Localization of Human Blood to Basophils[1] (35874)

RICHARD D. KUNSKE, JACOB J. PRUZANSKY, AND ROY PATTERSON

Allergy-Immunology Section of the Department of Medicine and the Department of Microbiology, Northwestern University Medical School, Chicago, Illinois 60611

Peripheral leukocytes from allergic individuals release histamine after incubation with specific antigens (1, 2). Early studies had attributed significant quantities of histamine to all the granulocytes (3). More recent studies have correlated the histamine of peripheral leukocytes with the basophil fraction (4, 5). Because of the increasing use of the leukocyte model of immediate hypersensitivity it seems important to confirm the cellular localization of histamine. The data of this investigation show that the distribution of histamine between two cellular fractions of leukocytes is correlated with the distribution of basophils between the two fractions and not with eosinophils or neutrophils.

Material and Methods. Buffer. An isotonic balanced buffer containing Tris, 0.025 *M;* NaCl, 0.12 *M*; and KCl, 0.005 *M* with the pH adjusted to 7.6 at 26° was used as standard diluent. Ethylenediaminetetraacetic acid (EDTA) was added to it to make 1% ED TA–Tris buffer. The $CaCl_2$ and $MgCl_2$ were added to standard diluent to final concentrations of 6×10^{-4} and 1×10^{-3} *M*, respectively, to make Tris–CM buffer.

Isopaque–ficoll (Ip-F) mixture. Ten milliliters of 33.9% isopaque (sodium 2,4,6-triiodo-*N*-methyl-3,5-diacetamido benzoate), 10.8 ml of 20% ficoll, and 13.1 ml of water were mixed.

Leukocytes. Thirty milliliters of blood were obtained from 15 healthy subjects and were processed as described by Pruzansky and Patterson (5). Their procedure provided two fractions of leukocytes after differential sedimentation on Ip-F for 40 min. One fraction of cells (A) was obtained from the plasma–Ip-F interface. The other fraction (B) was obtained as a sediment of erythrocytes and leukocytes. The two fractions were separated, washed with 15 ml of 1% EDTA–Tris buffer, and centrifuged at 400*g* for 30 min. Fraction A was resuspended in 6.5 ml of Tris–CM buffer.

Fraction B was further processed in a manner similar to that described by Archer (6) to produce an eosinophil-rich sample. Fraction B was resuspended by repeated aspiration in 15 ml of ice-cold distilled water and divided into two equal aliquots. To each aliquot was added 7.5 ml of 1% EDTA–Tris buffer. These aliquots were then centrifuged at 400*g* for 10 min and the supernatants were poured off. The sedimented cells were combined and resuspended in 6.5 ml of Tris–CM buffer.

Six milliliters of cell suspension A were divided into three 2-ml aliquots, and 3 ml of Tris–CM buffer were added to each. Cell suspension B after lysis was divided into three aliquots and diluted in a similar manner. All 6 samples were then centrifuged at 1000*g* for 10 min and the supernatant was poured into six tubes containing 0.5 ml of 4.4 *N* perchloric acid. To the sediment was added 5.0 ml of distilled water and 0.5 ml of 4.4 *N* perchloric acid, and the samples were then vigorously agitated.

Histamine assay. All 12 samples were analyzed for histamine using the techniques of Shore *et al.* (7) with the modifications of Pruzansky and Patterson (2). The median value of the three aliquots of each fraction was considered most representative of the true histamine content of the corresponding fraction.

Cell counts. Total cell counts for fractions A and B were obtained by counting the cells in the remaining 0.5 ml of cell suspensions A

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TABLE I. Distribution of Leukocytes (%) Within Fractions A and B.

Sample	Basophils	Eosinophils	Neutrophils	Mononuclear cells
		Fraction A		
1	2.6	0.0	4.1	93.9
2	4.0	0.6	17.4	78.0
3	1.6	0.6	26.6	71.2
4	2.2	0.4	17.8	79.6
5	2.4	0.2	8.8	88.6
6	3.0	0.0	2.8	94.2
7	1.8	0.0	2.6	95.6
8	1.4	0.1	4.7	93.8
9	1.6	2.2	4.0	92.2
10	1.4	0.6	8.7	89.3
11	1.3	0.0	7.7	91.0
12	2.4	0.0	3.8	93.8
13	3.7	0.0	3.1	93.2
14	1.7	0.0	1.7	96.6
15	2.3	0.0	1.8	95.9
		Fraction B		
1	1.0	38.6	35.6	24.8
2	1.8	16.7	75.7	5.8
3	2.8	59.0	7.2	31.0
4	1.8	85.4	1.8	11.0
5	0.4	11.9	85.8	1.9
6	1.1	7.4	76.8	14.6
7	0.6	54.9	11.2	33.3
8	1.0	41.1	8.6	49.3
9	3.8	30.4	15.0	50.8
10	3.0	43.0	12.6	41.4
11	1.8	30.7	29.5	38.0
12	3.5	56.2	12.6	27.7
13	1.9	39.2	26.5	32.4
14	1.6	15.5	71.9	11.0
15	2.0	58.3	16.1	23.6

and B using a Spencer hemocytometer. All counts were done in triplicate, and the median value used as most representative of the true count.

