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Research Article

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Living Histamine-Containing Cells from the Bronchial Lumens of Humans

DESCRIPTION AND COMPARISON OF HISTAMINE CONTENT WITH CELLS OF RHESUS MONKEYS

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ABSTRACT Cell populations obtained by bronchial lavage from human subjects were examined for the presence of cells related to the mast cell-basophil series. Such bronchial lumen histamine-containing cells (BLHCC) were identified. The BLHCC stained with toluidine blue may be identified by bright field or dark field microscopy. The BLHCC are alive as evidenced by ability to release histamine (H) after exposure to anti-IgE or calcium ionophore. Although H release from peripheral blood leukocytes by these two agents is potentiated by the presence of D_2O, H release from BLHCC of the same subjects by anti-IgE or calcium ionophore was not potentiated by D_2O. In studies comparing bronchial cell populations of humans and rhesus monkeys with peripheral blood leukocyte populations of the same subjects, the histamine content of the bronchial cell population was much higher in rhesus monkeys. IgE/Alb ratios of respiratory secretions and serum of the same human subjects were of the same order of magnitude in contrast to previous comparisons done on these fluids in rhesus monkeys.

INTRODUCTION

We have reported previously that free cells with morphological characteristics of mast cells and basophils occur in the lumen of bronchi of rhesus monkeys and dogs (1). Further studies demonstrated that these cells are living as evidenced by their ability to release histamine (H)[1] after immunologic stimulation (1) and that this H release mechanism is suppressed by agents which elevate 3',5' cyclic AMP (2). The cells with the appearance of mast cells and basophils from rhesus monkeys have been studied by electron microscopy and their characteristics more clearly defined.[2] The results demonstrated that individual H-containing cells from the bronchial lumens of rhesus monkeys have features of both lung mast cells and peripheral blood basophils but lack certain features of these cells. The bronchial mastocyte may be a third and separate type of cell of this series or an intermediate type of cell differing only in stage of development. In this report the bronchial lumen cells of this type will be referred to as bronchial lumen histamine containing cells (BLHCC). In addition to releasing H, the BLHCC of rhesus monkeys release a slow reacting substance of anaphylaxis after exposure to antigen and anti-IgE (3). Differences observed in release of H and slow reacting substance of anaphylaxis between this cell population and peripheral blood leukocytes (PBL) from the same animal have suggested further that there may be differences between these cells which can be detected by mediator releasing studies, particularly with the effect of D_2O as a differentiating agent (3).

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[1] *Abbreviations used in this paper*: BLHCC, bronchial lumen histamine containing cells; Ca I, calcium ionophore; H, histamine; PBL, peripheral blood leukocytes; RS, respiratory secretions; S, serum.

[2] Ts'ao, C., W. J. Metzger, R. Patterson, and I. M. Suszko. H-containing cells in bronchial lavage fluid. I. Ultrastructural characterization and comparison with three types of tissues of rhesus monkeys. *Int. Arch. Allergy Appl. Immunol.* In press.

Although these BLHCC have been demonstrated in monkeys and dogs, a major question remaining has been whether or not these cells are present in the bronchial lumens of human subjects. If these living cells are present in humans they may have relevance to clinical disease states such as IgE mediated asthma because of their location as the first mediator releasing cell in contact with inhaled antigen in the bronchial tree. The current studies were done to determine whether or not these living BLHCC are present in human subjects and whether they are alive and release H under appropriate stimulation. Finally, a comparison was made between the H content of human BLHCC and those from rhesus monkeys.

METHODS

BLHCC. Cells of this type from the bronchial lumens of human subjects were obtained by lavage during bronchoscopy.

Patient population. The patient population is outlined on Table I and II–IV and consisted of ambulatory male patients in a General Medical Hospital who had elective fiberoptic bronchoscopy for the evaluation of hemoptysis or a pulmonary radiological abnormality.

Protection of human rights. The research project reported here was reviewed and approved by the Human Subjects Research Committee of Northwestern University Medical School.

