

- in serum after allergen challenge of asthmatic patients and the effects of anti-asthmatic medication. *Int Arch Allergy Appl Immunol* 1988;87:306-12.
48. Bisgaard H, Groenborg H, Mygind N, Dahl R, Lindqvist N, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: effect of the glucocorticoid budesonide. *J ALLERGY CLIN IMMUNOL* 1990;85:891-5.
49. Masuyama K, Ishikawa T. Direct interaction of guinea pig eosinophils and adrenergic agents. *Int Arch Allergy Appl Immunol* 1985;78:243-8.
50. Hutson PA, Holgate ST, Church MK. The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leucocyte infiltration after allergen challenge of nonanesthetized guinea pigs. *Am Rev Respir Dis* 1988;138:1157-63.
51. Yukawa T, Ukena D, Kroegel C, et al. Beta₂-adrenergic receptors on eosinophils. Binding and functional studies. *Am Rev Respir Dis* 1990;141:1446-52.
52. Atkins PC, Norman ME, Zweiman B. Antigen-induced neutrophil chemotactic activity in man: correlation with bronchospasm and inhibition by disodium cromoglycate. *J ALLERGY CLIN IMMUNOL* 1978;62:149-55.
53. Nagy L, Lee TH, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N Engl J Med* 1982;306:497-501.
54. Kallenbach JM, Panz VR, Joffe BI, et al. Nocturnal events related to 'morning dipping' in bronchial asthma. *Chest* 1988;93:751-7.

IgE and adenosine 5' triphosphate receptors on immature murine mast cells are functionally linked to signal transduction mechanisms

Gian Luigi Rossi, MD, An Yen, MD,* and Kim Elaine Barrett, PhD

San Diego, Calif.

Calcium mobilization in response to IgE-receptor cross-linking by antigen was assessed in immature murine mast cells cultured from bone marrow to determine whether the early expression of IgE receptors on such cells may be of functional significance. IgE receptors were expressed by approximately 30% of cells after 1 week in culture and by an increased proportion at 2 and 3 weeks. The ability of a non-IgE-dependent stimulus, adenosine 5' triphosphate (ATP), to increase intracellular calcium in these cells was also tested. Calcium mobilization in large numbers of individual cells was monitored with use of a fluorimetric reagent and flow cytometry. Both antigen and ATP had significant effects on intracellular calcium in cells cultured for as little as 1 week with interleukin-3, when few cells exhibited morphologic or functional characteristics of mast cells. Longer times in culture were associated with an increase in the proportion of cells responding to these stimuli with calcium mobilization, but not with a change in the magnitude of the response. We conclude that the early expression of IgE receptors during mast cell development may be functionally significant, since these receptors appear to be linked to cellular signal transduction mechanisms. The data additionally imply a possible role for ATP in mast cell development. (J ALLERGY CLIN IMMUNOL 1992;90:765-71.)

Key words: Mast cells, calcium, signal transduction, IgE receptors, ATP

From the Divisions of Allergic and Immunologic Diseases and Dermatology,* Department of Medicine, University of California, San Diego, School of Medicine, San Diego, Calif.

Supported by grant no. AI 24992 from the National Institutes of Health.

Received for publication March 16, 1992.

Revised June 22, 1992.

Accepted for publication June 23, 1992.

Reprint requests: Kim E. Barrett, PhD, UCSD Medical Center, 8417, 225 Dickinson St., San Diego, CA 92103-8417.

1/1/40446

New aspects of mast cell biology have recently been elucidated, supporting the opinion that mast cells could play important roles in normal physiology in addition to their well-recognized involvement in disease.¹ Although a body of data exists regarding the biologic activity of mature mast cells and the various stimuli and factors that lead to mast cell differentiation, growth, and activation,¹ comparatively little is known about the functional capabilities of immature mast cells or mast cell precursors undergoing differ-

