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RESPIRATORY MAST CELLS AND BASOPHILOID CELLS

I. EVIDENCE THAT THEY ARE SECRETED INTO THE BRONCHIAL LUMEN, MORPHOLOGY, DEGRANULATION AND HISTAMINE RELEASE*

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SUMMARY

Studies of secretions obtained from the bronchi of dogs and monkeys by bronchial lavage demonstrated the presence of two types of cells with granules having the staining characteristics of mast cells or basophils. Larger pleomorphic granular cells appear to be mast cells. Smaller, round cells resemble basophils but are termed 'basophiloid' because their identity is not established. These two cell types were studied extensively in bronchial lavage preparations obtained from ragweed-allergic and normal dogs. The results demonstrate that there is a significant increase in the per cent of histamine release from these bronchial lavage cells after exposure to ragweed antigen when the cells were obtained from the bronchi of ragweed-sensitive dogs. This histamine release correlated with direct observation of the percentage of degranulation of the mast and basophiloid cells. These results show that viable, antigen-reactive cells are secreted into the lumen of the bronchi. They are significant as a source of cells for studies of IgE-mediated reactions and as cells which may be important in IgE-mediated respiratory responses.

INTRODUCTION

Studies of tissue obtained by brush biopsy from the bronchi of Rhesus monkeys and humans demonstrated the presence of viable mast cells (Patterson & Suszko, 1971). These mast cells could be maintained for periods of time in short term tissue culture preparations and were reactive to antigenic stimulus or to anti-IgE (Patterson *et al.*, 1972). This immunological reactivity was assessed by observing directly the morphologic changes which appeared as progressive intracellular degranulation in stimulated cells (Patterson & Suszko, 1971; Patterson *et al.*, 1972).

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In the course of experiments evaluating the type of cells obtained from bronchial lavage of dogs and monkeys, we observed two types of granular cells. The granules of these cells had the staining characteristics of mast cell and basophil granules (Burton, 1963). One type of granular cell was a large pleomorphic cell which appeared characteristic of the mast cells obtained from Rhesus monkey and human bronchi (Patterson & Suszko, 1971; Patterson *et al.*, 1972). Smaller, round cells resembling basophils were also present. Because their identity is not established, the term basophiloid is used to designate these cells. The current studies were done to determine if these respiratory mast cells and basophiloid cells are viable, if they are reactive to immunological stimuli as evidenced by morphological changes and if the mediator release from these cells occurs as evidenced by antigen-induced histamine release.

In order to study the above questions, dogs were selected as the model system since sufficient numbers of bronchial cells from these animals could be obtained. Two groups of dogs were available for study. These included dogs allergic to ragweed antigen (RWA) and normal, non-allergic dogs. The allergic dogs have been the subjects of previous studies which have characterized their clinical state (Patterson, 1969), the nature of their immediate-type respiratory response to RWA (Miyamoto *et al.*, 1968), the serum reaginic antibody against RWA (Patterson, Pruzansky & Chang, 1963) and provided evidence that the canine reaginic antibody is of the IgE class (Patterson, Roberts & Pruzansky, 1969). All dogs allergic to ragweed used for this study had typical canine ragweed pollenosis, immediate-type skin reactivity to RWA, immediate-type respiratory responsiveness to controlled laboratory exposure to aerosolized RWA and circulating reaginic antibody as demonstrated by the canine analogue of the human long-latency Prausnitz-Küstner reaction (Patterson, 1969).

MATERIALS AND METHODS

Animals

Four dogs with spontaneous ragweed allergy (sensitivity induced by natural environmental exposure to antigen) and four normal dogs were used in these studies. The characteristics of these animals are shown in Table 1. Young adult female Rhesus monkeys were used for study of monkey cells as previously described (Patterson & Suszko, 1971).

