PATHOMORPHOLOGY OF LYMPHOID TISSUE IN THE LATE STAGES AFTER ANAPHYLACTIC SHOCK

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Data on changes in the lymphoid tissue in anaphylactic shock (AS) relate only to the immediate period (2-3 h) after its development [7]. For medical practice, however, the most important problem for study is the pathomorphological state of lymphoid tissue in the immediate and late periods after AS.

This paper describes pathomorphological and morphometric investigations of lymph nodes and the thymus gland of rabbits in the early (2-3 days) and late (8-10 days) stages after AS. The rise in the frequency of AS in clinical practice and the lack of any comprehensive investigations into the state of the lymphoid tissue in the late stages after AS make these problems particularly urgent.

EXPERIMENTAL METHOD

Nineteen rabbits were sensitized by three subcutaneous injections of normal horse serum (NHS) in a dose of 6.4 mg/kg body weight. On the 18th-21st day after the last sensitizing injection, AS was induced in the rabbits by intravenous injection of the reacting dose of antigen (32 mg/kg). The classical picture of AS, of different degrees of severity, was observed in all the rabbits. To obtain lymphoid tissue the animals were killed by exsanguination under deep intravenous hexobarbital anesthesia (15 mg/kg) on the 2nd-3rd and 8th-10th days after AS (six animals were used at each period of the investigation). Lymph nodes from different functional groups were studied: somatic (popliteal), visceral (mesenteric), and the thymus. Intact animals receiving injections of physiological saline instead of sensitizing injections of NHS, served as the control; they received the reacting injection of serum in the same dose and in the same way as the experimental rabbits. Pieces of lymph nodes were fixed in Carnoy's fluid and of thymus in 10% neutral formalin by Lillie's method, after which they were dehydrated in alcohols of increasing concentration, cleared (the thymus in chloroform, the lymph nodes in xylol), and embedded in paraffin wax. Paraffin sections of lymphoid tissue, 5-6 μ thick, were stained with hematoxylin and eosin and with methyl green — pyronine by Brachet's method and to detect RNA, and treated with Schiff's reagents (after McManus) to detect aminoglycans and carbohydrates. Besides qualitative assessment of morphology of the lymphoid tissue, cells (lymphocytes, reticular cells, and pyroninophilic blast cells) in the follicles, paracortical zone, medullary cords, and sinuses (per unit area) and the PAS-positive cells were counted (in 10 fields of vision). The cells were counted by means of an MOV-1 ocular micrometer, with an ocular grid with a total area of 64 mm^2, under a magnification of 600. The width of the paracortical (T-dependent) zone and the diameter of the follicles (B-dependent zone) also were measured under a magnification of 120× (ocular 8, objective 15). The results of the measurements were expressed in microns. The numerical results were subjected to statistical analysis [4].

EXPERIMENTAL RESULTS

After AS widely different changes were observed in the structure of the lymph nodes (Tables 1 and 2).

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TABLE 1. Diameter of Follicles (DF), Width of Paracortical Zone (WPZ), and Density of Cells (per unit area) in Mesenteric Lymph Nodes of Rabbits in Early and Late Stages after AS (M ± m)

Parameter studied	Normal (n = 6)	After AS	
		2–3 days (n = 6)	8–10 days (n = 6)
Follicles			
Lymphocytes	27,50±2,43	12,33±2,54‡	10,83±1,89†
Blast cells	1,67±0,33	7,33±1,69†	5,50±0,76†
PAS-positive cells	3,33±1,12	5,67±1,31	8,17±1,70**
Reticular cells	2,17±0,48	3,50±0,56	6,00±0,45‡
Paracortical zone			
Lymphocytes	26,33±2,17	21,67±1,61	28,17±3,69
Blast cells	1,67±0,49	2,17±0,54	2,50±1,02
PAS-positive cells	2,67±0,67	5,67±1,21**	3,33±1,17
Reticular cells	2,00±0,37	2,67±0,42	3,33±1,20
Sinuses			
Lymphocytes	10,67±1,43	5,33±1,23*	5,17±1,38*
Blast cells	5,17±0,65	3,67±0,49	3,83±0,54
PAS-positive cells	3,17±1,05	9,33±1,58†	8,17±1,49*
Reticular cells	2,67±0,49	1,83±0,31	1,50±0,50
Medullary cords			
Lymphocytes	15,33±0,88	7,33±1,67†	9,67±1,45†
Blast cells	4,00±0,58	7,33±1,23*	4,33±0,59
PAS-positive cells	6,00±1,06	10,33±2,11	10,17±1,66**
Reticular cells	4,50±0,43	4,00±0,86	4,67±0,33
WPZ, μ	732,50±18,14	553,17±46,18†	480,83±58,10‡
DF, μ	572,50±59,84	330,50±36,90†	235,00±22,18‡

