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The receptor for immunoglobulin E on rat basophilic leukemia cells: Effect of ligand binding on receptor expression

(receptor turnover)

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ABSTRACT **We have evaluated the effect of ligand binding on expression of the receptor for IgE on rat basophilic leukemia (RBL) cells. RBL cells were grown in the presence or absence of ^{131}I-labeled IgE and sometimes were also surface labeled with ^{125}I. We found that cells grown in the presence of IgE continued to accumulate receptors at the surface and thus the apparent amount of cell-associated IgE continued to increase. The results obtained suggest that, in the presence of IgE, the elimination of the receptor from the surface was halted or slowed significantly (≈80%) while insertion into the membrane of previously synthesized receptor continued.**

Rat basophilic leukemia (RBL-1) cells have been shown (1) to have receptors that bind IgE tightly and specifically. Isersky and co-workers (2, 3) have reported that, in synchronized cultures, the number of receptors per cell varies with the cell cycle. During mitosis the receptors are equally divided between the two daughter cells, resulting in an apparent halving of the number of receptors per cell. The receptors are then rapidly replenished (≈13%/hr) in the G_1 phase of the cell cycle. In nonsynchronized cultures, the rate of increase in the number of receptors is slower and more variable, ranging between 0.5 and 6%/hr depending on the growth phase, with faster rates observed in the transition from exponential to stationary phase. Thus, in exponentially growing and stationary cultures, the rate of appearance of receptors at the cell surface equals or exceeds the rate of elimination of receptors from the surface. However, when cells from stationary cultures are resuspended in fresh medium at low density, up to 70% of the receptors may be lost within 2–12 hr.

In a separate study (4), Isersky *et al.* followed RBL-1 cells that had been incubated with IgE and then cultured in medium containing excess IgE. While the study was aimed at evaluating the fate of cell-bound IgE, it was assumed that to some extent the fate of the ligand reflects the fate of the receptor. Unlike other receptor–ligand complexes (5), the receptor-bound monomeric IgE was found to reside on the surface of RBL-1 cells for prolonged periods of time and to dissociate slowly in a biphasic fashion ($t_{1/2}$ of 7–12 and 130–250 hr). During mitosis, surface-bound IgE molecules were divided equally between the daughter cells, each of which proceeded to increase the number of receptors for IgE at the rate of ≈5%/hr. However, in the presence of IgE, the expected loss of receptors (4), previously shown to occur frequently on resuspension of RBL-1 cells in fresh medium (2), was not detected. Moreover, during the lag phase, the cells continued to accumulate receptors for IgE at their surface at the rate of 23%/24 hr. This could have been the result of prevention of the elimination of receptor from the cell surface. Alternatively the presence of IgE might have enhanced synthesis of the IgE receptor by RBL cells.

To evaluate critically the effect of ligand binding on the turnover of the receptor for IgE, we cultured RBL-HR$^+$-2H$_3$ cells (6) in the presence and absence of ^{131}I-labeled IgE. We found that the presence of IgE on the cells and in the medium effectively halted elimination of the receptor from the surface.

MATERIALS AND METHODS

Culturing and Handling of Cells. RBL-HR$^+$-2H$_3$ cells were grown as described and harvested 3–4 days after subculturing (6). The freshly harvested cells (8–10 × 10^5/ml) were incubated overnight in spinner bottles (2) at 37°C in Eagle's minimal essential medium (M. A. Bioproducts) containing 16% fetal bovine serum (GIBCO). This preliminary incubation enhanced the reliability of results obtained in prolonged experiments especially when using surface-iodinated cells (see below). During the experiment, cells (5–20 × 10^5/ml) were incubated at 37°C (4–32 hr) in spinner bottles in conditioned medium. The conditioned medium used in this study was supernatant obtained after 16–18 hr of growth of RBL cells (8–12 × 10^5/ml). In this study, cells were incubated during short-term experiments at high density (1.2–2.0 × 10^6 cells/ml). At this density cells are less likely to go into lag phase and divide and are more likely to remain in a stationary phase (2, 3). However, at this density they cannot be maintained at high viability for long periods of time. Thus, long-term observations were done at low cell density (5 × 10^5 cells/ml) under which cells might enter lag phase. Treatment with cycloheximide (Sigma) was done as described (7).

