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The Binding of IgM to B Lymphocytes: A Comparison of the Binding Characteristics of IgM Aggregates and EA_B (IgM) Complexes to Normal and Leukemic B Lymphocytes[1] (41700)

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Abstract. We have compared and contrasted the binding of Agg IgM and heavily sensitized EA_B (IgM) complexes to the Fc_μ receptor of normal and neoplastic human lymphocytes. Agg IgM binds uniformly to the entire $SmIg^+$ B cell population yet normal lymphocytes require culture in order to achieve binding of EA_B complexes to a subset of $SmIg^+$ B cells. In blocking studies IgM complexes and IgM aggregates appear to detect the same receptor and with both reagents binding is influenced by the presence of Mg^{2+} but not Ca^{2+} and is inhibited by EDTA.

The percentage of cells binding EA_B was highest in normal B lymphocyte fractions enriched for C_3^+ cells (CRL^+). EA_B binding to cells in the CRL^- fractions was negligible even though CRL^- fractions contained cells which were $SmIg^+C_3^-$. EA_B bound only to neoplastic chronic lymphocytic leukemia cells (CLL) that expressed a high percentage of C_3^+ cells. Clones lacking a C_3 receptor failed to bind EA_B. Thus, the binding of EA_B complexes to B lymphocytes appears to be associated principally with a subset that expresses a C_3 receptor whereas IgM aggregates bind to the entire $SmIg^+$ B cell population.

Both T and B lymphocytes have a receptor for the Fc portion of the IgM molecule (1–11). A rosetting assay utilizing bovine erythrocytes sensitized with IgM antibody [EA_B (IgM)] has been widely used for the detection of T_μ receptors. In spite of various modifications of technique the results of such assays vary considerably in the literature. In order to detect B lymphocyte Fc_μ receptors Dickler and colleagues and Ferrarini and colleagues modified the assay conditions and reagent surface antibody density (4, 8, 10). Even with these alterations the detection of a subset of SiG^+ B cells requires overnight incubation since the binding of EA_B (IgM) to freshly drawn B cells is highly variable. Paradoxically, certain neoplastic B lymphocytes derived from patients with chronic lymphocytic leukemia (CLL) or malignant lymphomas will bind EA_B (IgM) complexes when freshly drawn yet binding may diminish after overnight incubation (12–14).

In recent studies we have shown that heat-aggregated IgM will exclusively bind to the Fc_μ receptor of $SmIg^+$ B lymphocytes without binding to T cells (11, 15, 16). The binding characteristics of aggregates also differ from those of EA_B (IgM) complexes. IgM aggregates will bind to fresh as well as cultured B cells equally and, furthermore, the vast majority rather than a subset of SIg^+ B cells appear to bind aggregates.

In this investigation we have compared and contrasted the binding of heavily sensitized EA_B (IgM) complexes and IgM aggregates to normal and neoplastic lymphoid cells. Our data suggest that although both reagents may be detecting the same receptor, EA_B complexes appear to bind principally to a subset of B lymphocytes that express a receptor for C_3.

Materials and Methods. *Isolation and preparation of mononuclear cell suspensions.* Mononuclear cells from the blood of normal and leukemic donors were isolated on Ficoll–Isopaque density gradients. All leukemic donors had blood leukocyte counts in excess of 25,000/mm^3. In order to prepare purified lymphoid populations, gradient-derived cells populations were depleted of monocytes by incubation of cells in Hank's balanced salt solution (HBSS) on plastic dishes at 37°C for 0.5 hr. Monocyte contamination of cells suspensions was subsequently enumerated by the ability to phagocytize latex particles and morphologic identification with phase-constrast

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microscopy of living cells and Wright's-stained smears of fixed cells. Suspensions of normal lymphocytes characteristically contained <5% contaminating monocytes and often <1%. Neoplastic cell suspensions uniformly had <1% monocytes. All cell suspensions were incubated in serum-free medium for 0.5 hr at 37°C to remove cytophilic antibody.

Lymphocyte subset fractionation. Monocyte depleted lymphocyte preparations from normal human donors in HBSS were rosetted with neuraminidase-treated sheep RBC at a ratio of 1:100 and the rosetted cells were separated on a Ficoll–Isopaque gradient. Nonrosetting cells at the interface were collected and incubated with IgM- and C'_3-coated heterologous erythrocytes (19S EAC) for 30 min at 37°C on a rotator. The cells were then placed on a density gradient. Nonrosetting cells at the interface were complement rosette negative lymphocytes (CRL^-). The rosetting cells in the pellet were stripped of 19S EAC reagent by agitation, placed on another density gradient, and the cells at the interface held as the complement rosette-positive lymphocyte (CRL^+) fraction.

