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Immunology

Identification of a 145,000 M_r membrane protein as the C3d receptor (CR2) of human B lymphocytes

(complement/Raji cells/monoclonal antibodies)

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ABSTRACT **The C3d receptor (CR2) of human B lymphocytes mediates the binding to these cells of immune complexes that have activated the complement system and bear the fragments of C3, iC3b, C3d,g, and C3d. A 145,000 M_r membrane protein previously described as being recognized by the monoclonal antibody HB-5 and shown to be expressed only by B lymphocytes and B lymphoblastoid cell lines, such as Raji, was assessed for its possible identity as CR2. Treatment of Raji cells with HB-5 and goat F(ab')$_2$ anti-mouse IgG (GaM) diminished the capacity of these cells to form rosettes with sheep erythrocyte (E) intermediates bearing 130,000 molecules of iC3b or C3d, whereas treatment with the monoclonal antibody alone had no effect. The capacity of peripheral blood B lymphocytes to bind EC3d was similarly inhibited by the combination of HB-5 and GaM. The possibility that HB-5 may interact with a site on CR2 that is distinct from the ligand binding site permitted the direct analysis of the capacity of the HB-5 antigen to bind to the C3 fragments. Protein A-containing *Staphylococcus aureus* particles to which HB-5 had been bound were incubated with detergent lysates of Raji cells and B lymphocytes under conditions that had been shown to be associated only with the binding of the 145,000 M_r antigen. These particles bearing HB-5 and antigen derived from either cell type were shown to adhere specifically to EiC3b and EC3d, demonstrating that transfer of the HB-5 antigen from CR2-bearing cells to *S. aureus* particles led to the acquisition of CR2 function by the particles. The additional findings that the relatively weak capacity of Raji cells to form rosettes with EC3b was inhibited by HB-5 and that the *S. aureus* particles bearing immunoadsorbed HB-5 antigen bound to EC3b indicated that the C3b-binding function of the CR1-negative Raji cell resides in CR2, rather than in other membrane proteins.**

A most critical reaction in the complement system is the cleavage of the third complement protein, C3, that is mediated by the C3 convertases of the classical or alternative pathways of complement activation. The major cleavage fragment, C3b, covalently attaches to the antigen–antibody complex or bacterium bearing the C3 convertase by a transacylation reaction involving the glutamyl component of an internal thiolester (1–3). This C3b may then be hydrolyzed by factor I in the presence of cofactors to yield sequentially iC3b (4, 5) and C3d,g (6–8), which remain attached to the target via the covalent binding site. C3d,g is susceptible to further proteolysis by noncomplement-derived enzymes such as trypsin and neutrophil elastase to generate the C3d fragment (6).

These proteolytically generated fragments of C3 mediate the binding of complement-activating complexes to various cell types involved in immune and inflammatory reactions by interacting with three types of cellular receptors. The C3b receptor, also termed CR1, has primary specificity for C3b but may also bind iC3b (8, 9) and C4b (10), the major cleavage fragment of C4, and is present on erythrocytes, neutrophils, monocytes/macrophages, B cells, some T cells, and glomerular podocytes (11–14). CR1 has been shown to be a membrane glycoprotein having two allotypic forms of 250,000 M_r and 260,000 M_r (15, 16). A receptor for iC3b, known as CR3, binds this cleavage fragment of C3 and possibly C3d,g and is expressed by neutrophils, monocytes/macrophages, and large, granular lymphocytes having natural killing and antibody-dependent cell-mediated cytotoxic activity (17–20). CR3 has been identified as a glycoprotein having two polypeptide chains of 185,000 M_r and 105,000 M_r, respectively (19, 21). An additional type of receptor, designated the C3d receptor or CR2, binds the C3d region of iC3b and C3d,g and is found only on B lymphocytes and certain B-cell lines, such as the Burkitt lymphoma line Raji (22, 23). A 72,000 M_r protein that was isolated from culture supernatants of Raji cells has been reported to have CR2 function (24).

