# THE INTERACTION OF IgE WITH RAT BASOPHILIC LEUKEMIA CELLS

## I. Evidence for Specific Binding of IgE

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The initial and prerequisite event in IgE-mediated degranulation of mast cells and basophils is the binding of IgE to the surface membrane of these cells. The binding appears to be via the Fc region of the immunoglobulin (1, 2) leaving the Fab region free to react with antigens which then trigger degranulation.

Very little is known about the cellular receptors to which the Fc region of the IgE becomes bound. Our aim is to explore the IgE binding in detail; to study the binding parameters and to isolate and characterize the cellular receptor activity for IgE.

In this paper we present our initial studies on a rat basophil leukemia (3) which may be useful in this regard. The leukemic cells deplete reaginic passive cutaneous anaphylaxis (PCA)[1] activity from rat and mouse immune sera. Specific adsorption of rat IgE immunoglobulins was detected by the use of radiolabeled antilight-chain antibodies and IgE.

### *Materials and Methods*

*Rat Basophil Leukemia.*—A litter of 10 ICI Wistar rats (Imperial Chemical Industries, Ltd., Macclesfield, England) which had been subcutaneously inoculated at birth with leukemic basophils (3) 2 wk earlier, were received by us on 3/20/73. Daily blood smears were monitored and when leukemic basophils or tumors appeared the animals were sacrificed, and heparinized leukemic blood or minced tumor was transplanted subcutaneously.

After our initial experience (see Results) the following protocol has been routinely used: Basophilic tumor is removed from 2–3-wk old rats and minced in cold Tyrode's buffer (T). This buffer was adjusted to pH 7.6 at room temperature. After filtering through glass wool, 0.05–0.10 ml of a suspension containing 0.5–1 million cells/ml is inoculated subcutaneously, with a 26 gauge × ⅜ inch needle, into the back of the neck of newborn to 48-h old rats. Tumor develops in 10–15 days in about 70–80% of Wistar rats (ICI and WKY/N strains).

The leukemic cells were adapted to cell culture (see Results). They are now propagated

[1] *Abbreviations used in this paper:* PCA, passive cutaneous anaphylaxis; T, Tyrode's buffer; TCM, Tyrode's buffer with 1 mM $Ca^{++}$ and $Mg^{++}$.

according to the following protocol: Cells are grown in suspension in stationary Falcon flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) and are monitored by media pH, inverted microscopy, and cell counts. Eagle's Basal No. 2 Media supplemented with 15% heat-inactivated fetal calf serum[2] (Grand Island Biological Co., Grand Island, N. Y.), 4 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin is used. 50–70% of the medium is replaced every 2–3 days maintaining the cell density at 4–10 × $10^5$ cells/ml.

*Mouse Mastocytomas.*—$LAF_1$ mice with the ascitic mastocytoma of Furth (4) were obtained from P. Minard and D. Levy, Johns Hopkins University. The tumor was maintained by intraperitoneal (i.p.) transplantation of ascites every 7–10 days into 6–8-wk old $LAF_1$ mice (Jackson Laboratories, Bar Harbor, Maine).

Two Balb/c mice carrying the P815 ascitic mastocytoma (5) (generation 314) were provided by Dr. M. Potter, NCI, NIH. The tumor was maintained by weekly i.p. transplantation of 0.05-ml ascites plus 0.05 ml Ringer-Locke solution into $CDF_1$ mice (Balb/C × DBA/2).

*Rat Reaginic Serum.*—The pool of serum from rats injected with *Nippostrongylus brasiliensis* which was used in these experiments has been described elsewhere.[3]

*Mouse Reaginic Serum.*—Mouse reaginic serum (pool I, Tables I, II, and V) was prepared by injecting 2-mo old Swiss mice monthly with 1 μg ovalbumin (2× crystallized, Worthington Biochemical Corp., Freehold, N. J.) i.p. according to the method of Levine and Vaz (6). 1-wk after the fifth and subsequent injections the animals were bled and the high titered serums pooled. This pool had a PCA titer of 1:1024.

A pool of mouse reaginic serum (pool II, Table V) from $LAF_1$ mice injected with ovalbumin, ovomucoid, lysozyme, benzylpenicilloyl conjugates of bovine IgG, and serum albumin was a gift from Dr. R. Siraganian, NIDR, NIH. The pool had a titer of 1:1,280 vs. ovalbumin and ovomucoid, and a titer of <1:160 against the other immunogens.

*Purified Rat [$^{125}$I]IgE.*—Substantially purified rat [$^{125}$I]IgE was prepared as described elsewhere (footnote 3). For the experiments described here, preparations in which 65% of the counts were serologically identifiable as IgE were routinely used. The preparations contained approximately 25–50 μg of [$^{125}$I]IgE and 200–300 μg of uniodinated ovalbumin/ml.

An immunoglobulin-depleted preparation of the [$^{125}$I]IgE solution was obtained by passing the iodinated material through a rabbit antirat Fab column as described elsewhere (see footnote 3). This material contained 31% of the original counts and by radioimmunoassay only ~5% of the counts were precipitable by a goat antirat IgE serum.

*Inactivated Reaginic Serums.*—Reaginic serums were heat inactivated by incubation at 56°C for 4 h with complete loss of PCA titer. A reaginic fraction of rat serum purified by fractional ammonium sulfate precipitation, gel filtration, and ion-exchange chromatography[3] was dialyzed overnight vs. Tris 0.2 M, pH 8.6. One-half of the 1.36-mg sample which contained 8,000 PCA U was made 0.01 M in dithiothreitol, incubated at 37°C for 1 h, subsequently made 0.022 M in iodoacetamide and further incubated for ½ h. The reagents were added in the reverse order to another aliquot which served as a control. Both samples were dialyzed against T overnight. The treated sample had <250 PCA U/ml and the control 1,000 PCA U/ml.

