CYTOHISTOCHEMICAL CHARACTERISTICS OF IMMUNOPATHOLOGICAL CHANGES DURING INFLAMMATION OF THE LUNGS

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A cytochemical investigation of the state of the lysosomal membranes of the phagocytes and lymphocytes of the blood, trachea, and lungs of rabbits during immunization with adsorbed staphylococcal toxoid and with human serum albumin, during experimental inflammation of the lungs, and during pneumonia following immunization, is described. Changes as a result of immunization (systemic destabilization of the lysosomal membranes of the phagocytes and lymphocytes, microcirculatory disorders and infiltrative changes in the lungs) were shown to be premorbid relative to the pneumonia and to intensify the existing inflammation. It is suggested that these changes are a structural and functional manifestation of immunopathological reactions accompanying inflammation of the lungs.

KEY WORDS: phagocytes; lymphocytes; lysosomes; immunization; inflammation of the lungs.

The role of the immune response in the development of the cellular and tissue disturbances has been inadequately reflected in investigations of nonspecific inflammatory diseases of the lungs [5].

To study this problem a comparative investigation was made of the state of the lysosomal membranes of the phagocytes and lymphocytes of the blood, trachea, and lungs during immunization, experimental pneumonia, and inflammation following previous immunization, with simultaneous recording of the tissue changes in the lungs and the dynamics of the humoral immune response.

EXPERIMENTAL METHOD

Experiments were carried out on 71 rabbits (6 groups). The animals of groups 1 and 2 (20 in each group) were immunized with adsorbed staphylococcal toxoid (AST) and human serum albumin (HSA) respectively. Immunization entailed [1] three subcutaneous injections, at 5-day intervals, of increasing doses (0.1, 0.4, and 1.0 ml of AST and 0.8, 3.2, and 8 mg of HSA in 1.0 ml physiological saline) into the upper third of the hind limbs. The animals were killed 1 week after the 1st and 1, 3, and 5 weeks after the 3rd injection of the antigens. In the rabbits of group 3 (6 animals) inflammation of the lungs was induced by intratracheal insertion of a length of sterile nylon thread 8 cm long and 0.5 mm in diameter [2]. The animals of groups 4 and 5 (5 in each group) were immunized with AST and HSA in the same way as the rabbits of groups 1 and 2. One week after the end of immunization, inflammation of their lungs was induced by the method described above. The rabbits of groups 3-5 were killed 2 weeks after introduction of the thread. Control group 6 consisted of 15 healthy animals.

The blood sera were investigated by the quantitative precipitation test [3] and a quantitative variant of the complement fixation test [6]. Acid phosphatase was investigated in the cells of blood films and squash preparations from the trachea by Burstone's method (with naphthol phosphate AS and Fast blue BB). At least 100 cells were counted in each film. The results were subjected to statistical analysis by Student's t-test. Sections through the lungs were stained with hematoxylin-eosin and for acid phosphatase by Gomori's method. During the analysis of the films and tissue sections the structural character of distribution of the enzyme was assessed: granularity and diffuseness of formation of the reaction end product. In view of the known lysosomal localization of acid phosphatase [7], diffuse staining of the cytoplasm could be regarded as an indication of disturbed permeability of the lysosomal membrane [4].

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TABLE 1. Relative Percentages of Neutrophils, Lymphocytes, and Macrophages with a Diffuse Distribution of Acid Phosphatase in Blood and Trachea of Rabbits Immunized with AST and HSA

Cells	Group of animals	Control	Time of investigation			
			1 week after 1st injection of antigens	1 week after end of immunization	3 weeks after end of immunization	5 weeks after end of immunization
Blood neutrophils						
Sd	1-st	16,5 (15)	13,6 (5)	78,3 (5) ±4,65	42,1(5) ±5,63	25,2 (5)
P				<0,001	<0,001	
Sd	2-nd		6,0 (4) ±4,79	74,4 (3) ±6,04	77,3 (4) ±6,38	25,4 (4)
P			<0,05	<0,001	<0,001	
Blood lymphocytes						
Sd	1-st	20,3 (15)	25,2 (5)	67,1(5) ±4,01	55,2 (5) ±5,98	33,8 (5) ±4,80
P				<0,001	<0,001	<0,02
Sd	2-nd		10.3 (4)	83.0 ±5.73	68.0 (4) ±5,96	26,0 (4)
P				<0,001	<0,001	
Tracheal neutrophils						
Sd	1-st	25,2 (13)	17,7 (5)	41.6 (4) ±5.94	16.6 (5)	8,9 (5) ±5,31
P				<0.02		<0.01
Sd	2-nd		24,7 (5)	53.5 (5) ±5,20	18.9 (4)	13.0 (4) ±5.32
P				<0.001		<0,05
Tracheal lymphocytes						
Sd	1-st	23.1 (13)	20,1 (5)	43.9 (4) ±6.46	9.6 (5) ±5,63	18,0 (5)
P				<0.01	<0,05	
Sd	2-nd		19,6 (5)	45.1 (5) ±6,71	20.6 (4)	21.3 (4)
P				<0.01		
Tracheal macrophages						
Sd	1-st	24.7 (13)	24,0 (5)	33,7 (4)	19,2 (5)	31,3 (5)
	2-nd		26,8 (5)	61.4 (5) ±6.33	28.2 (4)	33,2 (4)
P				<0.001		