Bronchoscopy and lavage. The patients were premedicated after a 5–8 h fast with 50–75 mg of meperidine and 0.4–0.6 mg of atropine intramuscularly approximately ½ h before the procedure. Topical anaesthesia of the mouth and pharynx was achieved with Cetacaine spray (benzocaine and tetracaine), with the use of 4–5 ml of 4% Lidocaine solution for the larynx and tracheobronchial tree. Diazepam (3–10 mg) was given intravenously for sedation at the beginning of the procedure. An Olympus fiberoptic bronchoscope model BF 5B2 (Olympus Corporation of America, New Hyde Park, N.Y.) was introduced under direct vision through the oral cavity and pharynx and inserted between the vocal cords into the trachea. The flexible endotracheal tube surrounding the flexible portion of the bronchoscope was slipped into place over the bronchoscope (as previously described) (4).

The pulmonary lavage was done by injecting 15–20 ml of lactated Ringer's solution through the lumen of the bronchoscope into a peripheral bronchus, followed by the immediate suctioning of the fluid out of the lung through the bronchoscope with approximately 20 mm of negative pressure. The

TABLE I

Comparison of H Content of Bronchial Lumen Cells (BLC) and PBL Obtained Simultaneously by Bronchial Lavage or Venipuncture from the same Subjects

	H content*							
	Rhesus cells		Human cells				Ratio of bronchial cell H to PBL H	
	BLC	PBL	Patient no.	Age of patient	BLC	PBL	Rhesus cells	Human cells
				yr				
	0.228	0.021	1	21	0.199	0.200	10.85	0.99
	0.312	0.039	2	59	0.310	0.157	8.00	1.97
	0.363	0.019	3	50‡	0.179	0.064	19.15	2.84
	0.256	0.026	4	46	0.007	0.080	9.80	0.09
	0.124	0.016	5	45	0.109	0.244	7.77	0.45
	0.106	0.004	6	51	0.067	0.031	26.50	2.15
	0.217	0.018	7	61‡	0.232	0.030	12.05	7.70
	0.072	0.007	8	47	0.015	0.132	10.30	0.12
	0.141	0.012	9	52‡	0.033	0.041	11.80	0.80
	0.289	0.041	10	64‡	0.017	0.085	7.08	0.20
	0.224	0.100	11	63	0.029	0.120	2.24	0.24
	0.080	0.020	12	64‡	0.019	0.082	4.00	0.23
	0.480	0.040	13	64‡	0.022	0.061	12.00	0.36
	0.192	0.006	14	50	0.092	0.053	32.00	0.17
	0.072	0.009	15	61‡	0.030	0.150	8.00	0.20
	0.150	0.020	16	51‡	0.015	0.019	7.50	0.79
	0.460	0.080	17	81‡	0.015	0.048	5.75	0.32
	0.214	0.010	18	49‡	0.050	0.052	21.40	0.95
Mean±SD	0.22±0.12	0.03±0.03			0.08±0.09	0.09±0.06	12.01±7.86	1.14±1.87
	← →				← →		← →	
	$P < 0.001$				NS		$P < 0.001$	

* Content per total leukocyte population, μg H/10^6 cells.

‡ Final diagnosis of carcinoma.

TABLE II

*Differential Studies of Bronchial Lumen (BL) Cells from Human Subjects with Dried, Stained Cell Preparations**

Subject		Epithelial cells	Macrophages	Neutrophils	Eosinophils	Lymphocytes	Monocytes	Basophils or mast cells
1	BL	69	25	1	2	2.6	—	0.4
2	BL	24	70	2.3	0	3	—	0.7
3	BL	24	65	9	1.7	—	—	0.3
4	BL	25	35.7	7.5	24	7.5	—	0.3
5	BL	48	38	4	1.6	8	—	0.4

* 1,000 cells were counted.

resultant 5–10 ml of fluid was collected in a 50-ml Erlenmeyer flask with a side arm suction. In certain patients, at the time of bronchial washing a simultaneous heparinized sample of peripheral blood was collected for PBL H release. The material obtained by bronchial wash was centrifuged and the cells counted in a hemocytometer. The cells were divided into two to three portions and used for H release experiments.

PBL and BLHCC histamine release. Heparinized blood was mixed with 5% dextran (mol wt, 150,000) in 0.15 M NaCl to separate leukocytes from erythrocytes. Leukocytes were centrifuged, washed, and suspended in Tris-Ca++ Mg++ buffer, pH 7.6 (Tris-CM) as previously described (5). The BLHCC obtained by bronchial lavage with lactated Ringer's solution was centrifuged, washed with, and resuspended in Tris-CM buffer.

