

CELL CYCLE-ASSOCIATED CHANGES IN RECEPTORS FOR IgE DURING GROWTH AND DIFFERENTIATION OF A RAT BASOPHILIC LEUKEMIA CELL LINE

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The rat basophilic leukemia cells (RBL-1) have histamine-containing granules and receptors for IgE (1-3). The normal analogues of these cells (basophils and/or mast cells) are difficult to obtain in sufficient number and/or purity for detailed study; hence the tumor cell line may be useful for a variety of investigations. For example, the RBL-1 cells are so far the only ones in which a rigorous analysis of the IgE-membrane receptor interaction has been possible. Similarly, the RBL-1 cells are the most promising available for the isolation and characterization of the receptor, as well as an analysis of its organization within the membrane. This receptor is likely an important participant in antigen-induced IgE-mediated histamine release, yet nothing is known about its structure.

During the course of our studies, we observed that the IgE-binding capacity of cultured RBL-1 cells varied quite widely from culture to culture. The present study was undertaken to explore this phenomenon. We have found that the variations can be accounted for by changes in the number of receptors during the cell cycle and the distribution of cells in different phases of their cycle in cultures at various stages of growth.

Materials and Methods

Cell Culture. Rat leukemia basophils (RBL-1) were grown in spinner flasks in Eagle's minimum essential medium with Earle's salts (EMEM)¹ supplemented with 20% fetal calf serum (FCS) 0.06% glutamine, 100U/ml penicillin, and 100 µg/ml streptomycin. Three growth patterns were examined: (a) Cells from a stationary phase culture were resuspended in fresh medium and allowed to reach high density and remain in stationary phase for up to 72 h. (b) Cultures were maintained in exponential growth by daily dilutions with fresh medium at a ratio of 1:2 or 1:3. (c) Synchronized cultures were derived from exponential cultures by using a double thymidine block procedure (below). Cell viability was determined by 0.08% trypan blue exclusion. Cell counts and volume distribution curves were obtained with a Coulter Counter Model B standardized with mulberry pollen (12-14 µm in diameter) and equipped with a Coulter Channelizer and an x, y plotter (Coulter Electronics Inc., Hialeah, Fla.). The volumes given in this report are either the weighted average (for mixed populations) or the

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¹ Abbreviations used in this paper: EMEM, Eagle's minimum essential medium. FCS, fetal calf serum; RBL-1, rat basophilic leukemia cells.

mode (gradient-separated cells). Analysis of relative single-cell DNA content was performed by flow microfluorometry by the mithramycin method on a Los Alamos Scientific Laboratory multiparameter cell sorter (4-6). Details are given elsewhere.²

Thymidine Block and Synchronization. To achieve growth arrest in S phase (7), synchronization by double thymidine block was accomplished by a modification of the technique of Galvazi et al. (7) as described elsewhere.²

Separation of Cells by Velocity Sedimentation on Ficoll Gradients. The technique described for human lymphoid cells (8) was somewhat modified for RBL-1 cells.² A 5-10% linear continuous gradient (80 ml) was used with 5 ml of 5% Ficoll layered on top. $2-3 \times 10^7$ cells at 5×10^6 cells/ml were applied and the samples centrifuged for 30 min at 80 g at room temperature in a swinging bucket rotor.

Assessment of Receptor Activity. The relative IgE-binding capacity of RBL-1 cells under varying conditions was determined as follows. $0.145 \mu\text{g}$ [^{125}I]IgE were incubated for a fixed time (85 min) with a variable number ($2.4-14 \times 10^6$) of cells, and the percent of counts bound to the cells was plotted vs. the log cell number. At substantial levels of binding, a linear relationship was obtained (Fig. 1). This

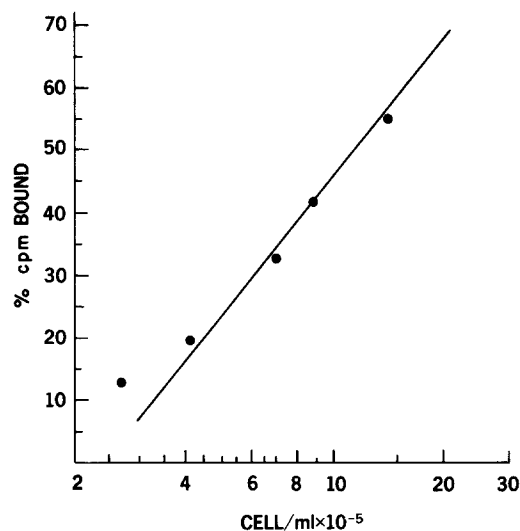


FIG. 1. Relationship between percent of added [^{125}I]IgE bound and receptor number. $0.145 \mu\text{g}$ [^{125}I]IgE was added to a series of tubes containing 1 ml and a variable number of cells from a single culture. Cell-bound IgE was determined after 85 min. The points are experimental data. The line is theoretical, assuming a K_a of $6 \times 10^9 \text{ M}^{-1}$, k_1 of $1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (2), and 8.4×10^5 receptors/cell. This curve was used as a standard curve to evaluate the (relative) receptor equivalents during the growth of several cultures. In such tests the percent of [^{125}I]IgE bound always fell in the 20-60% range.

standard curve could then be used to translate relative percent of bound counts to relative receptor number for test samples. Routinely 1×10^6 cells/ml were incubated for 85 min with $0.145 \mu\text{g/ml}$ [^{125}I]IgE.