Recently, a monoclonal antibody, HB-5, that is specific for a membrane protein of 145,000 M_r on human B cells was generated by immunization of mice with the human B-cell line SB (unpublished data; ref. 25). This monoclonal antibody was shown by Tedder *et al.* to bind only to mature B lymphocytes but not to T lymphocytes, granulocytes, monocytes, or erythrocytes (25). In the present study the CR2 function of peripheral blood lymphocytes and Raji cells is found to be inhibited by HB-5. In addition, the 145,000 M_r antigen extracted from detergent lysates of Raji cells and B lymphocytes by HB-5 is shown to bind to particles coated with iC3b and C3d, thereby directly demonstrating the CR2 function of this B-cell-specific membrane protein.

MATERIALS AND METHODS

Antibodies. The HB-5 monoclonal antibody (mouse, γ2a, κ) was produced by using the human B-cell line SB as an immunogen. It is reactive with all circulating B cells but unreactive with T cells, monocytes, neutrophils, and erythrocytes. The B-cell surface membrane antigen immunopreci-

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Abbreviations: C3, third component of complement; C3b, major cleavage fragment of C3; iC3b, first product of factor I cleavage of C3b; C3d,g, second product of factor I cleavage; C3d, trypsin-generated fragment of iC3b or C3d,g; CR1, complement receptor type 1 that has primary specificity for C3b; CR2, complement receptor type 2 that is expressed only by B lymphocytes and has primary specificity for iC3b and C3d; CR3, complement receptor type 3 that is found on myelomonocytic cells and has primary specificity for iC3b; iPr_2P-F, diisopropyl fluorophosphate; E, sheep erythrocyte(s); EC3b, E bearing C3b; EiC3b, E bearing iC3b; EC3d, E bearing C3d; GaM, F(ab')$_2$ fragment of goat anti-mouse IgG; NP-40, Nonidet P-40; SBTI, soybean trypsin inhibitor; HBSS, Hanks' balanced salt solution; GVB, Veronal-buffered saline containing 0.1% gelatin.

pitated with HB-5 has an apparent M_r of 145,000 (25). The antibody used in the present experiments was the 50% ammonium sulfate-precipitated fraction of mouse ascites fluid induced by the HB-5-producing hybridoma. Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis of this preparation revealed that the IgG2 monoclonal was the predominant protein, and protein concentration was determined by light absorbance at 280 nm.

An IgG2a murine myeloma protein, UPC10 (Bionetics, Kensington, MD), which is directed against levan and inulin, and the IgG2a monoclonal, W6/32, which is directed against a region common to HLA-A, -B, and -C (26) (provided by J. Strominger), were employed as controls. $F(ab')_2$ goat anti-mouse IgG (GaM) (Cappel Laboratories, Cochranville, PA) was employed as a second antibody for rosette inhibition studies. Rabbit $F(ab')_2$ anti-CR1 was prepared as described (11).

Cells. Neutrophils and mononuclear leukocytes were isolated by centrifugation of peripheral blood leukocytes on cushions of Ficoll-Paque (Pharmacia). Residual erythrocytes in the neutrophil preparation were hypotonically lysed and the neutrophils were suspended to 2×10^7 cells per ml in Hanks' balanced salt solution (HBSS) (KC Biological, Lenexa, KS) containing 0.1% bovine serum albumin (HBSS-albumin). The mononuclear cells were enriched for B lymphocytes by removing adherent cells and cells forming rosettes with sheep erythrocytes (E) that had been treated with aminoethylisothiouronium bromide hydrobromide (27).

The Burkitt lymphoma cell line Raji was cultured at 5×10^5 to 2×10^6 cells per ml in RPMI 1640 with 10% fetal calf serum, 2 mM glutamine, 50 units of penicillin per ml, and 50 μg of streptomycin per ml.