*Iodinated Rabbit Antirat Light-Chain Antibodies.*—The preparation of purified antirat light-chain antibodies has been described.[3] $F(ab')_2$ fragments of these antibodies were prepared by digesting a 0.85% solution with 2% wt/wt pepsin (Worthington Biochemical Corp, Freehold, N. J.) at pH 4.5, 37°C for 16 h (7). $F(ab')_2$ fragments were isolated by chromatography on Sephadex G-150 (Pharmacia Fine Chemicals, Inc, Piscataway, N. J.). Fab′ fragments were

[2] Either "gamma-globulin free" or conventional fetal calf serum have been used with no apparent difference in the cell growth.

[3] Isersky, C., A. Kulczycki, Jr., and H. Metzger. 1973. Isolation of IgE from reaginic rat serum. *J. Immunol.* In press.

prepared by reducing a 0.42% solution of $F(ab')_2$ fragments with 0.01 M dithiothreitol at pH 8.6 for 1 h and alkylating with 0.022 M iodoacetamide for 20 min, all at room temperature. Fab' fragments were purified by chromatography on Sephadex G-100.

The intact antibodies and their fragments were iodinated by a modification[3] of the procedure of McConahey and Dixon (8). Approximately $2 \times 10^{-3}$ mol $^{125}I$ were incorporated/mol of protein giving $1.0–1.5 \times 10^8$ cpm/mg when counted in a Packard Gamma Scintillation Spectrometer (Packard Instrument Co., Inc, Downers Grove, Ill.).

In order to approximate the antigenic valence of IgE with respect to the [$^{125}I$]antilight-chain antibodies, increasing amounts of the latter were added to a known amount of rat IgG. The complexes were then precipitated with excess rabbit antibodies specific for the Fc region of rat IgG. At saturation, approximately 3.5 mol of antilight-chain antibody were bound/mole IgG.

*Reagin Depletion and Binding Studies.*—Depletion and binding studies were performed with tumor minces or cultured cells alternatively. When tumors were used necrotic and capsular material was removed and the tumor minced in cold T. The suspension was filtered through glass wool and the cells washed at least once with cold T. When cultured cells were used they were simply centrifuged and washed at least once. In both cases the cells were counted in the presence of trypan blue. The tumor minces always contained moderate numbers of broken cells and showed only 60–70% viability of the intact cells. The cultured cells were essentially all intact and showed 95–100% viability.

For PCA depletion studies the cells were incubated with reaginic serum dilutions at 37°C and after an appropriate interval sedimented. Dilutions of the supernate were then assessed for PCA activity.

For studies involving the use of rabbit antirat light-chain antibodies, $2 \times 10^6$ washed minced tumor cells were incubated with serum or buffer for 1 h at 37°, and then centrifuged at 220 *g* for 15 min. The cells were resuspended in buffer and washed three times. The final cell pellet was suspended in an appropriate dilution of [$^{125}I$]rabbit antibody and incubated for 15–30 min at 0–4°C. The cells were again washed two to three times and the final pellet counted.

The binding of [$^{125}I$]IgE was assessed either by following the depletion of supernatant radioactivity or measuring the counts bound to cell pellets. For the depletion studies, the cells were washed once in T and then incubated with [$^{125}I$]IgE preparations at 37°C for 60–90 min. The cells were sedimented in a Sero-fuge (Clay-Adams, Inc., New York), for 2 min and measured aliquots of the supernates were counted. No corrections were made for the volume occupied by the cells since in most cases the error introduced by neglecting this correction was small.

For the assessment of cell-bound counts, 0.2 ml of cell suspension was layered over 0.2 ml of heat-inactivated fetal calf serum in plastic Microfuge tubes and centrifuged in a Microfuge (Beckman Instruments, Inc, Fullerton, Calif., Model 152) for 1 min. Supernatant fluid was aspirated and the tip of the tube containing the cell pellet snipped off and counted. The data reported in Tables V and VI and Figs. 2 and 3 were collected using this method.

*PCA Measurements.*—PCA titers of rat reaginic antibodies to the *Nippostrongylus brasiliensis* antigens were determined by standard methods (9). PCA titers of mouse reaginic antiovalbumin antibodies were determined in rats. ½ ml of a 2% ovalbumin solution was used for the antigen challenge. In all cases, the PCA units in 0.1 ml were expressed as the reciprocal of the highest dilution giving a reaction of 7 × 7 mm or greater. All PCA tests were made at least 48 h after the injection of reagins.

## RESULTS

*Propagation of Tumor.*—Newborn rats from eight Wistar strains (MNR/N, MR/N, NSD/N, RHA/N, RLA/N, SHR/N, WKY/N, and WN/N) were inoculated with suspensions of minced tumor (see Materials and Methods). Transplantation was successful in all strains, with 43–100% of a litter develop-

ing tumors. The clinical picture of leukemia (3) developed in all strains, and elevated peripheral basophils were present in the blood smears of three strains monitored (27%, 47%, and >50% in animals of one litter).

Transplantation was most successful in the strain of origin, in which 31 of 32 litters accepted the tumor, compared to 9 of 12 WKY/N litters. In both cases, ~70% of the animals in positive litters developed tumor and ~20% were found dead or had been cannibalized presumably because leukemia preceded the development (or the observation) of tumor.

4-wk old rats from four strains were given trochar implants of 10 × 2 × 2 mm tumor pieces, taken (whenever possible) from animals of the same strain. Initially, 20 of 25 litters accepted the transplant (109 of 175 animals) with tumors developing in 7–12 days, but the tumors decreased in size and many disappeared in 15–30 days.

