MEDULLARY CORD CYTOARCHITECTONICS OF REGIONAL LYMPH NODES OF RATS WITH CHRONIC MALATHION POISONING

V. N. Grigor'ev, N. K. Gainanova, and T. M. Zaiko

UDC 612.42:591.5-018.615.365

KEY WORDS: malathion; lymph nodes; cytoarchitectonics

Pesticides and, in particular, malathion, constitute a large group of factors which may affect man [1, 4], but without which modern agriculture would be inconceivable [1]. The starting point of this research was the idea that the most noticeable effect of exposure of mammals to the action of environmental factors can be detected in the lymph nodes, which are organs of peripheral immunity [2, 3, 7], controlling the systems of contact and exchange with the external environment.

In the investigation described below we investigated the mesenteric lymph nodes of experimental animals, being among the first such nodes to make contact with pesticides when ingested in excess with the diet, the hepatic lymph nodes, receiving lymph from the liver, to which the pesticide is carried via the system of the portal vein, and the popliteal lymph nodes, exposed to the action of the pesticide only indirectly.

EXPERIMENTAL METHOD

Experiments were carried out on 125 male Wistar albino rats weighing 190-210 g, divided into experimental groups as follows. Intact animals (25) constituted the normal group, 25 animals received an oily solution of malathion through a metal tube before taking food, in a dose of 2 ARD (ARD denotes allowable residual dose of pesticide in the food, which in the case of malathion is 0.033 mg/kg daily) daily for 1 month. Animals of the other group (25 rats) received the pesticide similarly in a dose of 4 ARD. The animals were studied immediately after the end of the month's course of feeding with the pesticide. To study the possibility of chemical imprinting the 50 remaining animals received an oily solution of malathion in a dose of 1 ARD for 1 month. After preliminary division of the animals into two groups 1 month after the end of this course, a 2nd month's course of malathion in a dose of 2 and 4 ARD respectively was given. Immediately after the end of poisoning, the animals were again investigated.

After decapitation one mesenteric, one hepatic, and one popliteal lymph node were removed from each animal and fixed in Bouin's fluid for 24 h, dehydrated in a series of alcohols, and embedded in paraffin wax. Paraffin sections 4-5 μ thick were cut on a sliding microtome and stained with Mayer's hematoxylin and eosin [4, 5, 7]. The cell composition of the medullary cords of the lymph nodes was studied in sections stained with azure II – eosin, using a covered square test system mounted in the microscope ocular. Statistical analysis was carried out by the method used for the normal distribution of features [4, 6]. Since one lymph node was taken from each situation and from each animal, and since one section cut through the hilus of the gland was studied from one lymph node, the number N in all cases was 25. The electron-microscopic preparative procedures were carried out by the traditional method [8, 9] and were used for general survey purposes.

Laboratory of Microlymphology, Research Laboratory of Clinical and Experimental Lymphology, Siberian Branch, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'nol Biologil i Meditsiny, Vol. 113, No. 3, pp. 315-318, March, 1992. Original article submitted April 19, 1991.

 0007-4888/92/0003-0412$12.50 ©1992 Plenum Publishing Corporation

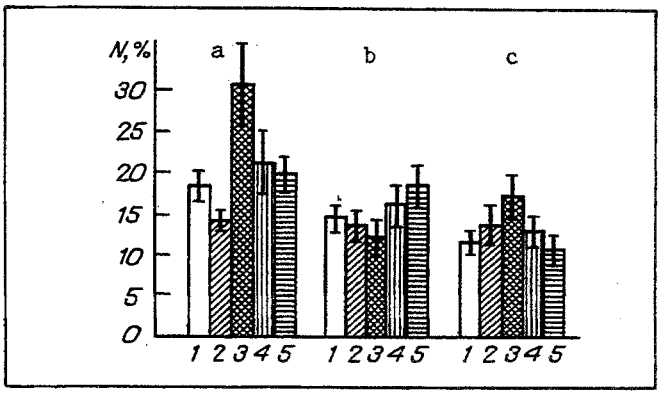

Fig. 1. Results of investigation of specific area of medullary cords of mesenteric, hepatic, and popliteal lymph nodes of rats poisoned with different doses of malathion: a) mesenteric, b) hepatic, c) popliteal lymph nodes; 1) normal state, 2) 2 ARD, 3) 4 ARD, 4) 2 ARD, 5) 4 ARD after imprinting.