Preparation of reagents. Ig. aggregates. IgM was purified from rabbit sera, fluorescein conjugated, and aggregated as previously described (15). Briefly, serum was precipitated with 50% saturated $(NH_4)_2SO_4$ and fractionated by sequential passage through Sephadex G-200 (Pharmacia, Uppsala, Sweden) and Bio Gel A-5M (Bio-Rad, Richmond, Calif.) chromatographic columns. Final purification employed a staph Protein A–Sepharose 4B column (Pharmacia, Uppsala, Sweden) to remove IgG and a Sepharose 4B affinity column coupled with CNBr to a high-titer goat anti-rabbit IgA antiserum to remove IgA from the rabbit protein. Human IgG was isolated from Cohn Fr II (Miles Labs, Elkhart, Ind.) on Cellex-D (Bio-Rad, Richmond, Calif.). Final purity of each Ig preparation was verified by immunodiffusion and sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. Freshly isolated Ig was conjugated to fluorescein isothyocyanate and excess FITC removed on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden). Preparations with o.d. 495/280 ratios in excess of 2.0 were used.

Ig solutions were heat aggregated as follows. Rabbit IgM (either FITC labeled or unconjugated) at a concentration of 15–20 mg/ml in phosphate-buffered saline (PBS), pH 7.0, was heated at 63°C for 0.5 hr in glass tubes in a water bath (17). After cooling, the solutions were diluted with PBS, pH 8.0, and centrifuged at 145,000*g* for 1 hr. The resulting pellet was resuspended in PBS with sodium azide. Working dilutions contained approximately 2.5 mg of protein/ml and were centrifuged at 1700*g* immediately before use to remove insoluble aggregates. The IgM reagents were used for 4–6 weeks (11) and the IgG reagent for 2 weeks (17).

Preparation of IgM antibody-coated bovine erythrocytes [EA(IgM)]. Maximum sensitization of bovine RBC was accomplished using a modification of the method described by Pichler (8). Bovine RBC were sensitized with a dilution of a chromatographically purified rabbit anti-bovine RBC antibody of the IgM class (protein concentration, 0.7 mg/ml; hemolysis titer, 1:1600). The precise dilution varied somewhat (usually approximately 1:20) and was determined as the dilution that gave optimal rosetting characteristics with EA_B $(IgM)^+$ cells from a control patient with CLL yet resulted in a minimal degree of agglutination. Washed packed bovine RBCs were first incubated with an equal volume of diluted antibody fraction at 37°C for 30 min, washed, and then resuspended at 1×10^9 cells/ml in HBSS + 1% BSA + gentamicin. The preparations were stored at 4°C and prepared fresh weekly.

Cells were assayed by incubating equal volumes of lymphocytes at 1×10^7 cells/ml and EA_B (IgM) reagent at 1×10^9 BRBC/ml at 37°C for 10 min followed by centrifugation at 200*g* for 5 min and further incubation at 4°C for 2 hr. Preparations were gently resuspended with trypan blue and assessed by light microscopy. The adherence of three or more bovine RBCs was considered positive and the characteristics of the rosettes were noted. Washed, unsensitized bovine RBCs served as a control for each experiment.

In order to determine whether our EA_B (IgM) complexes were contaminated with rabbit C'_3, we incubated the EA(IgM) complexes with goat antibody against rabbit C'_3 (Cappel Labs, Cochranville, Pa.) for 0.5 hr at 22°C. Cells were then washed and reincubated with fluorescein-conjugated rabbit anti-goat

IgG for 0.5 hr at 22°C. Cells were washed and when examined for fluorescence, none was detected. EA(IgM) complexes which had been incubated with anti-C'_3 antibody were also reacted with Fc_μ-positive, T-cell-depleted, normal lymphocytes which had been incubated overnight at 37°C. The presence of the anti-C'_3 antibody did not influence subsequent EA_B (IgM) binding.