Abbreviations used

ATP:	Adenosine 5' triphosphate
IL-3:	Interleukin-3
DNP:	Dinitrophenol
BSA:	Bovine serum albumin
V/B ratio:	Ratio of violet to blue Indo-1 fluorescence
[Ca ²⁺]:	Cytoplasmic calcium concentration
Fcε RI:	High-affinity receptor for IgE
IL-4:	Interleukin-4

entiation. However, it has recently been demonstrated that the expression of high-affinity receptors for IgE is an early event during the differentiation of mouse bone marrow-derived mast cells.^{2,3} These findings suggest that mast cells may have the ability to perform immunologically regulated functions before the development of a capacity to degranulate. Similarly, the purine nucleotide, adenosine 5' triphosphate (ATP), has been shown to mobilize intracellular calcium in neutrophil/monocyte progenitors and mouse mastocytoma cells.^{4,5} Since ATP has long been known to induce histamine release from mature rat peritoneal mast cells via a specific ATP⁴⁻ receptor,⁶⁻⁸ it is conceivable that purinergic receptors also are expressed at an early time point in the differentiation of mast cells and other hemopoietic cell types, and further, that they may, like IgE receptors, be functionally significant.

We have recently reported that flow cytometry and the fluorescent calcium indicator Indo-1 (Sigma Chemical Co., St. Louis, Mo.) can be used to monitor intracellular calcium mobilization in mature mast cells activated with both IgE-dependent and IgE-independent stimuli.⁹ A strength of this technique is that it permits the analysis of calcium mobilization events in large numbers of individual cells. In the present study we have applied this method to examine changes in intracellular calcium concentrations in mouse bone marrow mast cells at various stages of maturation. The cells were stimulated with either antigen or ATP to evaluate whether IgE or ATP receptors of immature mast cells are effectively linked to cellular signal transduction mechanisms. The findings are correlated with the proportion of cells expressing IgE receptors,

taining 20% conditioned medium from the WEHI-3B cell line (a constitutive producer of interleukin-3 [IL-3]) and fetal calf serum, as previously described.¹⁰ Cells were passaged weekly, at which time aliquots were removed for analysis.

Measurement of cytosolic [Ca²⁺]

Mast cells, cultured as described above, were washed free of tissue culture medium. They were then incubated (30 minutes at 37° C) in RPMI 1640 medium, at a density of 10⁷/ml, in the presence of antidinitrophenol (DNP) monoclonal IgE (10 µg/ml) and the cell permeant acetoxymethyl ester of Indo-1 (30 µmol/L). The cells were then washed in Hanks' balanced salt solution, resuspended at a density of 10⁶/ml in RPMI 1640 medium containing fetal calf serum (2%) CaCl₂ (1.8 mmol/L) and MgCl₂ (1 mmol/L), and held on ice in the dark until analysis. Analyses of Indo-1 fluorescence in individual cells were performed with use of an Ortho Cytofluorograph IIs (model 50H, equipped with a 2151 computer; Ortho Diagnostic Systems, Westwood, Mass.), with a technique based on that reported by Rabinovitch et al.¹¹ and previously described in detail.⁹ Cells (10⁶) in a volume of 1 ml were loaded into the cytofluorograph and were analyzed at 37° C at a rate of 500/sec, before and after the addition of antigen (50 to 200 ng/ml) or ATP (0.1 to 1 mmol/L).

Assessment of Fcε RI Expression

Cells were incubated with 1 µg/ml IgE (1 µg/10⁶ cells) for 1 hour at room temperature, with gentle agitation every 20 minutes. The cells were then washed and resuspended in calcium-free Tyrode's buffer containing FITC-conjugated sheep anti-IgE (250 µg/ml), and sodium azide (0.02%, to prevent cellular activation via cross-linking of IgE receptors). The cells were then incubated for 1 hour in the dark at room temperature. After three washes with calcium-free Tyrode's, the cells were analyzed for fluorescent intensity with use of a flow cytometer equipped with an argon laser (Ortho cytofluorograph IIs).

β-hexosaminidase assay

Mast cell degranulation was assessed by measuring the stimulated release of β-hexosaminidase.¹² In brief, cells were washed three times in Tyrode's buffer lacking divalent cations to remove tissue culture medium and sensitized at 37° C with anti-DNP IgE (1 µg/ml) for 30 minutes, followed by washing and challenge with either DNP-bovine serum albumin (BSA) antigen (200 ng/ml) or ATP (1 mmol/L) in complete Tyrode's buffer at 37° C. After 10

minutes, the cells were centrifuged at 200g for 10 minutes. The β-hexosaminidase content of each sample was measured enzymatically with use of p-nitrophenyl-β-D-glucosamide as substrate.¹³ Total cellular content of β-hexosaminidase was calculated by adding values from supernatant and pellet fractions, and the percent release of this mediator was calculated by comparing supernatant β-hexosaminidase with total cellular content.