The absolute IgE-binding capacity of the cells was determined by incubating 1×10^6 cells with excess [^{125}I]IgE ($2.9 \mu\text{g/ml}$) for 1 h. These conditions were calculated to give 97% saturation of the binding sites (2). 0.2 ml of the incubation mixture was pelleted through a layer of 0.2 ml FCS and centrifuged for 45 s in a Beckman 125 Microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The supernate was aspirated by suction and the tip of the micro test tube with the cell pellet

² Buell, D. N., Metzger, H., and C. Isersky. 1975. Cell cycle and morphologic changes during growth and differentiation of a rat basophilic leukemia cell line. Manuscript submitted for publication.

was snipped off and counted. The number of receptors per cell was calculated as previously described (2).

In both assays the cells were incubated with constant shaking at 37°C in the EMEM culture medium with 0.01 M HEPES and 20% FCS. The IgE used was isolated and iodinated as previously described (2) from sera of rats carrying an IgE-producing myeloma (9). The standard error of the mean for quadruplicate samples was lower than 1% in most cases and was never higher than 3% in both techniques. The presence of cold IgE in a 50-fold excess gave 95-98% inhibition of binding of [¹²⁵I]IgE.

To allow accurate estimation of the number of receptors on cells separated on Ficoll density gradients, (footnote 2) cells were first incubated for 35 min with excess [¹²⁵I]IgE (5×10^6 cells and 6 μ g IgE/ml). Special care was taken to remove the unbound IgE and avoid formation of clumps. This was achieved by incubating the cells in Eagle's spinner no. 1 medium (with 20% FCS and 0.01 M HEPES) and agitating the mixture gently on a Vortex mixer (Scientific Industries, Inc., Queens Village, N.Y.) every 15 min during the incubation period. Thereafter the 2 ml of cell suspension was layered on 20 ml FCS in a sterile tube and spun at 200 *g* at room temperature for 15 min. The serum was aspirated and the top inside walls of the test tube were wiped with a sterile cotton wool swab to remove traces of medium. The cell pellet was gently agitated before and during resuspension in fresh Eagle's spinner medium no. 1 without additives. The cell suspension was checked microscopically, and when free of clumps, was applied to the gradient. Preparation of the gradient itself, as well as centrifugation, fraction collection, cell counting, and volume determination were done as described above and elsewhere.² In addition, 1 ml from each fraction was counted in a Nuclear Chicago gamma counter (Searle Analytic Inc., Des Plaines, Ill.). A sample from each fraction was also spun at 2,000 rpm (700 *g*) for 30 min at 4°C and a measured portion of the supernate was counted to determine background. No counts above the machine background were detected. Because of the high viscosity of the Ficoll, samples were taken with a 1-ml automatic pipet with a detachable tip and both tip and sample were placed in a counting vial and counted. This technique of prelabeling, washing, and then separating the cells in a Ficoll gradient permitted accurate quantitation of IgE receptors in each of the gradient fractions. This experimental approach was possible because of the slow dissociation (half-life \geq 12 h) of bound IgE from the receptor (2).

Results

Interpretation of IgE-Binding Data. In subsequent paragraphs we describe changes in the IgE-binding activity of the RBL-1 cells during culture growth and transit through the cell cycle. The following assays were performed to test whether the observed variations were due to changes in the number of receptors or some other cause, e.g. changes in the amount of nonspecific binding or changes in the affinity of the binding sites. The binding of irrelevant proteins (iodinated insulin and rabbit IgG) was assessed with appropriate cell populations (Table I) to test for changes in specificity. In addition, the capacity of unlabeled rat IgE and human IgE to inhibit the binding of iodinated rat IgE was determined. In each instance the high specificity of the binding reaction for rat IgE was confirmed. The initial rate of IgE binding by cells showing substantial differences in IgE-binding capacity at equilibrium, was measured to test for quantitative changes in the receptor binding properties. As shown in Fig. 2, the initial rates were the same within experimental error when corrected for the number of binding sites. Similarly, the rate of dissociation of IgE was not significantly different when other cultures with large differences in receptor activity were examined (2). Because of these findings, we believe that the results to be described reflect changes in the number of available active receptors for IgE on the surface of the RBL-1 cells.

Variation in Receptor Number during Growth of Cell Cultures. Cells from

TABLE I
Specificity Testing of RBL-1 Cells

Cells*	Receptors per cell	Test protein	Protein bound
Double thymidine-blocked	1×10^6	$\mu\text{g/ml}$	$\mu\text{g}/10^6 \text{ cells}$
		[^{125}I]rabbit IgG (62)	Undetectable
		[^{125}I]insulin (4×10^{-4})	2.4×10^{-7}
		[^{125}I]rat IgE (2.9)	0.33
		[^{125}I]rat IgE (2.9) + $60 \times$ unlabeled rat IgE	0.023
		[^{125}I]rat IgE (2.9) + $56 \times$ unlabeled human IgE	0.32
Late stationary cells	1.6×10^6	[^{125}I]rabbit IgG (50)	Undetectable
		[^{125}I]rat IgE (2.9)	0.53
		[^{125}I]rat IgE (2.9) + $51 \times$ unlabeled rat IgE	0.011

* Cells at $10^6/\text{ml}$ in all tests.