19S EAC. A rabbit anti-sheep RBC antibody of the IgM class was obtained from Cordis Labs (Hialeah, Fla.). A subagglutinating titer of this antibody was determined by serial hemodilution experiments. An aliquot of washed, packed sheep RBCs was incubated with an equal volume of a subagglutinating titer (usually 1:250) of the antibody for 0.5 hr at 37°C. After washing, the cells were incubated with fresh mouse serum (source of complement) to equal 50% of the packed cell volume for 0.5 hr at 37°C. Cells were assayed for the presence of a C'_3 receptor after incubation of equal volumes of lymphocytes (1×10^7/ml) and 19S EAC (1×10^9/ml) for 30 min at 37°C on a rotating wheel as described by Bianco (18). EA(IgM) complexes lacking complement served as a control for each determination. Lymphocytes with a >3 adherent RBC were scored as positive.

Other surface markers. Cells were examined for E rosettes and surface membrane Ig as described in earlier publications (19, 20).

Results. *Comparison of Agg IgM and EA_B (IgM) binding to normal lymphocytes.* Lymphocytes from 12 random adult donors of all ages were studied (Table I). Cell isolates were initially examined for E rosette$^+$ and SmIg$^+$ cells. The percentages of cells binding AggIgM$^+$ and EA_B (IgM) were then compared. The percentage of SmIg$^+$ B cells in these monocyte-depleted isolated ranged from 5.2–26% (mean 11.5 ± 5.4). The percentages of AggIgM$^+$ cells were closely parallel, ranging from 4.4–23.2% (mean 9.3 ± 4.9). Values obtained with EA_B complexes were consistently lower, ranging from 2.0–7.7% (mean 2.6 ± 1.9). It is of interest that using EA_B complexes which have been heavily sensitized promotes minimal binding to fresh cells in many instances. As in previous reports, overnight incubation of cell isolates at 37°C in RPMI-1640 and 20% fetal calf serum (FCS) increased binding of EA_B complexes in each case (single exception donor No. 11) (3, 4, 8, 9). The percentage of AggIgM binding cells remained essentially unchanged following incubation. Although the post incubation values for EA_B rose, they were uniformly less than the values for aggregates and in 5 instances the value was significantly lower. The mean for aggregates was 8.9 ± 5.2% as compared to 6.6 ± 6.0% for complexes.

Blocking of EA_B binding with IgM aggregates. The difference in binding characteristics of aggregates and complexes to the Fc_μ receptor could be explained if there were separate

TABLE I. BINDING OF IgM AGGREGATES AND EA_B COMPLEXES TO NORMAL LYMPHOID CELLS: EFFECT OF PRIOR OVERNIGHT INCUBATION

Donor	Percentage E rosette$^+$	Percentage SmIg$^+$	AggIgM		EA_B	
			Fresh	p̄ incub.	Fresh	p̄ incub.
1	82	12.9	11.9	10.6	2.7	8.6
2	89	6.5	7.1	6.5	1.8	2.8
3	82	9.1	5.1	5.4	0.8	4.9
4	75	12.0	7.7	8.2	4.0	6.2
5	90	7.8	6.0	7.1	1.9	6.7
6	84	8.0	8.8	7.0	3.8	5.6
7	68	26.0	23.2	24.2	2.4	24.2
8	87	5.2	4.4	5.2	0.5	1.7
9	79.5	14.1	10.3	11.8	3.0	6.0
10	80	11.9	9.6	8.0	7.7	8.2
11	86.6	11.0	10.5	7.5	2.0	1.5
12	79	14.1	6.9	6.0	1.0	3.0
Mean ± SD		11.5 ± 5.4	9.3 ± 4.9	8.9 ± 5.2	2.6 ± 1.9	6.6 ± 6.0

TABLE II. BLOCKING OF EA_B (IgM) ROSETTING OF B CELL-ENRICHED FRACTIONS WITH AGGREGATED IgM[a]

Blocking reagent	Percentage EA_B rosettes
HBSS	21.6 (16.0–27.0)*
Agg BSA (4 ml/ml)	16.8 (12.5–22.5)
Agg IgG (4 mg/ml)	15.1 (10.0–20.0)
Agg IgM (0.1 mg/ml)	1.8 (1.0–4.0)
(0.2 mg/ml)	0
(0.4 mg/ml)	0

[a] Fractions derived from the blood of a patient with CLL. These fractions contained 89.2% IgM_R^+ cells and 90% SIg^+ cells.

