contrast, media from T-type HSB cells did not inhibit either EC3d or EC3b rosette formation. Second, media from Raji cells grown in [^{3}H]leucine contained a ^{3}H-labeled protein that bound to EC3d but did not bind to E, EC3b, or EAC14b (Fig. 1). Finally, 40-fold concentrated media from Raji and Daudi, but not HSB, inhibited the agglutination of EC3d by anti-C3d to a titer of 1600–3200.

Isolation of the C3d-Binding Protein from Raji Spent Culture Medium. Most of the C3d-binding activity and 20% of the protein was precipitated from 15 liters of Raji spent culture medium by addition of an equal volume of saturated ammonium sulfate and centrifugation at 15,000 × g for 30 min. After dialysis against 20 mM sodium phosphate buffer, pH 7.5, the dissolved precipitate was applied to a 2.6 × 60 cm column of DEAE-Sephacel (Pharmacia) equilibrated with the dialysis buffer. Bound proteins were eluted with a 3-liter linear gradient progressing to 0.3 M NaCl. Inhibition of anti-C3d agglutination activity was detected in four distinct eluted peaks (Fig. 2). The fractions composing each peak were separately pooled, concentrated (PM-10 membrane) to 15 ml, and chromatographed on a 5 × 90 cm column of Sephadex G-150 in phsphate-buffered saline (Fig. 3). When assayed by inhibition of anti-C3d agglutination, activity eluted in three peaks with calculated molecular weights of 50,000, 100,000, and >250,000. By contrast, with assays for inhibition of lymphocyte–EC3d rosettes, activity was restricted primarily to the excluded volume (>250,000 M_r). Each of the three Sephadex G-150 pools was dialyzed against pH 7.2 Veronal-buffered 3.2% dextrose/0.2% NaN_3/30 mM NaCl (6.0 mS at 22°C) and applied separately to a 0.5 × 2 cm column of C3d-Sepharose. After a 200-ml wash with 0.6 M NaCl/20% sucrose in 10 mM phosphate buffer, pH 7.5, the column was eluted with 0.1% NP-40/0.6 M NaCl in 10 mM phosphate buffer, pH 7.5. C3d-binding activity was recovered from each of the activity peaks from DEAE-Sephacel that were chromatographed on both Sephadex G-150 and C3d-Sepharose. The greatest specific activity and yield was obtained from processing the fourth peak from DEAE-Sephacel (Fig. 2) and the third peak from Sephadex G-150 (Fig. 3) through C3d-Sepharose, resulting in a 2-mg (A_{280}) yield.

Characteristics of the Isolated C3d-Binding Protein. After the various C3d-binding pools separated by DEAE-Sephacel

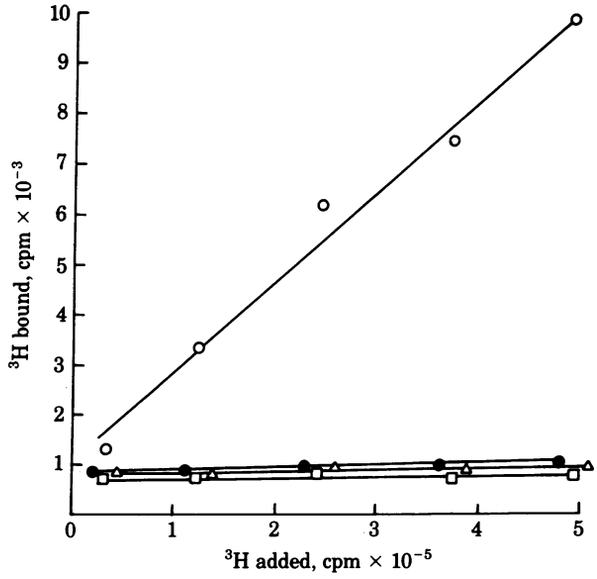

FIG. 1. Uptake of ^{3}H-labeled protein from Raji cell medium onto EC3d. Medium from Raji cells cultured in [^{3}H]leucine was added to EC3d (○), EC3b (●), EAC14b (■), or E (△) and then, after three washes, the amount of bound ^{3}H was determined.

