Mast Cells from Human Respiratory Tissue and Their in vitro Reactivity

Abstract. *Human respiratory mast cells, which were obtained coincidentally with diagnostic bronchial brush biopsy, were maintained under conditions for short-term tissue culture. Most mast cells were viable and degranulated on exposure to antibody to immunoglobulin E or to the mast cell degranulating agent compound 48-80. The degranulation of human mast cells is characteristically an intracellular process with no extracellular extrusion of granules.*

Reactions of mast cells are considered to be important in hypersensitivity responses of the immediate type. Of particular interest are the mast cells of the human respiratory tract; these cells are involved in reactions mediated by immunoglobulin E (IgE), which may produce clinical disease. Living mast cells have been obtained repeatedly from the respiratory tracts of rhesus monkeys (*1*). These primate mast cells reacted to exposure to immunologic stimuli including antiserum to IgE and ascaris antigen (*1*). They also reacted to exposure to a pharmacologic stimulus, the mast cell degranulating agent compound 48-80 (*1*). Examination of the mast cells by dark-field microscopy proved to be an improved technique for observation of cells and particularly of individual mast cell granules (*2*).

The respiratory mast cells from rhesus monkeys were obtained by bronchial brush biopsy. This technique has come into use as a diagnostic method for evaluation of human pulmonary lesions. Samples obtained during these routine diagnostic bronchial brush biopsies from patients were examined to determine if viable mast cells were present in the tissue samples. Mast cells were found, and a significant number were viable.

This report describes the identification, appearance, and the characteristic degranulation process of living human respiratory mast cells maintained under conditions for short-term tissue culture.

We did bronchial brush biopsies (*3*) on patients for diagnostic evaluation of pulmonary lesions. The respiratory cell preparations used for mast cell studies were obtained coincidentally to these diagnostic studies. At no time were bronchial brush biopsies done solely to provide tissue for mast cell studies.

Small amounts of respiratory tissue were teased on microscope slides and covered immediately with tissue culture medium 199 (TCM). The tissue was covered with cover slides and examined immediately or stored for no more than 3 hours at 4°C. At the time of cell examination the slides were maintained at 37°C (*1*). Respiratory tissue was stained lightly with a solution of 0.0005 percent toluidine blue in TCM. Reagents were added by the flow-through system previously described (*1*). Mast cells were observed continuously either by bright-field microscopy or dark-field microscopy or by both. Rabbit antiserum to IgE that was specific for the Fc piece of myeloma IgE was prepared (*4*). This antiserum bound 0.64 mg of IgE per milliliter, and was diluted 1 : 100 in TCM before use. Compound 48-80 was dissolved in TCM and used at a concentration of 50 μg/ml. Trypan blue was dissolved in TCM to provide a 0.4 percent solution (*6*).

Viability of respiratory cell preparations was determined by the observation of ciliary activity of columnar epithelial cells. Mast cells were identified initially by bright-field microscopy after they were treated with the dilute solution of toluidine blue. The mast cells were polymorphic with granules of uniform size. The nucleus was generally obscured by granules but occasionally could be identified by an unstained area in the cell. When the same cell was examined under dark-field microscopy, the granules were strikingly visible against the dark background. The granules of mast cells, which were stained lightly with toluidine blue, were light orange when they were seen by dark-field microscopy. With the conditions of staining described, the remainder of the cells did not have this orange hue, and mast cells were readily identifiable because of this characteristic. The individual granules of the mast cells were more easily observed and photographed by dark-field microscopy.

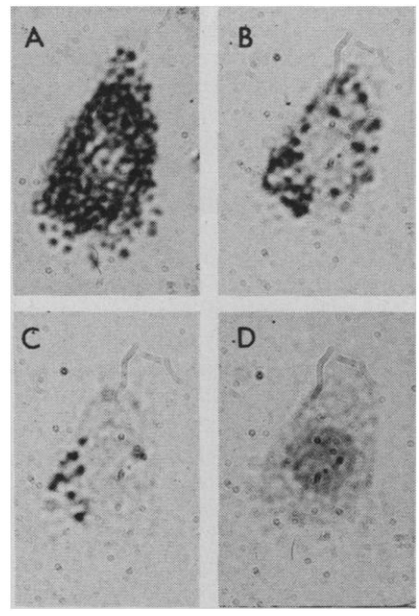

Fig. 1. Degranulation of a human mast cell after addition of antiserum to IgE at a dilution of 1 : 100. A normal respiratory mast cell is seen (A) before treatment, (B) 8 minutes after addition of antiserum, (C) 15 minutes after addition of antiserum, and (D) after addition of trypan blue (bright field microscopy; × 720).