40% of the H_2O was replaced by D_2O in the Tris-CM buffer when the effect of D_2O on specific histamine release was studied. Total cell numbers of both PBL and BLHCC in the cell suspensions were obtained with a hemocytometer.

Animals. Rhesus monkeys used in this study were the group of animals previously described (6). In brief, these animals were healthy young adult animals with or without defined respiratory responses to aerosol challenge with ascaris antigen. This respiratory reactivity has been evaluated over a period of years and each animal characterized in terms of its degree of cutaneous and respiratory responsiveness to antigen challenge (6).

H Measurement. H was extracted from the cell samples by standard methods, previously described (7). The H concentration was determined with an American Instrument Co., Inc. Bowman Spectrophotofluorometer. (Silver Springs, Md.)

Antisera and other pharmacologic agents. Rabbit anti-IgE was prepared against the Fc fragment of a myeloma IgE prepared as previously described (8). In preliminary experiments the use of this agent for H release from human PBL and BLHCC was evaluated and a 1:100 dilution of the anti-IgE found to be an effective concentration for studies of BLHCC and 1:1,000 for PBL H release.

D_2O was obtained from Sigma Chemical Co. St. Louis, Mo. In preliminary experiments with primate PBL cells, 40% D_2O was found to potentiate immunologic H release without nonspecific H release in the absence of the specific immunologic stimulus (5).

Ca++ ionophore A23187 (Ca I). This ionophore was generously supplied by Dr. R. J. Hosely, Eli Lilly and Company. (Indianapolis, Ind.) It was dissolved in absolute alcohol at 37°C by grinding against the side of a test tube with a glass rod. A dilution of the stock solution in pH 7.6 phosphate buffered saline contained 25 μg/ml Ca I. The fluorescence of this solution was determined at 444 nM after excitation at 373 nM with an American Instrument Co., Inc. Bowman spectrophotofluorometer and was used to standard-

TABLE III

*Studies with Living Human Cells Stained with Toluidine Blue for Identification and Trypan Blue Exclusion for Viability**

Bronchial cells							Percent
Experiment number	1	2	3	4	5	Total	cells viable
Number of cells staining with toluidine blue	1	6	2	3	4	16	
Number of cells viable	1	4	1	2	3	11	69
Percent of total cells which are H containing	0.1	0.6	0.2	0.3	0.4		
Peripheral blood cells							Percent
Experiment number	1	2	3	4	5	Total	cells viable
Number of cells staining with toluidine blue	16	11	6	17	2	52	
Number of cells viable	16	10	6	16	2	50	96
Percent of total cells which are H containing	1.6	1.1	0.6	1.7	0.2		

* 1,000 cells were counted.

TABLE IV

Calculated H Content (Picograms/Cell) with Toluidine Blue Stained Cells Examined by Dark Field Microscopy and Determination of H Content in Separate Cell Samples

Experiment number	1	2	3	4	5	6	7	8
Human bronchial cells	0.4	0.7	2.5	0.7	0.20	1.0	1.5	1.0
Human peripheral blood cells	0.5	0.1	0.2	0.25	0.25	0.75	0.4	0.4

ize the ionophore concentration in subsequent experiments. A stock H solution which was reacted with ortho phthaldialdehyde to form a fluorophore was used to compensate for instrument sensitivity changes. A working stock solution of Ca I in absolute alcohol which was saturated at −7°C was found convenient for daily use and contained 1.2 mg/ml. The concentration of Ca I could also be monitored by reading the optical density of the final solution at 280 nM. All cell samples incubated with Ca I were performed at a final concentration of 6 μg/ml and contained 0.5% ethanol. The presence of 0.5% ethanol in separate experiments was found not to induce H release nonspecifically nor did it inhibit release by the Ca I.

Microscopic studies. Freshly prepared cells in Tris-CM buffer were lightly stained with 0.0005% toluidine blue. The metachromatic granules of mast cells, basophils, and BLHCC observed by bright field microscopy also have a characteristic appearance when observed by dark field microscopy (1). By this technique, the metachromatic granules of mast cells and basophils appear a brilliant, yellow-orange, are easily identified, and are suitable for photomicroscopy. This was done with a Polaroid camera (Polaroid Corporation, Cambridge, Mass.) and type 57 Polaroid film. Cell viability was determined by exclusion of trypan blue.