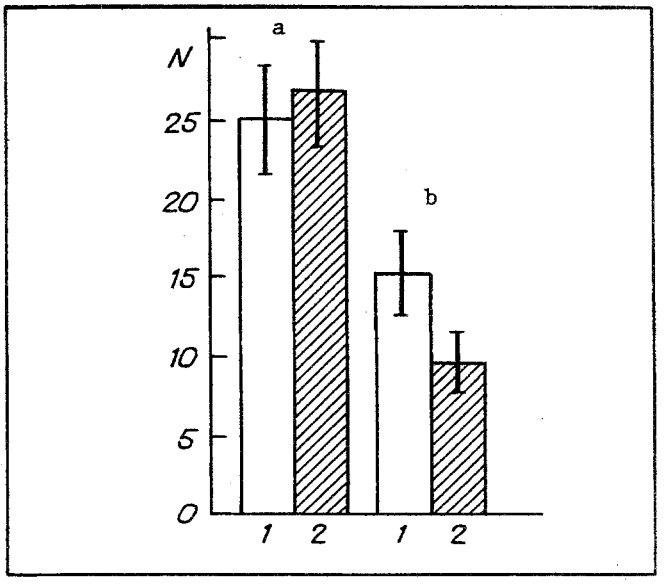

Fig 2. Results of investigation of numerical density of cells (per 1000 μ^2 area of section) of medullary cords of rat mesenteric lymph nodes 1 month after poisoning with malathion in a dose of 4 ARD: a) medullary cords, b) medullary sinuses; 1) normal state, 2) 4 ARD.

EXPERIMENTAL RESULTS

The preliminary study of the microanatomical organization of the lymph nodes showed the most marked changes in the medullary cords of the mesenteric lymph nodes of the rat 1 month after poisoning with malathion in a dose of 4 ARD: under these conditions their specific area was more than doubled compared with the control (Fig. 1). Analysis of the numerical density of the cells in the medullary cords of the mesenteric lymph nodes revealed no significant differences from normal, i.e., an increase was observed in the absolute number of cells in the population of this region (Fig. 2). Meanwhile the numerical density of cells in the medullary sinuses showed a significant decrease, and in conjunction with data in the literature [2, 8, 9], this suggested that cells may have migrated from the lumen of the sinus into the lymphoid parenchyma of the medullary cords. In fact, such pictures were found on electron-microscopic observation (Fig. 3).

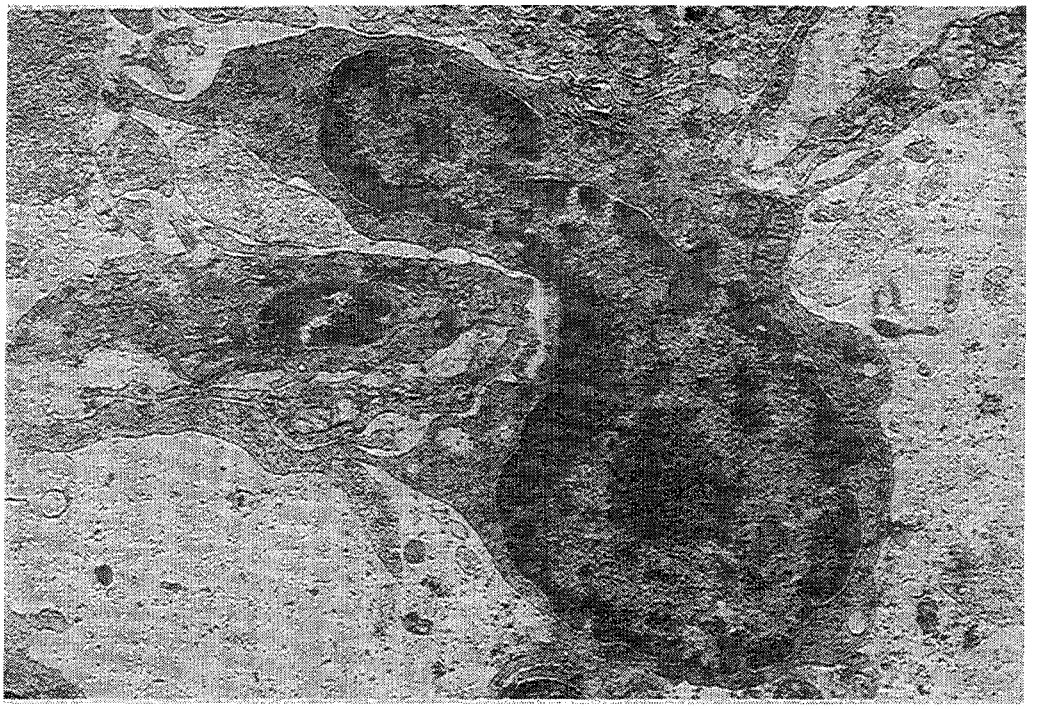

Fig. 3. Migration of medium-sized lymphocyte from lumen of medullary sinus of rat mesenteric lymph node into lymphoid parenchyma of medullary cord 1 month after poisoning with malathion in a dose of 4 ARD 14,500×.