The source of the mast cells obtained by diagnostic bronchial brush biopsies and the reactivities of these cells are shown in Table 1. Each cell was observed individually. In preparations with larger numbers of mast cells in adjacent fields, these mast cells were observed intermittently throughout the experiments. Of 67 cells, 3 reacted spontaneously and 17 did not show any degranulation. The remainder of the cells reacted to exposure to normal rabbit serum (NRS), antiserum to IgE, or compound 48-80.

The degranulation process of human respiratory mast cells appeared the same whether degranulation occurred spontaneously or after exposure to one of these substances. The characteristic response was the onset of degranulation within 2 to 5 minutes of exposure to the degranulating agent. The process of degranulation consisted of the intracellular loss of granules. Each granule disappeared suddenly. Disappearance of granules was random both with respect to time and to intracellular location. At no time did granules appear outside the mast cells. There was no evidence that granules located centrally in the mast cell migrated to the periphery of the cell for discharge through the cell membrane to the extracellular environment. Activity of mast cells increased and pseudopods were formed. These pseudopods filled with mast cell granules as a result of intracellular flow of the granules. The granules in these pseudopods could appear to be extracellular if

Table 1. Reactivity of mast cells. Degranulation is expressed as number of cells that reacted per number of cells exposed. Some cells were exposed sequentially to normal rabbit serum (NRS), rabbit antiserum to immunoglobulin E (IgE), and compound 48-80 until a reaction occurred.

Patient (No.)	Reason for brush biopsy	Mast cells found (No.)	Reactive mast cells (No.)	Degranulation			
				Spontaneous	In response to:		
					NRS (1 : 100)	Antiserum to IgE (1 : 100)	Compound 48-80 (50 μg/ml)
1	Chronic lung disease	14	8	0/14	0/1	0/11	8/13
2	Possible carcinoma of lung	27	23	0/27	4/19	14/27	5/10
3	Possible carcinoma of lung	2	2	1/2		1/1	
4	Possible carcinoma of lung	3	3	2/3	0/1	1/1	
5	Possible carcinoma of lung	4	4	0/4		4/4	
6	Possible carcinoma of lung	16	9	0/16	0/8	1/13	8/15
7	Obstructive lung disease; diagnostic bronchoscopy and brush biopsy	1	1	0/1		1/1	
1 to 7		67	50	3/67	4/29	22/58	21/38

the cell membrane was not as evident as it was in the preparations of living cells. Because our techniques permitted observation of the cell membrane, it was continually apparent that no granules were extruded from the cell. The appearance of a degranulating mast cell following exposure to antiserum to IgE is shown in Fig. 1.

Mast cells that were viable, as shown by their ability to degranulate, excluded trypan blue before degranulation. After degranulation, the mast cells were stained by trypan blue (Fig. 1D). The staining with trypan blue was observed with cells that degranulated after treatment with either compound 48-80 or antiserum to IgE.

Examination of mast cells by dark-field microscopy during the degranulation process facilitated observation of the individual granules. The degranulation of two mast cells, photographed by both bright-field and dark-field microscopy, is shown in Fig. 2.

Some mast cells did not degranulate completely, and the degree of degranulation could be estimated by visual or photographic comparison with the original cell. When a mast cell did degranulate completely, the degranulated cell had a round nucleus and the characteristic polymorphic shape (Figs. 1 and 2). However, the degranulated cell could be identified as a mast cell only because it had been under continuous observation during the degranulation process.

These studies demonstrate that living respiratory mast cells from humans can be obtained when bronchial brush biopsies are done for clinical diagnosis of pulmonary lesions. These mast cells are viable, as evidenced by their degranulation in response to pharmacologic or specific immunologic stimulation. By this criterion, over 70 percent of the cells in our samples were viable.

