
IMMUNOLOGY AND MICROBIOLOGY

Comparative Study of Eosinophils in the Blood and Induced Sputum during Bronchial Asthma

E. A. Gereng, I. V. Sukhodolo*, R. I. Pleshko*,
L. M. Ogorodova**, O. S. Kobyakova**,
Yu. A. Petrovskaya**, and Yu. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 1, pp. 59-61, January, 2004
Original article submitted November 26, 2002

Eosinophilia characterized by accumulation of low-density eosinophils and high functional activity of normal-density eosinophils was detected in the blood from patients with bronchial asthma. Low-density eosinophils are characterized by low content of granules. In patients with bronchial asthma morphological and functional characteristics of bronchial eosinophils were similar to those of blood low-density eosinophils.

Key Words: *eosinophils; cationic proteins; induced sputum*

Bronchial asthma (BA) is a serious medical and social problem. Epidemiological studies showed that 5-10% people in various countries suffer from BA [4]. Clinical and experimental studies performed in the past decade revised the notion of BA. It was hypothesized that eosinophils play a role in the pathogenesis of BA [1,4,6]. These cells play an effector role and synthesize and release granular proteins with high cytotoxic activity. Eosinophilic mediators were extensively studied during the search for new inflammatory biomarkers of BA [1,5,6]. Eosinophil count increases in the blood, induced sputum (IS), and lavage fluid from patients with BA [1]. It remains unclear whether the degree of activation is similar for circulating eosinophils and eosinophils localized in the respiratory tract. Here we compared functional activity of eosinophils in the blood and IS from patients with BA.

MATERIALS AND METHODS

We examined 10 patients (7 women and 3 men, 16-53 years) with exacerbation of moderate atopic BA ad-

mitted to the Department of Allergology (Tomsk Regional Clinical Hospital). The diagnosis of atopic BA was confirmed by anamnesis, positive skin tests with aeroallergens, typical clinical symptoms of the disease, and reversible bronchoobstruction in the test with broncholytics (more than 15%). The control group included 10 healthy volunteers (students of the Military Medical University).

Eosinophilic leukocytes were separated on a Percoll density gradient (1.070, 1.081, 1.095, and 1.105 g/cm³, JCN). The cells isolated on gradients 1.070 and 1.081 g/cm³ were attributed to low-density eosinophils (LDE). The population of normal-density cells included eosinophils isolated on gradients 1.095 and 1.105 g/cm³. We calculated the relative number of low- and normal-density cells isolated on each gradient. The content of intracellular cationic proteins reflecting functional activity of cells was measured [5,6].

IS was obtained after inhalation of a hypertonic solution in increasing concentrations (3, 4, and 5%) [1]. Cytologic preparations were examined to evaluate the absolute and relative number of eosinophils. For morphometric study images of eosinophils from the blood and IS were made using a digital camera and stored in the computer. The area of the cell, nucleus,

Central Research Laboratory; *Department of Morphology; **Department of Faculty Pediatrics, Siberian State Medical University, Tomsk

and cytoplasm was estimated using Adobe PhotoShop 5.0 software. The nucleus/cytoplasm ratio was calculated.

The results were analyzed by Mann-Whitney test.

RESULTS

The count of blood eosinophils in patients with BA markedly surpassed the control ($0.69 \pm 0.05 \times 10^6$ and $0.22 \pm 0.09 \times 10^6$ cells/ml, respectively, $p < 0.05$). In healthy donors more than 65% eosinophils were isolated on Percoll density gradients 1.095 and 1.105 g/cm³ (normal-density eosinophils). LDE were rarely found (Fig. 1).

The number of blood eosinophils isolated on gradients 1.070 and 1.081 g/cm³ increased in patients with atopic BA (19.10 ± 2.41 and $13.63 \pm 2.85\%$, respectively). In these patients the count of normal-density eosinophils was lower than in healthy donors ($p < 0.05$, Fig. 1).

Morphometry of eosinophils showed that normal-density cells in patients with BA are larger than in controls (6655 ± 256 and 5183 ± 500 pixels, respectively). It was associated with an increase in the area of the cytoplasm (4277 ± 275 vs. 2161 ± 95 pixels in the control, respectively). By contrast, the area of the nucleus in eosinophils of this population decreased. The observed changes were accompanied by a decrease in the nucleus/cytoplasm ratio from 1.96 to 0.50. Functional studies showed that in the majority of cells the number of cationic protein granules in the cytoplasm increased (Table 1).