MATERIAL AND METHODS**Material**

Mast cell culture. Bone marrow from femurs of BALB/c mice was cultured in RPMI 1640 medium con-

Cytochemical analysis

Cultured cells were spun onto glass slides in a cytocentrifuge (Cytospin; Shandon Southern Instruments, Sewickley, Pa.), air dried, and stained for 45 minutes with 0.1% alcian blue at pH 1, then counterstained with 0.5% Safranin (Sigma) for 30 minutes. The number of cells positive to alcian blue were counted and averaged in at least 10 high-power fields and expressed as a percentage of total cells.

RESULTS

Antigen-induced calcium mobilization

Cells from three different cultures of mouse bone marrow were analyzed after 1 week and 2 weeks of culture (see Methods for culture conditions); cells from culture no. 1 were also analyzed after 3 weeks. The cells were loaded with Indo-1 (30 μ mol/L), passively sensitized with IgE, and then challenged with specific antigen (DNP-BSA, 200 ng/ml) during flow cytometric analysis. Table I provides data for the increase in violet to blue fluorescence ratio (V/B ratio, reflective of cytoplasmic calcium concentration ($[Ca^{2+}]_i$) over baseline, and the proportion of cells responding to the antigenic stimulus. As cells were cultured for longer periods, there was a progressive increase in the average magnitude of the elevation of V/B ratio and an increase in the proportion of cells that responded to DNP-BSA with an increase in $[Ca^{2+}]_i$ (Table I). In the one culture examined at 3 weeks, approximately 52% of cells responded, which is not markedly dissimilar from the value previously reported by us, 80% responding cells, for mature cultured mouse mast cells studied under the same experimental conditions.⁹ It is apparent from Table I that the progressive increase in responsiveness in terms of the mean elevation in V/B ratio that occurred with longer periods of culture was actually wholly accounted for by the increase in the proportion of responding cells. In all cultures examined, an increase in the magnitude of the elevation in V/B ratio was accompanied by a parallel increase in the responding cell population. The ratio of V/B increase to percent of responding cells, an approximation of the average increase in $[Ca^{2+}]_i$ in those cells that responded to antigen, was similar at 1, 2, and 3 weeks of culture (2.4 ± 0.5 [3], 2.2 ± 0.1 [3] and 2.1 [1], respectively, mean $\pm$ SEM for the number of experiments in parentheses). Similarly, at all time points, the kinetics of calcium mobilization induced by antigen were similar. Thus addition of DNP-BSA increased the V/B ratio after a lag time of approximately 40 seconds; the peak value of V/B ratio was achieved after approximately 80 seconds. The V/B ratio slowly declined thereafter, but remained substantially ele-

TABLE I. Effect of antigen on calcium mobilization in murine bone marrow cells cultured for various times*

Time in culture (wk)	Increase in V/B ratio† (%)	Proportion of cells responding (%)
1	35.3 $\pm$ 19.4	14.3 $\pm$ 5.5
2	69.4 $\pm$ 19.7	32.1 $\pm$ 9.8
3	109.8	51.8

*Antigen was DNP-BSA at 200 ng/ml. Data are mean $\pm$ SEM for three experiments (1 and 2 weeks); only one culture examined at 3 weeks.

†Reflective of $[Ca^{2+}]_i$.

vated above baseline values for the remainder of the observation period (up to 400 seconds).

The ability of DNP-BSA to mobilize calcium was dose-dependent. Cells from two cultures were stimulated additionally with 50 mg/ml DNP-BSA at either 1 week or 2 weeks of culture. In both cases, the effect of this dose of antigen on V/B ratio was less than that observed with the same cells stimulated with 200 ng/ml of the antigen (data not shown).