To prepare E intermediates bearing different fragments of C3, 10^{10} E in 1.5 ml of half-isotonic Veronal-buffered saline containing 0.1% gelatin, 2.5% dextrose, 0.15 mM $CaCl_2$, and 5 mM $MgCl_2$ ($DGVB^{2+}$) were incubated for 3 min at 37°C with 25 μg of trypsin (Millipore, Freehold, NJ) and 3 mg of purified C3 (28) that had been radiolabeled with ^{125}I (29) to a specific activity of 63,000 cpm/μg. After addition of 0.5 mg of soybean trypsin inhibitor (SBTI; Sigma) in 0.5 ml of $DGVB^{2+}$, the E bearing small amounts of C3b were washed three times with $DGVB^{2+}$ and were then incubated with 100 μg of B (30), 20 μg of D (31), and 20 μg of P (31) in 1 ml of $DGVB^{2+}$ for 45 min at 30°C to form cell-bound amplification C3 convertase sites. These EC3b,Bb,P were washed once with ice-cold $DGVB^{2+}$ and were incubated for 30 min at 30°C with 2 mg of ^{125}I-labeled C3 and 20 μg of P in 1.5 ml of $DGVB^{2+}$ for deposition of additional C3b. Some of these EC3b were converted to E bearing iC3b (EiC3b) by incubation of 7.5×10^9 cells for 30 min at 30°C with human serum diluted 1:20 in Veronal-buffered saline containing 0.1% gelatin (GVB) and 40 mM EDTA (EDTA-GVB) (5). The diluted human serum had been previously adsorbed with 2×10^9 E per ml. The resulting EiC3b were washed three times with EDTA-GVB and stored in this buffer at 4°C. A portion of these cellular intermediates, 5×10^9 cells, was incubated with 5 μg of trypsin in 5 ml of EDTA-GVB for 10 min at 37°C to form E bearing C3d (EC3d) (5). After addition of 100 μg of SBTI in 0.1 ml of EDTA-GVB, the EC3d were washed three times with this buffer. To assure complete conversion of cell-bound C3b to C3d, the EC3d intermediates were subjected a second time to sequential treatment with adsorbed human serum and trypsin. The fragments of C3 bound to each of these cellular intermediates were analyzed by polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ (32) after treatment of erythrocyte membranes with 1 M hydroxylamine (1). Radiolabeled polypeptides of 115,000 M_r and 75,000 M_r were observed with EC3b, of 75,000 M_r and 65,000 M_r with EiC3b, and of 36,000 M_r, 33,000 M_r, and 31,000 M_r with EC3d (Fig. 1).

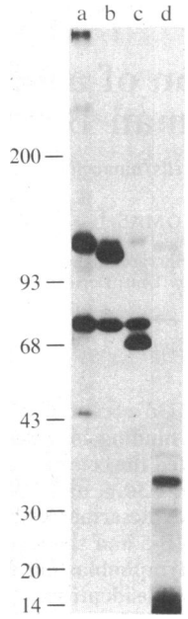

FIG. 1. Analysis of C3 fragments bound to E intermediates. ^{125}I-labeled C3 (lane a) and the covalently bound ^{125}I-labeled C3 fragments that were released with hydroxylamine from EC3b (lane b), EiC3b (lane c), and EC3d (lane d) were reduced with dithiothreitol and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis on a 5–15% polyacrylamide gradient followed by autoradiography. Molecular weights are shown as $M_r \times 10^{-3}$.