* Numbers in parentheses represent 95% confidence intervals.

membrane binding sites. In order to examine the possibility of two separate receptors, we prepared purified proteins to use in blocking experiments. B-cell-enriched lymphocyte fractions from a patient with CLL that bound complexes as well as aggregates both before and after overnight incubation at 37°C were used in these experiments. Cells were first incubated for 30 min at 4°C with the blocking protein followed by incubation with EA_B (IgM) complexes. The results are given in Table II. Preincubation of cells with aggregated bovine serum albumin (BSA) and medium alone (HBSS) had little or no effect on complex binding. AggIgG also had a similar effect on subsequent EA_B complex binding. However, aggregated IgM clearly blocked complex binding. Complete inhibition of EA_B binding was only observed with AggIgM in concentrations as low as 0.1–0.2 mg/ml.

Divalent cation requirement. In order to determine whether divalent cations influence binding, we prepared T-cell (E rosette$^+$) and monocyte-depleted lymphocyte fractions from several donors. Cells were cultured overnight, washed thoroughly, and resuspended in divalent cation-free medium (PBS) at 1×10^7 cells/ml. Graded concentrations of Mg^{2+} or Ca^{2+} were added to various aliquots of cells in PBS. Each aliquot was then tested for EA_B and AggIgM binding (Table III). In experiment 1, no aggregate binding and minimal EA_BIgM binding was seen with cation-free PBS. A Mg^{2+}-containing control showed 20.3% EA_B (IgM) rosettes and 92% $AggIgM^+$ cells. No change was seen following the addition of Ca^{2+}. In experiment 2, however, the addition of Mg^{2+} enhanced binding of both EA_B and IgM aggregates. After a steep increase between 2.5 and 5.0 m*M* added Mg^{2+}, binding plateaued. When a divalent cation chelater (EDTA) was added to PBS containing an optimal amount of Mg^{2+}, the values for EA_B and AggIgM binding decreased. In the case of EA_B complexes, it returned to the PBS control value. The addition of EDTA to the AggIgM system not only caused a decrease in per-

TABLE III. EFFECT OF DIVALENT CATIONS ON THE BINDING OF EA_B AND AGGIgM TO B LYMPHOCYTES

Divalent cation	EA_B (%)	AggIgM (%)	AggIgM Fluorescent intensity[a]
Experiment 1			
PBS[b]	2.6	0	—
PBS + 5 m*M* Ca^{+2}	3.9	0	—
10 m*M* Ca^{+2}	3.3	0	—
15 m*M* Ca^{2+}	4.4	0	—
PBS (+5 m*M* Mg^{+2})	20.3	92	2+
Experiment 2			
PBS[a]	7.1	8.0	1+
PBS + 2.5 m*M* Mg^{+2}	9.6	9.1	1–2+
5.0 m*M* Mg^{+2}	15.2	14.7	1–2+
7.5 m*M* Mg^{+2}	16.4	22.3	2–3+
10.0 m*M* Mg^{+2}	17.6	26.4	3–4+
15.0 m*M* Mg^{+2}	19.1	29.3	3–4+
PBS + 15 m*M* Mg^{+2} + 25 m*M* EDTA	7.1	16.7	1+

[a] Fluorescence graded 1–4+.

[b] PBS is divalent cation-free.

centage of fluorescent-labeled cells, but also resulted in a decrease in fluorescent intensity.

Binding of IgM to normal lymphocytes following subset fractionation. Our data and that in the literature suggest that EA_B (IgM) complexes detect a subset of the total B cell population. A commonly used method for preparing B-enriched fractions of normal blood lymphocyte populations employs density gradient centrifugation of lymphocytes rosetted to red cells sensitized with IgM antibody and complement (19S EAC) (8, 24). Utilizing this technique, a $SmIg^+C_3'^+$ (CRL^+)-enriched B-cell fraction is obtained. We tested the enriched and depleted fractions isolated in this manner in order to determine whether EA_B complexes bind preferentially to the B-enriched fraction. Binding of aggregates and complexes was compared in C_3'-enriched (CRL^+) and depleted (CRL^-)-fractions obtained from seven normal donors (Table IV). Cells were processed in three different ways: (i) no initial E rosette separation (ii) an initial E rosette separation and (iii) an initial E rosette separation utilizing neuraminidase-treated RBCs. Assays were performed on cells following an overnight incubation at 37°C. The CRL^+ fraction contained $SmIg^+C_3'^+$ cells which bound both EA_B complexes and aggregates. The values for both aggregates and EA_B were similar no matter which method of enrichment was used. The CRL^- fraction on the other hand, contained $SmIg^+$ B lymphocytes. These cells are usually $SmIg^+C_3'^+$. However, this fraction may contain $SmIg^+C_3'^+$ cells with a low density of C_3' receptors. Such cells lose their adherent RBCs during centrifugation and are separated into the CRL^- fraction. EAC rosetting cells were sought in four of the seven donors in the CRL^- fraction of small percentages (3.9–5.0%) were in fact found. Characteristically, these rosettes were fragile and usually consisted of three to four adherent RBCs.

