

## Comparison of Free Amino Acid Concentrations in Eosinophils and Neutrophils

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(Received December 6, 1984)

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*Key words: Glutamic acid, Eosinophils, Neutrophils*

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### ABSTRACT

Free amino acid concentrations were compared in eosinophils and neutrophils obtained from patients with eosinophilia. The level of glutamic acid was significantly higher in eosinophils than in neutrophils. This abundance of glutamic acid in eosinophils may be related to the formation of Charcot-Leyden crystals.

Eosinophilia is frequently present in immunologic, parasitic, and neoplastic conditions. However, the chemical mechanisms used by eosinophils to regulate immediate-type hypersensitivity reactions or to destroy antibody-coated, invasive parasites are, with rare exceptions, not yet known. The eosinophil clearly has many features in common with the neutrophil in morphogenesis, structure, and metabolism. It also has several distinctive features including surface antigenicity, certain unique enzymes, and greater longevity of the morphologically mature cell<sup>2)</sup>.

We have already reported the free amino acid concentrations in granulocytes and lymphocytes<sup>3,4)</sup>. The present study compares the free amino acid concentrations in eosinophils and neutrophils obtained from patients with eosinophilia.

### MATERIALS AND METHODS

#### *Preparation of leukocytes*

Heparinized venous blood was obtained from five children, aged seven to eleven years, with eosinophilia due to allergic rhinitis, bronchial asthma, urticaria, and eosinophilic granuloma. The leukocytes were initially obtained by sedimentation with 3% dextran (Dextran 150,000, Sigma Chemicals, St. Louis, Mo.) in phosphate-buffered saline (PBS), each liter of which contained 8.0 g of NaCl, 0.2 g of KCl, 2.9 g of Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, and 0.2 g of KH<sub>2</sub>PO<sub>4</sub>, for

30 min at room temperature. The leukocyte-rich supernatant was collected, and leukocytes were washed twice and suspended in heparinized PBS.

#### *Purification of leukocytes*

Leukocytes were isolated by a modification of the method of Vadas et al<sup>7)</sup>. We set up discontinuous gradients with metrizamide (Nyegaard & Co., A/S, Oslo). A stock solution of 30% metrizamide in Tyrode's solution, each liter of which contained 1.0 g of dextrose, 1.0 g of NaHCO<sub>3</sub>, 0.2 g of KCl, 8.0 g of NaCl, and 0.05 g of Na<sub>2</sub>HPO<sub>4</sub> (anhydrous), was diluted with heparinized Tyrode's solution to various densities (18, 20, 21, 22, 23 and 24% w/v). Gradients were prepared by carefully layering 2 ml volumes of decreasing densities of metrizamide solution into a 15 ml conical centrifuge tube (Falcon Plastics, Oxnard, Calif.), on top of which was placed 2 ml of a leukocyte suspension in PBS. The tube was centrifuged at 1,200 × g for 45 min at room temperature, and cells collected from each interface were counted.

#### *Chromatographic analysis of free amino acids*

For extraction of free amino acids from eosinophils and neutrophils, we used the ultrasonication method for granulocytes, as described in a previous report<sup>3)</sup>. Amino acids were analyzed with a Toyo Soda HLC-805 (Toyo Soda Co., Ltd., Tokyo, Japan) automatic amino acid

analyzer<sup>4</sup>).

Free amino acid concentrations in plasma obtained from patients with eosinophilia were also measured as described previously<sup>4</sup>).

#### Statistical analysis

Statistical analysis was performed by Student's t-test.

### RESULTS

Eosinophils were collected from the top of the gradient steps of concentrations of 22, 23, and 24%, while neutrophils were collected from the top of the gradient steps of concentrations of 20 and 21%. The purity of the eosinophil samples ranged from 83% to 100%, and that of the neutrophil samples from 84% to 94%.

Table 1 shows the free amino acid concentrations in eosinophils and neutrophils. Glutamic acid was significantly ( $p < 0.001$ ) more concentrated in eosinophils than in neutrophils. There was a less marked difference ( $p < 0.05$ ) between eosinophils and neutrophils in the concentrations of six other amino acids.

The free amino acid concentrations in the plas-

ma of our patients were all normal, including glutamic acid (Table 2).