Surface Radiolabeling of Cells and Immunoprecipitation. Raji cells were surface labeled by incubating 5×10^7 cells in 0.5 ml of HBSS for 30 min at room temperature with 1 mCi of $Na^{125}I$ (Amersham; 1 Ci = 37 GBq) in vials coated with 150 μg of Iodo-Gen (Pierce). The cells were washed once in HBSS and four times in HBSS-albumin and were lysed by suspension for 20 min in 2 ml of ice-cold phosphate-buffered saline (P_i/NaCl) containing 0.5% Nonidet P-40 (NP-40) (BDH), 0.5 mM EDTA, and 5 mM diisopropyl fluorophosphate (iPr_2P-F) (Sigma). After removal of detergent-insoluble material by centrifugation at 48,000 × *g* for 30 min, the lysate supernatants were precleared by sequential incubation with 50 μg of nonimmune rabbit IgG for 30 min at 0°C and with 0.15 ml of a 25% suspension of protein A-Sepharose CL-4B beads (Sigma) for 45 min at 0°C. The preclearance beads were removed by centrifugation and the supernatants were then incubated with 20 μg of HB-5, followed by addition of 0.15 ml of a 25% suspension of protein A-Sepharose CL-4B beads. Both preclearance and immune beads were washed five times with P_i/NaCl containing 0.5% NP-40 and adsorbed proteins were eluted by incubation of the beads with 0.15 ml of 1% $NaDodSO_4$ for 5 min at 100°C. The eluates were lyophilized, redissolved in sample buffer containing 0.1 M dithiothreitol, and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis on 5–15% polyacrylamide gels (32). Autoradiographs were prepared by exposing dried gels to XAR X-Omat film (Kodak).

Particle Adherence Assays. Twenty-five million EC3b, EiC3b, and EC3d were incubated at room temperature with 10^6 Raji cells, neutrophils, or adherent cell-depleted peripheral blood mononuclear cells, respectively, in 0.1 ml of HBSS-albumin for 60 min with gentle resuspension every 15 min. Wet-mount preparations of the cell mixtures were then examined by light microscopy for the adherence of the erythrocyte intermediates to the nucleated cell types.

Staphylococcus aureus cell wall particles, 3.2×10^7 (Bethesda Research Laboratories), were incubated for 30 min at 0°C with 40 μg of either HB-5 or UPC10 and were washed

three times with P_i/NaCl containing 0.5% NP-40, 0.5 mM phenylmethylsulfonyl fluoride, 5 mM iPr_2P-F, 0.02% sodium azide, and 1 mg of SBTI per ml (21). Each of the two types of monoclonal antibody-bearing *S. aureus* particles was incubated for 90 min at 4°C with lysates of 2×10^7 Raji cells or of 5×10^7 B-enriched mononuclear cells in 0.8 ml of the above buffer. The *S. aureus* particles were then washed once with this buffer, washed three times with GVB, and assessed for their capacity to bind to the C3 fragment-bearing E by incubation of 8×10^6 particles with 5×10^5 E, EC3b, EiC3b, and EC3d, respectively, in 0.05 ml of GVB for 2 hr at 37°C. The adherence of the particles to the cells was assessed by phase-contrast microscopy utilizing a Leitz Orthoplan microscope.

RESULTS

Inhibition of C3 Receptor Function of Raji Cells and B Lymphocytes by HB-5 Monoclonal Antibody. The possible function of the membrane protein recognized by the HB-5 monoclonal antibody as a C3 receptor was assessed initially by examining the capacity of HB-5 to inhibit the formation of rosettes between Raji cells and E bearing C3b, iC3b, or C3d. Preliminary experiments had indicated that Raji cells that had been pretreated with 40 μg of HB-5 per ml for 30 min at 37°C had no impairment of rosette formation with these E intermediates. Therefore, three replicate samples of 10^6 Raji cells per ml were cultured at 37°C in RPMI medium/fetal calf serum alone, in medium containing 20 μg of HB-5 per ml, a concentration 10-fold greater than that necessary for saturation, and in medium containing 24 μg of W6/32 per ml, a concentration of this IgG2a monoclonal antibody to HLA-A, -B, and -C that bound to 10-fold more sites on Raji cells than did excess HB-5. After 24 hr, each replicate sample of cells was washed and divided into two samples that were incubated for 30 min at 37°C with 10 μg of $F(ab')_2$ GaM in 0.05 ml of HBSS-albumin or with buffer alone. The six samples of Raji cells were then assessed for their capacity to form rosettes with the E intermediates bearing 130,000 molecules of C3b, iC3b, or C3d per cell. Forty percent, 92%, and 97% of Raji cells that had been cultured in medium alone bound EC3b, EiC3b, and EC3d, respectively (Table 1). Incubation of these cultured cells with GaM had no effect on their rosette-forming capabilities. Culture of Raji cells for 24 hr in the presence of HB-5 decreased to 8% the proportion of cells binding EC3b, but only slightly diminished the number binding EiC3b and EC3d. Greater inhibition was observed when the HB-5-treated Raji cells were also incubated with GaM so that only 5%, 26%, and 39% of the cells formed rosettes with EC3b, EiC3b, and EC3d, respectively. Identical treatment of Raji cells with W6/32 alone or followed by GaM did not alter the C3 receptor function of these cells.