Expression of Fc ϵ RI

Cells grown for 1, 2, and 3 weeks in culture were assessed for their expression of IgE receptors by flow cytometry. At 1 week, two populations of cells could be distinguished, large and small, on the basis of their forward scatter. The smaller cells had 16.3% that were positive for IgE receptor expression (mean of two determinations, range 15.7 to 16.9). Among the larger cells, 39.7% were positive for Fc ϵ RI (range 37.4% to 42.0%). Overall, 30.3% of the total cells (range 28.7 to 31.9%) were positive for Fc ϵ RI. At 2 weeks, a single population of cells was present as assessed by forward scatter. Of these, 64.8% (range 62.3% to 67.3%) were positive for Fc ϵ RI. At 3 weeks, the corresponding value was increased still further to 86.0% (range 85.2% to 86.8%).

ATP-induced calcium mobilization

Cells from the cultures tested for antigen-induced calcium mobilization were also examined at the same times for calcium mobilization in response to ATP (1 mmol/L). Table II provides data for the mean changes in V/B ratio and the percentage of responding cells at 1, 2, and 3 weeks of culture. As seen for the response of cells to antigen challenge, there was a progressive increase in the degree of calcium mobilization as cells were cultured for longer periods. Again, this was accounted for by an increase in the

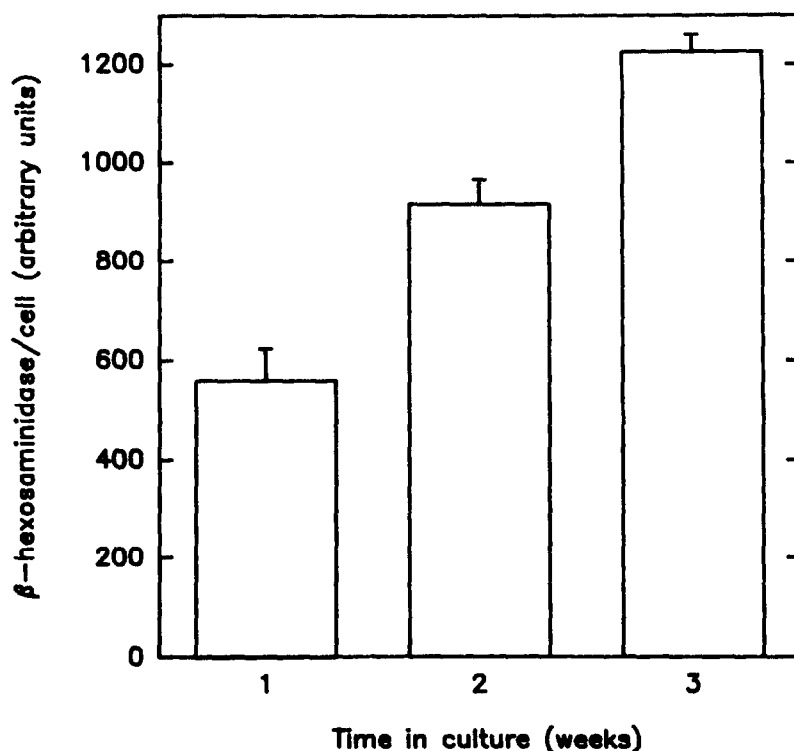


FIG. 1. Cellular content of β -hexosaminidase in mouse bone marrow cells grown for 1, 2, or 3 weeks in the presence of IL-3. Values are mean $\pm$ SEM for three determinations. The three means are significantly different ($p < 0.001$) by ANOVA.

TABLE II. Effect of ATP on calcium mobilization in murine bone marrow cells cultured for various times*

Time in culture (weeks)	Increase in V/B ratio† (%)	Proportion of cells responding (%)
1	38.3 $\pm$ 3.3	11.3 $\pm$ 2.4
2	143.2 $\pm$ 14.8‡	53.7 $\pm$ 13.6
3	129.8	57.1

*ATP dose was 1 mmol/L. Data are mean $\pm$ SEM for three experiments (1 and 2 weeks); only one culture examined at 3 weeks.

†Reflective of $[Ca^{2+}]_i$.

‡Significantly greater than corresponding value at 1 week of culture, $p < 0.05$ by Student's t test for paired samples.

proportion of cells responding to the stimulus. Of interest, however, is the evidence that the ability to respond to ATP may develop slightly more rapidly than that to antigen. Although 1-week-old cultures contained similar proportions of cells responding to antigen and ATP (compare Tables I and II), at 2 weeks there was a significantly greater increase in V/B ratio induced by ATP in these cells than by antigen (143.2 $\pm$ 14.8% vs 69.4 $\pm$ 19.7%, mean $\pm$ SEM, $n = 3$, $p < 0.05$ by Student's t test for paired

samples). The proportion of cells responding at 2 weeks was correspondingly greater when ATP was used as stimulus (53.7% $\pm$ 13.6% vs 32.1% $\pm$ 9.8%, mean $\pm$ SEM, $n = 3$), although not statistically significantly so.