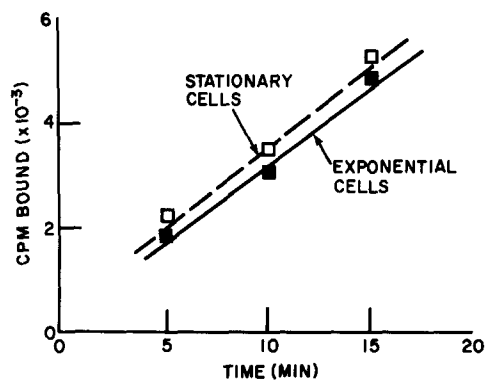


FIG. 2. Initial rate of [^{125}I]IgE binding to cells kept growing exponentially for 192 h (■—■) and to cells in the stationary phase for 48 h (□---□). Growth patterns of these two cultures are described in Fig. 4. The rates have been normalized on the basis of the number of binding sites determined at saturation.

cultures showing a stable, "stationary" cell number (about 1×10^6 cells/ml) were resuspended at low density ($3\text{--}5 \times 10^5$ cells/ml) in fresh medium. Although the cell number and viability remained unchanged (90–95%) for 16 h during this "lag" phase, a progressive loss in active receptors per cell (and therefore per culture) was observed (Fig. 3). This loss, which amounted to almost 70% in some instances, was evident as early as 2 h after the cells were resuspended in fresh medium (Fig. 3 I, 3 II) and was sometimes completed within 5 h (Fig. 3 I and Table II). The extent of the loss seemed to correlate with the length of time the cells had been maintained in the stationary phase, being more pronounced when

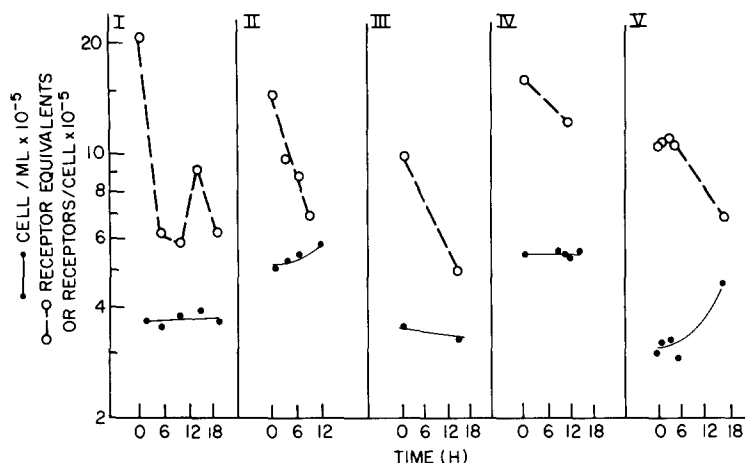


FIG. 3. Relationship between the progressive loss of receptor activity (O—O) during the lag phase and length of time cells were in the stationary phase before resuspension in fresh medium. In panels I-V the cells were respectively 72, 68, 62, 48, and 24 h in the stationary phase before they were resuspended in fresh medium.

TABLE II
Drop in Receptor Activity with Time after Resuspension of Cells at Low Density in Fresh Medium

Exp. no.	Time in stationary phase before resuspension	Length of lag*	Loss in receptor activity with time			
			5 h	6 h	10 h	12 h
I	<i>h</i> 72	<i>h</i> 26-37	—	65	—	68
II	68	12-24	—	38	—	52
III	62	16-24	—	23	—	46
IV	48	16-18	—	8	—	18
V	24	6-16	—	0	—	—
‡VIa	72	>10	37	—	28	—
VIb	48	>10	21	—	22	—
VIc	24	>10	3	—	10	—

* Lower number represents the time when cell count was last found unchanged; higher number represents the time when an increase in cell count was first noticed.

‡ 25 ml of cell suspension were removed at 24, 48, and 72 h from the same 500-ml culture. The cell number was about 1.2×10^6 on all 3 days and the viability remained 90-95%. The samples were spun down at 800 rpm at room temperature and resuspended in 90 ml of fresh medium.

the cells had been in stationary phase for 48-72 h (Fig. 3, Table II). Though the cells sometimes remained in lag phase for up to 24 h, no reaccumulation or further loss occurred in the latter part of the lag phase. This loss appeared to be completed before the cells entered the S phase. We have shown elsewhere² that at 9 h the cells from similar cultures have not yet entered the S phase and that only at 16 h has the percent of cells in S phase increased.

As the cells multiplied, the receptors per cell often showed a still further

decline. However, this was not accompanied by a decrease in cell-bound receptors in the total culture. Thus no net loss of IgE receptors occurred during proliferation (Fig. 4 I and II). As the number of cells grew, the number of receptors per cell progressively increased and continued to do so after the cells reached a stationary concentration. Up to 1.6×10^6 receptors/cell have been observed on cells maintained in such a stationary phase. The average volume of the cells in such stationary phase cultures varied in different experiments from 960 to $1,300 \mu\text{m}^3$. The rate of incorporation of [^3H]thymidine into such stationary phase cells was less than 6% of that observed in the exponential phase (Fig. 4 I, 116 h, and Fig. 4 II, 72 h). This is consistent with analyses of single cell DNA content that showed that over 90% of these cells have a DNA content expected for the G_1 phase of the cell cycle.