*Cell Culture.*—Tumors were removed from 2-wk old rats under sterile conditions, minced, and washed in the culture media (see Materials and Methods) with 1,000 U/ml penicillin and 1 mg/ml streptomycin. After filtration through sterile glass wool, 12 ml of a suspension containing $7 \times 10^5$ cells/ml were added to 30-ml Falcon flasks. After incubation in 10% $CO_2$ for 2 days, the cells were maintained in a 37°C incubator and growth began on the 3rd day.

In 15% fetal calf serum the cell doubling time was less than 48 h and cell densities of $2 \times 10^6$ cells/ml could be obtained. Fig. 1 shows a preparation of cultured cells 1 mo after initiation of cell culture.

*Depletion of PCA by Rat Leukemic Basophils.*—The capacity of leukemic basophils to deplete immune sera of PCA activity was assessed and representative experiments are shown in Table I. After 1 h incubation in either T or Tyrode's buffer with 1 mM $Ca^{++}$ and $Mg^{++}$ (TCM) substantial depletion occurred, approximately 10–20 PCA U being removed/$10^6$ cells. The presence of $Ca^{++}$, $Mg^{++}$, and up to 20% normal rat serum did not markedly affect this depletion. With longer incubation periods there seems to have been some further depletion although we do not have sufficient PCA depletion data to rigorously establish this point. As can be seen from Table I mouse reagins were also depleted with good efficiency. Since comparative values for PCA units per mole of rat and mouse IgE are unknown we cannot directly evaluate the significance of the apparent increased PCA depleting capacity of the cells for the mouse reagins.

In separate experiments $7 \times 10^6$ cells were incubated in TCM at 37° for 120 min. The cells were centrifuged and 0.7 ml of the supernate was incubated with 0.3 ml of diluted rat reaginic serum (160 PCA U/ml). After 60 min at 37° the solution was tested. No loss in PCA activity was found.

*Depletion of PCA by Mouse Mastocytoma Cells.*—For comparative purposes the capacity of mouse mastocytoma cells to deplete mouse and rat reagins was determined; representative data are given in Table II. The Furth tumor cells depleted both mouse and rat reaginic serums of PCA activity, but they were

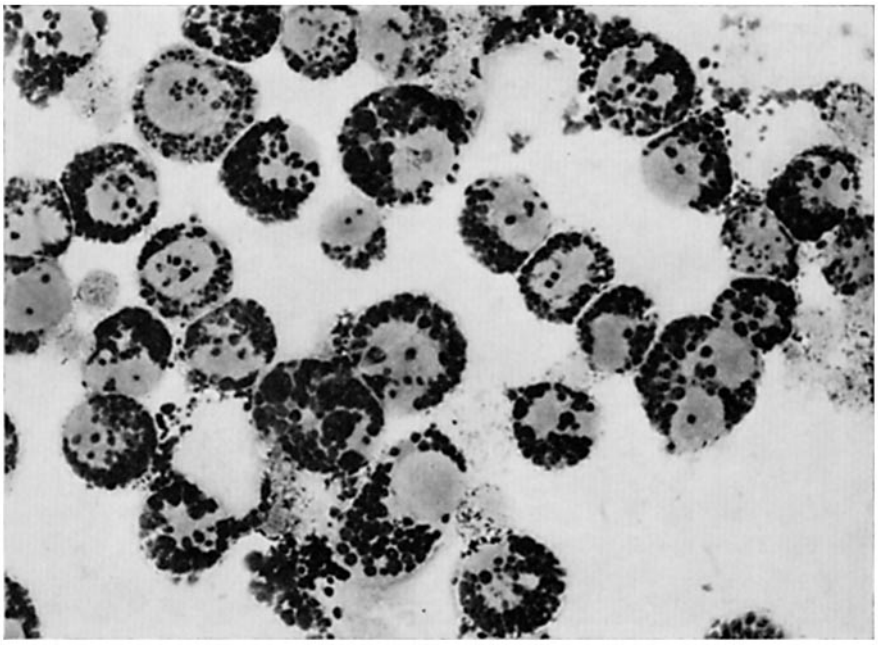

FIG. 1. Cytocentrifuged leukemic basophils from a suspension cell culture 1 mo after initiation of culture. The cells were stained with modified Wright's stain (Ames Co., Div of Miles Lab, Inc, Elkhart, Ind.). × 880.

considerably less effective than the rat cells (compare Tables I and II). Included in Table II are data published by Minard and Levy (10); our results are entirely consistent with theirs. These workers also showed that the medium of cell incubation mixtures did not in itself deplete PCA activity and we obtained similar results. Cells from the mouse mastocytoma P815 failed to exhibit PCA-depleting activity.

*Studies with Rabbit AntiRat Light-Chain Antibodies.*—Tumor cell suspensions were preincubated with reaginic sera or control solutions, washed, and incubated with $^{125}$I-labeled F(ab')$_2$ fragments of rabbit antirat light-chain antibodies.

Table III (Exp. A) shows that antilight-chain antibody was bound to cells preincubated with reaginic serum, but was bound only minimally to cells preincubated only with buffer or rat IgG. In separate experiments (not shown) we found that the specific binding was not affected by 0.1 % decomplemented rat serum present in the initial wash, by the number of washes (1–3) before preincubation with reagin, or by the presence of TCM.

Once incubated with reaginic serum, the cells did not lose their capacity to specifically bind antilight-chain antibody despite repeated washing before the addition of the iodinated antibody (Exp. B, Table III). Part of the small loss observed undoubtedly reflects a small loss of cells occurring during the multiple washes for which no correction was made.

