VIROLOGY

Morphofunctional Characteristics of Antigen-Presenting Cells in Lymph Node in Mice with Experimental West Nile Fever

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Pronounced transformation of cells in mesenteric lymph nodes, mainly in the thymus-independent zone and sinuses, was detected in albino mice experimentally infected with West Nile fever (strain 986). Maximum antigen-presenting activity was exhibited by activated macrophages, minimum activity — by dendritic cells of lymphoid follicles.

Key Words: *West Nile fever; antigen-presenting cells*

The first reports about antigen-presenting cells (APC) appeared in 1970s [2]. At present, APC are regarded as the most potent elements of the immune system playing the key role in the induction and regulation of primary adaptive immune response. APC include Langerhans cells of skin epidermis and other squamous epithelia, dendritic cells of the primary and secondary follicles in B-zones of the spleen and lymphoid tissue, interdigitate cells of the thymus, monocytes, B cells, and macrophages.

A unique role of APC is recognition and elimination of virtually all antigens. These cells accumulate and present antigens on their surface, thus protecting and regulating the adaptive immune response.

We studied antigen-presenting elements in lymph nodes of mice infected with West Nile (WN) virus.

MATERIALS AND METHODS

WN fever was modeled at the Laboratory of Arbovirus Infections, D. I. Ivanovskii Institute of Virology. Random-bred albino mice were intraperitoneally infected with WN virus (strain 986, 0.3 ml D_{50}). The animals were divided into 4 groups by the reaction to infection and were sacrificed under ether narcosis on days 3-4 (incubation period, group 1), 7-8 (group 3, manifest clinical symptoms), and 11-16 (group 4, asymptomatic period) of the experiment. Group 2 included animals dead at the peak of clinical manifestation (days 5-6 postinfection). Intact animals served as controls.

The material (intraperitoneal lymph nodes) was fixed in 10% neutral formalin and Carnoy fluid.

Micropreparations were stained with hematoxylin and eosin, Azur-eosin, according to Brachet, and according to Feulgen. The number of APC/100 μ^3 was counted by the stereometrical method [1]. The data were statistically processed (Table 1).

RESULTS

Plethoric capillaries, stasis, marginal state of leukocytes and monocytes, accumulations of monocytic cells around vessels were seen in group 1; the boundary between the cortical layer and medulla was blurred. Mature lymphocytes were scanty and formed a thin strip between the cortical matter and paracortical zone. Centroblasts, immunoblasts, lymphoplasmacytic cells, and activated macrophages (more basophilic on

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azur-eosin stained preparation and demonstrating positive Brachet reaction) predominated in the cortical matter and medulla.

Many light polygonal cells with large poorly stained nuclei, with minor and medium-sized lymphocytes on their surface were seen in subcapsular and medullary sinuses. Multinuclear (3-6 nuclei) cells with clear cytoplasm and light nuclei (small diffusely scattered chromatin granules) were seen mainly in the subcapsular sinuses. Desquamation of endothelial elements of lymphatic sinuses and their transformation (presumably, fusion) into multinuclear cells were seen. Weakly positive or no reactions were observed in Brachet- and Feulgen-stained multinuclear cells. Solitary polygonal dendritic cells with stretched processes and minor and medium-sized lymphocytes around them were seen in the cortical layer instead of follicles (Fig. 1). The phenomenon of minor and medium lymphocyte adhesion was more pronounced for activated macrophages situated in all compartments of the lymph node (in the sinuses, hilus, efferent lymph vessels). The number of phagocytic macrophages increased, particularly in the cortical zone, secondary follicles, marginal and medullary sinuses (Table 1).

Examination of the intraperitoneal lymph nodes from group 2 mice showed blurred interface between the cortical, paracortical, and medullary layers. Medium-sized and large lymphocytes with low density and slight Brachet and Feulgen reactions predominated. The contours of many medium and large lymphocytes were blurred, unclear, many lysed cells were seen. Macrophages were virtually absent. Numerous small blue granules (azur-eosin) were seen in lymphatic sinuses. The capillaries were collapsed and surrounded by erythrocytes. Free erythrocytes were found in pulp cords. Degenerative macrophages were seen in sinuses. Solitary dendritic cells, also degenerative, were seen in the subcapsular and paracortical zones. The number of phagocytosing macrophages decreased in all structural components of the lymph node (Table 1).