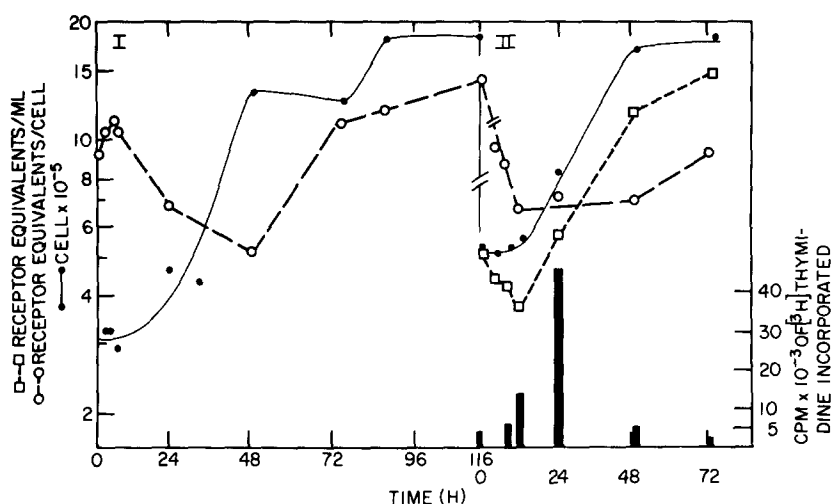


FIG. 4. Follow-up of the growth curves (●—●), expression of receptor activity per cell (O—O) and per culture (□—□) during growth of two consecutive cultures (I and II). Stationary cells (panel I, 0–116 h) were first followed and then resuspended in fresh medium and followed again through a second growth curve (panel II). The bars represent incorporation of [^3H]thymidine, assessed as previously described.² The data show that stationary cells in both panels I (116 h) and II (72 h) are not in S phase.

When the cells were maintained in exponential growth for 10–14 days by repeated dilution in fresh medium, the number of receptors per cell stabilized at about 6×10^5 (Fig. 5 A). This is about half the number observed on cells allowed to enter the stationary phase (Fig. 5 B).

Variation in Receptor Number during Growth of Synchronized Cell Cultures. Synchronization was achieved by a double thymidine block, which results in arrest of the cells in the early S phase of the cell cycle² (10, 11). In order to follow the dynamics of receptor expression during the entry of cells into such a block, thymidine was added to a culture and receptor activity was measured at 0, 6, 13, and 23 h. While the culture was entering the block, the average number of receptors per cell increased by about 25% (Fig. 6) until the cell number had stabilized. During the following 11 h the receptors per cell remained unchanged,

though the cell volume increased by another 33% (Fig. 6 II). After release from a double thymidine block, cells synchronously traversed the S and G₂ phases of the cycle within 10–12 h.² In four different experiments we found no increase in the number of receptors per cell (Fig. 7). A complete follow-up of a synchronized culture is described in Fig. 8. Again, while the cells were traversing the S and G₂ phases of the cycle, the cell number and viability remained unchanged and no increase in receptors per cell was observed (Fig. 8, 0–11 h), though the cell volume increased by 20%. The mean cell volume for this G₂ population was 2,500 μm^3 , more than double the volume of cells in a routine stationary culture (960–1,300 μm^3), and there were about 1×10^6 receptors/cell. Thus both cells in prolonged early S phase arrest and cells traversing S and G₂ phases of the cycle accumulated no new receptors for IgE, despite increasing cell volume.

An expected drop in mean cell volume and in the number of receptors per cell

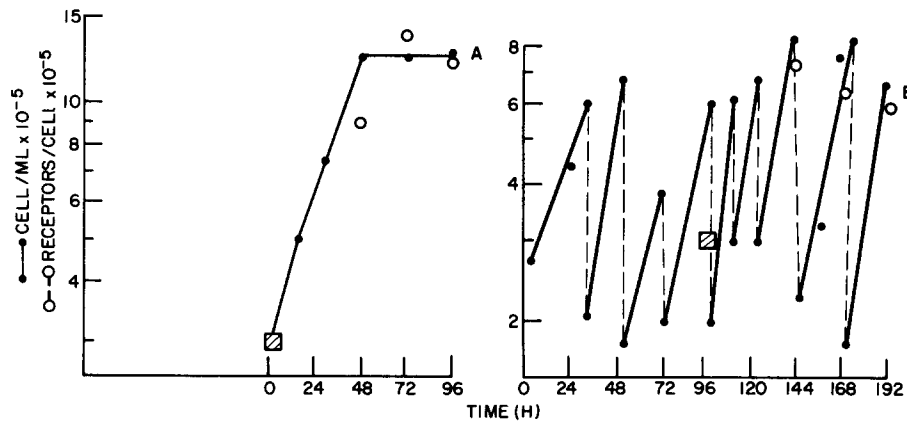


FIG. 5. Growth patterns (●—●) and expression of receptor activity (O) in a routine culture (A) and a forced exponential culture (B). The routine culture was derived from the exponential culture after 96 h of continued exponential growth.