Immunoglobulins. Rat myeloma IgE (IR162) (8) was isolated from rat ascitic fluid (9, 10). Rabbit anti-rat IgE was affinity purified as described (9). Normal rabbit IgG was isolated from pooled rabbit serum by using ammonium sulfate followed by ion-exchange and then by gel chromatography (9).

Radiolabeling. Iodination (^{131}I, Amersham) of rat IgE was carried out using the chloramine-T method (11). The specific activities obtained were 5–8 × 10^8 cpm/mg of protein. The radiolabeled IgE was kept in borate-buffered saline (pH 8.0) containing human serum albumin at 2 mg/ml. The ability of ^{131}I-labeled IgE to bind to RBL cells was estimated as described (10) and was found to be 50–80%. Cell surface iodination with ^{125}I (Amersham) was carried out as previously described (12).

Binding Assays and Loading of Cell Surface Receptors with IgE. RBL-HR$^+$-2H$_3$ cells were loaded with IgE by culturing at low density or high density in the presence of ^{131}I-labeled IgE (1.5 μg per 5 × 10^5 cells per ml or 6 μg per 2 × 10^6 cells per ml). Initially and at various subsequent time points, ali-

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Abbreviation: RBL, rat basophilic leukemia.

quots of cell suspensions from each culture were sampled and assessed to determine the number of receptors.

The number of receptors on cells incubated in the absence of IgE was estimated as described (3, 10). The cell suspensions were centrifuged, washed, and incubated with ^{131}I-labeled IgE (12 μg per 2 × 10^6 cells per ml) at 0–4°C for 1 hr. To assess the proportion of surface IgE in the total cell-associated IgE, the elution of the IgE at pH 3 was determined (13).

Extraction and Immunoprecipitation of ^{131}I-Labeled IgE–^{125}I-Labeled Receptor Complexes. ^{125}I-surface-labeled cells were incubated with ^{131}I-labeled IgE, and 5- to 20-ml portions of the cell suspensions were centrifuged. The cell pellets were washed once with medium and once with phosphate-buffered saline (pH 7.2) and then extracted with 1 ml of ice-cold 0.5% Nonidet P-40 in borate-buffered saline (pH 8.0) containing enzyme inhibitors (14). After 15 min of incubation (0–4°C), the extracts were centrifuged and the supernatants were kept at −80°C. The fraction of the ^{131}I-labeled IgE bound to the receptors was determined by precipitation of IgE–receptor complexes by addition of PEG. As reported previously (14), receptor–IgE complexes, but not unbound IgE, precipitate at 13% PEG. We found that 90 ± 5% of the ^{131}I-labeled IgE in the extract was bound to the receptor.

The IgE–receptor complexes were immunoprecipitated essentially as described (14). Briefly, the extracts were incubated (1 hr, 0–4°C) with normal rabbit IgG (100 μg/ml) and PEG (final concentration, 4%). After centrifugation, unlabeled IgE (5 μg/ml) was added to the supernatants, which were further incubated (1 hr, 0–4°C) with affinity-purified anti-IgE (100 μg/ml). More than 90% of the ^{131}I-labeled IgE was immunoprecipitated under these conditions. The precipitates were washed twice with borate-buffered saline (pH 8.0)/0.5% Nonidet P-40/4% PEG and once with 0.08 M Tris buffer (pH 6.5). The washed pellets were solubilized in 0.08 M Tris buffer/2% $NaDodSO_4$, incubated for 2 min in boiling water, and analyzed by $NaDodSO_4$/PAGE.