Immunoglobulin and albumin determinations. Immunoglobulin and albumin concentrations were measured in plasma and respiratory secretions. IgG and albumin quantitations were measured by radial immunodiffusion plates (Meloy Laboratories Inc., Springfield, Va.). IgE concentrations were determined by the double antibody radioimmunoassay technique of Gleich et al. (9).

Respiratory secretions were concentrated to 1 ml before

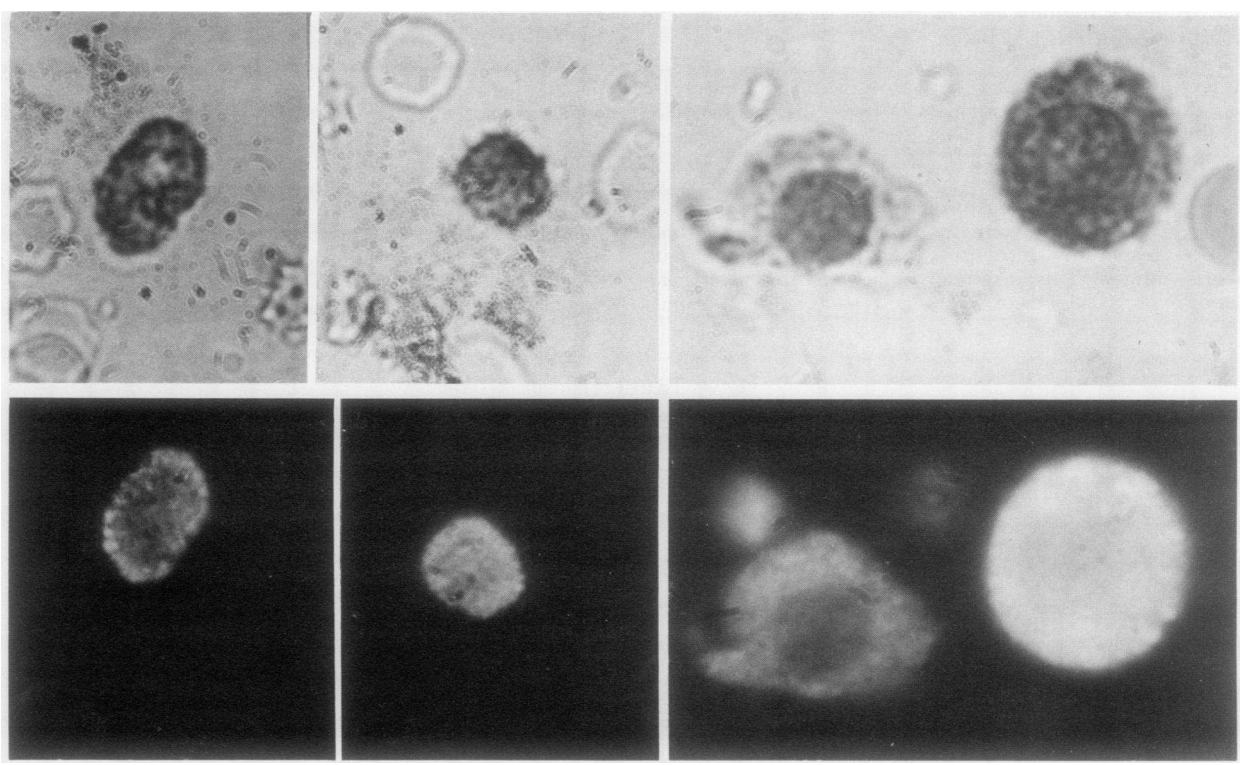

FIGURE 1 Human BLHCC obtained by bronchial lavage. These are living cells stained with toluidine blue. Left, the same cells shown by both bright field (top) and dark field microscopy (bottom) are shown ×800. Right, a higher magnification of a BLHCC (BL) and another cell, probably a macrophage with granules which do not stain with toluidine blue, are seen. Top, bright field microscopy; bottom, dark field microscopy.