TABLE I

*Depletion of PCA by Rat Basophil Leukemia Cells*

| Exp. | Cell-type* | Cells/ml × $10^{-6}$ | Incubation conditions‡ | Species of PCA | PCA U/ml | | | PCA depleted/$10^6$ cells |
|---|---|---|---|---|---|---|---|---|
| | | | | | Initial | Final | Δ | |
| A | — | 0 | 1 | Rat | 20 | 20 | 0 | — |
| | | 0 | | | 40 | 40 | 0 | — |
| | | 0 | | | 160 | 160 | 0 | — |
| | C | 4.9 | | | 20 | 0 | 20 | >4.1 |
| | | 4.9 | | | 40 | 10 | 30 | 6.1 |
| | | 4.9 | | | 160 | 160 | 0 | (<16) |
| | | 8.9 | | | 29 | 0 | 29 | >3.3 |
| | Tu | 11 | | | 20 | 0 | 20 | >1.8 |
| | | 11 | | | 40 | 0 | 40 | >3.6 |
| | | 11 | | | 160 | 40 | 120 | 11 |
| B | — | 0 | 2 | Rat | 40 | 40 | 0 | — |
| | | 0 | | | 80 | 80 | 0 | — |
| | | 0 | | | 160 | 160 | 0 | — |
| | C | 4.7 | | | 40 | 0 | 40 | >8.5 |
| | | 4.7 | | | 80 | 20 | 60 | 13 |
| | Tu | 2.9 | | | 80 | 28 | 52 | 18 |
| | | 2.9 | | | 160 | 80 | 80 | 28 |
| C | — | 0 | 3 | Rat | ND§ | >320 | 0 | — |
| | | 0 | | | ND | >160 | 0 | — |
| | Tu | 7.0 | | | ND | 80 | >240‖ | >34 |
| | | 7.0 | | | ND | 40 | >120‖ | >17 |
| | — | 0 | 3 | Mouse | 2600 | 2600 | 0 | — |
| | | 0 | | | 640 | 640 | 0 | — |
| | | 0 | | | 160 | 160 | 0 | — |
| | Tu | 7.0 | | | 2600 | <160 | >2400 | >340 |
| | | 7.0 | | | 640 | <40 | >600 | >340 |
| | | 7.0 | | | 160 | <10 | >150 | >21 |

* C, cultured cells and Tu, tumor mince.

‡ 1 = TCM buffer plus 0.1% normal rat serum, vol 1 ml, 60 min, 37°C; 2 = T buffer, vol 3 ml, 60 min, 37°C; 3 = TCM buffer, plus 20% normal rat serum, vol 1 ml, 120 min, 37°C.

§ ND, not determined.

‖ Calculated by subtraction from cell-free control.

TABLE II

*Depletion of PCA by Mouse Mastocytoma Cells*

| Exp. | Cell type* | Cells/ml × $10^{-6}$ | Species of PCA | PCA U/ml | | | PCA depleted/$10^6$ cells |
|---|---|---|---|---|---|---|---|
| | | | | Initial | Final | △ | |
| A | — | 0 | Mouse | 40 | 40 | 0 | — |
| | Furth | 120 | | 80 | >40 | <40 | <0.34 |
| | | 120 | | 40 | <40 | >0 | >0 |
| | | 240 | | 80 | 40 | 40 | 0.17 |
| | | 240 | | 40 | 20 | 20 | 0.083 |
| | | 490 | | 80 | 20 | 60 | 0.12 |
| | | 490 | | 40 | <20 | >20 | 0.041 |
| B | — (2)‡ | 0 | Mouse | 20 | 20 | 0 | — |
| | (2) | 0 | | 10 | 10 | 0 | — |
| | Furth (2) | 58 | | 20 | 10 | 10 | 0.17 |
| | (2) | 58 | | 10 | 0 | 10 | 0.17 |
| | (2) | 115 | | 20 | 10 | 10 | 0.087 |
| | (2) | 115 | | 10 | 0 | 10 | 0.087 |
| C§ | Furth | 10 | Mouse | 20–40 | 20–40 | (0) | (0) |
| | | 50 | | 20–40 | 16–32 | 4–8 | 0.08–0.16 |
| | | 100 | | 20–40 | 4–8 | 16–32 | 0.08–0.16 |
| D | — (2) | 0 | Rat | 20 | 20 | 0 | — |
| | (2) | 0 | | 10 | 10 | 0 | — |
| | Furth (2) | 100 | | 40 | 20 | 20 | 0.2 |
| | (2) | 100 | | 20 | 10 | 10 | 0.1 |
| | (2) | 100 | | 10 | <10 | >0 | >0 |
| E | — (3) | 0 | Rat | 80 | ~80 | ~0 | — |
| | Furth (2) | 100 | | 80 | ~80 | ~0 | — |
| | (2) | 200 | | 80 | >40 | <40 | <0.2 |
| | (2) | 400 | | 80 | 40 | 40 | 0.1 |
| | (2) | 800 | | 80 | 20 | 60 | 0.075 |
| F | — | 0 | Rat | 80 | 80 | 0 | — |
| | | 0 | | 40 | 40 | 0 | — |
| | | 0 | | 20 | ~20 | ~0 | — |
| | Furth | 110 | | 80 | >40 | <40 | <0.36 |
| | | 110 | | 40 | <40 | >0 | >0 |
| | | 110 | | 20 | 10 | 10 | 0.091 |
| | P815 | 110 | | 80 | 80 | 0 | 0 |
| | | 110 | | 40 | 40 | 0 | 0 |
| | | 110 | | 20 | ~20 | ~0 | ~0 |

* Furth mastocytoma cells and P815, Dunn-Potter mastocytoma.

‡ Numbers in parentheses indicate duplicates, etc.