Gel Electrophoresis. The solubilized immunoprecipitates were analyzed by electrophoresis on 12.5% $NaDodSO_4$/polyacrylamide slab gels (1.5 mm thick) (15). Molecular weight standards were obtained from Bio-Rad. The procedures used were the same as described (12, 14). Two-millimeter-thick slices of the stained, washed, and dried gels were assayed for radioactivity (12). The amounts of ^{131}I-labeled IgE-related radioactivity detected in each lane were summed and, from this value, we calculated the fractional recovery of IgE from the original extract. The ^{125}I counts in the respective lanes were divided by this value to account for potential loss of ^{125}I-labeled receptor in each sample.

RESULTS

Effect of IgE on Expression of Receptor Activity. We first evaluated the effect of ^{131}I-labeled IgE on the accumulation of IgE–receptor complex by RBL cells (Table 1). The cells were suspended at low density in the presence or absence of ^{131}I-labeled IgE (1.5 μg/ml), and the number of IgE receptors was determined. In the absence of IgE, in two out of three cultures, the cells lost 20–36% of the receptors in 4 hr and up to 50% in 8 hr (experiment A, cultures 1 and 2). This loss occurred in the absence of cell division. In contrast, when the same cells were cultured in the presence of IgE, no loss occurred and in fact, by 8 hr, cells in both cultures bound 34% more IgE (experiment A, cultures 4 and 5). In culture 3, only an insignificant loss (8% in 8 hr) was observed in the absence of IgE, but cells cultured in the presence of IgE (culture 6) expressed 24% more receptors in 8 hr (experiment A, cultures 3 and 6, respectively). After the initial 8 hr in culture and in the next 16–24 hr, cells grown in the absence of IgE also acquired receptors as evidenced by the 40–80% increase in the number of receptors (experiment A, cultures 1–3). In contrast, cells grown in the presence of IgE continued to accumulate ^{131}I-labeled IgE, and in 28–32 hr the amount of IgE carried by these cells increased to 2.5 times that bound by the original cells. During the first 32 hr in culture there was no significant change in the number of cells per milliliter in cultures 1, 2, 4, and 5, although there was some increase (20 and 36%, respectively) in cultures 3 and 6.

Table 1. Changes in number of IgE Fc receptors on RBL cells

Time, hr	Level of receptor activity, % zero time					
	Culture without IgE			Culture with IgE		
	1	2	3	4	5	6
	Experiment A					
4	81	64	100	100	134	123
8	47	54	92	134	134	124
24	73	78	86	201	263	216
28	86	89	—	242	231	—
32	—	—	139	—	—	264
	Experiment B					
4	100	89	—	134	162	—

Cells were cultured at low density (0.5 × 10^6/ml, experiment A) or at high density (1.2–2 × 10^6/ml, experiment B), in the presence or absence of ^{131}I-labeled IgE. Results found at each time point are expressed as [(number of IgE receptors at indicated time/number of IgE receptors at 0 time) × 100]. At each time point, these results represent a mean of duplicate samplings of duplicate incubation mixtures. The SD was calculated for each set and was found to not exceed 4% of the mean.

As shown in Table 1 (experiment B, cultures 1, 2, 4, and 5), cells that were resuspended in fresh medium at 1.2–2.0 × 10^6/ml in the absence of IgE did not gain or lose significant numbers of receptors within 4 hr. However, in the presence of IgE the numbers of receptors increased steadily, and the cells acquired 30–70% additional receptors within 4 hr. During this period, we detected no cell division and no change in viability of either culture.

To assess whether the presence of IgE enhanced the rate of receptor synthesis, we incubated cells for 4 hr with or without IgE in medium containing cycloheximide (7). As shown in Table 2, the presence of IgE increased the number of IgE receptors but this increase was not inhibited by cycloheximide. Nor did the presence of cycloheximide have any effect on the number of receptors expressed by cells incubated in the absence of IgE. However, protein synthesis, as measured by [^{3}H]leucine incorporation, was almost completely (96%) inhibited (Table 2).