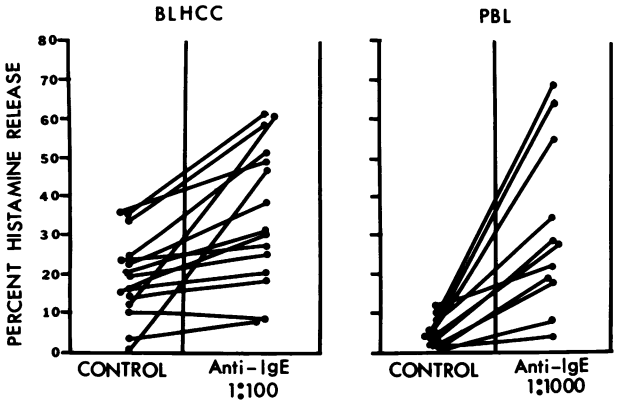

FIGURE 2 H release from human BLHCC (left) and PBL (right) after exposure to anti-IgE.

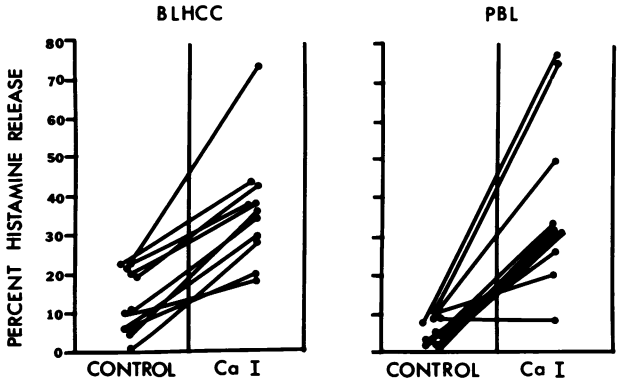

FIGURE 3 H release from human BLHCC (left) and PBL (right) after exposure to Ca I.

protein measurements by a propellent-pressurized ultra filtration cell, model 10PA, with a type XM50 membrane (American Instrument Co., Inc., Lexington, Mass.).

RESULTS

Identification of BLHCC from human bronchi. Cells in washes of human bronchi were stained with toluidine blue and examined by bright field and dark field microscopy. These cells were similar in appearance to those of the BLHCC previously observed in rhesus monkeys (1). The granules stained metachromatically and were identified by bright field microscopy as well as by the yellow orange color with dark field microscopy (Fig. 1). In wet preparations the cells are round or oval and the granules have the same staining characteristics as those previously described for cells obtained from the bronchial lumens of rhesus monkeys (1). The illustration of cells in Fig. 1 is not intended to imply that all of the cells are identical in appearance since pleomorphism and variations in numbers of granules between cells was observed. The details of the morphology of the cells is the subject of a separate study.

H release from human BLHCC and PBL. After identification of the BLHCC from human bronchi, the next experiments were done to demonstrate that these cells were living as demonstrated by their ability to release H after appropriate stimuli. Because the cell donors were not selected or evaluated for the presence of IgE mediated allergic disease, the stimuli for H release were anti-IgE and Ca I. In preliminary experiments 1:100 and 1:1000 anti-IgE were found most effective for BLHCC and PBL H release, respectively. After exposure to anti-IgE the H content of the cell supernate was compared with the H content of control cell supernatant solutions. The results were expressed as percent increase of H due to anti-IgE over H in the control samples. The results (Fig. 2) show that in majority such experiments there was increased release of H after exposure to anti-IgE. The greater percent increase in release of H from PBL is in part due to the lower control levels in PBL as described below but a greater reactivity of PBL is suggested by the markedly increased concentration of H released despite a 10-fold higher dilution of anti-IgE.

Preliminary experiments with Ca I in which normal volunteers were tested showed that a concentration of 6 μg/ml was consistently effective in releasing H from PBL. This concentration of Ca I was used to test the BLHCC and PBL from subjects in this study. The results (Fig. 3) demonstrate that there was a significant release of H from both BLHCC and PBL after exposure of BLHCC or PBL to Ca I. The percent increase in release of H from PBL due to Ca I was higher than that from BLHCC, again in part resulting from the higher degree of spontaneous

