GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Possible Mechanisms for the Regulation of Neutrophil Apoptosis during Allergic Inflammation

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The neutrophil-mediated inflammatory response is regulated via activation of the apoptosis program, which decreases the degree of tissue alteration. In rabbits with allergic inflammation a significant negative correlation was revealed between the intensity of neutrophil apoptosis and blood interferon-γ concentration.

Key Words: *neutrophils; apoptosis; γ-interferon; regulation*

The notions of blood granulocytes were revised in recent times. They are considered as a special population of cells with a wide range of functions. They first detect and recognize the antigenic structure of foreign pathogens. Blood granulocytes are involved in cell-cell cooperation and modulate the function of other cells providing the succession of the immune processes [2,3]. Biogenesis of phagocytosis and phagolysosomes with subsequent apoptosis is a fundamental biological and general pathological process, which plays a role in the maintenance of homeostasis, regulation of inflammation, pathogen elimination, and processing and presentation of the adoptive immunity against antigens characteristic of these pathogens to cells [1].

The blood system of polymorphonuclear neutrophilic leukocytes is of particular interest in this respect. These cells initiate a variety of normal immunological and immunopathological reactions.

MATERIALS AND METHODS

Experiments were performed on 15 male Chinchilla rabbits weighing 2.5-3.0 kg. Periodontitis in rabbits of the treatment group (n=10) was induced by local administration of 1 mg/ml *E. coli* lipopolysaccharide to the gingival tissue. Control rabbits (n=5) received 50 μl physiological saline at 48-h intervals. The studies were conducted weekly for 1-5 weeks.

Phagocytic activity of macrophages was evaluated by their ability to engulf and release ^{14}C-labeled typhoid fever vaccine. Experiments were performed with a suspended cultures of viable peritoneal macrophages obtained by peritoneal lavage. Radioactivity was measured on a Beckman spectrometer. Chemotaxis was studied in macrophage suspension in isotonic NaCl. Optical density was measured on a photoelectrocolorimeter at 670 nm. The number of peritoneal cells (by protein concentration) was estimated at each time point. The intensity of reactive oxygen species generation by macrophages was determined by the reduction of nitroblue tetrazolium to diformazan with oxygen radicals. The respiratory burst in neutrophil was studied by chemiluminescence (Luminometer-1250, LKB) after preactivation with formyl-methionyl-leucine-phenylalanine.

For evaluation of neutrophil apoptosis (NA), leukocytes were isolated and cultured in RPMI-1640 medium. Cell concentration and viability were evaluated. The percent of cells permeable for supravital dye propidium iodide (percent of dead cells)

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TABLE 1. Dynamics of Test Parameters during Inflammation

Parameter	Control	Weeks				
		1	2	3	4	5
IFN-γ, pg/ml	4.87±0.10^{+}	5.93±0.10^{+}	21.47±1.75^{+*}	29.07±0.91^{+*}	39.28±1.52^{+*}	20.34±1.08^{+*}
IL-4, pg/ml	1.39±0.02	1.48±0.03^{+}	1.51±0.03^{+*}	1.58±0.04^{+}	2.93±0.10^{+*}	1.74±0.11^{+*}
Intensity of macrophage phagocytosis, cpm/mg protein	9424±156	10 475±172^{+}	11 520±203^{+*}	12 084±201^{+}	13 892±231^{+*}	12 506±209^{+*}
Intensity of macrophage chemotaxis, mg protein/ml	0.050±0.001	0.050±0.001	0.060±0.001^{+*}	0.070±0.001^{+*}	0.080±0.001^{+*}	0.070±0.001^{+*}
Oxidative burst in macrophages, rel. units	1.40±0.02	1.54±0.02^{+}	1.70±0.01^{+*}	2.040±0.025^{+*}	2.38±0.05^{+*}	1.92±0.05^{+*}
NA, %	50.90±0.81	41.10±0.87^{+}	37.2±1.3^{+*}	31.6±0.6	29.70±0.63^{+*}	35.30±1.49^{+*}

Note. $p<0.05$: $^{+}$compared to the control; *compared to the previous term.

was measured by flow cytometry. After DNA extraction and DNA-specific fluorescent staining, apoptotic cells were identified as cells with degraded chromatin localized in the subdiploid peak of the DNA histogram.

The concentrations of interleukin-4 (IL-4) and interferon-γ (IFN-γ) in blood plasma were measured using commercial kits for enzyme immunoassay (Tsitokin).