To ascertain that all IgE molecules bound to the cells during culture with IgE were indeed at the surface, we assessed their accessibility to the extracellular environment by using

Table 2. Effect of cycloheximide on receptor accumulation

Exp.	Cyclo-heximide	[^{3}H]Leucine incorporation, cpm × 10^{-4}/ 1 × 10^6 cells	Level of receptor activity, % zero time	
			Without IgE	With IgE
1	−	ND	92	173
	+	ND	112	193
2	−	21.6	114	171
	+	0.9	94	166

Cells (2 × 10^6/ml) were resuspended in medium with or without ^{131}I-labeled IgE in the presence or absence of cycloheximide at 10 μg/ml (7). At 0 time and at 4 hr, ^{131}I-labeled IgE binding activity and/or amount of cell-bound ^{131}I-labeled IgE was measured. Results represent means of duplicate samplings of duplicate incubation mixtures, and the SD did not exceed 4% of the mean. ND, not determined.

elution at acid pH (13). This was done at 4, 8, and 24 hr. More than 90% of the IgE bound to such cells could be eluted at acid pH. To ascertain that all the surface-bound IgE was indeed bound to the receptor, we extracted the cells with buffer containing Nonidet P-40 and assessed whether the ^{131}I-labeled IgE present in the extracts would precipitate at 13% PEG (14). Essentially all of the ^{131}I-labeled IgE (90 ± 5%) precipitated, indicating that it was bound to the receptor. We concluded that all the additionally bound IgE was at the surface and bound to the receptor. Thus, the presence of IgE on the cells and in the medium caused a 130–160% increase in the number of IgE receptors in 24–32 hr.

Effect of IgE on Elimination of Its Receptor from the Cell Surface. To determine whether the presence of IgE on the cells and in the medium had any effect on the rate of elimination of the receptor from the surface, we first radiolabeled the cell surface proteins with ^{125}I and then cultured the cells at high (2×10^6/ml) or low (5×10^5/ml) density in the presence or absence of ^{131}I-labeled IgE. After 4–32 hr in culture, the amounts of ^{125}I-labeled receptor remaining on the cell surface were measured by $NaDodSO_4$ gel electrophoresis and these values were compared with the amounts of receptor present at time 0. As shown in Fig. 1 *A* and *B*, when cells were grown at high density for 4 hr, very little loss of ^{125}I-labeled receptor occurred in the presence or absence of IgE. Cells grown in the presence of IgE lost only 10% of the ^{125}I-labeled receptors, while those grown in the absence of IgE lost 20% of the ^{125}I-labeled receptors. During this time cells grown in the absence of IgE lost 11% of their binding activity while cells grown in the presence of IgE accumulated 62% additional IgE at the surface (Table 1, experiment B, cultures 2 and 5). However, this additional binding was not associated with any change in the pattern obtained on gel electrophoresis (Fig. 1 *A* and *B*).