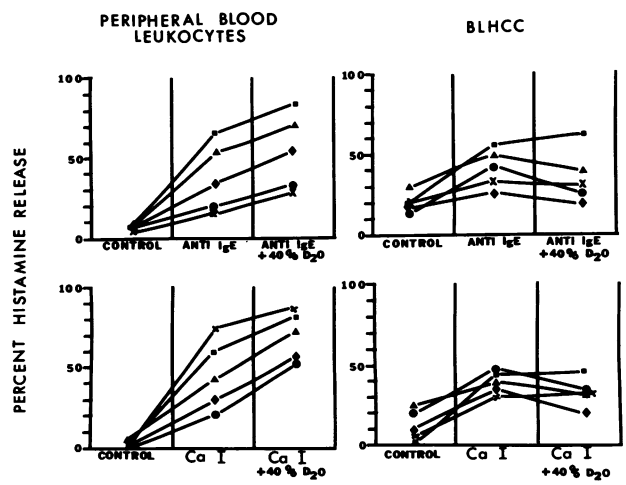

FIGURE 4 H release from human PBL (left) and BLHCC (right) after exposure to either anti-IgE or Ca I. The effect of 40% D_2O on H release from both types of cells is shown.

TABLE V
Comparative Studies of Rhesus BLHCC by Different Staining Techniques and the H Content of BLHCC

Experiment number	Percent of bronchial cells which are H-containing cells determined by dark field microscopy*	μg H per 10^6 cells	pg H per BLHCC	Percent of bronchial cells which are H-containing cells determined by bright field microscopy‡	μg H per 10^6 cells	pg H per BLHCC
1	0.50	0.076	1.50	0.60	0.076	1.39
2	2.20	0.336	1.50	1.40	0.336	2.40
3	2.00	0.317	1.58	2.50	0.317	0.63
4	0.30	0.066	2.20	0.40	0.066	1.60
5	1.10	0.171	1.65	0.60	0.171	2.88
Mean ±SD	1.22±0.76		1.68 ±0.26	1.10±0.77		1.78 ±0.78

* 1,000 cells counted. Cells stained with Toluidine blue.
‡ 1,000 cells counted. Cells stained with Hansel's stain.

release observed with the BLHCC as will be described below.

Effect of D_2O on potentiation of H release from human BLHCC and PBL. The demonstration that D_2O potentiates antigen induced H release from human PBL (10) was followed by the demonstration that D_2O potentiated the immunologic release of H from rhesus PBL but had a significantly lower potentiating effect on immunologic release of H from rhesus BLHCC (3). The effect of D_2O on the release of H from human PBL due to anti-IgE and Ca I was studied to determine whether similarities or differences could be detected. The results are shown in Fig. 4 and demonstrates the following. H release due to anti-IgE and Ca I from PBL occurred and there was a significant potentiation of H due to the presence of 40% D_2O (Fig. 4, left). The differences observed with BLHCC are that there are higher control levels of release of H probably due to the trauma occurring during collection of BLHCC (Fig. 4, right). Although H release due to anti-IgE and Ca I from BLHCC occurs, this is less than that observed with PBL. Finally, no significant potentiation of H release from BLHCC due to D_2O was observed with either anti-IgE or Ca I. (Fig. 4, right).

Histamine content of human and rhesus BLHCC. Previous studies of H release from rhesus BLHCC due to antigen and anti-IgE (3) demonstrated results similar to those described herein with human BLHCC with Ca I and anti-IgE. Further studies were done in this series of experiments to determine whether there were other similarities or differences between human and rhesus BLHCC and PBL. The total H content of bronchial cells and of PBL were compared. The cell counts were counts of total cells not just the H containing cells. The H content of the cell populations expressed as microgram histamine per 10^6 total cells is shown in Table I. These results demonstrate significant differences in H content of these cell populations. The major difference is the high ratio of cellular content of histamine of the bronchial cells of rhesus monkeys compared with rhesus PBL, human bronchial cells, or human PBL. The histamine containing cells in human bronchial fluid and peripheral blood and the viability of these cells are shown in Tables II–IV. Histamine content per peripheral blood basophil and bronchial lumen histamine containing cell was of a similar order of magnitude.

The number of basophils in peripheral blood of rhesus monkeys was extremely difficult to enumerate and there were insufficient cells found to provide significant data. The histamine containing cells from the bronchial lumen were evaluated with dark field and bright field microscopy and histamine content per cell calculated (Table V). The results with either microscopy technique were similar and the histamine content of rhesus bronchial lumen histamine containing cells appeared to be somewhat higher than the analagous human cells.