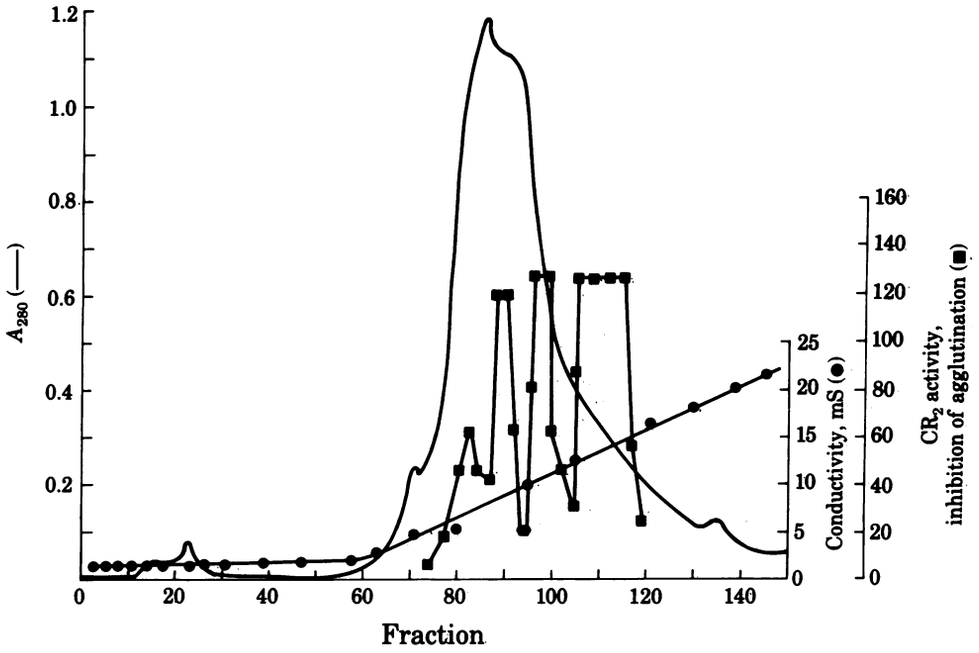

FIG. 2. DEAE-Sephacel column chromatography of the 50% ammonium sulfate precipitate of Raji cell medium.

and Sephadex G-150 had been eluted from C3d-Sepharose, each was analyzed by $NaDodSO_4$ gel electrophpresis. With each pool, primarily only one protein was observed of 50,000 M_r without reduction and 72,000 M_r with reduction of disulfide bonds (Fig. 4). When this single gel protein band was analyzed for carbohydrates by gas/liquid chromatography, three out of six sugars present were identified with available standards as galactose, mannose, and glucosamine.

Preparation and Characteristics of Antibody Specific for the C3d-Binding Protein. Two rabbits were immunized weekly with 100 μg of purified C3d-binding protein emulsified in Freund's complete adjuvant. Immune sera obtained after 6 weeks, but not preimmune sera, inhibited completely EC3d rosette formation with all lymphocyte types (Table 1). Multiple absorptions of the two antisera with immobilized human serum (18) had no effect on the lymphocyte–EC3d rosette inhibition titer. $F(ab')_2$ antibody at 300 μg/ml inhibited lymphocyte–EC3d rosette formation completely, whereas at 3.0 mg/ml it did not inhibit EC3b rosette formation with lymphocytes or neutrophils. A range of 0.3–3.0 mg/ml of this antibody inhibited lymphocyte–ECbi rosette formation by only 50–60% but had no effect on neutrophil–EC3bi rosette formation.

The radiolabeled and solubilized lymphocyte surface and cytoplasmic components eluted from $F(ab')_2$ anti-C3d binding protein-Sepharose were analyzed by $NaDodSO_4$ gels. With either BF cells labeled with ^{125}I and lactoperoxidase or Raji cells labeled endogenously with [^{3}H]leucine, only a single antigen