The degree of calcium mobilization induced by ATP was dose-dependent. In one culture examined at 2 weeks, 1 mmol/L ATP increased the V/B ratio by 154.3%, with 80.9% of cells responding, whereas addition of 0.1 mmol/L ATP to the same cells increased V/B ratio by only 64.6%, with 44.2% of cells responding.

Development of capacity to degranulate

We measured cellular content of β -hexosaminidase, a representative granule-associated mediator, and its release induced by antigen (DNP-BSA) or ATP in mouse bone marrow cells cultured for 1, 2, and 3 weeks. Culture of bone marrow cells was associated with a progressive increase in their content of β -hexosaminidase on a per cell basis (Fig. 1). The increase in content of this mediator was statistically significant as assessed by analysis of variance (ANOVA) ($p < 0.001$). Although even 1-week-old cultures contained appreciable amounts of β -hexosaminidase, mediator release from these immature cultures could not be

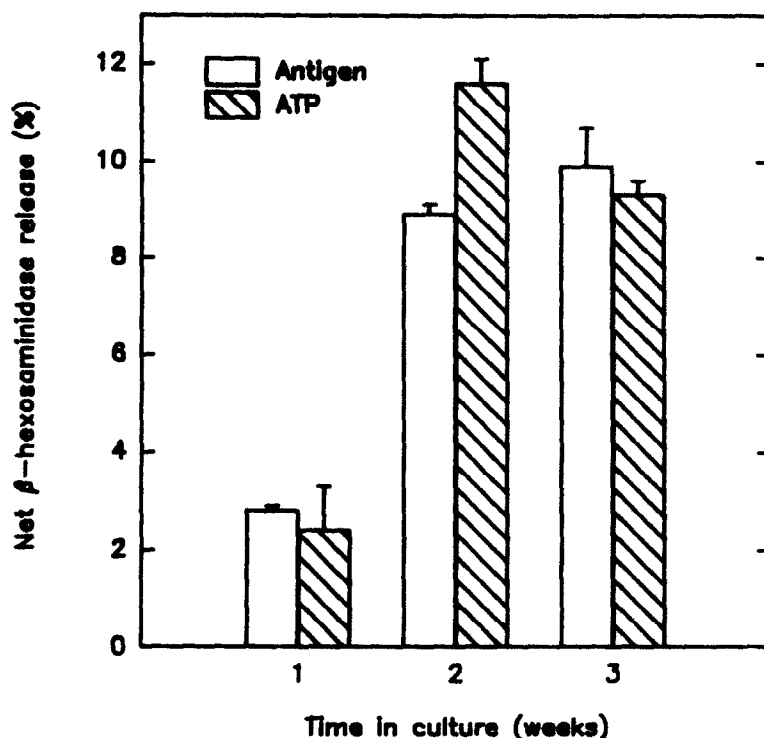


FIG. 2. Net release of β -hexosaminidase induced by DNP-BSA (200 ng/ml; "antigen") or ATP (1 mmol/L) from mouse bone marrow cells grown for 1, 2, or 3 weeks in the presence of IL-3. Values are mean $\pm$ SEM for three determinations.

specifically stimulated by either DNP-BSA (200 ng/ml) or ATP (1 mmol/L) (Fig. 2). There was a high degree of spontaneous release of this mediator from 1-week-old cultures ($10.2\% \pm 0.6\%$, mean $\pm$ SEM, $n = 3$), but little or no net release induced by the receptor-dependent stimuli. In contrast, cells examined at 2 and 3 weeks of culture had low levels of spontaneous β -hexosaminidase release ($1.1\% \pm 0.1\%$, 2 weeks; $1.6\% \pm 0.1\%$, 3 weeks; mean $\pm$ SEM, $n = 3$) and net release could be stimulated by either DNP-BSA or ATP (Fig. 2). Of interest, release induced by ATP appeared to be maximal at 2 weeks, whereas that induced by antigen was slightly higher at 3 weeks than at 2 weeks.