Bronchial lavage

Dogs were anaesthetized with sodium pentobarbital. Ninety millilitres of Lactated Ringer's (Hartmann's solution) was introduced in 20-ml aliquots into the tracheobronchial tree through a bronchoscope. The Lactated Ringer's was aspirated. The bronchial solution was centrifuged at $350 \times g$ and aliquots of cells were suspended in tissue culture medium (TCM) which was Medium 199 (Grand Island Biological Co., Grand Island, New York) for morphological studies or Tris buffer, pH 7·6, molarity 0·025 for histamine release studies. Bronchial lavage of Rhesus monkeys was identical except that aliquots of 10 ml were used.

Cell preparations

Total cell counts were done using the standard technique for counting leucocytes using a haemocytometer (Hepler, 1965). Cell differential counts were done by preparation of smears stained with Methylene Blue and Eosin (Burton, 1963). Five hundred cells were

counted and classified as epithelial cells, macrophages, lymphocytes, neutrophilic or eosinophilic leucocytes, mast cells or basophiloid cells. Cells which could not be identified were listed as unclassified cells.

Mast cell and basophiloid cell degranulation

Techniques used were those previously described for studies of respiratory mast cells (Patterson & Suszko, 1971; Patterson *et al.*, 1972). Briefly, cells suspended in TCM were lightly stained with 0·0005% Toluidine Blue solution. Cell suspensions were maintained at 37°C during microscopic observation. The preparations were examined by both light field and dark field microscopy. Mast cells were identified by their characteristic appearance (Patterson & Suszko, 1971; Patterson *et al.*, 1972).

TABLE 1. Characteristics of ragweed-sensitive and normal dogs

	Ragweed-sensitive				Normal
Animal number	1	2	3	4	5–8
History of seasonal ragweed symptoms	+	+	+	+	Not available: pound dogs
Cutaneous titre to ragweed antigen *	10^4	10^6	10^5	10^5	None
Onset of symptoms after aerosol challenge with ragweed (minutes) †	6	1·5	—	2·6	None
48-hour PCA titre ‡	4	64	64	16	None

* Reciprocal of highest serial 10-fold dilution of a 1:100 RWA giving an immediate-type skin test (Patterson, 1969).

† Time of onset of dyspnoea after aerosol exposure to solution of ragweed extract containing 10,000 Protein Nitrogen Units per ml.

‡ Reciprocal of highest serial 2-fold dilution of serum giving positive passive transfer to homologous skin (4).

Histamine release studies

These studies used methods established for antigen-induced histamine release from leucocytes of human subjects with pollen sensitivity (Pruzansky and Patterson, 1966). RWA was a 1:100 weight/volume (5000 Protein Nitrogen Units/ml) extract of Ambrosia elatior (Hollister–Stier Laboratories). RWA was dialysed against phosphate-buffered saline at pH 7·35 prior to use.

RESULTS

Identification of cells and tabulation of cells

Differential counts of cells obtained from bronchial lavage are shown in Table 2. The greatest percentage of cells in the bronchial wash solutions were either cells of the macrophage type or ciliated columnar epithelial cells. Neutrophilic leucocytes constituted no more than 4·4% of cells in any preparation studied and the average was 1·3% of cells in the

nineteen studies. Two types of cells contained granules staining metachromatically. One type was a large, pleomorphic cell with the characteristic appearance of a mast cell (Fig. 1a). The second cell with metachromatic granules in shown in Fig. 1b. This cell is approximately 12 μm in size, mononuclear, round or slightly oval in shape. On the basis of these characteristics, the cell resembles a basophil more than a mast cell. The granules of the smaller cells appear larger than those of a basophil or mast cell and fill the cell more than the granules of a peripheral blood basophil (Fig. 1c). Because of the similarities and the differences in appearance of the smaller bronchial metachromatic granular cells and basophils they are designated as basophiloid cells until their identity is established. Both mast cells and

TABLE 2. Differential counts of cells obtained from canine and Rhesus monkey bronchi

