Effects of Secretory Products of Activated Neutrophils on Morphological Composition and Functional Activity of Peritoneal Exudation Cells during Inflammation of Staphylococcal Origin

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Substance A_5 isolated from supernatants of activated neutrophils from donors significantly increases the percentage of neutrophils and macrophages in the peritoneal exudation of mice on days 3 and 7 of staphylococcal inflammation and stimulates functional activity (lysosomal, phagocytic, and NBT-reducing) of these cells, reduced as a result of inflammation, on days 3, 7, and 14 of the inflammatory process.

Key Words: *staphylococcal inflammation; secretory products of neutrophils; immunoreactivity*

Study of cooperation processes between immunocompetent cells during inflammatory process is a pressing problem of modern immunology [1,5]. The role of neutrophils and their secretory products in the regulation of the immune system under conditions of developing inflammation remains poorly studied. Neutrophils are known to release bioactive products with immunostimulating effects [2].

We studied the effects of neutrophil mediators on morphological composition and functional activity of peritoneal exudation cells during the development of staphylococcal inflammation.

MATERIALS AND METHODS

Experiments were carried out on adult CBA mice from the Ural Research and Practical Center of Radiation Medicine.

Secretory products of donor neutrophils were isolated by the method developed by I. I. Dolgushin *et al.* [3]. Neutrophils were isolated from donor blood in Ficoll-verograffin double density gradient (Pharmacia, Spofa) by the method proposed by L. Wong *et al.* [8]. Granulocytes were suspended in medium 199 to a concentration of 5×10^6 cells/ml. Neutrophils were incubated for 1 h at 37°C with 1.7-μ latex microspheres (50 particles per cell). Supernatants of activated neutrophils (SAN) were prepared by 10-min centrifugation at 3000 rpm followed by filtration through 0.24-μ membrane filters (Millipore). Isolated donor neutrophil mediators were separated on Sephadex G-15 column and A_5 peptide-containing fraction with pronounced immunostimulating characteristics was obtained. Peptide substance, called A_5 substance, with NH_2 terminal leucin [2] was isolated by high-performance liquid chromatography from this fraction on modified silica gels in acetonitrile gradient.

Experimental staphylococcal inflammation was modeled by 3 intraperitoneal injections of 24-h *Staphylococcus aureus* culture, strain Cowan 209 (2×10^8 microbial bodies in 1 ml normal saline per mouse). The number of injections and Staphylococcus dose were determined in previous experiments, which showed that 3 intraperitoneal injections of *S. aureus* suspension in a dose of 2×10^8 microorganisms per mouse significantly suppressed immunoreactivity of experimental animals.

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In order to detect the effect of A_5 substance from donor SAN on morphological composition and functional activity of peritoneal exudation under conditions of staphylococcal inflammation 1 h after the last injection of *S. aureus* suspension, the animals were 3 times (with 24-h intervals) injected intraperitoneally with donor SAN A_5 substance in a dose of 10^{-7} mg/mouse in 0.1 ml normal saline. The dose and number of injections of the neutrophil secretory products were determined previously [2].

The effects of A_5 substance of donor SAN on morphological composition and functional activity of peritoneal exudate cells were evaluated on days 3, 7, and 14 of staphylococcal inflammation, which corresponded to certain phases of the inflammatory process. The study of morphological composition included evaluation of the percentage of neutrophils, macrophages, and lymphocytes in the peritoneal exudate. The functions of peritoneal neutrophils and macrophages were studied by evaluating lysosomal, phagocytic [6], and NBT-reducing [4,7] activities of these cells. Peritoneal exudation cells were collected by washing the abdominal cavity with cold (0°C) medium 199 with heparin (10 U/ml).

Two control groups were used: 1) intact animals receiving normal saline instead of donor SAN A_5 substance according to the same protocol and 2) mice injected with suspension of *S. aureus* and normal saline.

The data were processed using STATISTICA software.

RESULTS

Analysis of morphological composition of peritoneal exudation cells showed a significant increase in the percentage of neutrophils and macrophages on day 3 of staphylococcal infection in mice. Later (on days 7 and 14) the percentage of these cells decreased, but remained above the normal (Table 1).

Changes in immunological reactivity under conditions of staphylococcal inflammation pass through 3 stages. Early immunosuppression (day 3) developing during the first day of inflammation is characterized by suppression of lysosomal and NBT-reducing activities of neutrophils and macrophages and phagocytic activity of neutrophils. This was followed by compensation (immunoreactivity increased) by such parameters as lysosomal and phagocytic activities of neutrophils and macrophages, but not spontaneous and latex-induced NBT reduction activity of neutrophils and macrophages, which still decreased on day 7. On day 14 the immune system was suppressed by all the studied parameters (Tables 2, 3).

Injection of secretory products of neutrophils to mice with staphylococcal infection led to a significant increase in the percentage of neutrophils and macrophages in the peritoneal exudate on days 3 and 7 of inflammation. On day 14 the composition of peritoneal exudation approached the normal, while in control group 2 the percentage of neutrophils remained elevated in comparison with intact animals, which attested to persisting inflammation (Table 1).

Injection of donor SAN substance A_5 stimulated the immunoreactivity suppressed by inflammation, increased lysosomal activities of neutrophils and macrophages (days 3, 7, 14 of inflammation), activity and intensity of phagocytosis (days 3 and 14), and parameters of spontaneous and latex-induced NBT reduction of neutrophils and macrophages (days 3, 7, and 14 of inflammation). In some cases these parameters even surpassed the normal (Tables 2 and 3).