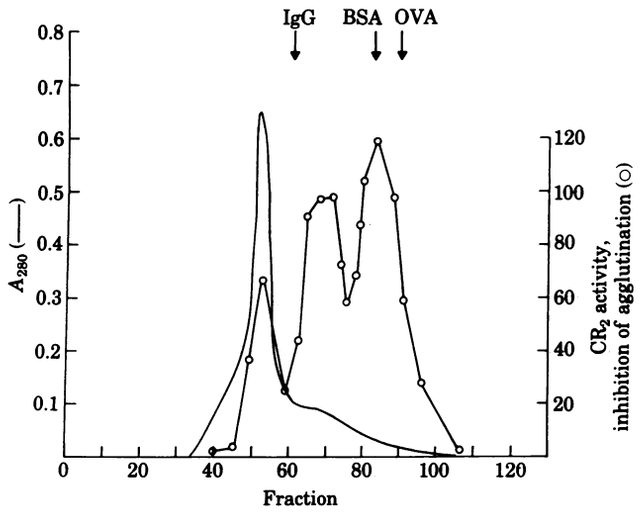

FIG. 3. Sephadex G-150 column chromatography of the last eluting activity peak obtained from DEAE-Sephacel (see Fig. 2). The molecular weight of each activity peak was determined from a previous calibration run with known markers: human IgG (150,000 M_r), bovine serum albumin (BSA, 68,000 M_r), and ovalbumin (OVA, 43,000 M_r).

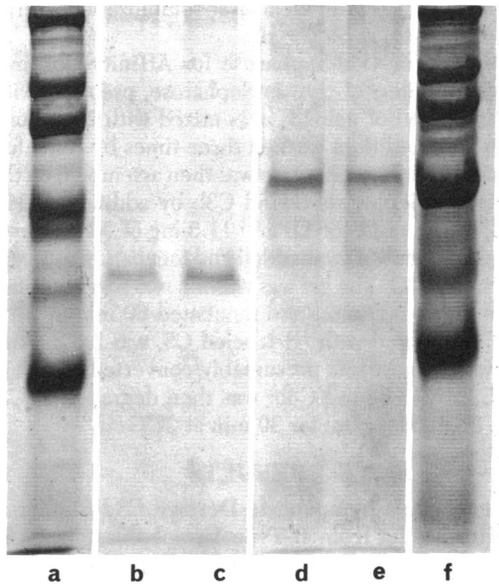

FIG. 4. Analysis of the isolated C3d-binding protein by $NaDodSO_4$/polyacrylamide gel electrophoresis. The outer tracks contained known molecular weight markers: myosin (200,000 M_r), β-galactosidase (116,500 M_r), phosphorylase b (94,000 M_r), bovine serum albumin (68,000 M_r), and ovalbumin (43,000 M_r). Tracks a–c contained unreduced proteins and d–f, reduced. Two different pools of C3d-binding activity were electrophoresed unreduced (b, c) and reduced (d, e).

Table 1. Inhibition of lymphocyte and neutrophil EC3 rosette formation by F(ab')$_2$ anti-C3d-binding protein

Antibody-treated C-receptor cell	Inhibition of rosette formation, %		
	EC3b	EC3bi	EC3d
Blood lymphocytes	0	62	100
Tonsil lymphocytes	0	42	100
Lymphoblastoid cells			
Raji (CR_1^- CR_2^+)	NT	51	100
Daudi (CR_1^- CR_2^+)	NT	52	100
BF (CR_1^+ CR_2^+)	0	64	100
Blood neutrophils	0	0	NT

NT, not tested because the C-receptor cells lacked either CR_1 (Raji and Daudi) or CR_2 (neutrophils) and did not form EC3b or EC3d rosettes, respectively.

was detected (Fig. 5); it had identical molecular weight characteristics as the isolated C3d-binding protein (Fig. 4).

Immunofluorescence with Anti-C3d-Binding Protein. Immunofluorescence assays with F(ab')$_2$ anti-C3d-binding protein gave results similar to those obtained by parallel EC3d rosette assays. With normal blood lymphocytes, anti-C3d-binding protein fluorescence (fluorescein) was observed only on anti-Ig-rhodamine-positive B cells and not on sheep E-rosette-positive T cells. Neutrophils and monocytes did not form EC3d rosettes nor stain with F(ab')$_2$ anti-C3d-binding protein.