Analysis of the cytoarchitectonics of the mesenteric lymph nodes of rats after a month's course of malathion in a dose of 2 ARD revealed a significant decrease in the relative number of small lymphocytes in the structure of the lymphoid parenchyma of the medullary cords and an increase in the number of medium sized lymphocytes and immunoblasts (Table 1). The increase in the number of plasma cells was accompanied by a decrease in the relative number of plasmablasts, which were evidently involved in the process of more rapid differentiation to different forms of plasma cells [2, 3, 5]. The number of macrophages, degenerating cells, erythrocytes, and mitoses increased, but not significantly, and mast cells appeared. Poisoning with a dose of 4 ARD led to more marked changes in the cytoarchitectonics of the lymphoid parenchyma of the medullary cords of the mesenteric lymph nodes. There was a sharp increase in the relative number of medium-sized lymphocytes, immunoblasts, and plasmablasts. The number of immature plasma cells increased, whereas the number of mature plasma cells decreased appreciably, indicating a disturbance of maturation of cells of the plasmacytic series, and the timely transition of different forms into the next stage of development [1, 3, 4, 7]. There was a sharp increase in the number of macrophagelike forms of reticular cells and macrophages against the background of an increase of many times in the relative number of degenerating cells. Besides mast cells, neutrophils and eosinophils appeared and mitotic activity was sharply reduced (Table 1).

Imprinted animals responded to subsequent malathion poisoning in a much more restrained manner; a dose of 2 ARD, moreover, was accompanied by the development of a compensatory reaction, in the form of accumulation of many small lymphocytes in the medullary cords [1, 2]. Poisoning with malathion in a dose of 4 ARD enabled the imprinted animal only to reduce substantially the adverse effects of this aggression on the lymphoid parenchyma of the medullary cords of the rats' mesenteric lymph nodes (Table 1).

Whereas a plasma-cell response of the lymphoid parenchyma of the medullary cords to poisoning by the pesticide predominated in the mesenteric lymph nodes, the macrophagal system gave the maximal response in the hepatic lymph nodes. The severity of the tissue damage and the reaction of the imprinted animals followed the same trend as in the mesenteric lymph nodes. It is remarkable that whereas malathion in a dose of 2 ARD caused no increase in the relative number of macrophages in the medullary cords of the unimprinted animals, the imprinted animals gave a violent macrophagal response, despite the fact that differences in the numbers of degenerating cells from the normal state were not significant. This indicates the greater potential of the macrophages for completion and subsequent reproduction of many

TABLE 1. Changes in Cytoarchitectonics of Medullary Cords of Mesenteric, Hepatic, and Popliteal Lymph Nodes of Rats Poisoned with Various Doses of Malathion ($\bar{x} \pm S_{\bar{x}}$)