Cytochemical analysis

Cytospin slides of 1-, 2-, and 3-week-old cultures were prepared, stained with alcian blue/safranin, and the proportion of cells scored as either ungranulated (no alcian blue-stained material in the cytoplasm), sparsely granulated (cell with less than 10 blue granules), or well-granulated (cell with greater than 10 alcian blue positive granules, often obscuring the nucleus), was determined by an observer blinded as to the age of the culture. As shown in Fig. 3, and described previously by others, growth of mouse bone marrow in the presence of IL-3 led to a progressive

increase in the proportion of cells displaying the cytochemical and morphologic characteristics of mast cells. Of note, at 1 week in culture, less than 8% of cells were positive to alcian blue despite the fact that approximately 15% of such cells responded to IgE-dependent stimulation with an increase in cytoplasmic calcium levels (c.f. Table I), and approximately 30% expressed Fc ϵ RI.

DISCUSSION

In the present study we have shown that mouse bone marrow cells, cultured in the presence of IL-3, develop the ability to respond to both an IgE-mediated stimulus (antigen) and to ATP at a very early stage of differentiation into mast cells. To assess whether responsiveness had developed, we monitored the ability of the named stimuli to mobilize intracellular Ca²⁺ in large numbers of individual cells with use of flow cytometric techniques. Even after 1 week of culture, a significant proportion of cultured cells ($>10\%$) were responsive to both antigen and ATP, and longer periods in culture were associated with a progressive increase in the proportion of cells displaying a response to these stimuli. In contrast, 1-week-old cultures had a low content of β -hexosaminidase, did not release this mediator specifically in response to stimulation, and contained few cells with morphologic

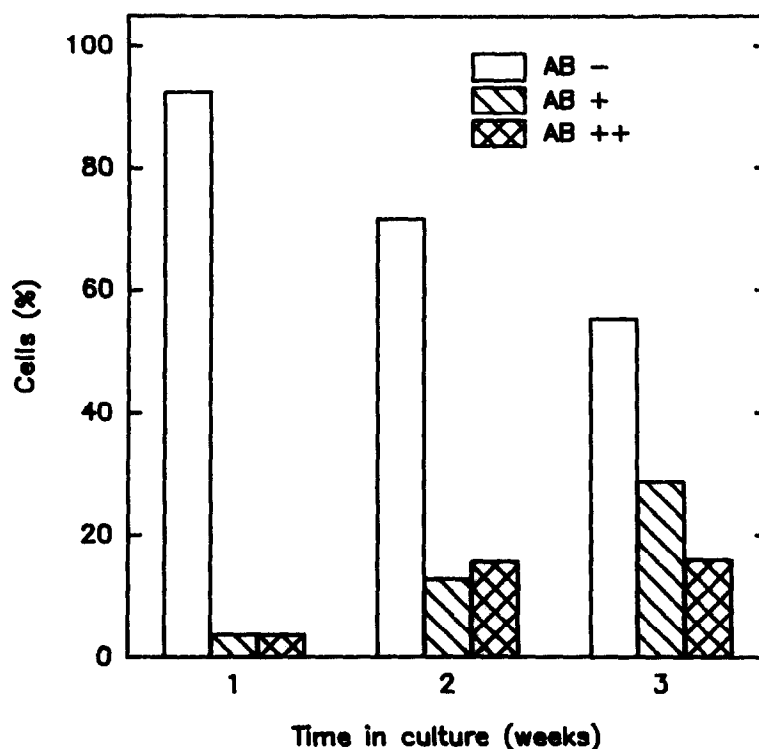


FIG. 3. Proportion of cells either negative for alcian blue staining (ungranulated, *AB* -), containing less than 10 alcian blue-positive granules (sparsely granulated, *AB* +), or containing greater than 10 alcian blue-positive granules (well-granulated, *AB* ++) in cytocentrifuge preparations of mouse bone marrow cells grown for 1, 2, or 3 weeks in the presence of IL-3. Values are from a single experiment representative of three similar experiments.

characteristics consistent with their identification as mature mast cells.