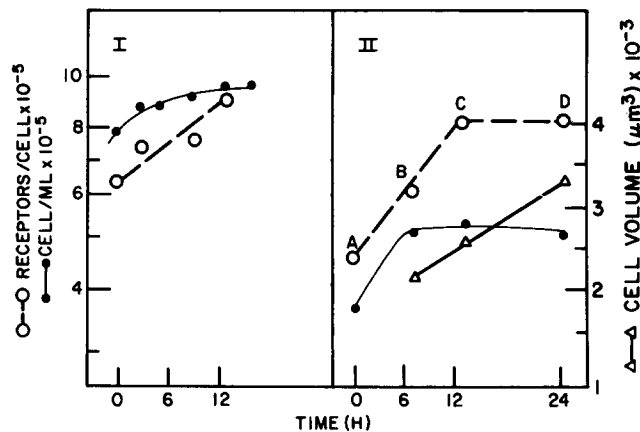


FIG. 6. Changes in cell number (●—●), receptor activity (O---O), and cell volume (Δ — Δ) in the presence of excess thymidine. Panels I and II represent experiments at two different initial cell concentrations.

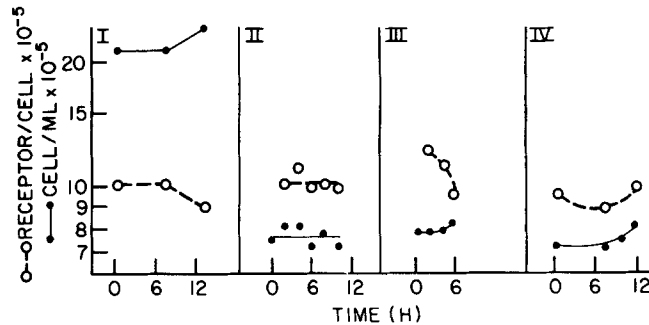


FIG. 7. Growth curves (●—●) and expression of receptor activity (O---O) after release from thymidine block. In experiments I and IV, cells at two different initial concentrations were exposed twice for 8 h to 2 mM thymidine with a 12-h interval. In experiments II and III cells were exposed twice for 9 h to 2.5 mM thymidine with a 16-h interval.

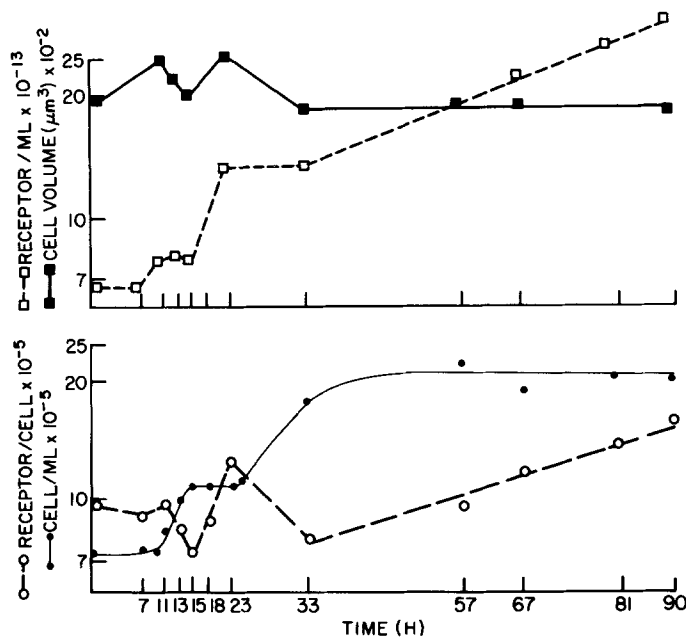


FIG. 8. Growth curve (●—●), receptors per cell (O---O), receptors per culture (□---□), and cell volume (■—■) in a synchronized culture.

was observed during the first wave of cell division (Fig. 8, 9–15 h). The number of cell-bound receptors per culture remained constant, however, thereby clearly indicating that there was no accumulation of receptors per cell during mitosis. This was followed by a 6-h interval during which there was no change in number of cells per milliliter but during which the number of receptors per cell (and per culture) increased by 40% and the mean cell volume increased by 20%. As a second wave of division proceeded, the number of receptors per cell again fell, while cell-bound receptors per culture again remained constant (Fig. 8, 21–33 h). As the culture became stationary the number of receptors per cell gradually

doubled. During the latter period the average cell volume remained constant. Late in the stationary phase the culture had an average volume of $1,700 \mu\text{m}^3$ and 1.6×10^6 receptors per cell. Both volume and number of receptors per cell are slightly higher than usually observed in nonsynchronized cultures. The accumulation of IgE receptors in stationary phase cultures was always associated with a constant or decreasing average cell volume for the population.

Separation of Cells in Ficoll Gradients. The RBL-1 cells separate according to their volume when sedimented through a Ficoll gradient. The larger cells have been shown to have the DNA content expected for the G_2 phase of the cell cycle, the intermediate ones mainly that expected for the S phase, and the smallest that expected for G_1 .² A comparative analysis of the various fractions yielded the extreme values for volumes and receptors per cell listed in Table III.