Animal number	Number of cells/ml (millions)	Total number cells (millions)	Differential count (%)							
			Mac.	Epith.	Lym.	Eos.	Neut.	Unid.	B.	M.
Rhesus monkey										
1	0·93	18·6	80·3	11·4	5·4	0·6	0	2·0	0	0·3
2	0·84	21·0	67·0	18·6	3·0	2·0	0	9·4	0·2	0
3	0·87	19·8	68·8	18·0	10·6	1·2	0·8	0·4	0·2	0
Ragweed-sensitive dogs										
1	0·48	13·4	37·7	18·1	6·0	44·2	0	2·7	0·5	0·8
	0·96	26·0	28·8	13·9	17·0	28·5	0·7	10·0	0·6	0·5
2	7·8	101·4	39·6	17·0	25·8	9·0	3·2	5·0	0·4	0
	2·6	46·8	40·4	28·5	26·0	0·6	0·7	3·5	0·2	0·1
3	2·4	48·0	27·3	50·3	15·0	3·4	0·9	2·9	0·1	0·1
	0·87	52·2	47·0	15·2	8·0	16·2	2·8	10·6	0	0·2
4	0·74	29·6	45·0	23·0	21·0	3·7	2·7	4·0	0·3	0·3
	0·52	28·08	41·0	24·0	20·7	2·1	1·5	10·0	0·5	0·2
Normal control dogs										
1	0·96	24·0	32·5	45·0	15·0	3·4	1·9	1·8	0·2	0·2
	0·64	19·2	40·9	38·5	7·8	7·1	1·2	4·2	0·2	0·1
2	0·43	15·02	30·0	34·2	22·1	7·6	0·7	4·5	0·4	0·5
	1·37	34·25	40·0	55·3	1·7	2·3	0	0	0·33	0·33
3	0·45	20·25	60·0	32·4	3·4	0·6	1·4	2·0	0·2	0
	0·86	21·50	32·2	57·2	1·8	1·2	4·4	3·0	0·2	0
4	0·92	30·80	48·0	6·0	21·0	18·0	2·0	5·0	0	0
	0·98	56·84	19·8	58·3	8·7	8·7	0·7	2·6	0·6	0·6

Mac. = macrophage; epith. = epithelial cell; lym. = lymphoid cell; eos. = eosinophil; neut. = neutrophil; unid. = unidentified cell; B = basophil-like cell; m = mast cell.

basophiloid cells were identified in Rhesus monkey and canine bronchial lavage preparations (Table 2). The incidence of the cells is approximately the same although minor variations were apparent in individual experiments. In all but one of nineteen monkey or canine bronchial cell preparations either mast cells or basophiloid cells were detected.

Cell viability

The exclusion of Trypan Blue (0·4% solution) was used as one test of cell viability. In five experiments, at least 100 cells of the basophiloid and mast cell types were examined.

Over 90% of the basophiloid cells excluded Trypan Blue in all experiments. 30–40% of the mast cells excluded Trypan Blue in these experiments, indicating a lower viability or a higher permeability of the cell membrane of the mast cells in these studies.

Histamine release from bronchial cells

The histamine content of respiratory and blood cells of several species is shown in Table 3. Preliminary experiments using bronchial cells of ragweed-sensitive dogs indicated that histamine release occurred from these cells maximally with concentrations of 5000 Protein Nitrogen Units of RWA per ml. This concentration of RWA was 50% of that used for