Thompson et al.³ recently reported that transcripts for the subunits (α , β , and γ) comprising the high-affinity receptor for IgE (Fc ϵ RI) are present in the cytoplasm of murine bone marrow cells within 1 week of culture in the presence of IL-3. Moreover, they reported that 75% of cells express membrane-associated Fc ϵ RI at this time point, whereas only 25% of the cells were identified as mast cells by the criteria used by these authors. Accordingly, the authors suggest that the early appearance of IgE receptors during mast cell differentiation may confer an ability to respond to IgE-dependent stimuli, in ways other than degranulating, before acquiring a significant complement of granule-associated mediators. We also found that significant numbers of murine bone marrow cells cultured with IL-3 express Fc ϵ RI at 1 week, although the proportion of cells in our study calcium (30%) was lower than that reported by Thompson et al.,³ as was the proportion of cells identified as mast cells by alcian blue staining.

A number of recent investigations have highlighted the ability of mast cells to act as a source of multiple cytokines.^{14, 15} In some cases, synthesis of such mol-

ecules may be triggered via Fc ϵ RI. For example, Fc ϵ RI positive mouse bone marrow cells were reported to produce IL-4 in response to an IgE-dependent stimulus.¹⁶ Likewise, bone marrow mast cell secretion of endothelin could be triggered via the IgE receptor but was independent of the release of granule constituents.¹⁷ Our present finding that IL-3-dependent mouse bone marrow cells respond to antigen with mobilization of intracellular calcium, before being able to degranulate, is in keeping with the hypothesis that immature mast cells could play a physiologic role during their development. It is tempting to speculate that triggering of immature mast cells via their IgE receptors is directed toward the generation of cytokines such as IL-3 or IL-4, which in turn could stimulate cell growth and differentiation via a positive feedback loop.

We have also shown here that immature mast cells are responsive to an IgE-independent stimulus, ATP. In fact, there was evidence that such responsiveness may develop somewhat more briskly than that to antigen. Although an intracellular role for ATP is well-recognized, its extracellular functions are less well-understood, although it can influence many biologic processes including neurotransmission, vascular tone,

platelet aggregation, and, notably, mast cell secretion.¹⁸ A specific membrane receptor has been described in the rat mast cell, which is activated by the tetrabasic free acid ATP⁴⁻ (a minor component of total ATP) and linked to phosphatidylinositol turnover and consequently calcium mobilization.^{7, 8} Of interest, Cowen et al.⁴ reported that extracellular ATP is a potent inducer of calcium mobilization in neutrophil/monocyte progenitors, raising the possibility that activation of the inositol phospholipid signaling system by ATP may play a role during cell differentiation. We found that intracellular ATP induces calcium mobilization in a significant proportion of immature mouse mast cells as early as 1 week in culture, and that after 2 weeks the proportion of responding cells was greater than 40% in all cultures, with a maximum of 80.9% in one culture. There is accordingly a possibility that ATP might also be involved in mast cell development. There are a number of conditions under which extracellular release of ATP is likely to occur. For example, ATP can be copackaged in both adrenergic and cholinergic neurotransmitter granules and released during neurotransmission into synaptic spaces or stored with serotonin in platelet granules and released locally during platelet activation.¹⁹⁻²¹ Given that the existence of mast cell/nerve interactions is now well-established,²² it is possible that ATP could be released in significant amounts in proximity to the mast cell membrane. Alternatively, stromal cells might release ATP in the microenvironment of the bone marrow, providing an effective signal to progenitor cells during differentiation, as suggested by Cowen et al.⁴

In conclusion, we have found that immature mast cells are able to respond to both IgE-mediated and non IgE-mediated stimuli at early stages of maturation. This response is manifest as an active calcium signal transduction mechanism, and appears to develop before the ability to store and release appreciable quantities of granule-associated mediators. Further studies are necessary to clarify the biologic relevance of this ability.

We thank Dennis Young for performing the FACS analysis. G.L.R. was the recipient of a grant from the Clinica del Lavoro Foundation, IRCCS, Medical Center of Pavia, Italy. A.Y. is the recipient of a postdoctoral fellowship from the Dermatology Foundation sponsored by Dermik Laboratories.