Fig. 9 I depicts the results of a gradient experiment performed on cells maintained in exponential growth for 14 days. The smallest cells had a mean

TABLE III
*Comparison of Exponential and Stationary Cultures**

	Forced exponential	Early stationary
Mean receptors per cell	4×10^5	14×10^5
Average cell volume, μm^3	1,500	960
Small cell volume, μm^3	960	672
Receptor per small cell	3.5×10^5	9.5×10^5
Large cell volume, μm^3	1,850	1,440
Receptor per large cell	4.8×10^5	15.6×10^5
Cells in G_1 phase, %‡	41	80

* The data in this table were derived from two cultures fractionated on a Ficoll gradient.

‡ Determined by flow microfluorometry by the mithramycin technique. Details of method given in Buell et al.²

volume of $960 \mu\text{m}^3$ and bore 3.5×10^5 receptors/cell. The largest cells had an average volume almost twice as great ($1,850 \mu\text{m}^3$) but only about 33% more receptors (4.8×10^5 /cell). Significantly, cells in fraction 6 (Fig. 9 I), which showed a 23% increase in volume when compared to cells from fraction 8, showed no significant change in the average receptors per cell. We have elsewhere shown that the larger cells were mainly G_2 cells;² therefore, this experiment again suggests that the passage from S phase into and through G_2 phase was not associated with an increase in receptors per cell. When cells from a routine stationary culture were separated on a Ficoll gradient (Fig. 9 II), the smallest cells (fraction 13) had a volume of $672 \mu\text{m}^3$ and bore 9.5×10^5 receptors, and intermediate size cells in fraction 10 had a volume of $960 \mu\text{m}^3$ and bore the maximal number of receptors detected in this culture (1.5×10^6). Cells in fraction 8 had a larger volume ($\times 1.5$), but carried the same number of receptors, and the largest cell had a volume of $1,440 \mu\text{m}^3$ and still bore 1.5×10^6 receptors/cell. DNA content analysis revealed that more than 90% of the cells in fractions 11–13 were G_1 cells, while in fraction 9 there were 62% S-phase cells. Since the increase

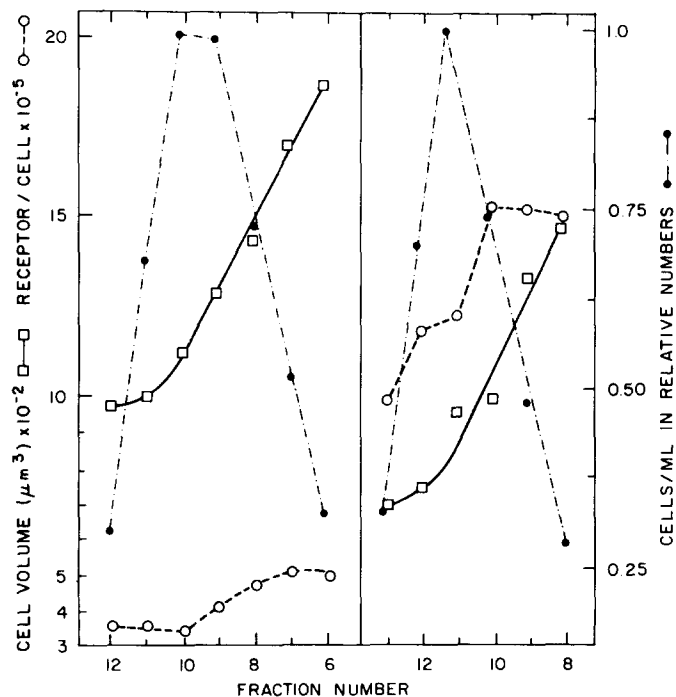


FIG. 9. Ficol gradient fractionation of cells. Cell count (●—●), cell volume (□—□), and number of receptors per cell (O---O). I (left-hand panel), forced exponential culture; II (right-hand panel) stationary culture.

in receptors per cell was observed only between fractions 13 and 10, we conclude that the increase in receptors per cell occurred while cells were passing through the G_1 phase and before they entered the S phase.

Cells from exponentially growing and from stationary cultures are compared in Table III. The smallest cell from a stationary culture carried nearly three times as many receptors for IgE as the smallest cell from an exponentially growing culture. Similarly, the largest cell in stationary cultures had threefold as many receptors per cell as cells from a forced exponential culture. By combining these results with those from flow microfluorometry, which indicate the relative number of cells in the cell-cycle phases, a relatively greater accumulation of smaller G_1 phase cells is seen, with relatively many receptors per cell in the stationary phase cultures.

Discussion

The foregoing results appear to indicate that the number of detectable IgE receptors on the cultured rat basophils varies significantly during the cell cycle and that the average number of receptors expressed by cells in a given culture could be controlled by varying the growth conditions. The changes in numbers of observed receptors per cell were not artifactual. This was documented firstly by demonstrating that the specificity of binding of the cells did not change (Table I). Secondly, there were no changes in the rate of association (2, Fig. 2) or in the

rate of dissociation (2) which could have led to false estimates during the binding analyses. Although lag phase cells have not been examined in detail, exponential and stationary cultures, showing substantial differences in receptor activity, have been. The kinetic data are most consistent with a variable number of uniform receptors. In the absence of structural data, we can of course only talk about a variable number of receptors in operational terms. How the rate of receptor synthesis or insertion into the membrane varies is unknown.