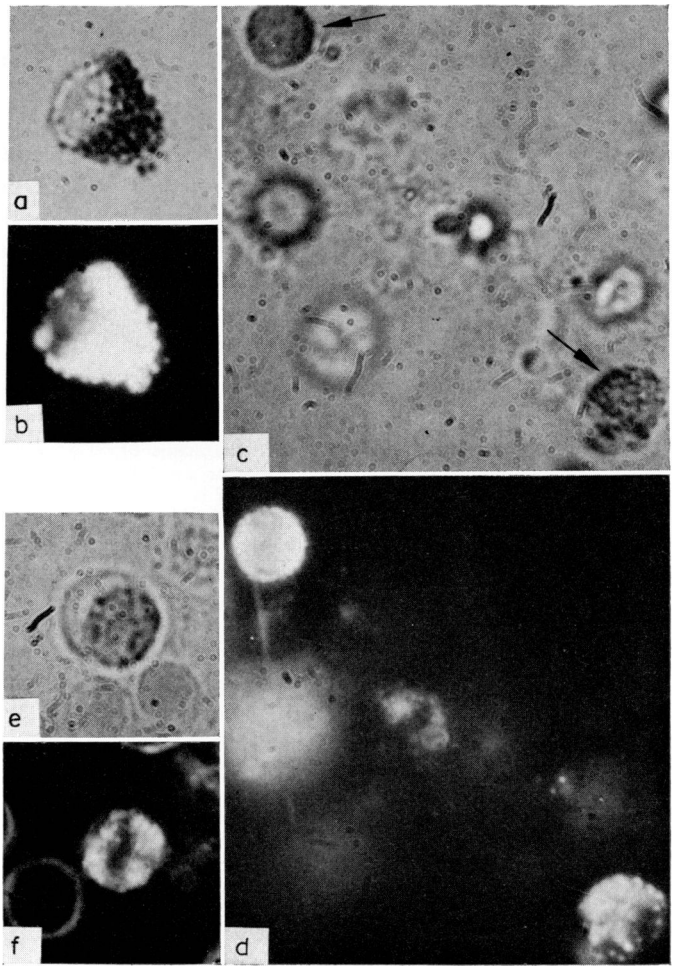

FIG. 1. Live cells stained lightly by Toluidine Blue. The same cells are photographed by both bright field and dark field microscopy. (a), (c) and (e) bright field; (b), (d) and (f) dark field. (a) and (b) canine bronchial mast cells, (c) and (d) canine bronchial basophiloid cells, (e) and (f) canine peripheral blood basophil. (Magnification ×800.)

TABLE 3. Histamine content of canine and primate blood and respiratory cells

		Dog		Rhesus monkey		Human	
		Number of cells	μg H	Number of cells	μg H	Number of cells	μg H
Peripheral blood	1	10^7	0·0544	ND *	ND	10^7	0·04
	2	10^7	0·0476	ND	ND	10^7	0·11
Respiratory cells	1	10^7	0·0324	10^7	0·0305	ND	ND
	2	10^7	0·0450	10^7	0·0205	ND	ND

* ND = not done.

aerosol challenge of RWA-sensitive dogs and did not produce cutaneous reactivity or respiratory responses in normal dogs.

The addition of RWA to bronchial cells of RWA-sensitive dogs resulted in a significantly higher percentage of histamine release than occurred from cells obtained from normal control dogs (Fig. 2a). The histamine release from bronchial cells of ragweed-sensitive dogs was equal to or greater than that which occurred from peripheral blood leucocytes of the same animals (Fig. 2a). The percentage histamine release from bronchial cells and peripheral blood leucocytes of ragweed and control dogs after exposure to Tris buffer is shown in Fig. 2b. The results demonstrate that no significant difference exists between the reactivity of the cells to Tris buffer whether or not these cells were obtained from RWA-sensitive animals or control subjects.