Table 1. Inhibition by HB-5 of rosette formation between Raji cells and E intermediates bearing fragments of C3

Antibody treatment		% rosette formation*			
24 hr	30 min	E	EC3b	EiC3b	EC3d
		Raji cells			
None	None	0	40	92	97
None	GaM	0	38	98	93
HB-5	None	0	8	75	81
HB-5	GaM	0	5	26	39
W6/32	None	0	37	93	94
W6/32	GaM	0	37	91	96
None	Anti-CR1	0	39	91	89
		Neutrophils			
	None	ND	57	ND	ND
	Anti-CR1	ND	9	ND	ND

*Two-hundred nucleated cells were counted for each sample. Raji cells with greater than three E bound and neutrophils with greater than two E bound were scored as positive for rosette formation. ND, not determined.

Table 2. Adherence to E intermediates of *S. aureus* particles primed with monoclonal antibody and exposed to NP-40 lysates of Raji cells and of B-cell-enriched peripheral blood lymphocytes

Monoclonal antibody on *S. aureus*	Lysate	% E with adherent *S. aureus*			
		E	EC3b	EiC3b	EC3d
HB-5	Raji	1	39	99	98
UPC10	Raji	0.4	0	1	2
HB-5	B cell	1	19	48	74
UPC10	B cell	1	1	3	3

Two-hundred E were assessed for adherence of *S. aureus* in each sample.

Raji cells that had been cultured in RPMI medium/fetal calf serum and incubated for 30 min at room temperature with 5 μg of $F(ab')_2$ anti-CR1 in 0.05 ml of HBSS-albumin showed no diminution in their capacity to form rosettes with EC3b, whereas identical treatment of 10^6 neutrophils with $F(ab')_2$ anti-CR1 decreased the proportion of these cells forming rosettes with EC3b from 57% to 9% (Table 1).

Adherent cell-depleted, peripheral blood mononuclear cells were treated with HB-5 or W6/32 for 30 min at room temperature, followed by GaM, and assessed for their capacity to form rosettes with EC3d. Six percent of the lymphocytes that had been incubated with buffer alone bound EC3d. Treatment of the lymphocytes with either HB-5, W6/32, or GaM alone did not alter the rosette reaction. The addition of GaM to the lymphocytes that had been preincubated with 0.5, 1, 2, 4, and 8 μg of HB-5 per ml caused 40%, 27%, 60%, 57%, and 76% inhibition, respectively, of rosette formation with EC3d. Addition of GaM to lymphocytes that had been treated with comparable concentrations of W6/32 did not inhibit binding of EC3d by these cells.

Binding of the Antigen Recognized by HB-5 to the Erythrocyte Intermediates Bearing Fragments of C3b, iC3b, and C3d. The capacity of HB-5 to inhibit C3 receptor function on Raji lymphoblastoid cells and CR2 function of peripheral blood B lymphocytes only in the presence of second antibody suggested that the monoclonal antibody bound to an epitope on the receptor that was distinct from the ligand binding site. To assess more directly the C3 binding activity of the membrane protein recognized by HB-5, protein A-containing *S. aureus* particles bearing HB-5 or UPC10, an anti-levan IgG2a, were treated with NP-40-solubilized proteins of Raji cells, washed, and assessed for binding to E intermediates. The control *S. aureus* particles that had been primed with UPC10 did not adsorb a constituent of Raji cells that led to the binding of these particles to the E intermediates (Table 2). In contrast, the *S. aureus* particles bearing HB-5 and exposed to the detergent lysate of Raji cells had acquired the capacity to bind to EiC3b, EC3d, and, to a lesser extent, EC3b (Fig. 2). The specificity of immunoadsorption by HB-5 and insolubilized protein A was demonstrated by immunoprecipitation from ^{125}I-labeled Raji membrane constituents of a single protein having the reported molecular weight of the HB-5 antigen (25)—namely, 145,000 M_r (Fig. 3). Additionally, *S. aureus* particles bearing HB-5, but not UPC10, were able to bind to these erythrocyte intermediates after incubation with an NP-40 lysate of normal B-enriched peripheral blood lymphocytes (Table 2). The suboptimal binding observed with these particles may indicate the presence of less HB-5 antigen and may indicate a rank order of binding of C3d > iC3b > C3b.