§ Data taken from published results of Minard and Levy (10).

In Exp. C, Table III, ~2.2 × $10^6$ cells were preincubated with increasing numbers of PCA units; corresponding increases in the amount of bound antilight-chain antibodies were found.

The amount of bound antilight-chain antibodies also was influenced by the concentration of the rabbit antibodies to which the cells were exposed (Exp. D, Table III). Substantial increases in the amount of rabbit antibody adherent to unsensitized cells were also observed at the higher concentrations.

In view of the latter results it was relevant to examine the binding of labeled

TABLE III

*Binding of [$^{125}I$]F(ab')$_2$ Fragments of Rabbit Antilight-Chain Antibodies to Rat Leukemic Basophils**

| Exp. | Buffer | Preincubation | | Incubation | | Counts bound/cell × $10^3$ | |
|---|---|---|---|---|---|---|---|
| | | PCA/ml | Washes | Antibodies/vol | Washes | Gross | Net |
| | | | | *μg/ml* | | | |
| *A*‡ | TCM | 4000 | 3 | 6.75/0.9 | 3 | 10 | 9.2 |
| | | 320 | | | | 8.6 | 7.8 |
| | | 0§ | | | | 1.8 | 1.0 |
| | | 0 | | | | 0.80 | — |
| B | T | 320 | 2 | 3.75/0.5 | 3 | 7.1 | 5.2 |
| | | 0 | 2 | | | 1.9 | — |
| | | 320 | 4 | | | 6.8 | 4.9 |
| | | 0 | 4 | | | 1.9 | — |
| | | 320 | 6 | | | 5.8 | 4.1 |
| | | 0 | 6 | | | 1.7 | — |
| | | 320 | 8 | | | 5.8 | 4.3 |
| | | 0 | 8 | | | 1.5 | — |
| C | TCM | 640 | 3 | 7.5/1.0 | 2 | 7.6 | 5.5 |
| | | 320 | | | | 6.3 | 4.2 |
| | | 224 | | | | 6.1 | 4.0 |
| | | 160 | | | | 5.3 | 3.2 |
| | | 64 | | | | 4.6 | 2.5 |
| | | 32 | | | | 3.7 | 1.6 |
| | | 0 | | | | 2.1 | — |
| D‡ | T | 1600 | 3 | 3.75/0.5 | 2 | 12 | 10 |
| | | 320 | | | | 10 | 8.4 |
| | | 0 | | | | 1.6 | — |
| | | 1600 | | 13.5/0.5 | | 24 | 19 |
| | | 320 | | | | 18 | 13 |
| | | 0 | | | | 4.9 | — |
| | | 1600 | | 37.5/0.5 | | 33 | 22 |
| | | 320 | | | | 29 | 18 |
| | | 0 | | | | 11 | — |

* $2.2 \times 10^6$ (±10%) washed minced tumor cells were preincubated at 37°C for 1 h in reaginic serum dilutions or buffer, washed in cold buffer, and incubated at 0–4°C for 15–20 min with labeled F(ab')$_2$ fragments of antilight-chain antibodies.

‡ Cells minced in presence of 0.1% decomplemented rat serum.

§ 70 μg/ml rat IgG.

rabbit antibodies of various specificities (Table IV). (The data in Table III are not directly comparable to those in Table IV since the antibodies used in the latter experiments did not have the same specific activity as those used previously.) Compared to rabbit antilight-chain antibody or its fragments only small amounts of anti-γ heavy-chain antibody and nonspecific rabbit IgG be-

TABLE IV

*Binding of [$^{125}I$]Rabbit Antibodies and Fragments to Rat Leukemic Basophils**

| Preincubation (PCA/ml) | Incubation | | Counts bound/cell × $10^3$ | | Net rabbit molecules bound/cell |
|---|---|---|---|---|---|
| | [$^{125}$I]rabbit antibody specificity | Molecular form | Gross | Net | |
| 320 | Light chain | Fab' | 8.0 | 7.4 | 1,100,000 |
| 0‡ | " " | " | 1.4 | 0.8 | 130,000 |
| 0 | " " | " | 0.6 | — | — |
| 320 | " " | $F(ab')_2$ | 34 | 30 | 1,500,000 |
| 0‡ | " " | " | 7.8 | 3.5 | 180,000 |
| 0 | " " | " | 4.3 | — | — |
| 320 | " " | IgG | 53 | 46 | 1,200,000 |
| 0‡ | " " | " | 12 | 4.7 | 120,000 |
| 0 | " " | " | 7.3 | — | — |
| 320 | γ Heavy chain | IgG | 3.8 | 2.0 | 57,000 |
| 0‡ | " " | " | 4.3 | 2.5 | 71,000 |
| 0 | " " | " | 1.8 | — | — |
| 320 | None | IgG | 1.5 | −1.9 | −57,000 |
| 0‡ | " | " | 1.3 | −2.1 | −62,000 |
| 0 | " | " | 3.4 | — | — |

* $8.4 \times 10^6$ washed tumor basophils were preincubated with 4 ml of rat reagin dilutions or controls at 37°C × 1 h. and washed three times in cold buffer. Aliquots were then incubated with 7.5 μg of labeled rabbit antibody or fragments of known specific activity. After three washes, the pellet was counted and rabbit molecules/cell were calculated. (Net binding to cell pellet for Fab', $F(ab')_2$ and IgG antilight chain was 0.16, 0.42, and 0.53 μg, respectively.)

‡ A dilution of normal serum comparable to the reaginic serum dilution was used.

came bound to the sensitized cells. Moreover the anti-γ heavy-chain antibody and nonspecific rabbit IgG did not distinguish between cells incubated with reaginic or normal serum.