Cell distribution. Distribution of cellular components in fractions A and B were obtained by doing differential cell counts on dried preparations stained with May-Grünwald-Giemsa stain. Counts were done on 500–2000 cells/preparation. No attempt was made to differentiate mononuclear cells.

Results. When leukocytes were prepared and processed as described, two cellular fractions were obtained. Table I shows the distribution of cells within each fraction. Fraction A consisted mostly of mononuclear cells (71.2–96.6%) and relatively small numbers of other components. Of the cells in A, 1.3–4.0% were basophils, 1.8–26.6% were neutrophils, and 0.0–2.2% were eosinophils. In eight samples no eosinophils were found in fraction A.

Fraction B was composed of variable combinations of all cellular components. Using the method described, a relatively high concentration of eosinophils was obtained (7.4–85.4%). The percentage of basophils was essentially the same as that found in fraction A (0.4–3.8%). A wide distribution of neutrophils (1.8–85.8%) and mononuclear cells (1.9–50.8%) was obtained. Figure 1 demonstrates the distribution of histamine between fractions A and B compared to the distribution of basophils between fractions A and B. There is a nearly linear relationship

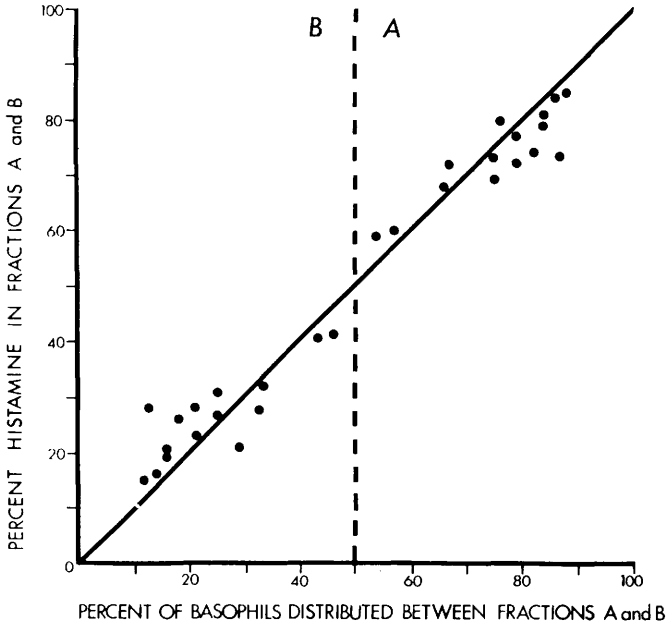

FIG. 1. Percentage distribution of histamine and basophils between fractions A and B prepared from blood of 15 donors.

between these measurements which approximates a 1:1 ratio. As the distribution of basophils was varied between the two fractions, the distribution of histamine varied correspondingly. Fraction A contained 53.7–87.8% of the basophils and 59.3–84.9% of the histamine. Fraction B contained 12.2–46.3% of the basophils and 15.1–40.7% of the histamine.

Figure 2 demonstrates the distribution of histamine between the two fractions as a function of neutrophil distribution between the two fractions. No relationship between the measurements could be determined. Although the distribution of neutrophils varied from 2.2–94.2% in A and 5.8–97.8% in B, the percentage of histamine in the corresponding fractions varied over a much narrower range and the variations were unrelated to neutrophil distribution.

Figure 3 demonstrates the distribution of histamine between the two fractions as a function of eosinophil distribution between the two fractions. No correlation between these measurements could be determined. In the 8 samples with 0.0% eosinophils, 69.1–84.9% of the histamine was present.

TABLE II. Percentage of Histamine Lost into Supernatant After Cells in Fraction B Were Lysed with Distilled Water.

Sample	Histamine (%) in supernatant	Neutrophils (%) lysed
1	7.4	88.5
2	3.3	73.2
3	5.2	95.3
4	6.1	96.7
5	8.2	27.3
Mean	6.0	76.2

In order to determine the amount of histamine that was lost when the cells of the Ip-F sediment used to prepare fraction B were lysed with distilled water, the supernatant after lysis was analyzed for histamine. Table II contains the results of the five samples that were analyzed. Only 3.3–8.2% (mean = 6.0%) of the total histamine was lost into the supernatant even though 27.3–96.7% (mean = 76.2%) of the neutrophils were lysed. Exposure of erythrocytes, neutrophils, eosinophils, and basophils to distilled water resulted in lysis of primarily neutrophils and erythrocytes and produced a fraction relatively enriched in eosinophils and basophils. The