DISCUSSION

The specificity of the HB-5 monoclonal antibody for an antigen expressed only by B lymphocytes and not by other pe-

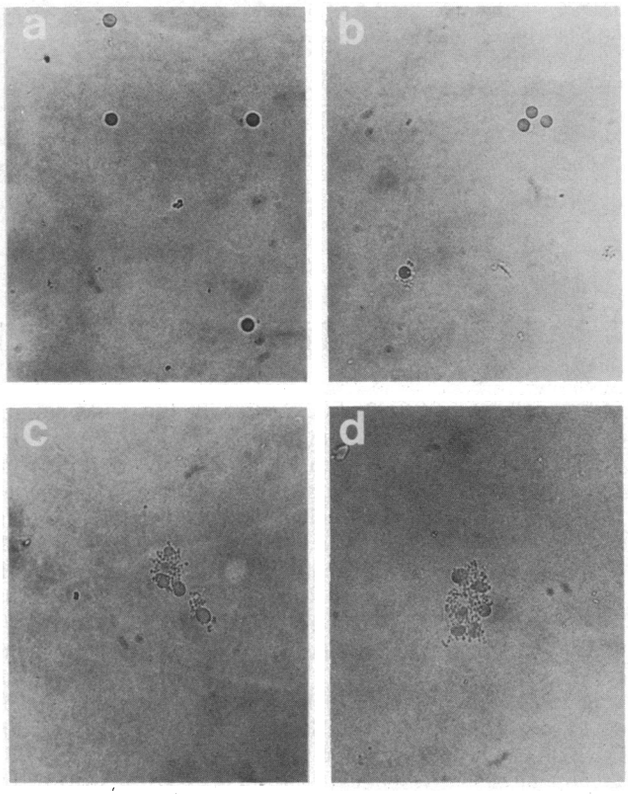

FIG. 2. Phase-contrast micrographs of the adherence of *S. aureus* particles bearing the HB-5 antigen to E (*a*), EC3b (*b*), EiC3b (*c*), and EC3d (*d*). These reaction mixtures are identical to those assessed in Table 2.

ripheral blood or tissue leukocytes (25) and the presence of the C3d receptor, termed CR2, only on B lymphocytes (22, 25) prompted our examination of the possibility of identity between the HB-5 antigen and CR2. As this monoclonal antibody had been selected based on its reactivity with B cells rather than its specificity for CR2, we considered that it might bind to an epitope that was sufficiently distant from the ligand binding site of CR2 to not interfere with receptor function. Indeed, incubation of Raji cells and peripheral blood mononuclear cells with HB-5 alone had minimal inhibitory effects on the binding by these cells of E bearing the defined fragments of C3, C3b, iC3b, and C3d (Fig. 1 and Table 1). Definite interference of C3 receptor function by HB-5 occurred after uptake of a second antibody, GaM, on the cell-bound HB-5 (Fig. 1 and Table 1) and may have been secondary to greater steric interference with the ligand binding site of HB-5 antigen or to an altered topographic distribution, shedding, or internalization of the HB-5 antigen. That less specific effects of HB-5 and GaM may have accounted for the rosette inhibiion was at least partially excluded by the absence of inhibition by treatment of Raji cells and peripheral blood lymphocytes with GaM and W6/32, an anti-HLA monoclonal antibody that bound to 10-fold more sites per cell than HB-5, as determined by cytofluorographic analysis. Thus, the HB-5 antigen either had C3 receptor function or was uniquely associated with the receptor.