Legend. Here and in Table 2: *$P < 0.02$, †$P < 0.01$, ‡$P < 0.001$, **$P < 0.05$; mean number of cells per field of vision is shown, obtained by counting in 15 fields of vision in each preparation; number of PAS-positive cells counted in 10 fields of vision of each preparation (mean number of PAS-positive cells per field of vision shown).

By the 2nd-3rd days after AS a decrease in diameter of the follicles and widening of their germinative centers were observed. In the follicles the number of cells with wide pyroninophilic cytoplasm and a hyperchromic nucleus, rich in chromatin (blast cells) was increased, whereas in the group of mesenteric lymph nodes the number of reticular cells also was increased on the 8th-10th day after AS. Besides the large number of mitoses in the reticular and blast cells, a marked decrease in the number of lymphocytes was observed in the lymph node follicles; changes were observed in these cells — pycnosis, rhexis, and lysis of their nuclei with phagocytosis of the fragments isolated in the form of macrophages by the reticular cells. In the paracortical zone of the follicles branching cells with large granules, giving a positive reaction for aminoglycans (PAS-positive cells), which can be regarded as evidence of destruction of the connective-tissue component of the lymph nodes, could be seen.

Changes in the paracortical zone (plateau) of the lymph nodes were less marked: a reduction in its width, more especially in the group of mesenteric lymph nodes, an increase in the number of PAS-positive cells. In the group of popliteal lymph nodes the number of reticular cells are reduced. The visible changes in cells of the plateau were slight and inconstant. Changes in the medullary cords and sinuses of the lymph nodes took the form of multiplication of histiocytes and of blast and plasma cells, their separation from the cord and accumulation in the lumen of the sinus, and an increase in the number of PAS-positive cells. Littoral cells at different stages after shock were partly in a state of necrosis, and frequently detached. In the sinuses and cords of the mesenteric nodes there were fewer lymphocytes at all stages of the investigation. On the 2nd-3rd day and, in particular, on the 8th-10th day after AS immature and mature plasma cells were found more frequently and were located at the periphery of the follicles and beneath the capsule in large groups and continuous areas. They were even more numerous in the medullary cords and medullary and marginal sinuses.

TABLE 2. Diameter of Follicles (DF), Width of Paracortical Zone (WPZ), and Density of Cells (per unit area) in Popliteal Lymph Nodes of Rabbits in Early and Late Stages after AS (M ± m)