RESULTS

In control animals, phagocytic activity of macrophages, NA, and cellular and humoral immune factors remained unchanged at various terms of the study.

In treated animals, the test parameters progressively increased (Table 1), peaked during the 4th week, and then slightly decreased (except NA, which peaked after 1 week, then decreased, and increased again during the 5th week). A cross-correlation analysis of the mean values was performed in the dynamics of this process and for each term of the study for evaluation of the cause-effect relations between the test parameters. A strong positive correlation was found between the intensity of phagocytosis and determinant factors (chemotaxis, r=0.97; and accumulation of free radicals in macrophages, r=0.97). No correlation between the test parameters was revealed during individual periods of study. Hence, these processes are characterized by specific variations in each specimen. However, this relation becomes obvious in determining the resultant value for the group.

Parameters of cellular (IFN-γ) and humoral immunity (IL-4) were mutually related and correlated with NA and intensity of phagocytosis.

The correlation was strong, positive, and significant (Table 2), except the correlation between IL-4 concentration and intensity of chemotaxis, which was insignificant. Blood IFN-γ concentration was in a strict reliable negative correlation with the severity of NA. The correlation with IL-4 was medium-strength and insignificant (Table 2). Analysis of IFN-γ correlations for different terms of the study revealed no specific features, which probably reflects the so-called "search response".

Comparative study of plasma IFN-γ concentration and severity of NA (Fig. 1) demonstrated high discordance between these parameters (strong negative correlation, r=-0.9). Hence, IFN-γ decreases the intensity of NA.

These data suggest that IFN-γ and IFN-γ-dependent mechanisms regulate apoptosis, which provides the possibility of modulating these regulatory processes for controlling the intensity of apoptosis. Various cytokines (IL, IFN) play an important role in the regulation of apoptosis in immune cells. NA regulates neutrophil-mediated reactions by maintaining the balance between function and safe elimination of cells. The interferon cascade plays a central role in the events regulating differentiation and apoptosis [1]. In the majority of cases immediate early response genes (*i.e.*, genes characterized by increased transcription in the absence of

TABLE 2. Correlation of Immune Parameters with IFN-γ and IL-4 in the Dynamics of the Process

Parameter	Correlation with IFN-γ	Correlation with IL-4
Intensity of phagocytosis	0.94*	0.82*
Intensity of chemotaxis	0.96*	0.79
Oxidative burst	0.96*	0.85*
NA	-0.9*	-0.63

Note. *$p<0.05$.

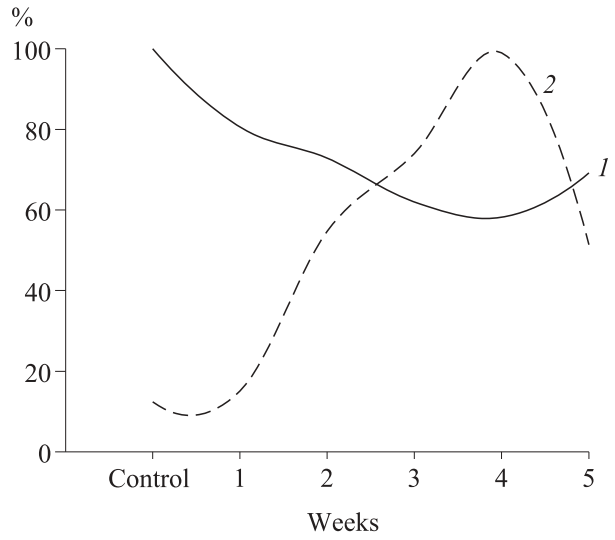

Fig. 1. Intensity of NA (*1*) and plasma IFN-γ concentration (*2*). Maximum value of each parameter is taken as 100%. The correlation coefficient is calculated from the absolute values.

de novo protein synthesis) are regulated by the family of transcription-regulating signal proteins (signal transducer and activator of transcription, STAT). They are rapidly activated in response to ligand-receptor interaction and are recruited in the intracellular domain of the apoptotic receptor. The critical activating components of STAT are an IFN-γ-sensitive cell cycle regulator and IL-6 response to acute phase proteins [4]. Differences in the cell response are typical of various target cells and probably depend on their maturation and development. Specific features of polymorphonuclear cells and their mitochondrial compartment modify the apoptotic response to typical inductors [5]. Studying of the dependence of NA on IFN-γ will allow us to understand the pathogenetic mechanisms of inflammatory diseases, improve differential diagnostics, and develop new methods of therapy (pharmacologically induced apoptosis).

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