DISCUSSION

The possibility of shed C receptors in lymphoblastoid cell culture media was previously suggested by the finding of EAC rosette-inhibiting activity in B but not T cell media, and by the demonstration that C receptors were regenerated rapidly during membrane turnover (19). In the present study this rosette-inhibiting activity was shown to be due to a lymphocyte-derived glycoprotein that bound to EC3d but not to EC3b or EAC14b, and resembled monovalent Fab anti-C3d in that it inhibited bivalent anti-C3d agglutination of EC3d. During isolation of the C3d-binding activity from media, EC3d-rosette inhibition was compared to inhibition of anti-C3d agglutination of EC3d. The anti-C3d inhibition assay not only was more sensitive but also detected both charge and size heterogeneity in the C3d-binding activity. C3d-Sepharose affinity chromatography and NaDodSO$_4$ gel analysis of the various C3d-binding activities isolated by differences in size or charge indicated that all C3d-binding activity was probably associated with the same 72,000 M_r protein. Thus, the probable explanation for the size and charge heterogeneity of C3d-binding activity is that C3d-binding protein was shed from cell membranes as both individual molecules and multivalent complexes that included various other membrane components. The rosette inhibition assay was apparently sensitive primarily to these large complexes of C3d-binding protein, whereas the anti-C3d inhibition assay was sensitive to the monovalent C3d-binding protein that eluted from Sephadex G-150 with an apparent M_r of 50,000. Because Sephadex G-150-isolated monomers bound to C3d-Sepharose in a similar manner as did larger C3d-binding protein complexes, binding to the C3d matrix did not require multipoint attachment. Although the C3d-binding protein was not eluted from C3d-Sepharose by high-salt washes, complete elution required only NP-40 in low salt (5 mS) buffer and was not enhanced by the 0.6 M NaCl included in the NP-40 elution buffer. Thus, binding to C3d apparently involves only hydrophobic interactions, and in this way differs from CR_1 binding to C3-Sepharose, which involves both salt and hydrophobic interactions (5).

Analysis of the C3d-binding protein by NaDodSO$_4$ gel electrophoresis indicated a single polypeptide chain of 50,000 apparent M_r unreduced and 72,000 M_r after reduction of disulfide bonds. This 44% increase in apparent M_r after reduction probably indicates extensive intrachain disulfide bonds. Preliminary

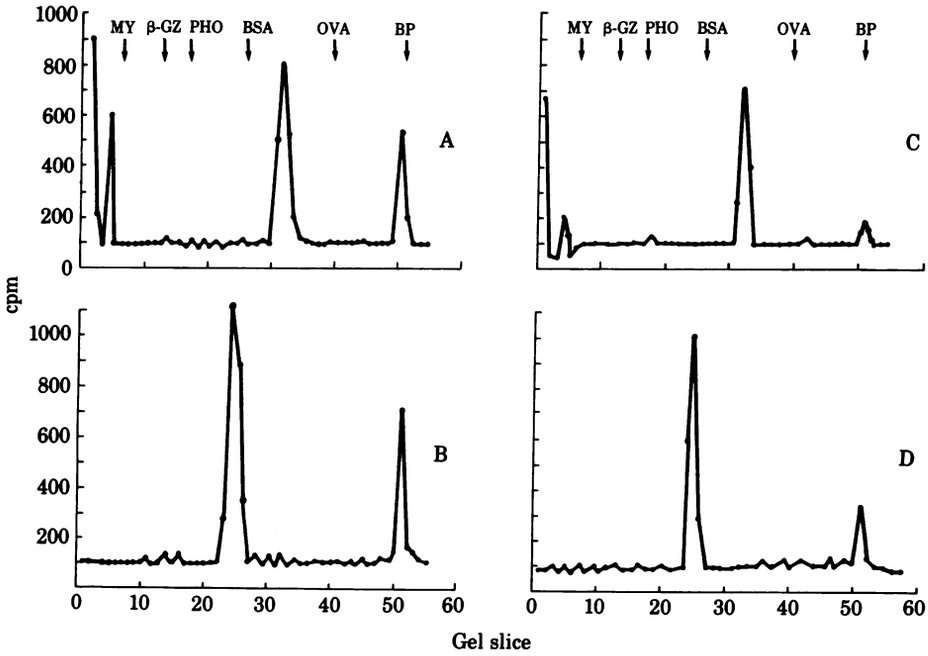

FIG. 5. Analysis of NaDodSO$_4$/7% polyacrylamide gel electrophoresis of solubilized lymphocyte antigens eluting from Sepharose-F(ab')$_2$-anti-C3d-binding protein. The proteins shown in *A* (unreduced) and *B* (reduced) were from BF cells (CR_1^+ CR_2^+) labeled with ^{125}I and lactoperoxidase. *C* (unreduced) and *D* (reduced) show material from Raji cells (CR_1^- CR_2^+) endogenously labeled with [^{3}H]leucine. Control eluates prepared with radiolabeled cells and preimmune IgG contained <10% as much radioactivity as did the F(ab')$_2$ antibody elutes and were not electrophoresed. Molecular weight markers: myosin (MY, 200,000 M_r), β-galactosidase (β-GZ, 116,500 M_r), phosphorylase b (PHO, 94,000 M_r), bovine serum albumin (BSA, 68,000 M_r), ovalbumin (OVA, 43,000 M_r), and bromphenol blue tracking dye (BP).

carbohydrate analysis indicated the presence of galactose, mannose, and glucosamine. The glycoprotein nature of the C3d-binding protein was a further indication of its outer membrane surface origin.