A few cells degranulated spontaneously, and others degranulated after exposure to NRS. A progressively higher number of cells degranulated after exposure to antiserum to IgE and compound 48-80. Since the latter was the final agent added to cells that did not react to NRS or to antiserum to IgE or to both these substances, we think that a much higher percentage of cells would react to compound 48-80 than is shown here. Further, we suggest that those mast cells that do not react to compound 48-80 at the concentration used (50 μg/ml) are probably not viable.

The mast cells were obtained from a selected patient population that was drawn largely from an older age group. Several subjects had presumptive diagnoses of carcinoma. The results must

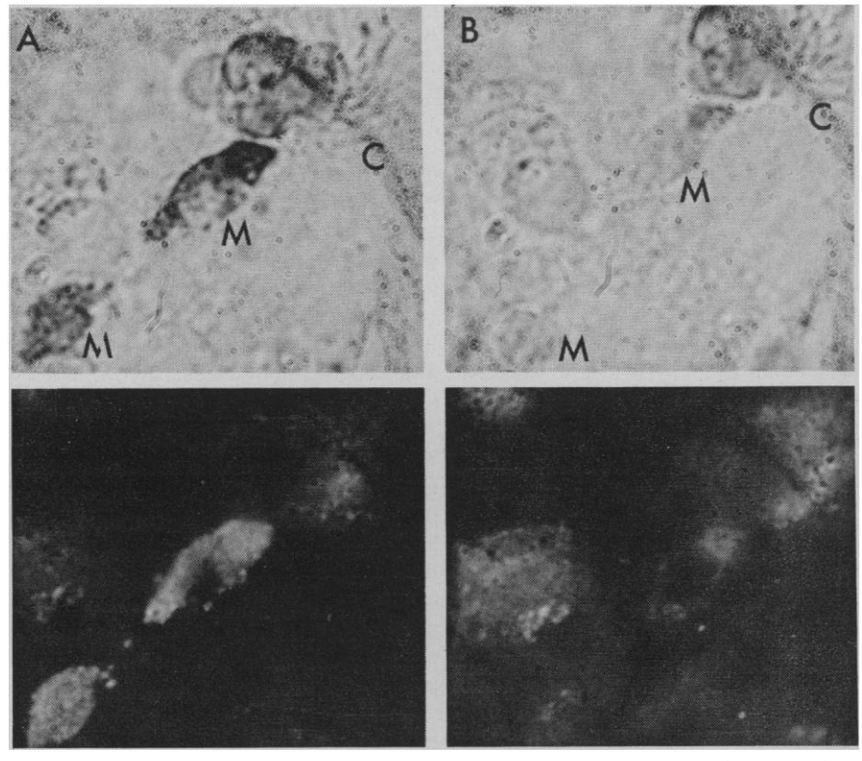

Fig. 2. Degranulation of two mast cells by compound 48–80; photographed by bright-field microscopy (top) and dark-field microscopy (bottom). Abbreviations are: *M*, mast cell; and *C*, ciliated columnar epithelial cell. (A) Mast cells are seen before addition of compound 48-80. (B) After addition of compound 48-80, cells are degranulated (× 680).

be considered in relation to the patient's age; a higher percentage of degranulation after treatment with antiserum to IgE might occur in a population of cells obtained from younger patients.

We describe a means of obtaining living human respiratory mast cells coincidentally and without risk to human patients. The method has two limitations. (i) Only a small number of cells can be obtained and studied at any time. (ii) Cells can be obtained only from patients who are undergoing brush biopsies for other reasons; this limitation will remain unless the diagnostic indications for examination of mast cells are expanded in the future. It is unlikely that samples taken at different times from the same patient will be available.

The characteristics of the degranulation process of these respiratory mast cells is remarkably similar to that described for rhesus cells obtained in the same manner. Parallel studies with the latter system are appropriate, since the rhesus cells can be obtained repeatedly and under controlled conditions from the same animal (*1, 2*).

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7. Supported by PHS training grant AI00057 and the Ernest S. Bazley grant.

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Phosphodiesterase in Dictyostelium discoideum and the Chemotactic Response to Cyclic Adenosine Monophosphate

Abstract. *A phosphodiesterase with a low Michaelis constant for cyclic adenosine monophosphate was found in the membrane fraction of the cellular slime mold. This activity was highest during the aggregation stage. Enzyme with similar properties was also secreted by the cells. Dithiothreitol inhibited both enzymes and potentiated the cellular response to cyclic adenosine monophosphate.*

During the aggregation phase of their development, cellular slime molds are attracted to one another by a chemotactic hormone (acrasin). In *Dictyostelium discoideum*, this hormone is adenosine 3′,5′-monophosphate (cyclic AMP) (*1*). After aggregation the cells undergo multicellular differentiation, and cyclic AMP also affects the rate and possibly the outcome of this process (*2*).