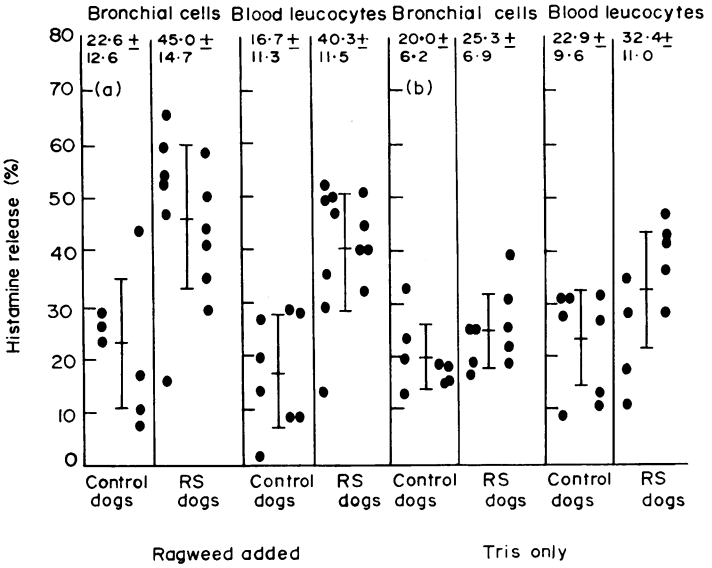

FIG. 2. Percentage histamine release from bronchial cells and peripheral blood leucocytes of ragweed-sensitive (RS) and control dogs. (a) Following exposure to RW antigen; (b) following exposure to Tris buffer.

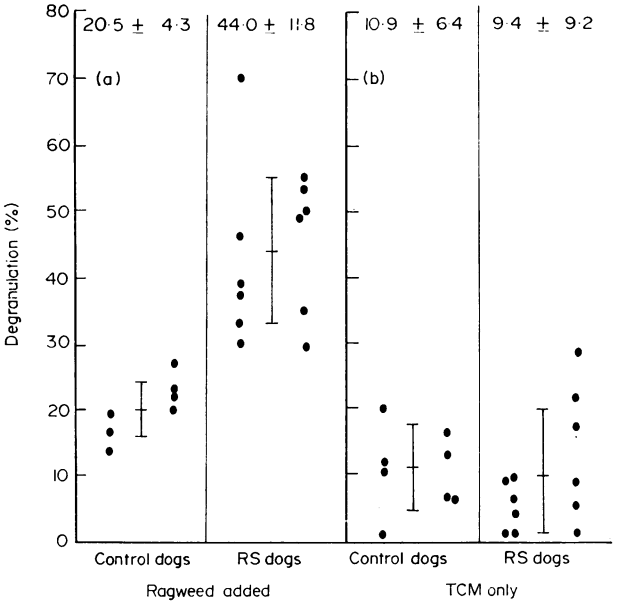

FIG. 3. Percentage degranulation of bronchial basophiloid and mast cells from ragweed-sensitive (RS) and control dogs. (a) Following exposure to RWA; (b) following exposure to tissue culture medium (TCM).

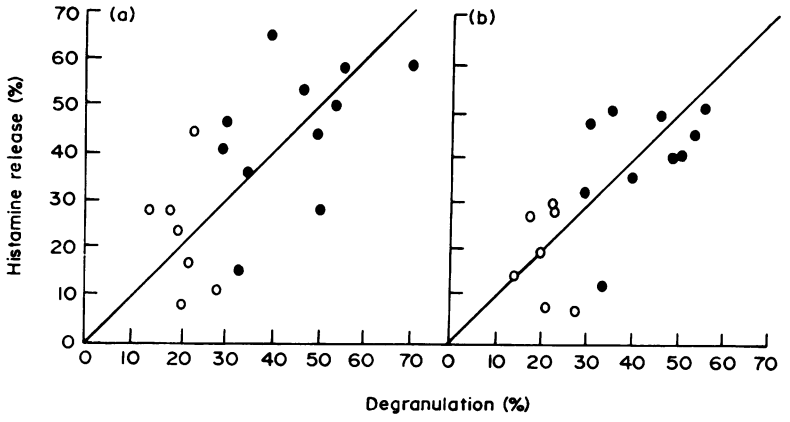

FIG. 4. Correlation of histamine release and cell degranulation. (●) Ragweed-sensitive dogs; (○) control dogs. (a) Percentage histamine release from bronchial cells correlated with percentage degranulation of bronchial mast cells and basophiloid cells after exposure to ragweed (RW). $r = 0{\cdot}8166$; $t = 9{\cdot}807 > t_{16}(0{\cdot}001) = 4{\cdot}015$. (b) Percentage histamine release from peripheral blood leucocytes correlated with percentage degranulation of bronchial mast cells and basophiloid cells after exposure to RW. $r = 0{\cdot}7351$; $t = 5{\cdot}441 > t_{16}(0{\cdot}001) = 4{\cdot}015$.