Antisera from rabbits immunized with the C3d-binding protein inhibited formation of B cell rosettes with EC3d but not with EC3b or EAC14b. Analysis of antibody specificity for intact cell antigens demonstrated a single protein on $NaDodSO_4$ gels with an apparent M_r of 50,000 unreduced and 72,000 reduced. Because the same result was obtained with either surface-iodinated or endogenously ^3H-labeled cells, it is probable that the cell-bound antigen is identical to the C3d-binding protein, and the possibility of a larger cytoplasmic precursor is apparently ruled out.

Because the C3d-binding protein bound to C3d and not to C3b or C4b, and because C3d-binding protein-specific antibody both inhibited B cell EC3d rosette formation and reacted with an apparently identical B cell membrane surface protein, it was concluded that the C3d-binding protein probably represented membrane-shed CR_2. Further evidence for the CR_2 origin of the C3d-binding protein was the finding of equivalent EC3d rosette formation and immunofluorescence staining with $F(ab')_2$ anti-C3d-binding protein.

CR_1 and CR_2 are distinct and separate membrane components. CR_2 is smaller than CR_1 (4, 5), and no common determinants have yet been detected with either anti-CR_1 or anti-CR_2 sera. Furthermore, CR_1 and CR_2 are probably not adjacent on the cell surface, because saturation of B cell membranes with Fab' anti-CR_1 inhibited only CR_1 rosettes (4, 5) and saturation with $F(ab')_2$ anti-CR_2 inhibited only CR_2 rosettes. Finally, even though most normal B cells express both CR_1 and CR_2 simultaneously, a low proportion of normal B lymphocytes also express either CR_1 or CR_2 individually (9, 14).

Monocytes and neutrophils lacked detectable CR_2 by both rosette and immunofluorescence assay. In previous reports of CR_2 on phagocytes (8, 20, 21), complexes thought to be EAC1–3d were in fact EAC1–3bi and contained little C3d (7, 22). C3bi attaches to neutrophil and monocyte CR_3 components that are distinct from CR_1 and CR_2 and unreactive with C3b, C3d, or C4b (7, 22). Phagocyte EC3bi rosettes were unaffected by amounts of either anti-CR_1 (5) or anti-CR_2 that completely inhibited EC3b and EC3d rosettes with neutrophils and Raji cells, respectively (Table 1).

Early studies of lymphocyte C receptors also used EAC1–3bi thought to be EAC1–3d (1, 19). However, inhibition studies with fluid-phase C3d (9) indicated that EAC1–3bi complexes were at least partially bound to CR_2, although it was not previously possible to assay lymphocytes specifically for CR_3 by an EC3bi rosette assay. In the present study, however, the finding that lymphocyte EC3bi rosettes were inhibited only partially by complete blockade of membrane CR_2 with anti-CR_2 (Table 1), suggested that EC3bi complexes were bound to lymphocytes simultaneously by way of CR_2 and a distinct C3bi-specific receptor (CR_3) that was distinct from CR_1 and CR_2.

The function of lymphocyte C receptors is presently unknown. Not only do lymphocytes express three distinct types of C3 receptors, but also lymphocytes express surface-bound C5 (3) and a receptor for β1H (2). Cleavage of lymphocyte surface C5 leads to release of endogenous β1H and C3bINA* and possibly also blastogenesis.† In either case, β1H and C3bINA act upon any nearby bound C3b. When the C3b is bound to an alternative pathway-activating surface, it is relatively resistant to C3bINA and remains as C3b complexed to factor Bb and P (23). By contrast, when the C3b is bound to normal tissue, then it is rapidly converted into C3bi. C-activating surfaces will thus bind to lymphocyte CR_1 by way of C3b, and this will bring the C3b,Bb,P enzyme into contact with surface C5. Subsequently, the C5 would be cleaved, possibly inducing blastogenesis,† and little CR_2- and CR_3-reactive C3bi would be generated. By contrast, when normal tissue-bound C3b is presented to a lymphocyte, the C3b is converted rapidly to C3bi that is bound subsequently to CR_2 and CR_3 (2, *). Accordingly, it would be predicted that whereas cleavage of surface C5 might lead to lymphocyte blastogenesis, the engagement of CR_2 and CR_3 might suppress lymphocyte activation.