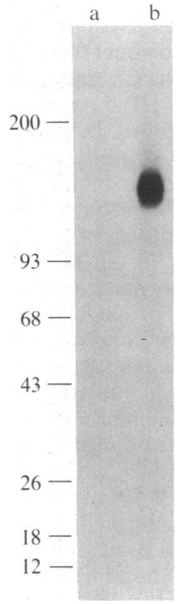

FIG. 3. Autoradiograph of $NaDodSO_4$/polyacrylamide gel electrophoresis of ^{125}I-labeled membrane proteins immunoprecipitated from Raji cells by nonimmune rabbit IgG (lane a) and HB-5 (lane b). Molecular weights are shown as $M_r \times 10^{-3}$.

This evidence that the monoclonal antibody did not react with the ligand binding site of CR2 permitted a direct analysis of the ligand binding activity of the HB-5 antigen by a method previously employed in the identification of monocyte receptors binding iC3b (21). Protein A-bearing *S. aureus* particles were primed with HB-5 and reacted with solubilized proteins of Raji cells under conditions that were associated with the uptake only of a 145,000 M_r polypeptide (Fig. 3), which was identical in size to the antigen found on normal B cells (25). Not only did the immunoadsorbed Raji membrane protein mediate the binding of the *S. aureus* particles to the erythrocyte intermediates (Fig. 2) but these particles bound to the different intermediates in the same rank order as did intact Raji cells—i.e., EC3d = EiC3b > EC3b (Tables 1 and 2). Thus, transferring the HB-5 antigen from Raji cells to *S. aureus* conferred on these particles a complement receptor function that was identical to that of the Raji cell. Acquisition of CR2 function by the *S. aureus* particles also occurred when normal B lymphocytes served as the source of the HB-5 antigen (Table 2).

The conclusion that the HB-5 antigen is CR2 is based on its capacity to bind iC3b and C3d and its expression only by B lymphocytes (22, 25) and B-lymphoblastoid cell lines, the latter characteristics serving to distinguish it from CR3, which has a different cellular distribution (19). The additional findings that HB-5 inhibited the binding by Raji cells of EC3b (Table 1) and that the antigen mediated the binding of *S. aureus* particles to EC3b (Fig. 2 and Table 2) explains the apparently discrepant findings from several laboratories that Raji cells form rosettes with particles bearing large amounts of C3b (9, 33, 34), despite lacking CR1 (12, 35). The C3d region in C3b may be sufficiently exposed for weak interaction with CR2. Sepharose-C3b has been reported to absorb 140,000 M_r membrane proteins from Raji cells (36) and B lymphocytes (37). Based on the present finding of a similar molecular weight for CR2 and the capacity of CR2 to bind C3b, the adsorbed proteins may have represented CR2. In support of this conclusion is the recent finding that a 140,000 M_r membrane protein of lymphocytes that was adsorbed by Sepharose-C3 also bound to Sepharose-C3d and was recognized by a monoclonal antibody that inhibited CR2 function of B lymphocytes (38). The previous identification of CR2 as a 72,000 M_r protein that was present in Raji cell culture supernatants may indicate either that an additional membrane protein on these cells is capable of mediating CR2 function or that the 72,000 M_r protein represented a proteolytically degraded fragment of the HB-5 antigen (24).

Although B lymphocytes have been known to express

CR1 and CR2 for a decade, the definition of their role in the reactions of these cells requires the identification of the membrane proteins having receptor function (11). For example, antibody to the C3b receptor has been shown recently to enhance the maturation into antibody-secreting cells of B lymphocytes suboptimally stimulated with pokeweed mitogen (39). The recognition of the 145,000 M_r protein recognized by HB-5 as CR2 will permit similar approaches to an understanding of the role of this C3 receptor in B-cell functions.

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