IgE content of human respiratory secretions (RS). Previous studies of the respiratory secretions of rhesus monkeys (6) demonstrated that a significantly higher ratio of IgE/albumin than IgG/albumin in these respiratory tract secretions in contrast to these ratios in serum. The same study was conducted on 10 unselected samples obtained by bronchial lavage from human subjects. The results are reported in Table VI and Fig. 5. These show that the IgE/Alb ratios of RS are approximately similar in magnitude to those of serum. The IgG/Alb ratio of RS is less than half of that of the IgG/Alb ratio of serum from the same subjects.

Previous studies of IgE/Alb contrasted with the IgG/

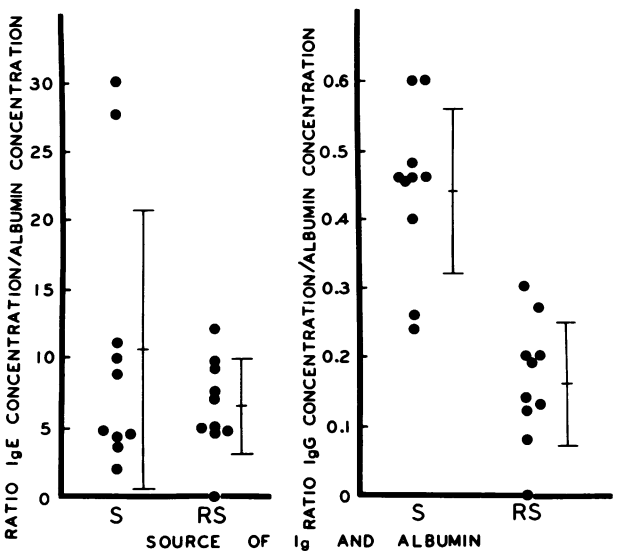

FIGURE 5 Ratios of IgE concentration to albumen concentration in S and RS of humans (left) and rhesus monkeys (right).

Alb ratios in serum and RS of rhesus monkeys provided definitive evidence that IgE is a secretory Ig in that species (6). The results of the current study provide support for the concept of IgE as a respiratory secretory Ig because of the dissociation of relative IgE content in serum (S) and RS of the same subjects and the comparisons of the IgE/Alb and IgG/Alb ratios in S and RS of the human subjects (Table VI and Fig. 5). The final histologic diagnosis of the patients are shown in Table VI. No consistent differences in the Ig content of S or RS of patients with or without malignant neoplasms were observed.

DISCUSSION

The studies reported here demonstrate that H-containing cells of the mast cell-basophil series reside free in the lumen of human bronchi. These cells are alive as evidenced by their ability to release H, which has been shown to be a function of living human basophils (11). The cells have been shown to release H after exposure to two stimuli, anti-IgE, and

TABLE VI
Comparison of IgE, IgG, and Albumen Concentrations in S and RS of Human Subjects

Patient no.	Diagnosis	IgE		IgG		Alb		Ratio IgE/Alb		Ratio IgG/Alb	
		S	RS	S	RS	S	RS	S	RS	S	RS
		μ/ml		mg/ml		mg/ml					
1	Bronchitis	200	5.0	11.6	0.08	48	1.0	4.2	5.0	0.24	0.08
2	Bronchogenic carcinoma	265	6.0	14	0.12	30	0.80	8.8	7.5	0.46	0.14
3	Bronchogenic carcinoma	900	8.5	14	0.24	30	1.20	30	7.0	0.46	0.20
4	Bronchogenic carcinoma	360	6.0	8.6	0.07	33	0.50	11	12	0.26	0.13
5	Bronchogenic carcinoma	135	8.0	18	0.45	30	1.65	4.5	4.8	0.60	0.27
6	Liver scan defect	330	3.0	15.4	0.13	33	0.67	10	4.5	0.46	0.19
7	Carcinoma on mediastinotomy	145	6.0	12	0.08	30.4	0.65	4.8	9.2	0.40	0.12
8	Lung abcess	840	3.0	14.6	0.13	30.4	0.60	27.6	5.0	0.48	0.20
9	Mild endobronchitis	83	5.0	19.2	0.05	42.4	0	2	0	0.45	0
10	Hemoptysis	107	6.5	18	0.21	30	0.67	3.6	9.7	0.60	0.30
	Mean±SD	336.5 ±296.2	5.7 ±1.8	14.54 ±3.29	0.16 ±0.12	33.72 ±6.3	0.77 ±0.44	10.65 ±10.02	6.47 ±3.38	0.44 ±0.12	0.16 ±0.09
								$P < 0.2$ (NS)		$P < 0.001$	