Legend. Values of Sd (mean error of difference between arithmetic means) and of P in Tables 1 and 2 are given for results differing statistically significantly from control. Number of films tested shown in parentheses.

TABLE 2. Relative Percentage of Cells with a Diffuse Pattern of Acid Phosphatase Distribution in Blood and Trachea of Unimmunized and Immunized Rabbits with Experimental Pneumonia Lasting 2 Weeks

Group of animals		Blood cells		Tracheal cells		
		neutrophils	lymphocytes	neutrophils	lymphocytes	macrophages
3-rd	Sd	55,4 (5) ±4,86	53,3 (5) ±4,06	68,5 (4) ±7,10	55,8 (4) ±8,41	45,8 (4) ±6,93
	P	<0.001	<0,001	<0,001	<0,01	<0,01
4-th	Sd	85,1 (5) ±5,85	74,6 (5) ±6,26	79,9 (5) ±6,20	67,5 (5) ±6,41	68,0 (5) ±7,86
	P	<0,001	<0,001	<0,001	<0,001	<0,001
5-th	Sd	79,9 (4) ±4,96	84,2 (4) ±5,32	80,1 (4) ±5,66	67,4 (4) ±7,25	74,9 (4) ±7,38
	P	0,001	<0,001	<0,001	<0,001	<0,001
Control		16,5 (15)	20,3 (15)	25,2 (13)	23,1 (13)	24,7 (13)

EXPERIMENTAL RESULTS

In the course of immunization of the animals of group 1 the complement consumption increased from $C'H_{50}$ 8.82 in the control to $C'H_{50}$ 278.4 on the 7th day after the 3rd injection of AST, after which it fell to regain its original level by the end of the experiments. Activity of the antialbumin sera also reached a maximum on the 7th day after the end of immunization. At this time, in the zone of equivalence, 1 ml serum bound on average 204.6 μg of antigen. Later the antibody level in the sera of the rabbits of group 2 fell. Inflammation of the lungs for 2 weeks in the animals of groups 4 and 5 caused no significant change in the dynamics of elimination of serum antibodies.

A single injection of antigens had no significant effect on the state of the lysosomal membranes of the phagocytes and lymphocytes (Table 1). Only after administration of HSA was a fall observed in the number of neutrophils with a diffuse distribution of acid phosphatase in the blood. At the height of the immune response the number of cells with disturbed permeability of their lysosomal membranes in the blood and trachea increased significantly (excluding tracheal macrophages in the rabbits of group 1). Three and 5 weeks after the end of immunization a decrease in the activity of the sera was accompanied by a decrease in the number of phagocytes and lymphocytes with destabilized lysosomal membranes, initially in the trachea and later in the blood also.

Moderate signs of diffuse edema and of infiltration chiefly by macrophages and lymphocytes were observed in the lungs after the 1st injection of AST and HSA. The character of distribution of acid phosphatase in the various tissue cells was unchanged. The edema and infiltration were intensified 1 week after the end of immunization, the number of neutrophils was increased (especially in the lungs of rabbits of group 2), and the lysosomal membranes of the phagocytes, lymphocytes, and bronchial epithelial cells were destabilized. Single focal concentrations of neutrophils with central zones of destruction also were found in the lungs of the animals of group 1. Later in the investigation the structure of the lung tissue was restored and the lysosomal membranes became stabilized.

The development of experimental inflammation of the lungs in the nonimmunized rabbits (group 3) in the course of 2 weeks was characterized by destabilization of the lysosomal membranes of all three types of cells (Table 2). Solitary foci of marked neutrophilic infiltration typologically connected with the bronchi and moderately severe destructive changes were observed in the lungs. The permeability of the lysosomal membranes was disturbed in the cells of the foci of infiltration and bronchial epithelium.