### DISCUSSION

In this study we compared the free amino acid concentrations in eosinophils and in neutrophils obtained from patients with eosinophilia and found that the level of glutamic acid is three times higher in eosinophils. Charcot-Leyden crystals are found in a variety of conditions associated with eosinophilia, and the protein of these crystals has a high content of glutamic acid<sup>5,6</sup>. Since the plasma glutamic acid level is normal in patients with eosinophilia, one may assume that the abundance of glutamic acid in eosinophils is related to the formation of Charcot-Leyden crystals. Although the biochemical significance of glutamic acid in eosinophils is not clear, this amino acid may play an important role in eosinophil functions. Since eosinophils obtained from patients with eosinophilia are reported to be significantly activated<sup>1,8</sup>), one cannot assume that the glutamic acid level is also high in normal eosinophils.

Table 1. Free amino acid concentrations in eosinophils and neutrophils

Amino acid	Concentration (nmol/10 <sup>7</sup> cells) <sup>1</sup>	
	Eosinophil	Neutrophil
Taurine	93.46 ± 19.31	105.25 ± 5.99
Aspartic acid	10.84 ± 2.32 <sup>2</sup>	7.53 ± 0.42
Threonine + glutamine	5.46 ± 1.23 <sup>2</sup>	7.90 ± 1.37
Serine + asparagine	4.33 ± 1.03	4.32 ± 1.11
Glutamic acid	27.87 ± 5.89 <sup>3</sup>	8.77 ± 2.05
Glycine	6.79 ± 1.83	5.24 ± 0.28
Alanine	4.26 ± 0.69 <sup>2</sup>	3.29 ± 0.21
Valine	0.98 ± 0.17 <sup>2</sup>	0.74 ± 0.06
Methionine	0.77 ± 0.26	0.65 ± 0.20
Isoleucine	0.53 ± 0.13	0.46 ± 0.03
Leucine	0.70 ± 0.13	0.72 ± 0.12
Tyrosine	0.93 ± 0.20 <sup>2</sup>	0.67 ± 0.12
Phenylalanine	0.40 ± 0.08	0.45 ± 0.12
Histidine	1.37 ± 0.37	1.40 ± 0.30
Ornithine	0.64 ± 0.31 <sup>2</sup>	1.16 ± 0.33
Lysine	0.92 ± 0.44	0.84 ± 0.21
Arginine	0.98 ± 0.26	0.65 ± 0.28

<sup>1</sup>Data expressed as mean ± SD.

<sup>2</sup>Significantly different from neutrophil;  $p < 0.05$ .

<sup>3</sup>Significantly different from neutrophil;  $p < 0.001$ .

**Table 2.** Free amino acid concentrations in plasma obtained from patients with eosinophilia

Amino acid	Concentration (nmol/ml) <sup>1</sup>	
	Patient	Control <sup>2</sup>
Taurine	44.20 ± 11.08	38.61 ± 4.24
Aspartic acid	5.28 ± 0.87	5.84 ± 0.97
Threonine + glutamine	756.44 ± 87.89	736.99 ± 165.14
Serine + asparagine	172.00 ± 20.58	182.50 ± 36.05
Glutamic acid	70.91 ± 11.60	72.20 ± 12.83
Glycine	281.64 ± 40.44	282.04 ± 67.01
Alanine	399.22 ± 65.61	386.95 ± 86.52
Valine	254.33 ± 66.38	252.68 ± 62.52
Methionine	31.28 ± 13.12	29.32 ± 12.22
Isoleucine	65.35 ± 11.51	74.60 ± 27.35
Leucine	119.54 ± 16.29	137.15 ± 38.64
Tyrosine	66.77 ± 14.68	84.75 ± 23.54
Phenylalanine	66.44 ± 11.12	71.05 ± 14.80
Histidine	93.26 ± 13.59	81.60 ± 11.53
Ornithine	70.60 ± 6.86	67.69 ± 17.21
Lysine	160.76 ± 28.41	188.01 ± 45.33
Tryptophane	66.35 ± 13.85	63.10 ± 14.83
Arginine	66.45 ± 5.09	78.13 ± 18.53

<sup>1</sup>Data expressed as mean ± SD.

<sup>2</sup>Age-matched control (n = 10).

#### ACKNOWLEDGEMENTS

We are grateful to Dr. Alice S. Cary for assistance in preparing this manuscript.

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