The authors acknowledge the assistance of Dr. Elizabeth Fowler of the University of North Carolina in preparation of [^3H]leucine-labeled Raji cells and spent culture media, Ms. Carmen B. Lee of the University of North Carolina for the carbohydrate analyses by gas/liquid chromatography, Ms. Mandy Dozier, Ms. Joyce Knapp, and Ms. Sharon Bleu for their excellent technical assistance, and Ms. Cathy Miller for preparation of the manuscript. This work was supported by research grants from the National Cancer Institute (5R01-CA25613-02) and the American Heart Association (80 766). G.D.R. is an Established Investigator of the American Heart Association (78 155) and both J.D.L. and N.J.D. are supported by postdoctoral fellowships from the Department of Medicine, University of North Carolina.

† Sundsmo. J. S. & Gotze, O. (1980) *Abstracts of the 4th International Congress on Immunology*, July 1980, Paris, abstr. 15.3.28.

1. Ross, G. D., Polley, M. J., Rabellino, E. M. & Grey, H. M. (1973) *J. Exp. Med.* **138**, 798–811.
2. Lambris, J. D., Dobson, N. J. & Ross, G. D. (1980) *J. Exp. Med.* **152**, 1625–1644.
3. Sundsmo, J. S. & Müller-Eberhard, H. J. (1979) *J. Immunol.* **122**, 2371–2378.
4. Fearon, D. T. (1980) *J. Exp. Med.* **152**, 20–30.
5. Dobson, N. J., Lambris, J. D. & Ross, G. D. (1980) *J. Immunol.* **126**, 693–698.
6. Dierich, M. P. & Reisfeld, R. A. (1975) *J. Immunol.* **114**, 1676–1682.
7. Ross, G. D. (1980) *J. Immunol. Methods* **37**, 197–211.
8. Ross, G. D., Jarowski, C. I., Rabellino, E. M. & Winchester, R. J. (1978) *J. Exp. Med.* **147**, 730–744.
9. Ross, G. D. & Polley, M. J. (1975) *J. Exp. Med.* **141**, 1163–1180.
10. Ly, I. A. & Mishell, R. A. (1974) *J. Immunol. Methods* **5**, 239–247.
11. Ross, G. D. & Winchester, R. J. (1980) in *Manual of Clinical Immunology*, eds. Rose, N. R. & Friedman, H. (Am. Soc. Microbiol., Washington, DC), 2nd Ed., pp. 213–228.
12. Ross, G. D. & Polley, M. J. (1976) *Scand. J. Immunol.*, Suppl. 5, **5**, 99–111.
13. Tack, B. F., Dean, J., Eilat, D., Lorenz, P. & Schechter, A. (1980) *J. Biol. Chem.* **255**, 8842–8847.
14. Ross, G. D., Winchester, R. J., Rabellino, E. M. & Hoffman, T. (1978) *J. Clin. Invest.* **62**, 1086–1092.
15. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
16. Fox, A., Schwab, J. & Cochran, T. (1980) *Infect. Immun.* **29**, 526–531.
17. Lambris, J. D. & Papamichail, M. (1980) *J. Immunol. Methods* **34**, 287–293.
18. March, S. C., Parikh, I. & Cuatrecasas, P. (1974) *Anal. Biochem.* **60**, 149–152.
19. Rosso di San Secondo, V. E. M., Meroni, P. L., Fortis, C. & Tedesco, F. (1979) *J. Immunol.* **122**, 1658–1662.
20. Reynolds, H. Y., Atkinson, J. P., Newball, H. H. & Frank, M. M. (1975) *J. Immunol.* **114**, 1813–1819.
21. Ehlenberger, A. G. & Nussenzweig, V. (1977) *J. Exp. Med.* **145**, 357–371.
22. Ross, G. D. & Rabellino, E. M. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1467 (abstr.).
23. Fearon, D. T. & Austen, K. F. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1683–1687.