*Direct Binding Studies with [$^{125}I$]IgE.*—As reported elsewhere[3] we have partially purified IgE from reaginic serum and radiolabeled such preparations with $^{125}$I. These preparations are free of all immunoglobulins other than IgE. In the experiments reported here preparations in which approximately 50% of the counts (80% of the IgE-related counts as determined by radioimmunoassay) became bound to the leukemia cells at high cell:IgE input ratios ($>7 \times 10^5$ cells/PCA U) were used.

Fig. 2 illustrates a typical binding curve observed when 10 PCA U of [$^{125}$]IgE were incubated with $1.55 \times 10^6$ cells at 37°C for varying time periods. The specificity of the reaction was determined by adding a variety of inhibitors to the incubation mixture before adding the cells and measuring the amount of

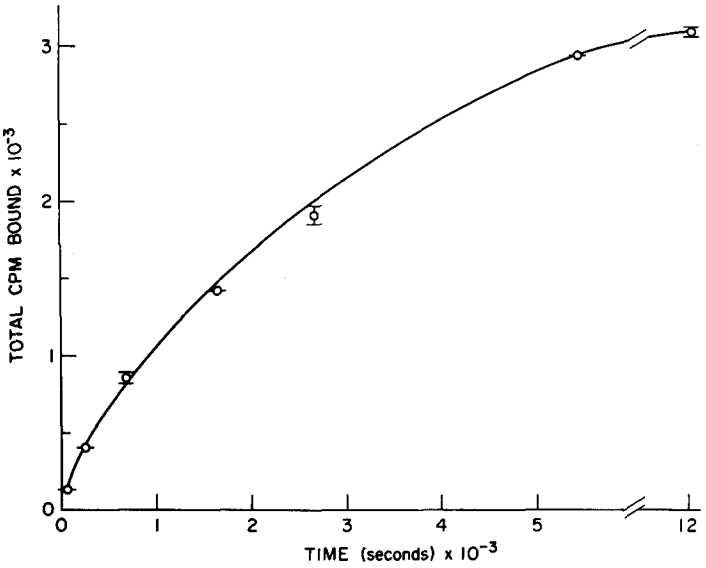


FIG. 2. [$^{125}$I]IgE binding to cultured basophils. 0.2-ml samples were removed at the times indicated from incubation mixtures each of which contained 1.55 × $10^6$ cells and partially purified [$^{125}$I]IgE(Prep S, 10 PCA U) in a final volume of 1.0 ml. Temp.: 37°C. The cross-bars indicate the range of values for duplicate samples.

IgE bound after several time intervals. Equilibrium results are tabulated in Tables V and VI. Typical data are further illustrated in Fig. 3.

The binding of the [$^{125}$I]IgE was inhibited more than 90% by adequate amounts of reaginic rat serum. Normal rat serum caused only slight inhibition and there was no clear-cut dose dependence. Reaginic and normal mouse serums gave corresponding results. A mouse reaginic serum with higher reaginic activity (pool II) gave better inhibition than a less active serum (compare Exp. A and B, Table V). A purified preparation of a human IgE myeloma failed to inhibit the binding of rat IgE significantly (Exp. C, Table V; Fig. 3).

Reaginic serum which had been heated for 4 h at 56°C and which was devoid of 48-h PCA activity, failed to inhibit the binding of [$^{125}$I]IgE (Table VI). Similarly, reduction and alkylation of a reaginic serum fraction caused a 75% drop in PCA activity compared to a control (in which the reducing and alkylating reagents were added in reverse order) and a comparable reduction (71–88%) in inhibitory capacity (Exp. B, Table VI).

As a further test of the specificity of binding, the partially purified [$^{125}$I]IgE preparation was depleted of immunoglobulin by passing it over a rabbit antirat Fab column. Only ~5% of the total radioactivity of this material was associated with IgE as determined by radioimmunoassay, and only 7–10% of the counts became bound under conditions where 50% of the counts in the unabsorbed preparation were adsorbed by the cells.

TABLE V

*Inhibition of [$^{125}$I]IgE Binding by Rodent and Human IgE**

| Exp. | Cells/ml × $10^{-6}$ | Inhibitor | | Percent inhibition (vs buffer control) |
|---|---|---|---|---|
| | | Type | Amount | |
| | | | *μl/ml* | |
| A | 1.46 | Normal rat serum | 200 | 1.2 |
| | | | 40 | 8.5 |
| | | | 8 | 15 |
| | | Reaginic rat serum | 200 | 91 |
| | | | 40 | 65 |
| | | | 8 | 28 |
| | | Normal mouse serum | 200 | −3.6 |
| | | | 40 | −0.7 |
| | | Reaginic mouse serum I | 200 | 49 |
| | | | 40 | 20 |
| B | 2.86 | Normal rat serum | 823 | 26 |
| | | | 264 | −0.7 |
| | | | 68 | −18 |
| | | | 14 | −6.5 |
| | | Reaginic rat serum | 823 | 95 |
| | | | 264 | 88 |
| | | | 68 | 57 |
| | | | 14 | 31 |
| | | Normal mouse serum | 823 | 16 |
| | | | 264 | −3.1 |
| | | | 68 | −16 |
| | | | 14 | −3.1 |
| | | Reaginic mouse serum II | 823 | 84 |
| | | | 264 | 64 |
| | | | 68 | 28 |
| | | | 14 | 19 |
| | | | *mg/ml* | |
| C | 1.46 | Human myeloma IgE (PS) | 0.99 | 8.1 |
| | | | 0.10 | 4.5 |
| | | | 0.01 | 3.7 |

* In Exp. A the cells were incubated with 10 PCA U of [$^{125}$I]IgE and either rat normal or reaginic (8,000 PCA/ml) serum, or with mouse normal or reaginic (10,240 PCA/ml) serum. In Exp. B the cells were incubated with 20 PCA U of [$^{125}$I]IgE and either rat normal or reaginic (8,000 PCA/ml) serum, or with mouse normal or reaginic (aggregate of >25,000 PCA/ml) serum.