LDE differed from normal-density cells in low area of the cell (3166 ± 402 and 5183 ± 500 pixels, res-

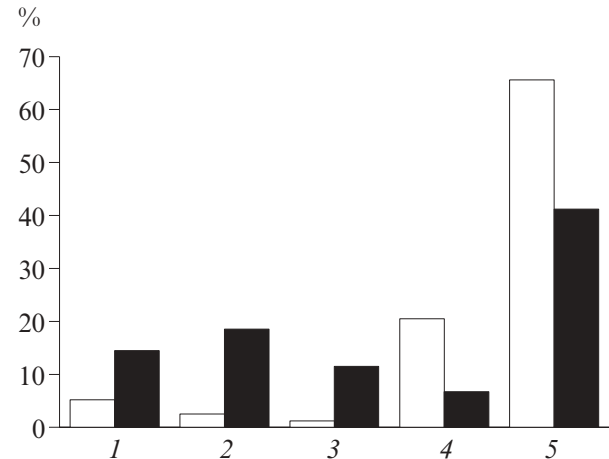


Fig. 1. Density of blood eosinophils from patients with bronchial asthma (BA): eosinophil count (1); density gradients 1.070 (2), 1.081 (3), 1.095 (4), and 1.105 g/cm³ (5). Light bars: control. Dark bars: patients with BA.

pectively) and decrease in the area of the cytoplasm and nucleus. The nucleus/cytoplasm ratio in LDE was lower than in normal-density cells (0.45 and 0.53, respectively). The population of LDE was presented by small number of protein-positive cells with low content of cationic protein granules (Table 1).

It was impossible to isolate fractions of eosinophils from IS. Therefore, morphological and functional characteristics of bronchial eosinophils were compared with those of LDE and normal-density cells circulating in the blood. Eosinophils of IS were similar to LDE, but differed from normal-density cells (Table 1). Eosinophils present in IS and blood differed in the size (2522 ± 152 and 3166 ± 402 pixels, respectively), area of the nucleus and cytoplasm, ratio of protein-

TABLE 1. Morphofunctional Parameters of Eosinophils in the Blood and IS from Patients with BA ($M \pm m$)

Parameter	Atopic BA		Control
	blood	sputum	blood
Eosinophil count, %	$15.10 \pm 0.39^*$	$19.7 \pm 1.1^*$	5.10 ± 0.39
Number of protein-positive cells, %	43.7 ± 3.3 (I)	$37.50 \pm 3.83^+$	0 (I)
	$84.0 \pm 2.8^*$ (II)		65.5 ± 2.5 (II)
Average number of granules	2.89 ± 0.20 (I)	$2.75 \pm 0.34^+$	0 (I)
	$11.6 \pm 0.8^*$ (II)		5.52 ± 0.41 (II)
Cell area, pixels	3166 ± 402 (I)	$2522 \pm 152^+$	0 (I)
	$6655 \pm 256^*$ (II)		5183 ± 500 (II)
Cytoplasm area, pixels	1893 ± 295 (I)	$1608 \pm 100^+$	0 (I)
	$4277 \pm 275^*$ (II)		2161 ± 95 (II)
Nucleus area, pixels	846.0 ± 84.3 (I)	$813.0 \pm 31.4^+$	0 (I)
	$2134 \pm 113^*$ (II)		4248 ± 301 (II)

Note. I) density gradient 1.070; II) density gradient 1.105. $p < 0.05$: *differences between normal-density eosinophils in the blood from healthy donors and patients with BA; +differences between normal-density blood eosinophils and bronchial eosinophils from patients with BA.

positive cells (37.50 ± 3.83 and $43.70 \pm 3.39\%$, respectively), and content of cationic protein granules reflecting their functional activity.

Our results indicate that eosinophilia with increased number of low-density eosinophils and high functional activity of normal-density eosinophils are typical of patients with BA. LDE are characterized by small number of protein-positive cells and low content of granules. The appearance of these cells is regulated by mediators of allergic inflammation (interleukin-3, interleukin-5, and granulocyte/macrophage colony-stimulating factor) [2,3]. These cytokines coordinate maturation, differentiation, and activation of eosinophilic leukocytes. In contrast to LDE, normal-density cells are characterized by high potential activity. Secretion of cationic proteins in response to specific allergenic stimulation is followed by a decrease in the density of cells. These peculiarities probably contribute to accumulation of LDE in the blood during BA. Bronchial eosinophils were morphologically and functionally similar to LDE. It should be emphasized that bronchial eosinophils belong to the fraction of low-density blood cells. Moreover, IS contains eosinophils

that underwent degranulation in the bronchial wall. W. W. Busse *et al.* showed that the content of the major base protein and ratio of low-density cells increase in the bronchial fluid [5].

It can be hypothesized that eosinophils in the sputum are morphologically and functionally similar to low-density cells in the blood. The number of granules in these cells is low, which indirectly indicates that cationic proteins present in the cytoplasm of eosinophils cause damage to the bronchial epithelium.

REFERENCES

1. S. N. Avdeev, E. Kh. Anaev, and A. G. Chuchalin, *Pul'monologiya*, No. 2, 81-86 (1998).
2. E. Kh. Anaev, M. V. Samsonova, A. L. Chernyaev, and A. G. Chuchalin, *Ter. Arkhiv*, No. 3, 23-25 (1997).
3. E. Kh. Anaev, A. L. Chernyaev, A. R. Tatarskii, and L. M. Voronina, *Pul'monologiya*, No. 4, 82-85 (1994).
4. A. G. Chuchalin, *Ter. Arkhiv*, No. 3, 3-8 (1994).
5. W. W. Busse, M. Nagata, and J. B. Sedgwick, *Eur. Respir. J. Suppl.*, **22**, 132-135 (1996).
6. H. P. Kuo, T. R. Ju, and C. T. Ju, *Ibid.*, **7**, No. 8, 1452-1459 (1994).