Three lines of evidence suggest that RBL-1 cells express more receptors in the G_1 phase of the cell cycle: (a) Cells that were kept growing exponentially have fewer receptors than cells in stationary cultures. Such exponential cultures contain relatively fewer cells in the G_1 phase of their cycle. (b) When exponentially growing cells were allowed to reach high densities, they ceased to divide, entered the stationary phase, and began to accumulate receptors. Cells that survived for up to 72 h in stationary cultures continued to accumulate receptors. The vast majority of cells in such cultures have the DNA content of G_1 cells.² Other cells lines are also believed to be arrested in the G_1 phase of the cycle in the stationary phase of growth (10, 11). Furthermore, gradient separation of such cultures showed that the accumulation of receptors was occurring primarily in the G_1 phase cells. (c) The smaller G_1 cells from stationary cultures contained three times more receptors than similar cells from exponential cultures. This is consistent with the evidence that cells traverse the G_1 phase more rapidly in the exponential as compared to the stationary cultures (10, 11).

Other evidence suggests that receptors do not accumulate in other phases of the cell cycle. Thymidine block experiments, designed to allow a closer look at the cells in the S and G_2 phases, indicated that the cells do not accumulate receptors during these phases. Although it is generally believed that synchronization with excess thymidine does not change the rate of synthesis of RNA and most proteins (12, 13), some perturbation of cell cycle events have been described (14, 15). Consequently, from these synchronization experiments one cannot categorically exclude the possibility that receptors may accumulate in early S phase of unblocked cells. Ficoll gradient cell separations of exponential cultures also indicated that the G_2 phase is definitely not associated with an increase in receptors for IgE. Some of the intermediate fractions could contain cells completing the G_1 phase, as well as cells entering the S phase; therefore, gradient fractionation of exponential cultures cannot be used exclusively to confirm the observation that cells in S phase do not accumulate receptors. Receptors do not accumulate in the latter part of the lag phase, when the cells traverse the S and G_2 phases before division, indicating again that the cells do not accumulate receptors in the S and G_2 phases of the cycle. Finally, cell division in synchronized RBL-1 cultures resulted in a drop in number of receptors per cell, while the number of receptors per culture remain unchanged. Thus mitosis in RBL-1 cells is not associated with either net gain or loss of receptor but simply with distribution of receptors to daughter cells.

It seems reasonable to conclude that RBL-1 cells accumulate receptors primarily and probably exclusively in the G_1 phase of the cell cycle. Since our assay is based upon detecting [¹²⁵I]IgE bound to a receptor, we detect only active receptors and we cannot exclude the possibility that synthesis and/or insertion of

the receptors occur at some other time and that in the G_1 phase the receptor is only unmasked or completed. There is evidence from other systems that membrane components are actually being synthesized in the G_1 phase. Both L cells (16) and KB cells (17) were shown to synthesize surface membrane components throughout the cycle, with a sharp increase in the rate of synthesis during the G_1 phase immediately after cell division and a further increase in the stationary phase (18). Onodera and Sheinin (19) have shown that surface components in 3T3 cells are synthesized at a higher rate immediately after cell division and that the rate of synthesis declined as the cells passed through S phase. Graham et al. (20), working with NIL-2-HSV cells and P815Y cells, have concluded that various membrane components are synthesized and inserted throughout the interphase at an even pace, with the exception of carbohydrates, which are synthesized and integrated at a slightly higher rate in the G_1 phase. In view of the above and the fact that the RBL-1 cells continue to accumulate receptors in a prolonged G_1 arrest, it is not unlikely that these receptors are actually synthesized in the G_1 phase.

The relationship between phases in cell cycle and degrees of expression of membrane markers has been studied in a number of different systems. According to some (21), the expression of *H-2* determinants in P815Y murine cells, as measured by cytolysis decreased during the G_1 -S period, was lowest at S phase and restored in the G_2 phase. Others (22-24) claim that the expression of *H-2* determinants as measured in three other murine cell lines by immunofluorescence was highest in the G_1 phase of the cycle. Accumulation of acetylcholine receptor in mouse neuroblastoma cells was associated with cessation of cell division and higher cell densities (25). The expression of HL-A antigen on surface membranes of human RPMI 8866 cells (26) and human WI-L2 cells (27, 28) was maximal in the G_2 and S phases of the cycle. An increase in expression of H blood group determinants on surface membranes of HeLa cells (29) occurred immediately before and during cell division. The contradiction between the various observations may be related in part to differences between the cell lines and the experimental procedures. Thus, for example, H-determinant-positive cells found by Kuhns and Bramson (29) during a prolonged mitotic wave might have belonged to the G_1 fraction. Graham et al. (20) suggested that interphase cells are less susceptible to immunocytolysis because of a nonspecific decrease in cell fragility. Therefore, their finding that the G_2 cells had increased sensitivity in the immunocytolysis assay may not be directly related to actual increase in number of receptors but rather to increased fragility.