REFERENCES

1. Wasserman SI. Mast cell biology. *J ALLERGY CLIN IMMUNOL* 1990;86:590-3.
2. Bender JG, Van Epps DE, Stewart CC. Characterization of granulocytes and mast cells in cultures of mouse bone marrow stimulated with interleukin-3. *J Cell Physiol* 1988;135:71-8.
3. Thompson HL, Metcalfe DD, Kinet JP. Early expression of high-affinity receptor for immunoglobulin E (Fcε RI) during differentiation of mouse mast cells and human basophils. *J Clin Invest* 1990;85:1227-33.
4. Cowen DS, Lazarus HM, Shurin SB, Stoll SE, Dubyak GR. Extracellular adenosine triphosphate activates calcium mobilization in human phagocytic leukocytes and neutrophil/monocyte progenitor cells. *J Clin Invest* 1989;83:1651-60.
5. Negishi M, Hashimoto H, Ichikawa A. Differential regulation of thrombin- or ATP-induced mobilization of intracellular Ca²⁺ by prostacyclin receptor in mouse mastocytoma cells. *Biochem Biophys Res Commun* 1991;176:102-7.
6. Gomperts BD. Current status review. Exocytosis: the role of Ca²⁺, GTP and ATP as regulators and modulators in the rat mast cell model. *J Exp Pathol* 1990;71:423-31.
7. Cockcroft S, Gomperts BD. The ATP⁴⁻ receptor of rat mast cells. *Biochem J* 1980;188:789-98.
8. Tatham PER, Cusack NJ, Gomperts BD. Characterization of the ATP⁴⁻ receptor that mediates permeabilisation of rat mast cells. *Eur J Pharmacol* 1988;147:13-21.
9. Rossi GL, Young DJ, Wasserman SI, Barrett KE. Calcium mobilization in activated mast cells monitored by flow cytometric analysis. *Agents Actions* 1990;31:257-62.
10. Marquardt DL, Walker LL. Inhibition of mast cell adenosine responsiveness by chronic exposure to adenosine receptor agonists. *Biochem Pharmacol* 1986;36:4297-302.
11. Rabinovitch PS, June CH, Grossman A, Ledbetter JA. Heterogeneity among T cells in intracellular free calcium responses after mitogen stimulation with PHA or anti-CD3. Simultaneous use of indo-1 and immunofluorescence with flow cytometry. *J Immunol* 1986;137:952-61.
12. Marquardt DL, Walker LL, Wasserman SI. Cromolyn inhibition of mediator release in mast cells derived from mouse bone marrow. *Am Rev Respir Dis* 1986;133:1105-9.
13. Schwartz LB, Austen KF, Wasserman SI. Immunologic release of beta-hexosaminidase and beta-glucuronidase from purified rat serosal mast cells. *J Immunol* 1979;123:1445-50.
14. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of Fcε RI or to calcium ionophores. *Nature* 1989;339:64-7.
15. Burd PR, Rogers HW, Gordon JR, et al. Interleukin-3-dependent and -independent mast cells stimulated with IgE express multiple cytokines. *J Exp Med* 1989;170:245-59.
16. Seder RA, Plaut M, Barbieri S, Urban J Jr, Finkelman FD, Paul WE. Purified Fcε R⁺ bone marrow and splenic non-B, non-T cells are highly enriched in the capacity to produce IL-4 in response to immobilized IgE, IgG_{2a}, or ionomycin. *J Immunol* 1991;147:903-9.
17. Ehrenreich H, Burd PR, Rottem M, et al. Endothelins belong to the assortment of mast cell-derived and mast cell-bound cytokines. *New Biologist* 1992;4:147-56.
18. Gordon JL. Extracellular ATP: effects, sources and fate. *Biochem J* 1986;233:309-19.
19. Morel N, Meunier FM. Simultaneous release of acetylcholine and ATP from stimulated cholinergic synaptosomes. *J Neurochem* 1981;36:1766-73.
20. Rojas E, Pollard HB, Heldman E. Real-time measurements of acetylcholine-induced release of ATP from bovine medullary chromaffin cells. *FEBS Lett* 1985;185:323-7.
21. Ingberman CM, Smith JB, Silver MJ. Direct measurement of platelet secretion in whole blood. *Thromb Res* 1979;16:335-45.
22. Bienenstock J, MacQueen G, Sestini P, Marshall JS, Stead RH, Perdue MH. Mast cell/nerve interactions in vitro and in vivo. *Am Rev Respir Dis* 1991;143:S55-8.