Pronounced hyperplasia of germinative centers was observed in lymph nodes of group 3 mice: medium-sized and large lymphocytes predominated and only few minor lymphocytes were seen at the periphery of the follicle. The paracortical zone was poorly discernible. Hypertrophic dendritic cells (3-4 per visual field) were seen in the follicle centers; minor and medium lymphocytes were seen along these cells' processes. Many active macrophages were seen mainly in the efferent sinuses of the lymph node (Fig. 2). Macrophages were enlarged, their cytoplasm contained clear bubbles and solitary dense droplets well stained with eosin. Minor and medium lymphocytes formed peculiar rosettes on the surface of macrophages.

TABLE 1. Number of APC/100 μ^3 in Intraperitoneal Lymph Nodes of Albino Mice Infected with WN Virus (*M*±*m*, *n*=6)

Structural components	Dendritic cells in groups					Phagocytosing macrophages in groups				
	control	1	2	3	4	control	1	2	3	4
Zone cortical	0	8.0±1.8*	0	8.0±1.2*	4.00±0.07*	16.0±0.4	44.0±1.7*	8.0±0.5*	29.0±1.2*	34.0±1.8*
paracortical	10.0±0.8	24.0±1.4*	4.00±0.03*	28.0±1.7*	28.0±2.4*	7.00±0.03	18.0±0.9*	3.00±0.07*	24.0±0.9*	22.0±1.1*
Pulp cords	11.0±1.1	8.0±1.1	3.00±0.02*	7.0±1.1*	6.0±1.1*	24.0±0.7	40.0±1.9*	8.00±0.07*	34.0±1.7*	44.0±1.4*
Follicles										
primary	7.0±0.6	12.0±1.3*	8.0±0.8	6.0±1.1	12.0±1.7*	8.0±0.1	19.0±1.1*	4.00±0.07*	27.0±1.3*	30.0±1.1*
with reactive centers	8.0±1.1	7.0±1.1	4.00±0.03*	14.0±1.8*	17.0±1.4*	21.0±0.7	49.0±2.1*	11.00±0.17*	39.0±1.9*	37.0±1.7*
Sinuses										
marginal	0	0	0	0	0	12.0±0.3	34.0±2.1*	17.0±1.8*	39.0±2.4*	18.0±1.7*
intermediate	0	0	0	0	0	8.00±0.07	13.00±0.06*	2.00±0.01*	18.0±1.4*	12.0±1.1
medullar	0	0	0	0	0	14.0±0.4	32.0±3.4*	18.0±1.4	36.0±1.7*	28.0±1.9*
portal	0	0	0	0	0	6.00±0.08	5.00±0.07	12.0±1.2*	29.0±2.1*	32.0±1.9*

Note. *p<0.05 compared to normal.