Morphological changes in mast cells and basophiloid cells following exposure to RWA

Cells lightly stained with Toluidine Blue were observed directly by dark field microscopy before, during and after the addition of RWA. Certain cells underwent morphological changes which consisted of the disappearance of the intracellular granules. The percentage degranulation of cells from ragweed-sensitive and normal dogs following exposure to RWA was determined. A significantly higher percentage of mast cells and basophiloid cells from RWA-sensitive dogs underwent degranulation than did bronchial cells from non-RWA-sensitive dogs (Fig. 3a). This pattern of degranulation did not occur when cells of RWA-sensitive and control dogs were exposed to TCM in the absence of RWA (Fig. 3b).

Correlation between respiratory mast cell and basophiloid cell degranulation, respiratory cell histamine release and leucocyte histamine release

The percentage histamine release from respiratory cells of RW-sensitive dogs and non-allergic dogs is plotted against the percentage degranulation of mast cells and basophiloid

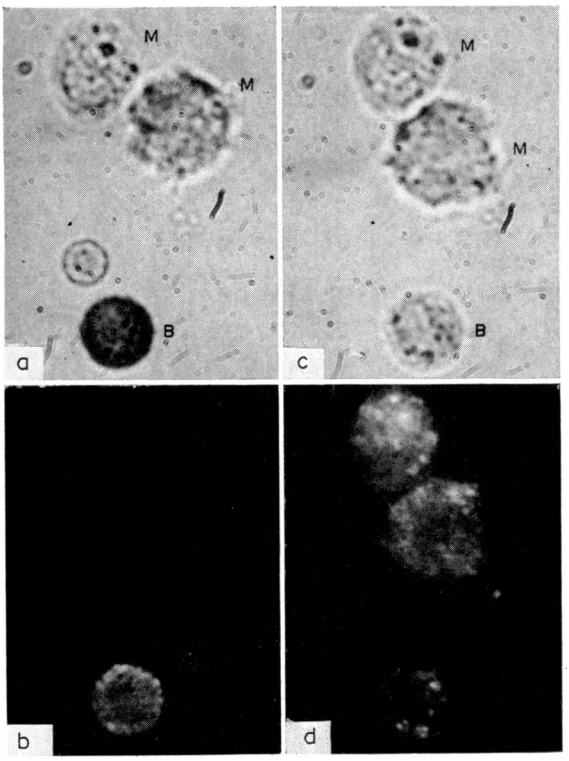

FIG. 5. Degranulation of living bronchial basophiloid cell after exposure to RWA. (a) and (b) cell lightly stained with Toluidine Blue prior to exposure to RWA; (c) and (d) the same cell after degranulation; (a) and (c) bright field microscopy; (b) and (d) dark field microscopy; M = macrophage; B = basophiloid cell. Less photographic exposure was required to show the basophiloid cell by dark field microscopy prior to degranulation. Under these conditions macrophages are hardly visible (b). (Magnification × 800.)

cells from the same animals in Fig. 4a. There is a significant correlation between the percentage histamine release and cell degranulation comparing the RW-sensitive and normal dogs. A similar correlation between histamine release from peripheral blood leucocytes and respiratory basophiloid and mast cell degranulation of the same two groups of animals is shown in Fig. 4b.

Morphological changes in respiratory mast cells and basophiloid cells

The morphological changes of the mast cells and basophiloid cells which had the appearance of intracellular degranulation were identical with those previously described for the degranulation of respiratory mast cells obtained by bronchial brush biopsy (Patterson and Suszko, 1971; Patterson *et al.*, 1972). The changes occurred 2–15 min after addition of RW to the cells. The appearance of cells before and after the degranulation process is shown in Fig. 5.