It has been suggested that the G_1 phase is that part of the cycle in which specialized products and traits of differentiation preferentially appear (30). Immunoglobulin synthesis (31) and the appearance of myosin and myofibril fusion in muscle differentiation (32) are examples. Other culture systems have been described where differentiation occurs only after high-density growth arrest in a G_1 state. Such systems include S protein synthesis in rat neuroglial cells (33) and the synthesis of chondroitin sulfate in chondrocytes (34). RBL-1 cultures behave similarly with minimal differentiation in terms both of basophilic granules and the IgE receptor during rapid cycling exponential growth and of marked differentiation occurring only after G_1 growth arrest.

A number of reports deal with the relationship between the expression of a membrane marker and cell volume. Cikes and Friedberg (22) felt that an inverse relationship existed between cell volume and *H-2* surface antigen expression in the TLS-V9-MLV cells. The expression of *H-2* determinant (21) on P815Y cells varied independently of cell volume. The data presented by Pellegrino et al. and Ferrone et al. (27, 28) on W-1 and L2 cells and

by Everson et al. on RPMI 8866 cells (26) indicates that the expression of *H-2* determinants increased with the volume. Both groups felt that it may be wrong to relate these data to density of the determinant per square unit of surface area, since the cells investigated were not perfect spherical forms. In the RBL-1 cell culture the changes in number of receptors per cell do not appear to be directly related to changes in cell volume. Thus cells in stationary cultures accumulated receptors in the absence of any corresponding change in volume, while some increases in volume occurring during the G_1 phase in exponentially growing cells could be associated with an increase in the number of receptors per cell. Further increases in volume occurring during the late S phase and the G_2 phase of cell cycle were not associated with an increase in number of receptors per cell. Scanning electronmicroscopy studies³ indicate that RBL-1 cells have approximately the same number of folds and microvilli on cells derived from a stationary culture (more than 80% G_1 cells) and on cells derived from a culture in which more than 50% of the cells were in the S phase. Thus, unlike CHO cells, shown to have more peripheral processes in the G_1 phase and less in the S phase (35, 36), RBL-1 cells seem to have the same surface morphology in S and G_1 phase and therefore a relatively constant, albeit nonlinear, relationship between volume and surface area. Consequently, our data suggest a higher density of receptors per unit area in the stationary cells. However, a more direct and rigorous determination of the surface area would be required to prove this point.

A significant and rapid drop in receptor activity occurs upon resuspension of stationary cells in fresh medium at low densities. This drop starts and is probably completed before the cells enter the S phase. We are uncertain whether the observed drop in receptor activity is due to shedding of the receptor or to some other cause, e.g., changes in susceptibility. Studies are under way to examine these various possibilities. Similar phenomena have been described by other investigators. Stimulation of resting bone marrow-derived murine lymphocytes with a mitogenic lipopolysaccharide from *Escherichia coli* (37) was associated with a sharp decrease in surface-bound Ig determinants within 20 min of exposure to the mitogen. Stout and Coons (38) have found that the exposure of both spleen and thymus-derived murine lymphocytes to synthetic nucleotides and dibutyryl cyclic AMP caused significant reduction in the ability of these cells to bind antigen. At the same time these cells were found to have increased protein and RNA synthesis. A number of other changes in membrane functions (enzyme activity, ion transport) have been associated with the reentry of various resting cells into the growth cycle (15).

We have demonstrated that the changes in the number of receptors for IgE on RBL-1 cells form an orderly and self-consistent pattern related to cell cycle events. The highest number of receptors were found on cells from stationary cultures; these cells were shown to be arrested in the G_1 phase of the cycle.² Such stationary cells also undergo marked morphological changes, as judged by a significant increase in the size and number of basophilic granules, which are nearly extinct in cells from a culture maintained in exponential growth.² Therefore, two differentiation markers characteristic of this cell line, the IgE receptor and the basophilic granules, are expressed most in the stationary cells. The cyclic changes in expression of IgE receptor in RBL-1 cells makes this a useful system for studying the incorporation (and in the lag phase, loss) of a defined component into the membrane. Such studies would be more definitive if

³ Scanning electron micrography was kindly performed by Dr. Raul Braylan, National Cancer Institute, National Institutes of Health.

the receptor were also structurally defined, and we are working to this end. The present results may be useful in this regard since they suggest methods for maximizing the yield of receptor and the incorporation of radioactive amino acids or carbohydrates into the receptors. Also, the loss of receptor activity during the lag phase is worth additional exploration.

Summary

The rat basophilic leukemia cell line (RBL-1) showed an inverse relationship between growth rate and expression of receptor activity for IgE. After prolonged exponential growth, the number of receptors per cell stabilized at $4-6 \times 10^5$. Cells in stationary cultures, which are arrested in the G_1 phase of the cell cycle, continued to accumulate up to $0.9-1.7 \times 10^6$ receptors/cell with no increase in volume. Upon resuspension in fresh medium at low density, these cells were shown to lose up to 70% of the receptor activity within 4 h. Assessment of cultures synchronized by double thymidine block and cells fractionated by centrifugation on a Ficoll gradient indicated that the RBL-1 cells acquire receptors in the G_1 phase of the cell cycle. No accumulation of active receptors occurred during the S and G_2 phases, though the average cell volume increased. Cell division resulted in a drop in number of receptors per cell while the number of cell-bound receptors in the culture remained unchanged. This indicates that during mitosis receptors were simply distributed to daughter cells.

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