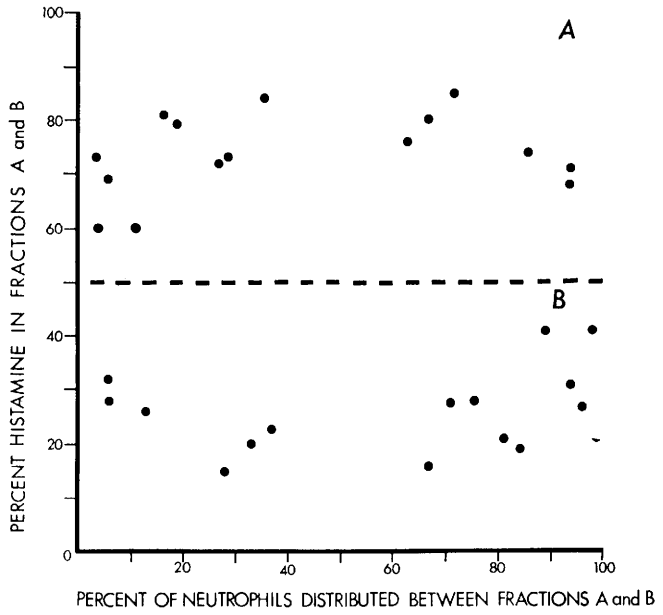

FIG. 2. Percentage distribution of histamine and neutrophils between fractions A and B prepared from blood of 15 donors.

small number of basophils lysed during this process could account for the minimal amount of histamine in the supernatant.

Discussion. Using the methods described, a variable distribution of human leukocyte types between two fractions was obtained. One fraction contained most of the basophils and very few, or no, eosinophils. The other

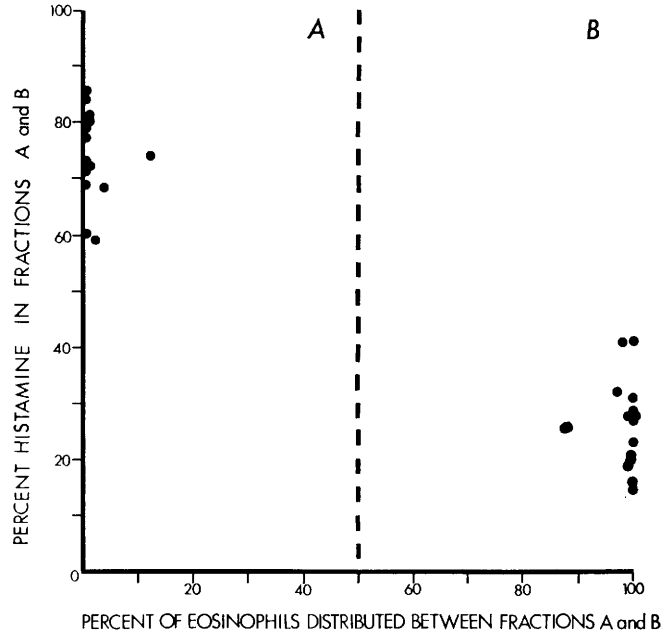

FIG. 3. Percentage distribution of histamine and eosinophils between fractions A and B prepared from blood of 15 donors.

fraction contained almost all of the eosinophils but few basophils. The neutrophils were distributed in a somewhat random fashion between the two fractions. Since there are no confirmed reports of significant quantities of histamine in mononuclear cells, this investigation has concerned itself solely with the granulocytes.

A nearly linear relationship between the distribution of histamine and basophils which approximates a 1:1 ratio was obtained. These data support the hypothesis that histamine released from human peripheral leukocytes comes only from the basophil leukocytes. Similar results had been found by Sampson and Archer (4) and Pruzansky and Patterson (5). Although Graham *et al.* (3) had attributed 49% of the histamine in human leukocytes to the nonbasophil leukocytes, water lysis of up to 96.7% of the neutrophils could account for no more than 8.2% of the histamine. The methods employed in this investigation provided a fraction enriched with eosinophils. There was no correlation between the distribution of histamine and eosinophils between the fractions. Those samples with no eospinophils had 69.1–84.9% of the histamine, while those samples with 100.0% of the eosinophils had only 15.1–30.9% of the histamine.

Ishizaka *et al.* (8) have recently reported localization of IgE only on the human basophil. This investigation confirms the exclusive localization of histamine in the same cells. Therefore, the reaction of cell bound IgE class antibody with an antigen to activate the release of histamine from leukocytes can only involve the basophils.

Summary. Human peripheral leukocytes from 15 subjects were separated into two fractions. One fraction contained most of the basophils and very few eosinophils. The other fraction contained all or almost all of the eosinophils but few basophils. The neutrophils were distributed in a random fashion between the two fractions.

The histamine in each fraction was measured and found to correlate only with the basophil leukocytes. Variations of eosinophil distribution had no effect on histamine levels. Lysis of neutrophils produced only minimal amounts of histamine. These results demonstrate that histamine found in human peripheral leukocytes is localized to the basophil leukocytes.

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