The Regulatory Function of Neutrophils in Patients with Atopic Dermatitis

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A defect in the regulation of the phagocytic component of immunity is revealed in patients with atopic dermatitis with more than 70% skin involvement. This defect manifests itself in the lack of production of stimulating neutrophilokines and impaired receptor capacity of monocytes. The regulation of not only peripheral cells, but also their bone marrow precursors was impaired.

Key Words: *neutrophilokines; neutrophils; cluster formations; atopic dermatitis*

Studies of the regulation of the immune system in health and disease are of special importance in patients with chronic immunodeficiency states. One such disease is atopic dermatitis [1,4,5].

Meanwhile, the structure of chronic immunodeficiency in such patients and the regulation of immune disorders are still largely unexplored. Data on the contribution of neutrophils and their peptide products to the regulation of various immune systems in health and disease (infection, injury, and stress) have been reported in recent years [2,3]. However, the regulatory effects of neutrophils in chronic diseases, specifically, in atopic dermatitis, are still unknown.

MATERIALS AND METHODS

The secretory function of neutrophils and the receptor capacity of peripheral blood monocytes were studied in 35 patients with atopic dermatitis with more than 70% skin involvement and in 30 healthy donors. The method of investigation has been described previously [2,3].

The stimulation/suppression index was assessed by the ratio of the cell activity index in supernatant of neutrophils of a patient or donor to the cell activity index in medium 199 (control).

The regulatory function of cells was studied on the model of phagocytosis of monodispersed polystyrene latex particles 1.7 μ in diameter and by the capacity of neutrophils to reduce nitroblue tetrazolium (NBT) to diformazan.

Supernatants of latex-activated neutrophils of 10 patients (experiment) and 10 donors (control) were combined and injected to CBA mice in a dose of 0.02 ml three times at 48-h intervals, and on day 10 after the last injection bone marrow cells were isolated by washing from diaphyses in DME medium with 5% fetal calf serum (FCS) and bicarbonate to attain a final concentration of 8 mM. The bone marrow thus obtained was washed in RPMI-1640 medium with 5% FCS and bicarbonate. Mononuclears were isolated in a Ficoll-Verographin density gradient (g=1.077 kg/liter). Cells were incubated for 30 min in RPMI-1640 medium with FCS and bicarbonate in plastic dishes at 37°C to remove adhesive cells. The cells were counted in Goryaev's chamber, and the concentration was brought to 20 pl/ml in RPMI-1640 medium with HEPES solution, L-glutamine, 15% FCS, 8 mM bicarbonate, 10% conditioned medium of mouse splenocytes [6], 0.5% agarose (Sigma), 5 mg/ml bovine serum albumin, 100 U/

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TABLE 1. Secretory Function of Neutrophils ($M \pm m$)

Group	Stimulation/suppression index			
	phagocytosis		NBT test	
	activity	intensity	activity	intensity
Patients ($n=35$)	0.95±0.102**	0.886±0.129***	1.202±0.148*	1.198±0.202*
Donors ($n=30$)	1.37±0.017	1.62±0.051	1.49±0.06	2.03±0.22

Note. Here and in Tables 2 and 3: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with control.

TABLE 2. Receptor Capacity of Monocytes ($M \pm m$)

Group	Stimulation/suppression index			
	phagocytosis		NBT test	
	activity	intensity	activity	intensity
Patients ($n=35$)	1.19±0.09**	1.207±0.141**	0.0997±0.048**	1.039±0.084**
Donors ($n=30$)	1.37±0.017	1.62±0.051	1.49±0.06	2.03±0.2

TABLE 3. Effect of Supernatants of Activated Neutrophils (SAN) of Patients on Cluster and Colony Formation in CBA Mice

Groups	Cluster formation	Colony formation
SAN ($n=5$)	31.67±3.62	3.33±0.8
SAN donors ($n=5$)	45.75±2.24*	6.0±0.8*
Control (CBA mice, $n=5$)	31.25±3.6	3.25±0.8

ml penicillin, and 100 U/ml streptomycin. Culturing was carried out in 24-well Flow Lab. plates for cell cultures, 0.2 ml cell suspension per well at 37°C, absolute humidity, and 5% CO_2 in ambient air for 8 days. After incubation the cells were dried, fixed in methanol, and stained after Romanowsky-Giemsa. Clusters (20 cells) and colonies (21 or more cells) were counted under a light microscope (×150) and cell morphology was assessed (×600). The results were processed by variational statistics method.

RESULTS

Patients with atopic dermatitis developed manifest disorders of the neutrophil secretory function (Table 1), which were expressed in the absence of activating products in the neutrophil supernatant of patients (stimulation/suppression index 0.8 to 1.2).

The monocytes of patients could not be stimulated by peptide products of donor neutrophils causing stimulation of normal donor monocytes. This indicates a gross defect of regulation in the neutrophil-monocyte cooperation system (Table 2).

Injection of supernatants of such patients to intact mice did not lead to changes in the bone marrow colonies and cluster formations. By contrast, donor supernatants of stimulated neutrophils markedly stimulated the proliferation of granulocyte-macrophage precursors (Table 3).

The results indicate that such patients develop a marked defect in the regulation of the phagocytic component of immunity in the absence of the production of stimulating neutrophilokines, as well as a disturbed receptor capacity of monocytes. The regulation of not only peripheral cells, but also their bone marrow precursors is deranged.

REFERENCES

1. B. G. Glukhen'kii and S. A. Grando, in: *Immunity-Related Dermatoses* [in Russian], Kiev (1990), pp. 225-230.
2. I. I. Dolgushin, *Lab. Delo*, № 2, 7-9 (1988).
3. A. V. Zurochka, *Immunobiological Characteristics of Secretory Products of Neutrophils (Neutrophilokines)*, Author's Abstract of Doct. Med. Sci. Dissertation [in Russian], Chelyabinsk (1991), p. 286.
4. K. N. Suvorova, *Atopic Dermatitis* [in Russian], Saratov (1989), p. 169.
5. N. P. Toropova and O. A. Sinyavskaya, *Eczema and Neurodermatitis in Children* [in Russian], Ekaterinburg (1993), p. 447.
6. I. L. Chertkov and O. A. Gurevich, *The Hemopoietic Stem Cell and Its Microenvironment* [in Russian], Moscow (1984), p. 211.