At the same stage of inflammation phagocytes and lymphocytes with destabilized lysosomal membranes were more numerous in the blood and trachea of the immunized rabbits than of the animals of groups 1, 2 (at the height of the immune response) and 3. This increase was statistically significant: for neutrophils ($Sd = \pm 9.09$; $P < 0.01$), lymphocytes ($Sd = \pm 8.33$; $P < 0.05$), and macrophages ($Sd = \pm 12.12$; $P < 0.05$) in the trachea of the animals of group 4 compared with group 1; for neutrophils ($Sd = \pm 5.52$; $P < 0.001$) of the trachea of the rabbits of group 5 compared with group 2; for neutrophils ($Sd = \pm 9.02$; $P < 0.01$ and $SD = \pm 6.40$; $P < 0.01$) and lymphocytes ($Sd = \pm 9.07$; $P < 0.05$ and $SD = \pm 5.77$; $P < 0.001$) of the blood of the animals of groups 4 and 5 respectively compared with group 3; for tracheal macrophages of the rabbits of group 5 compared with group 3 ($Sd = \pm 11.58$; $P < 0.005$).

The course of inflammation in the lungs of the immunized animals was characterized by a greater extent and intensity of infiltration and destructive changes, leading to the formation of small and large abscesses. Destabilization of the lysosomal membranes of the phagocytes and lymphocytes was considerably intensified, as reflected in the diffuse amorphous staining of the foci of infiltration with end products of the reaction for acid phosphatase. A special feature distinguishing the inflammatory changes in rabbits immunized with HSA was the abundance of hemorrhages.

From the comparative aspect it can be concluded from these results that the response of the body to immunization with AST and HSA, with reference to certain cellular and tissue changes (systemic destabilization of the lysosomal membranes of the phagocytes and lymphocytes, microcirculatory disorders and infiltrative changes in the lungs), can be regarded as a premorbid state relative to the pneumonia, and on the other hand, as a factor intensifying inflammation. In this connection it can be postulated that these cellular and tissue disturbances are immunopathological.

Differences in the dynamics of the cellular changes in the trachea and blood during immunization by AST and HSA are evidently due to the specific character of elimination of particles and injured cells in the air passages and to differences in the properties of the antigens used, although these do not radically change the basic principles mentioned above.

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EFFECT OF EXTIRPATION OF THE CERVICAL SYMPATHETIC GANGLIA ON POSTNATAL DEVELOPMENT OF THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS IN RATS

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Experimental data on the role of adaptive and trophic influences of the sympathetic nervous system in the postnatal development of the hypothalamus are described. It was shown by karyocytometry that after extirpation of the cervical sympathetic ganglia (CSG) in rats ontogenetic development of the neurosecretory cells of the paraventricular nucleus (PVN) is delayed and changes are observed in the blood vessels. Manifestation of the effect of sympathectomy coincided with the onset of intensive growth of the cytoplasm of PVN cells. Sympathectomy was more effective in the case of extirpation of the CSG from young rats than from adult animals.

KEY WORDS: gangliectomy; development of the hypothalamus; paraventricular nucleus; neurosecretory cells; karyocytometry.

Several morphological investigations have been devoted to the study of the effect of the cervical sympathetic ganglia (CSG) on hypothalamic function in adult rats [1, 5, 7, 10]. However, there is as yet no information in the literature on the role of the CSG in the ontogenetic development of the hypothalamus.

The object of the investigation described below was to discover whether the CSG affect hypothalamic development and, in particular, the postnatal development of the paraventricular nucleus (PVN). Interest in this problem is due to the fact that when the CSG are extirpated in young rats, growth of the animals and the development of their motor activity are delayed [8, 9, 17], and later the generative functions are disturbed, and the hypothalamic PVN is known to be concerned in the regulation of these functions [13, 18].

EXPERIMENTAL METHOD

Experiments were carried out on 26 intact and 20 gangliectomized male albino rats taken for investigation on the 10th, 20th, and 30th days after birth and at the age of 2, 3, and 5 months. The intact animals were investigated additionally at the ages of 1 and 5 days. One-stage bilateral extirpation of CSG (superior and middle) was carried out under ether anesthesia on young rats aged 1-5 days. Horner's syndrome was well marked in the sympathectomized rats during 5 months of observation.

The brain was fixed in Bouin's fluid and embedded in paraffin wax. Serial frontal sections (6 μ) were stained with cresyl violet. The development of PVN in the intact and sympathectomized rats was compared by a morphometric method. According to data in the literature, in the course of ontogenetic maturation the volume

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