Ca I. The release of H after exposure to anti-IgE is a reverse passive type of immunologic reaction which requires IgE on the surface of the mediator releasing cell. It is likely that a similar release of H would be demonstrated from cells actively sensitized by IgE antibody if the appropriate antigen were used for challenge as has already been demonstrated with BLHCC from bronchial lumens of ragweed sensitive dogs (2) and ascaris sensitive rhesus monkeys (6). These BLHCC constitute a population of cells which are available within the bronchial lumen to react with inhaled antigens and release vasoactive agents in allergic humans and to respond to nonspecific immunologic stimuli such as the complement peptides C3a and C5a (12) and even poorly defined environmental irritant substances which might not be expected to cross the basement membrane of the bronchial mucosa. Such cells might participate significantly in acute IgE mediated smooth muscle reactions of the human airway (particularly in patients with asthma, a disease associated with hyperreactivity to H) or potentiate the inflammatory reactions resulting from inhaled protein against which IgG antibody is directed.

Rat mast cells (13) and human blood basophils (14) have been shown to be stimulated to secrete H by Ca I A23187. This process seems to be similar in many respects to antigen specific IgE-mediated histamine release. The use of ionophore circumvents the immunologic stimulus and provides another modality of inducing secretion of H. In this study the nonimmunologic induction of mediator release from PBL and BLHCC followed the same pattern as the immunologic release with anti-IgE even with respect to D_2O potentiation in PBL and not in BLHCC and in the greater sensitivity of PBL. The high spontaneous reactivity of human bronchial cells (control panels of Fig. 4) produced a lower net release of H than in monkey cells treated in a comparable fashion (6). The human bronchial cells were obtained from patients who had received numerous medications but were conscious. The cell stability of these cells could have been compromised by these medications or trauma during the aspiration of bronchial lavage fluid.

The results of the D_2O experiments are parallel to the results observed with rhesus PBL and BLHCC which demonstrated that the release of H from PBL due to anti-IgE was significantly potentiated by D_2O but that the release of H from BLHCC due to anti-IgE was not (6). This led to further support for the suggestion that activities of different populations of the releasing cells of the mast cell-basophil series may be differentiated by biochemical in addition to morphologic characteristics (15).

Unless the populations of human cells studied in these experiments are not representative of human populations of cells as discussed above, a definitive species difference between human and rhesus H containing cells has been observed. Based on the same total number of cells the H content of rhesus BLHCC is high and that of PBL is low. By contrast the H content of human BLHCC and PBL are of similar magnitude.

A further difference noted between the human results reported here and those observed in rhesus monkeys (6) is that the IgE/Alb ratio of RS in the monkey was approximately 10-fold that of the IgE/Alb ratio in S. The ratios were found to be of similar magnitude in these human studies. The only explanation at this time is that this variation is due to a difference in species. It is apparent that in either species the free IgE of RS provides a source of IgE to which the BLHCC are exposed during the period of time they are in the bronchial lumen of the respiratory tract.

The population of cell donors used for these studies did not consist of an unselected, random population of adult humans because a majority of cell donors are older subjects who had pulmonary lesions of a nature requiring fiberoptic bronchoscopy and cytologic studies to evaluate the possibility of neoplasia. Without the availability of a totally random, unselected population of human subjects of all ages the absolute generality of the results obtained here cannot be determined. The majority of the patients with malignancy did not have far-advanced debilitating disease. Inspection of the results of Tables I and VI do not indicate any apparent definitive differences in the results observed in those patients with an established diagnosis of neoplasm and those patients in whom such a diagnosis was not established. We believe the results reported are sufficiently consistent to be representative of human subjects in general with the possible exception that a younger population may have more reactive cells and possibly more histamine containing cells in the bronchial lumen. The results of these limited experiments are similar to those already reported for rhesus monkeys (6). Because rhesus monkeys and dogs offer a source of BLHCC which are available from animals with characterized IgE mediated sensitivity (6, 16), can be obtained repeatedly from the same animal for serial studies and can be obtained in larger amounts from these anesthetized animals, the major significance of the studies reported here is that the results obtained using the rhesus monkey and canine systems probably have relevance to human events.

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