TABLE VI

*Inhibition of [$^{125}$I]IgE Binding by Heated and Reduced Rat Reaginic Serum**

| Exp. | Cells/ml × $10^{-6}$ | Inhibitor | | Percent inhibition vs buffer control) |
|---|---|---|---|---|
| | | Type | Amount | |
| | | | *μl/ml* | |
| A | 1.53 | Native normal serum | 200 | −1.7 (±1.9)‡ |
| | | Heated normal serum | 200 | −8.6 (±1.2) |
| | | Native reaginic serum | 200 | 90 (±0) |
| | | Heated reaginic serum | 200 | 2.2 (±4.2) |
| B | 2.82 | Native reaginic serum | 820 | 95 |
| | | | 260 | 88 |
| | | | 68 | 57 |
| | | | 14 | 31 |
| | | Reduced reaginic serum fraction | 260 | 26 |
| | | | 130 | 10 |
| | | | 66 | 11 |
| | | | 26 | −6.3 |
| | | Reduced reaginic serum fraction control | 260 | 91 |
| | | | 130 | 85 |
| | | | 66 | 75 |
| | | | 26 | 54 |

* In Exp. A the cells were incubated with 10 PCA U of [$^{125}$I]IgE and normal serum (native or heated), or heated reaginic serum with no PCA activity, or native reaginic serum (8,000 PCA/ml). In Exp. B the cells were incubated with 20 PCA U of [$^{125}$I]IgE and either reaginic (8,000 PCA/ml) serum, or a reduced and alkylated fraction (<250 PCA/ml), or a control (1,000 PCA/ml).

‡ Range of duplicates.

## DISCUSSION

The rat basophil leukemia line induced by a β-chlorethylamine (ICI no. 42464) and serially transplanted in a closed colony of Wistar rats (3) has been transplanted into eight other Wistar strains. Transplantation characteristics and cell morphology are equivalent to those originally described (3). The cell line has been transplanted for over 3 yr in England and for 7 mo in our laboratory with no evidence of change. It therefore seems sufficiently stable to warrant extensive exploration.

The cells were adapted to a suspension cell culture by routine methods. After 4 mo in culture, the basophil leukemia cells do not differ in microscopic appearance or in IgE-binding capacity from either the original cultured cells or the minced tumor cells. The cultured cells (and frozen cultured cells) initiated basophil tumors and leukemia similar to those initiated by tumor minces upon subcutaneous injection into newborn rats.

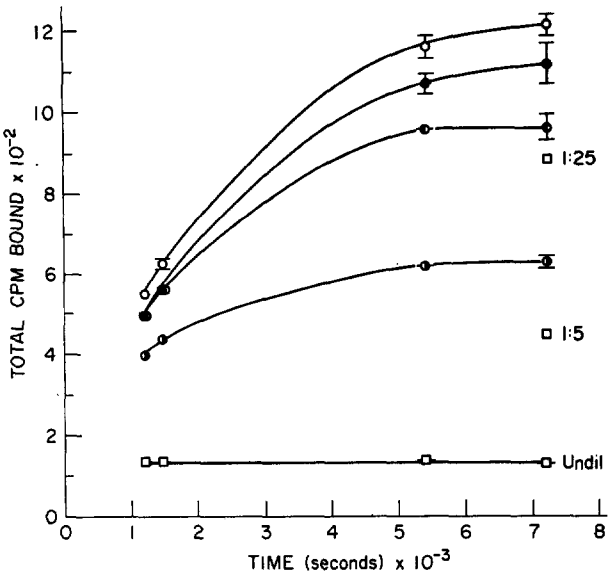


FIG. 3. [$^{125}$I]IgE binding to cultured basophils. At the times indicated samples were taken from the incubation mixtures each of which contained: 1.46 × $10^6$ cells, partially purified [$^{125}$I]IgE (Prep V, 10 PCA U), 0.2-ml inhibitor and a total volume of 1.0 ml. Temp.: 37°C. The cross-bars indicate the range of duplicate points where taken. □, rat reaginic serum (8,000 PCA/ml), dilutions indicated; ◐, mouse reaginic serum (10,240 PCA/ml) undiluted; ◐, mouse reaginic serum (10,240 PCA/ml) diluted 1:5; ●, human IgE myeloma (PS), 5.0 mg/ml; O, Tyrode's buffer.

Both cultured cells and tumor suspensions were found (in collaboration with Dr. R. Siraganian, NIDR, NIH) to contain 0.6–0.9 μg histamine/$10^6$ cells. This is much lower than the histamine content previously reported for the cells (3) but is equivalent to that present in normal human and monkey basophils (11, 12). We do not know the reason for the discrepency between our own and Eccleston et al. (3) results in this respect. It is worth noting that we have not so far been able to obtain histamine release either with compound 48/80[4] (12) or with antigen or antilight-chain antibodies using cells preincubated with IgE.

In addition to the obvious advantage of cultured cells (homogeneity, manipulability, etc.) these preparations are most amenable for binding studies. While tumor minces routinely showed many broken cells and 30–40% of intact cells were nonviable the cultured cells appear largely intact (Fig. 1) and are routinely 95–100% viable.

Our experiments demonstrated that these cells bind rat and mouse IgE immunoglobulins. This was ultimately shown by direct binding studies using

[4] Compound 48/80 is a condensation product of formaldehyde and p-methoxyphenylethylmethylamine (12).