Table 1. Enzyme activity and secretion during early development. Pellet activity is units of enzyme per milligram of protein; a unit is 1 nmole of cyclic AMP hydrolyzed per minute. Enzyme secretion is units secreted per milligram of cellular protein per hour. Protein was determined by the method of Lowry *et al.* (*13*).

Developmental stage	Pellet activity	Enzyme secretion
Amoeba	4.00	2.62
Early aggregation	14.7	1.46
Late aggregation	6.03	0.72

Phosphodiesterase activity—cleavage of cyclic AMP to adenosine 5′-monophosphate—has been reported in filtrates from *D. discoideum* cultures. The enzyme has a broad specificity for cyclic nucleotides, and the Michaelis constant (K_m) for cyclic AMP is high, 1 to 2 m*M* (*3*). In this report we describe a phosphodiesterase that has a much lower K_m (10 to 15 μM) for cyclic AMP (*4*). This sensitivity, together with the pattern of enzyme activity and secretion during development, suggests that this form of phosphodiesterase is involved in modulating the response of the cells to cyclic nucleotides. This interpretation is supported by our observation that inhibition of the enzyme enhances the chemotactic response to cyclic AMP.

Amoebas of *D. discoideum* were grown in the presence of *Escherichia coli* on nutrient agar sheets. The amoebas were washed free of bacteria (*5*) and plated on 2 percent agar that contained 0.01*M* phosphate buffer at *p*H 6.5, 0.001*M* ethylenediaminetetraacetic acid (EDTA), and streptomycin sulfate (500 μg/ml). At appropriate stages of development, the cells were removed by rinsing the agar with salt solution (*6*) buffered with 0.01*M* tris(hydroxymethyl)aminomethane (tris), *p*H 7.5. The suspension was centrifuged at 1500*g* for 5 minutes at 0°C, and a portion of the centrifuged cells was resuspended in an equal volume of tris buffer and frozen.

The remaining cells were suspended in 10 times their volume of salt solution that was buffered with 0.01*M* phosphate at *p*H 6.5, and the suspension was swirled on a rotating platform for 1 hour at 24°C. The cells were removed by centrifugation. To the supernatant, solid ammonium sulfate was added to 90 percent saturation. The material thus precipitated was redissolved in 0.01*M* tris, *p*H 7.5, and dialyzed overnight against the same buffer. This preparation was designated the secreted fraction.

The frozen cell suspension was thawed and centrifuged at 27,000*g* for 15 minutes, and the pellet was resuspended in tris and centrifuged again. The washed pellet was resuspended in tris and stored at −80°C; this was called the pellet fraction. The supernatants from the two centrifugations were combined and designated the intracellular fraction.

Enzyme activity was measured in a mixture that contained extract; 0.1*M* tris, *p*H 7.5; alkaline phosphatase (from *E. coli*), 25 μg/ml; adenosine deaminase (from intestinal mucosa), 0.02 μg/ml; glutamic acid dehydrogenase (from beef liver), 0.25 mg/ml; 5.5 m*M* α-ketoglutaric acid; 0.1 m*M* EDTA; 0.25 m*M* nicotinamide adenine dinucle-

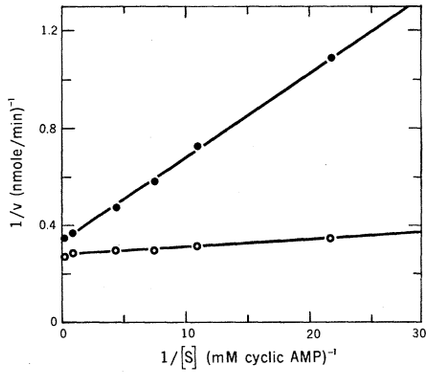

Fig. 1. Kinetic behavior of secreted enzyme in the presence (solid circles) and absence (open circles) or 1 m*M* DTT. Graph is according to the method of Lineweaver and Burk (*14*).