TABLE 1. Dynamics of the Effect of A_5 Substance of Donor SAN on Morphological Composition of Peritoneal Exudate in Mice Infected with *Staphylococcus* (*M*±*m*, *n*=7)

Group	Cell composition, %		
	neutrophils	macrophages	lymphocytes
Control I (intact mice+normal saline)	4.86±0.59	18.00±1.51	77.14±1.79
Day 3			
Control II (staphylococcus+normal saline)	7.71±0.52*	22.29±0.81*	70.00±1.15*
Staphylococcus+A_5	10.00±0.44*+	26.57±1.43*+	63.43±1.36*+
Day 7			
Control II (staphylococcus+normal saline)	6.29±0.52	20.57±0.84	73.14±1.14
Staphylococcus+A_5	8.00±0.44*+	24.57±0.95*+	67.43±1.04*+
Day 14			
Control II (staphylococcus+normal saline)	6.86±0.74	20.57±0.57	72.57±1.13
Staphylococcus+A_5	4.57±0.57+	18.86±0.74	76.57±1.04+

Note. Here and in Tables 2 and 3: $p<0.05$ *compared to control I, +compared to control II.

TABLE 2. Dynamics of the Effect of A_5 Substance of Donor SAN on Functional Activity of Neutrophils in Peritoneal Exudate of Mice Infected with *Staphylococcus* (*M*±*m*, *n*=7)

Group	Index of summary lysosomal fluorescence, arb. units	Phagocytosis		Spontaneous NBT test		Induced NBT test	
		activity, %	intensity, arb. units	activity, %	intensity, arb. units	activity, %	intensity, arb. units
Control I (intact mice+normal saline)	226.5±38.3	71.3±1.9	179.0±21.9	99.3±0.7	1.68±0.04	100±0	1.64±0.08
Day 3							
Control II (staphylococcus+ normal saline)	56.7±11.9*	55.2±3.8*	132.0±12.3	86.40±0.77*	0.940±0.015*	76.8±5.4*	0.870±0.069*
Staphylococcus+A_5	245.30±25.16$^+$	64.0±3.2	145.00±11.65	100±0$^+$	1.800±0.039$^+$	94.70±3.87$^+$	1.680±0.097$^+$
Day 7							
Control II (staphylococcus+ normal saline)	170.0±9.7	71.40±3.35	237.6±14.6	81.30±5.03*	0.920±0.046*	69.60±6.15*	0.750±0.077*
Staphylococcus+A_5	273.0±42.7$^+$	65.60±3.08	155.2±20.0$^+$	96.7±1.4$^+$	1.240±0.058*$^+$	99.40±0.56$^+$	1.780±0.077$^+$
Day 14							
Control II (staphylococcus+ normal saline)	25.5±2.9*	57.0±6.8	125.00±13.59	64.0±2.9*	0.72±0.05*	68.0±4.5*	0.770±0.045*
Staphylococcus+A_5	93.30±13.65*$^+$	65.30±10.24	146.70±30.03	100±0$^+$	1.490±0.077$^+$	97.40±1.54$^+$	1.290±0.062*$^+$

TABLE 3. Dynamics of the Effect of A_5 Substance of Donor SAN on Functional Activity of Macrophages in Peritoneal Exudate of Mice Infected with *Staphylococcus* (*M*±*m*, *n*=7)

Group	Index of summary lysosomal fluorescence, arb. units	Phagocytosis		Spontaneous NBT test		Induced NBT test	
		activity, %	intensity, arb. units	activity, %	intensity, arb. units	activity, %	intensity, arb. units
Control I (intact mice+normal saline)	166.0±50.5	61.0±2.2	189.7±9.5	100±0	1.79±0.07	100±0	1.70±0.05
Day 3							
Control II (staphylococcus+ normal saline)	140±63	60.80±6.15	185.6±30.8	83.2±4.6*	1.09±0.12*	75.2±4.6*	0.980±0.077*
Staphylococcus+A_5	334.7±27.7*$^+$	80.7±3.2*$^+$	318.0±30.1*$^+$	100±0$^+$	2.230±0.097*$^+$	92.0±3.87$^+$	1.79±0.13$^+$
Day 7							
Control II (staphylococcus+ normal saline)	292.5±79.0	73.00±3.35*	256.0±13.8*	77.6±3.1*	0.89±0.05*	69.00±5.03*	0.820±0.056*
Staphylococcus+A_5	404.0±93.2	65.60±3.85	206.4±24.6	94.70±3.49$^+$	1.680±0.085$^+$	99.40±0.56$^+$	1.930±0.031*$^+$
Day 14							
Control II (staphylococcus+ normal saline)	21.0±1.9*	56.7±4.5	176.00±15.38	72.7±4.5*	0.75±0.07*	62.00±1.94*	0.700±0.058*
Staphylococcus+A_5	74.70±17.75$^+$	86.80±3.85*$^+$	352.00±32.69*$^+$	100±0$^+$	2.04±0.20$^+$	100±0$^+$	1.570±0.062$^+$

Hence, substance A_5 of donor SAN modulated morphological composition of peritoneal exudate during staphylococcal inflammation.

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