TABLE IV. PERCENTAGE OF NORMAL LYMPHOCYTES BINDING IgM FOLLOWING GRADIENT SEDIMENTATION OF 19 S EAC-TREATED CELLS

	Fraction			
	C′ enriched (CRL^+)		C′ depleted (CRL^-)	
Donor	AggIgM	EA_B (IgM)	AggIgM	EA_B (IgM)
1	7.8	12.6	12.0	2.0
2	6.0	17.0	15.5	0
3	12.5	16.3	15.9	<1.0
4	13.5	6.5	14.5	0
5	63.0	49.0	15.0	2.0
6	31.8	33.3	33.6	4.9
7	88.5	69.0	20.7	1.7

Note. All assays performed on cells incubated overnight at 37°C. Samples 1 and 2 were not subjected to an initial E rosette separation, 3–5 were separated by differential centrifugation, and neuraminidase-treated sheep cells were used in the initial separation in samples 6 and 7.

As anticipated, cells in the CRL^- fraction continued to bind IgM aggregates in percentages that paralleled the $SmIg^+$ population (Table IV). In contrast, EA_B binding was negligible in the CRL^- fraction in all patients. Three of seven samples essentially showed no binding and the remainder exhibited low percentages (1–4.9%) thus indicating that in CRL^- (depleted) fraction, $SmIg^+C_3'^-$ cells remain which still bound aggregated but did not bind EA_B complexes.

IgM binding to neoplastic B lymphocytes. Monoclonal (neoplastic) B-cell isolates derived from patients with chronic lymphocytic leukemia were studied in order to (i) compare and contrast the binding characteristics of complexes and aggregates and, (ii) test the hypothesis that complexes bind preferentially to an $SmIg^+C_3'^+$ subset in contrast to cells which express an $SmIg^+C_3'^-$ phenotype.

We only studied cell isolates that were predominantly neoplastic in character ($SmIg^+ > 70\%$ of total cells). Binding of aggregates and complexes was measured in fresh isolates and after overnight incubation at 37°C in RPMI-1640 + 20% FCS. SmIg and 19S EAC rosetting were determined in both samples as well.

As can be seen in Table V, fresh cells characteristically bound aggregated IgM and the percentages paralleled the $SmIg^+$ population. After incubation, there was little change in the percentages of cells binding aggregates. Fresh cells from the majority of cases bound complexes as well. With overnight incubation at 37°C in medium, the percentage of cells binding complexes dropped in most instances tested.

The relationship of IgM binding to a C_3' receptor was also examined in these cell iso-

TABLE V. BINDING OF IgM TO NEOPLASTIC B LYMPHOCYTES

Donor	Percentage $SmIg^+$	Percentage $C'_3{}^+$	Percentage $AggIgG^+$	Percentage $AggIgM^+$		Percentage $EA_B{}^+$	
				Fresh	$\bar{p}$ incub.	Fresh	$\bar{p}$ incub.
CLL							
1	95	37	10[a]	95	95	60	22
2	85	30	85	85	NT[b]	65	NT
3	85	70	85	85	80	50	14
4	70	61.6	60	76	80	72	21
5	97	32.6	90	97	97	44	15
6	95	50	95	79	NT	60	NT
7	95	34	95	95	85	50	5
8	95	65	95	95	95	75	6.5
9	95	30	50	95	52.7	50	30.3
10	95	51	90[a]	95	NT	70	NT
11	85	0	30[a]	60	45	0	0
12	95	9.3	95	95	95	3.4	<1

[a] Faint staining.
[b] NT = not tested.

lates. Two clones (Nos. 11, 12) expressed a complement receptor in low percentage (<15%). These two clones bound aggregates in high percentage (60 and 95%), yet EA_B were not bound (0, 3.4%). In each case where a high percentage of cells expressed a C'_3 receptor, complexes were bound by a large fraction of the cells. There were no clones that expressed a receptor for EA_B complexes in the absence of a receptor for IgM aggregates.