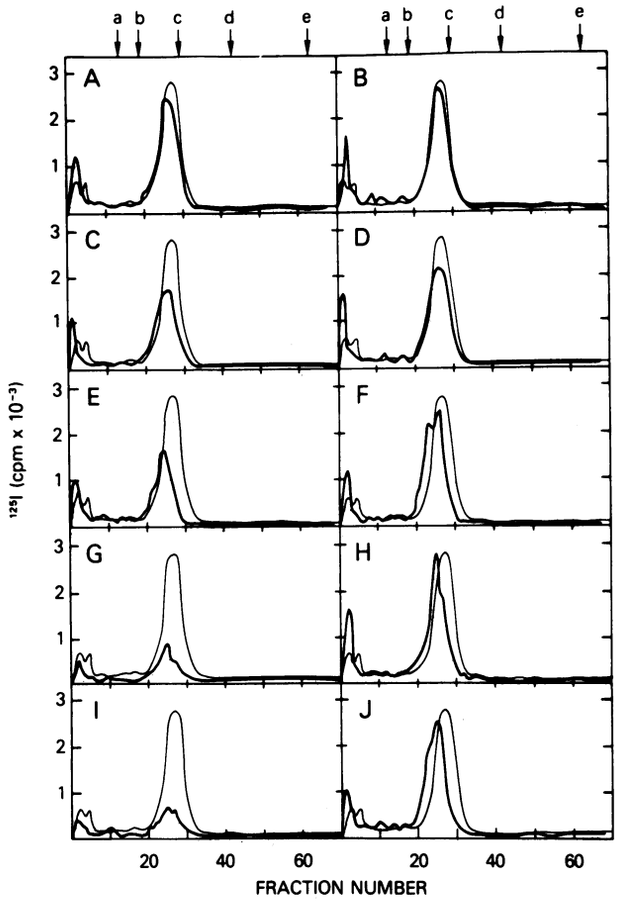

FIG. 1. Effect of IgE on elimination of its receptor from the surface of RBL cells. Cell surface proteins were radiolabeled with ^{125}I, and the washed cells were resuspended at 2×10^6/ml (*A* and *B*) or at 5×10^5/ml (*C–J*) in the absence (*A*, *C*, *E*, *G*, and *I*) or the presence (*B*, *D*, *F*, *H*, and *J*) of ^{131}I-labeled IgE and cultured for 4–32 hr. The results (heavy lines) represent the corrected ^{125}I cpm detected on each segment. The thin lines represent findings for 0 time. Measurements were made at 4 (*A*, *B*, *C*, and *D*), 8 (*E* and *F*), 24 (*G* and *H*), and 32 (*I* and *J*) hr. The molecular weight standards (a–e) were as follows: a, phosphorylase B (92,000); b, bovine serum albumin (69,000); c, ovalbumin (46,000); d, carbonic anhydrase (30,000); and e, lysozyme (14,300).

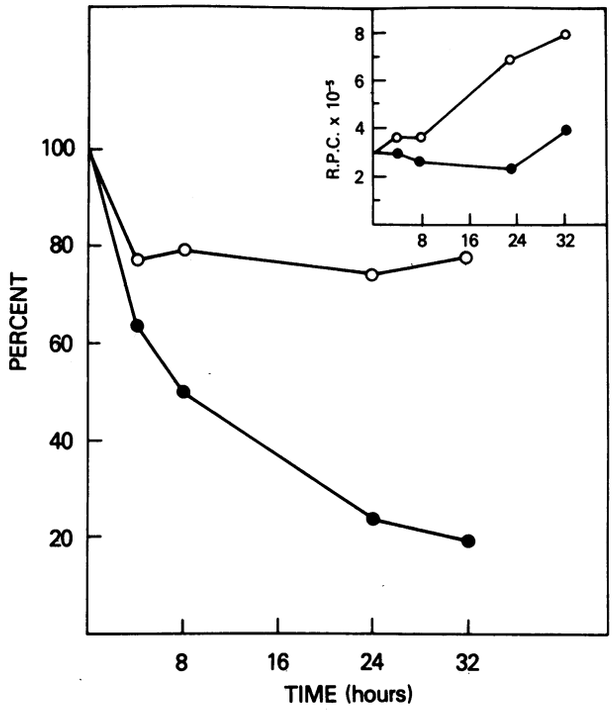

FIG. 2. Effect of IgE on the rate of elimination of its receptor from the surface of RBL cells. The ratio between the corrected sum of ^{131}I counts and the sum of ^{125}I counts for each lane was calculated. The differences between the ratio obtained at the various time points (lanes) and at 0 time are expressed as percentage loss of ^{125}I-labeled receptor-related radioactivity in the absence (●) or presence (○) of IgE. (*Inset*) Number of receptors per cell (R.P.C.) obtained at each of the time points in cultures with or without IgE.

We performed a similar experiment with cells that were resuspended at low density following the iodination. As shown in Fig. 1 *C*, *E*, *G*, and *I*, in the absence of IgE, there was considerable loss of ^{125}I-labeled receptors from the surface. In the presence of IgE (Fig. 1 *D*, *F*, *H*, and *J*), however, there was no significant change in the amount of ^{125}I-labeled receptor. Again, regardless of the amount of ^{125}I-labeled receptor detected, the apparent molecular weight distribution of the receptors revealed only insignificant changes (Fig. 1), which could have been caused by the technique used.