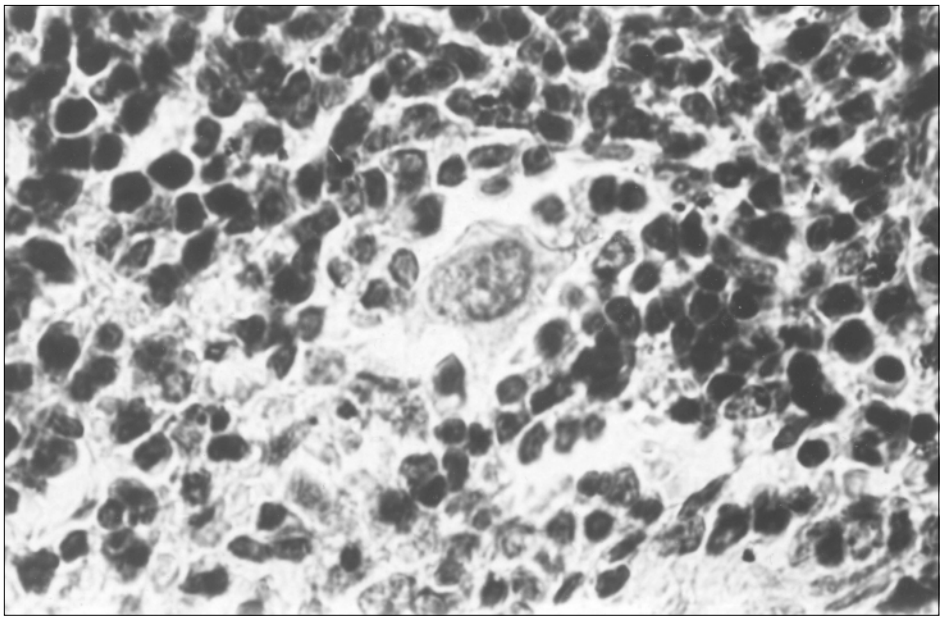

Fig. 1. Intraperitoneal lymph node (day 4 of experiment). Cortical layer, central part of secondary follicle. Group of dendritic cells with minor and medium lymphocytes around them. Azur-eosin staining, ×680.

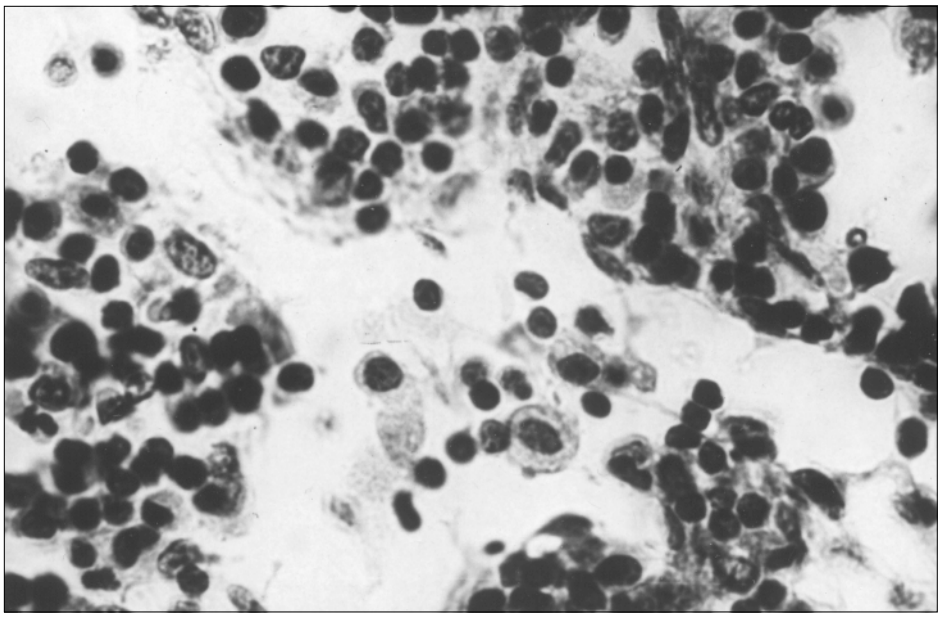

Fig. 2. Intraperitoneal lymph node (day 8 of experiment). Efferent sinus of the lymph node. Numerous activated macrophages with adhering lymphocytes. Azur-eosin staining, ×680.

A similar histological picture was observed in group 4. However the follicles were more clearly seen in this group, the contours of the paracortical zone were clear-cut, and the efferent lymphatic sinuses contained more active macrophages without signs of degeneration.

Hence, transformation of lymph nodes at the early stage of infectious process (incubation period and peak of clinical manifestations) involves mainly the thymus-independent zone and cells of the sinus, which is characteristic of primary antigenic stimulation. APC include mainly activated macrophages and, to a lesser extent, dendritic cells. On days 11-15 of the disease the thymus-dependent zone of the lymph node is activated and the number of phagocytizing macrophages sharply increases. A characteristic feature of morphological changes in the lymph nodes of mice dead during the experiment was weak reaction of macrophages and endothelial cells in the marginal, intermediate, medullary, and portal sinuses, predominance of non-activated lymphocytes (monomorphic picture), pronounced degenerative and necrotic changes in lymphoid elements, and the absence of APC activation.

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