Discussion. The demonstration of the Fc_μ receptor on B lymphocytes utilizing EA_B (IgM) has been hampered by difficulties in preparing a standardized reagent and clearly defining those conditions which will promote preferential binding to the B-cell receptor. The study of Pichler and Knapp (14) has suggested that heavily sensitized erythrocytes might achieve this goal. Based on these observations we have prepared an EA_B (IgM) reagent which appears to detect the B-cell Fc_μ receptor yet retains cross-reactivity with T cells. The major purpose of this study was to compare the binding of EA_B complexes to the IgM aggregate reagent which we have developed that is specific for the B-cell Fc_μ receptor and does not cross-react with T cells. In this study we have shown that the characteristics of the binding of EA_B (IgM) complexes and IgM aggregates to the Fc_μ receptor of B lymphocytes differ in several important respects. In our previous work and in this study as well, aggregate binding tends to be more consistent under varying conditions and aggregates detect virtually all $SmIg^+$ B cells. In our hands and those of other investigators, binding of EA_B complexes requires overnight incubation and only a subset of $SmIg^+$ B cells is detected (20). In spite of these differences in binding characteristics, both reagents may be detecting the same surface receptor moiety. In our blocking experiments with various aggregated proteins, only IgM completely blocked EA_B binding to the Fc_μ receptor.

Binding of EA_B and IgM aggregates to human lymphocytes is influenced by the presence of Mg^{2+} but not Ca^{2+}. This result is similar to findings recently reported in the mouse (22). Murine lymphocytes express an Fc_μ receptor and the Mg^{2+}-dependent binding of EA(IgM) complexes can be depressed by addition of divalent cation chelaters. This is in contrast to mouse macrophages which exhibit Ca^{2+}-dependent EA(IgM) complex binding (23). The observation that the binding of both EA_B and aggregates to mouse and human lymphocytes is Mg^{2+} dependent further suggests that these reagents may be detecting a single species of surface receptors.

Our results indicate that maximally sensitized EA_B complexes detect a subset of B lymphocytes. Since earlier studies reported a high percentage of EA_B-binding cells present in B-cell fractions enriched by density centrifugation of 19S EAC rosetted cells (8, 24), we investigated the possibility that the binding of

EA_B complexes to B lymphocytes is associated with the expression of a C'_3 receptor. Our data support such a concept. Lymphocytes in the CRL^+ fraction bound EA_B and IgM aggregates equally as well and the percentages approached that of $SmIg^+$ cells. In contrast, the CRL^- fraction which still contained $SmIg^+$ cells that lack a C'_3 receptor, bound IgM aggregates but did not bind EA_B complexes. Furthermore, if EA_B complexes detect $SmIg^+C'^+_3$ cells, then lymphoid tumors which are monoclonal expansions might be expected to mimic normal cells in their binding characteristics. Neoplastic clones with the phenotype $SmIg^+C'^+_3$ should bind complexes while $SmIg^+C'^-_3$ clones should not. Of the 12 neoplastic clones studied, 2 bound both 19S EAC and EA_B (IgM) in low percentage, yet bound IgM aggregates. In the remaining donors, whenever a C'_3 receptor was expressed, a large fraction of the cells bound EA_B.

The apparent correlation between EA_B binding and the presence of a C'_3 receptor deserves further comment. We examined the possibility that this merely reflected an artifact resulting from the presence of a C'_3 bound to EA_B complexes and an augmentation effect of binding through a C'_3 receptor. No C'_3 could be detected on the complexes when looked for directly or in blocking experiments. A possible explanation for the preferential binding of EA_B to C'_3 receptor-positive cells may relate to the affinity of EA_B for the receptor and the density of surface membrane Fc_μ receptors (7). Binding would be increased if the density of surface Fc_μ receptors were high and cross-linking could be maximized. It may be that C'^+_3 receptor cells have a high density of Fc_μ receptors and C'^-_3 cells have a relatively low density. Under these circumstances, the presence of a C'^+_3 phenotype correlates with EA_B binding.

A similar correlation between Fc_γ receptors and C'3 receptors has been shown to exist for mammalian lymphocytes (1). Presumably the coexistence of antigen–antibody complex and C'3 receptors on the B-cell surface is related to the generation of B-cell activation signals. The data in this study suggest that there exists a subset of SIg^+-positive B cells that bear Fc_μ and Fc_γ receptors as well as a receptor for C'3. It is by no means clear how these surface membrane structures interact *in vivo* when B cells are activated, but it seems likely that they are intimately involved in triggering B-cell responses to antigen.

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