[$^{125}$I]IgE, studies which confirmed the interpretations of experiments involving PCA depletion and the use of antilight-chain antibody binding.

The PCA-depleting activity of the rat leukemia cells is considerably greater than that described by Minard and Levy for the mouse mastocytoma of Furth (10) and confirmed by us (Table II). The activity is similar to that described for purified normal rat peritoneal mast cells as determined by Bach and Brashler (13). That cell-conditioned medium did not cause any PCA depletion suggesting that the depletion was not simply due to the destruction of IgE antibodies by proteases, which might have been released by the cells. That depletion occurred in the presence of a 40-fold excess of normal over reaginic serum (Exp. C, Table I) testifies to the specificity of the phenomenon.

Cells incubated with reaginic-serum bound much more rabbit antilight-chain antibodies than cells incubated with buffer or normal serum (Tables III and IV). The small amount of binding seen in the latter instances could be due to some IgE already present on the cells (these experiments were performed before the time cell culture preparations were available) or due to some residual homocytotropic rat IgG (14). No significant binding of normal rabbit IgG (absorbed on a rat serum protein column to remove potential "natural" antibodies to rat immunoglobulin) was observed and only an insignificant difference in binding between intact rabbit-IgG antibodies, and $F(ab')_2$ fragments of such antibodies was evident (Table IV). This suggests that these cells have little if any binding activity for the Fc regions of immunoglobulins in general.

The most convincing evidence for specific IgE-binding activity comes from the experiments using partially purified [$^{125}$I]IgE, particularly from the inhibition of binding studies. The radiolabeled IgE accounts for only an insignificant fraction ($<0.1\%$) of the total protein in those experiments. The binding was inhibited by reaginic rat and mouse serums almost completely, but hardly at all by normal serums. On the other hand, a purified human IgE myeloma protein which was known to bind avidly to human basophils (15), inhibited the binding of the rat IgE only slightly if at all. From the estimated concentration of rat IgE in our reaginic serum pool ($\sim$0.14 mg/ml)[3] and the relative inhibitory activity of that serum and the human IgE, we estimate that the latter is bound at least 1,000-fold less avidly than the rat IgE by the leukemic cells. Conflicting data on the capacity of normal rat mast cells to bind human IgE have been reported (16–20).

Some IgG immunoglobulins appear to compete for the IgE-binding sites on rat and mouse mast cells (13, 19, 20). Our failure to observe inhibition of IgE binding by normal serums may be due to inadequate concentrations of the relevant IgG subclass in the serums we used or to differences in the receptor on the leukemic cells. Studies are underway to clarify this point.

Studies with rat mast cells and human blood basophils indicate that $Ca^{++}$ and $Mg^{++}$ are required for IgE-mediated histamine release but not for the initial binding of IgE antibodies to the target cells (21, 22). Our studies with the rat

leukemic cells also indicate that $Ca^{++}$ and $Mg^{++}$ do not influence the binding of IgE.

In all species where IgE-like antibodies have been observed, PCA activity is lost when the reaginic serum is heated or reduced; our studies with the leukemic cells suggest that this is due to the failure of IgE to bind to the IgE receptors (Table VI). Studies with human IgE led to a similar conclusion (24).

We are presently investigating the binding parameters of the IgE receptors on these cells. Our initial studies on the kinetics and concentration dependence of binding are consistent with a second order, reversible reaction and more extensive studies than are reported here will be necessary to obtain accurate values for the number of receptors for IgE on these cells. However, some of the experiments reported here on PCA depletion, antilight-chain binding, and [$^{125}$I]IgE binding were performed under roughly equivalent conditions (IgE and cell concentration, temperature, and time). Furthermore, we can estimate the amount of IgE/PCA unit,[3] the number of antilight-chain antibodies which can bind to each IgE molecule (see Materials and Methods), and the specific activity of our iodinated IgE. We can therefore determine whether the results obtained by the three different analyses are consistent with each other. Such calculations show that in each instance several hundred thousand molecules of IgE became bound/cell. Since these molecules were detected by the antilight-chain antibodies, it appears that the major portion of the bound IgE is retained on the surface of the cell. Preliminary autoradiographic studies on cells incubated with [$^{125}$I]IgE (performed with the help of Dr. A. Rosenthal, NIAID, NIH) are consistent with this interpretation. These latter studies also indicated highly uniform labeling of the cell culture preparation.

## SUMMARY

A rat basophilic leukemia cell line originally described by Eccleston et al. (3) has been successfully transplanted into eight Wistar strains and adapted to suspension cell culture without noticeable morphological changes.

The cells deplete the reaginic activity of mouse and rat immune sera to an extent roughly equivalent to that reported for normal rat mast cells. Studies utilizing radioiodinated antilight-chain antibodies and radioiodinated partially purified rat IgE indicate that the cells bind IgE to their surface membrane with high specificity. Heating or mildly reducing the rat IgE destroyed its binding activity. The binding is unaffected by the presence or absence of $Ca^{++}$ and $Mg^{++}$, and is markedly inhibited by reaginic but not by normal rat or mouse serums. The affinity of these cells for human IgE, if present at all, is substantially lower than for rat IgE.

We are grateful to Dr. B. J. Leonard for providing us with tumor, the requisite breeding pairs of rats, and advice concerning tumor transplantation. Dr. Don Buell helped us adapt the tumor to suspension culture. Ms. Patricia Minard gave us much useful advice in respect to the mouse mastocytoma experiments. Dr. Hansen was helpful in regard to providing us with a

variety of Wistar strain newborn rats. Finally we are much indebted to Mr. George Poy for his dedicated assistance in transplanting and maintaining the tumors.

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