The relative amounts of ^{125}I-labeled receptor at various times on cells grown at low density are shown in Fig. 2. In the absence of IgE, 50% of the ^{125}I-labeled receptor disappeared from the cell surface within 8 hr, and only 20% of the receptors were associated with the cells after 24–32 hr. However, these cells maintained the ability to bind comparable amounts of IgE during the 32-hr period (Fig. 2 *Inset*). The results indicate that the cells should have expressed new receptors that replaced a fraction of the radiolabeled receptors. These observations taken together indicate that 50% of the IgE receptors on the original cells were replaced by new receptors within 8 hr. In contrast, cells incubated with IgE

lost 20% of the ^{125}I-labeled receptor within the first 4 hr but no further loss occurred through the next 28 hr. Furthermore, during this time, the IgE binding ability increased by 170% (Fig. 2 *Inset*). This increase is precisely accounted for by the combined effect of halting the elimination from the surface of the labeled receptors by IgE and the expression of new receptors at the rate of 50%/8 hr, which is comparable with that observed in the absence of IgE.

DISCUSSION

This study confirms (3, 4) that the association of IgE with IgE receptors affects the rate of their elimination from the cell surface. The results show that the increase in IgE binding activity detected during incubation of RBL cells with ^{131}I-labeled IgE is due to the combined effect of halting the elimination from the surface and the continued transport of new receptors to the surface. The increase in number of receptors during 4 hr of incubation with IgE could be explained without any increase in the rate of receptor synthesis. Unlike the IgE receptor on B lymphocytes (16), the increase in number of IgE receptors in RBL cells was not inhibited in the presence of cycloheximide. The results suggest that *de novo* synthesis of receptor molecules may have occurred following cell division (3) and that the accumulation of cell surface receptors during stationary phase is due to the incorporation of previously synthesized receptors into the membrane. Thus, the actual rate of synthesis following cell division may be faster than that estimated from the measurement of IgE binding activity by whole cells (2) and closer to the rates suggested from incorporation of radioactive amino acids into IgE receptors (17).

In this work, we have analyzed either the radiolabeled α chain of the receptor (18) or the binding activity as it is expressed at the surface. We did not analyze extracts for additional binding activity because cells grown in nonsynchronous culture do not seem to contain cryptic receptors (19). We cannot rigorously exclude the possibility that receptors that were eliminated from the surface were not degraded and perhaps recycled to the surface. However, the results shown in Fig. 2 indicate that this did not occur in RBL cells. Our present and previous data indicate that expression of new receptors on RBL cells at the surface equals or exceeds elimination of preexisting receptors, except in the lag phase of growth that follows resuspension at low density (refs. 2–4 and Table 1). These results suggest that the efficiency of incorporation of radioactive amino acids into the cell surface receptors, as well as the rate of their elimination (refs. 2 and 3 and Fig. 1 *A* and *C*), depends on the growth conditions of the culture and might explain the low rate of biosynthetic labeling encountered in previous studies (18). Nevertheless, our previous work has shown that the rate of receptor insertion into or accumulation at the membrane is not enhanced by induced endocytosis of up to 70% of the receptors (20).

It has been believed that the turnover of IgE receptors on RBL cells is slow, if it occurs at all (18). However, the present experiments clearly showed turnover of a substantial fraction (50%) of the receptors within 8 hr, if the receptors are not occupied by IgE. More importantly, most of the receptor can be protected from elimination from the cell surface by occupation with the ligand. These findings complement our previous observations (4) that up to 75% of the cell-bound IgE remains at the cell surface for extended periods (up to 130–250 hr) and that the cell-bound IgE has a prolonged survival when compared with serum IgE. Although results obtained with tumor cell lines may not always be directly related to findings with normal cells, taken together with previous observations (3, 4, 16, 18, 20, 21), our present study with RBL cells may explain the mechanisms underlying the prolonged persistence of passive sensitization (21).

Note: Since completion of these experiments, we have learned that as part of an independent study R. Quarto, J. P. Kinet, and H. Metzger (personal communication) have performed experiments similar to some of those described here. Their data on the kinetics of degradation of unliganded vs. liganded receptors and on the acquisition of excess receptors by cells loaded with IgE agree well with our own.

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