Parameter studied	Normal (n = 6)	After AS	
		2–3 days (n = 6)	8–10 days (n = 6)
Follicles			
Lymphocytes	23,83±2,33	8,66±3,11†	8,50±0,76‡
Blast cells	4,17±0,70	12,83±2,52†	12,67±2,64*
PAS-positive cells	3,00±0,82	9,50±1,43†	3,00±0,63
Reticular cells	5,50±0,43	5,83±2,14	3,50±0,43*
Paracortical zone			
Lymphocytes	25,67±1,28	25,33±2,09	21,00±2,05
Blast cells	3,17±0,87	2,00±0,63	3,67±0,92
PAS-positive cells	2,67±0,56	6,67±0,97†	3,50±0,96
Reticular cells	4,33±0,61	2,83±0,31**	2,33±0,49**
Sinuses			
Lymphocytes	10,83±1,80	10,00±1,44	7,17±2,29
Blast cells	2,00±0,52	6,83±0,75‡	6,17±1,22†
PAS-positive cells	4,83±1,11	6,67±1,45	10,17±1,54*
Reticular cells	1,50±0,43	2,33±0,49	3,00±0,45**
Medullary cords			
Lymphocytes	6,00±0,68	5,67±0,49	5,00±1,06
Blast cells	5,33±1,12	9,83±1,62**	11,00±2,56*
PAS-positive cells	5,17±0,67	9,50±1,23†	8,67±0,84†
Reticular cells	4,50±0,43	4,33±1,02	4,17±0,48
WPZ, μ	587,17±51,21	625,67±52,95	390,33±60,45**
DF, μ	564,33±40,30	251,67±18,30‡	285,00±13,26‡

In the late stages after AS reticular cells separated from the reticulum, round in shape, with a pale oval nucleus, could be seen in the cords and sinuses. Hyperemia, swelling, and desquamation of the endothelium were very conspicuous in the venules of the capsule and in the trabeculae.

Microscopic examination of the thymus showed only a significant increase in the number of pyroninophilic blast forms of thymocytes: on the 2nd-3rd day 6.67 ± 0.76 compared with 2.33 ± 0.42 under normal conditions ($P < 0.01$), and on the 8th-10th day 5.33 ± 0.93 ($P < 0.05$). Thickening of the cortex was observed on account of an increase in the number of lymphocytes. Characteristically no signs of accidental involution (rhexis, lysis, pycnosis, phagocytosis, migration of thymocytes, inversion of layers, multiplication of Hassall's corpuscles) were present in either experimental group. In most cases what was observed was lymphoid hyperplasia rather than involution of the organ.

When the possible causes of the changes in the lymphoid tissue are discussed, the first point to consider is the effect of factors such as corticosteroids, hypoxia, and the direct damaging action of the immune complex and of mediators of immediate allergy released under these circumstances.

Large doses of steroids are known to cause diffuse karyorhexis and destruction of cells in all lymphoid tissues (especially T lymphocytes) [10, 11, 12]. Since a high corticoid sensitivity is characteristic of thymocytes and T lymphocytes [1, 13], preservation of the T-dependent zone of the lymph node and absence of accidental involution in the thymus in the present experiments indicate that in the late stages after AS no hypersecretion of corticosteroids is observed. At the same time, during the first 1-1.5 h after the beginning of AS the blood corticosteroid level undoubtedly rises [3, 5].

An experimental study of the effect of various types of hypoxia on lymphoid tissue revealed selective injury to follicle cells, with a decrease in their size and number and a decrease in the number of lymphocytes in the paracortical zone [13]. Destructive and atrophic changes taking place selectively in the follicles also have been described during a study of

the lymphoid tissue of persons dying from shock and from dogs with experimental endotoxin and hemorrhagic shock [12, 13].

Disturbances of the microcirculation and the accompanying hypoxia, as a result of the antigen–antibody reaction and pathochemical changes developing in this situation are evidently decisive factors in the changes arising in lymphoid tissue during AS.

Lymphocyte proliferation in the germinative centers of the follicles, their blast transformation, and plasmatization of the lymph nodes, primarily of the follicles and medullary cords, in the present experiments belong to the humoral type of immune reaction. Proof of the action of a humoral factor of antigenic nature in the late stages after AS on the lymphoid tissue is given by the presence of blast cells not only in the lymph nodes, but also in the thymus.

When the plasma-cell response in lymphoid tissue after AS is discussed it must also be recalled that this response, reflecting specific immunologic adaptation of the organism in response to antigenic stimulation, is also one feature of the general adaptation syndrome, i.e., a stereotypic nonspecific adaptive response to injury [2]. Evidence in support of this view is given by the increase in the number of pyroninophilic (plasma) cells in the lymphoid tissue of experimental animals with stress caused by electrical stimulation, by subcutaneous injection of formalin, and by operative trauma [6, 8, 9].

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