DISCUSSION

These studies have demonstrated that there are free cells of the mast cell and basophil type which appear in the lumen of the bronchial tree. Although some variation in the ratio of mast cells to basophiloid cells appears in the initial results (Table 2), tabulation of percentages of these cells in all preparations suggest that the mast cells and basophiloid cells appear in approximately equal numbers. The identity of the basophiloid cells is not established. Possible identities include: (i) peripheral blood basophils secreted into the bronchial lumen from the peripheral blood; (ii) tissue basophils migrating into the bronchial lumen; (iii) cells of the mast cell type, either in an early stage (pro mast cell) or a late, degenerating mast cell. The markedly higher ratios of these basophiloid cells to neutrophils in the bronchi in comparison to the basophil:neutrophil ratio in peripheral blood indicated that these cells are not merely basophils transferred from peripheral blood. If they are basophils from peripheral blood they are transferred preferentially to the bronchi. That these cells are of the same series but in different developmental stages or that one of these cell types may be a degenerating cell should be considered. For several reasons it appears likely that the mast cells and basophiloid cells are the cells that contain and release histamine specifically *in vitro* in the histamine release experiments described in this report. Studies of human peripheral blood leucocytes have shown that only basophils decrease after addition of antigen (Pruzansky & Patterson, 1970) and that histamine in human peripheral blood leucocytes is localized to basophils (Kunske, Pruzansky & Patterson, 1971). Further, Ishizaka, Tomioka and Ishizaka (1970) have shown that IgE localizes only to basophils of peripheral blood. Although the other bronchial cell types observed (Table 2) have not been excluded as reactive cells because of species and tissue differences, none of the cells of the other types has been shown to either contain histamine or to fix IgE. The correlation of cell degranulation with histamine release provides support for the following interpretation. The most logical conclusion for the studies described here is that canine IgE anti-ragweed is fixed to receptors on the mast and basophiloid cells and that these cells release histamine as a result of addition of RW antigen.

The presence of free, antigen-reactive cells which release histamine in the lumen of the respiratory tract has potential interest in relation to IgE-mediated, immediate-type reactions of the respiratory tract. These cells are obtainable repeatedly from the same animal. It is possible or even probable that these cells participate in immediate-type respiratory reactions

in the canine and other species. If these cells are not involved in production of IgE-mediated respiratory reactions because of insufficient numbers of cells or other factors, they at least provide a source of histamine-releasing cells from the respiratory tract available for study. The preliminary studies of Rhesus monkey bronchial cells have shown that cells similar to the canine bronchial mast cell and basophiloid cells occur in bronchial lavage solutions obtained from Rhesus monkeys. These results with Rhesus monkey bronchial cells indicate that the presence of mast and basophiloid cells in the bronchial lumen is not unique for the canine species.

The potential importance of the mast and basophiloid cells described in these studies is as follows. If sufficient amounts of mediators such as histamine are released into the bronchial lumen from these antigen-reactive IgE-sensitized cells they may be significant participants in the induction of immediate-type respiratory reactions. It is even possible that mediators released from the bronchial lumen mast and basophiloid cells are of importance in explaining such phenomena as the rapid onset of the respiratory response in dogs and monkeys which occurs within minutes of antigen delivery to the airway (Miyamoto *et al.*, 1968). The respiratory response in dogs and monkeys begins at approximately the same time (5 minutes) after challenge with either histamine, specific antigen or anti-IgE. Passage of each of these agents through the bronchial mucosa might be expected to occur in inverse relationship to its molecular weight if similar quantities of each were present. Anti-IgE, which is used in the reverse passive respiratory response of monkeys (Patterson, Talbot & Roberts, 1972), has a molecular weight four times that of ragweed antigen E and over a thousand times that of histamine. That the temporal development of symptoms is the same for each of these agents is consistent with but does not prove the possibility that intraluminal cells produce the mediators which result in respiratory symptoms.

Finally, the action of certain poorly absorbed drugs such as disodium cromoglycate, presumed to inhibit antigen-induced mast cell degranulation (Cox, 1967), might be better explained by their effect on the intralumenal mast and basophiloid cell population with which it would come into close contact following inhalation of antigen.

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