Cells (N)	Normal state	Feeding with malathion for 1 month		Feeding imprinted animals for 1 month with malathion	
		2 ARD	4 ARD	2 ARD	4 ARD
1	2	3	4	5	6
Mesenteric lymph nodes					
Small lymphocytes	24,5±2,38	4,90±0,52*	8,63±0,82*	31,93±2,75*	18,22±2,34
Medium-sized lymphocytes	8,02±0,73	10,90±1,00*	4,27±0,53*	7,52±0,83	10,54±0,95*
Immunoblasts	1,07±0,06	3,27±0,31*	0,67±0,08*	2,63±0,25*	2,27±0,28*
Plasmablasts	6,29±0,47	3,44±0,35*	0,27±0,03*	4,95±0,53*	7,79±0,82
Immature plasma cells	9,79±0,87	12,80±0,93*	16,70±1,80*	5,74±0,62*	6,67±0,53*
Mature plasma cells	34,26±2,73	44,50±3,27*	26,90±2,57*	32,73±3,47	29,59±3,05
Mott's cells	0,32±0,02	0,59±0,06*	0,62±0,06*	0,25±0,03	—
Reticular	11,94±1,27	12,84±1,32	26,76±1,43*	9,73±0,95	16,57±1,52*
Monocytes	0,65±0,04	0,54±0,06	0,54±0,06	1,02±0,12*	1,54±0,13*
Macrophages	1,07±0,06	1,58±0,12*	6,57±0,64*	0,93±0,09	2,86±0,31*
Neutrophils	—	—	0,82±0,08*	—	0,52±0,05*
Eosinophils	—	—	0,95±0,09*	—	0,47±0,05*
Mast cells	—	0,29±0,03*	0,76±0,07*	—	0,25±0,03*
Erythrocytes	0,20±0,02	0,67±0,07*	0,98±0,08*	—	0,20±0,03
Degenerating	0,97±0,05	2,14±0,22*	4,99±0,53*	0,85±0,08	1,52±0,14*
Mitoses	1,20±0,11	1,54±0,13*	0,52±0,05*	1,72±0,16*	0,99±0,12
Hepatic lymph nodes					
Small lymphocytes	27,90±3,14	29,65±3,14	14,05±1,27*	31,40±3,52	24,93±2,56
Medium-sized lymphocytes	7,24±0,70	8,67±0,72	6,53±0,67	7,53±0,82	7,74±0,75
Immunoblasts	0,69±0,08	0,94±0,08*	1,56±0,12*	1,12±0,12*	0,99±0,09*
Plasmablasts	3,95±0,40	3,52±0,41	5,67±0,52*	4,73±0,42	6,82±0,75*
Immature plasma cells	5,67±0,74	4,86±0,53	1,52±0,12*	5,12±0,53	4,17±0,42
Mature plasma cells	28,90±2,25	26,75±2,84	29,54±3,53	24,12±2,53	20,31±2,42*
Mott's cells	—	0,56±0,06*	0,64±0,07*	0,37±0,04*	0,47±0,05*
Reticular	16,73±1,82	14,53±1,25	18,71±2,36	12,10±1,12*	17,52±1,82
Monocytes	2,16±0,24	1,87±0,19	0,52±0,05*	1,93±0,21	3,24±0,36*
Macrophages	3,56±0,37	4,12±0,43	13,63±0,77*	8,75±0,93*	9,74±0,97*
Neutrophils	0,52±0,05	0,67±0,07	1,29±0,12*	0,62±0,07	0,51±0,06
Eosinophils	—	0,52±0,05*	0,14±0,02*	—	—
Mast cells	0,14±0,02	0,12±0,01	0,87±0,09*	—	0,27±0,03*
Erythrocytes	0,37±0,04	0,45±0,05	0,95±0,09*	0,29±0,04	0,37±0,04
Degenerating	1,52±0,16	2,03±0,14*	3,53±0,32*	1,37±0,14	2,00±0,10
Mitoses	0,65±0,06	0,74±0,07	0,86±0,09	0,55±0,06	0,92±0,09*
Small lymphocytes	50,18±5,23	63,96±6,66	61,73±5,72	39,70±4,13	32,12±3,57*
Medium-sized lymphocytes	8,93±0,76	6,54±0,62*	5,37±0,54*	7,53±0,76	8,32±0,86
Immunoblasts	0,75±0,08	0,27±0,03*	0,32±0,04*	1,12±0,13*	0,95±0,09
Plasmablasts	2,44±0,25	0,87±0,09*	0,93±0,09*	2,75±0,31	1,95±0,24
Immature plasma cells	2,76±0,31	1,56±0,17*	1,43±0,15*	3,14±0,29	2,79±0,31
Mature plasma cells	25,72±2,46	15,27±1,63*	16,27±1,75*	28,56±3,14	32,75±3,00
Mott's cells	—	—	—	—	0,52±0,05*
Reticular	7,54±0,76	6,74±0,73	8,27±0,95	9,29±0,95	10,36±0,92*
Monocytes	0,35±0,04	0,55±0,05*	0,67±0,06*	1,12±0,10*	1,53±0,15*
Macrophages	0,52±0,06	0,47±0,05	0,63±0,07	1,23±0,11*	1,32±0,14*
Neutrophils	—	—	—	0,27±0,03*	0,32±0,03*
Eosinophils	—	1,27±0,11*	2,13±0,22*	2,93±0,32*	3,84±0,41*
Mast cells	—	0,95±0,09*	0,82±0,08*	1,52±0,16*	1,76±0,18*
Erythrocytes	—	—	—	—	0,32±0,03*
Degenerating	0,54±0,06	0,63±0,06	0,67±0,07	0,52±0,05	0,67±0,07
Mitoses	0,27±0,03	0,92±0,09*	0,76±0,08*	0,32±0,03	0,48±0,05*

Legend. Asterisk indicates values differing significantly ($p < 0.05$) from normal; N) relative number of cells (as a percentage of their total number).

trace reactions [2, 5, 7]. In the popliteal lymph nodes of the rats malathion poisoning led to marked inhibition of the plasma-cell branch, which was virtually independent of the dose of the pesticide. Whereas the number of degenerating cells and macrophages in this region did not differ significantly from normal, the number of eosinophils and mast cells was very great (Table 1). It is remarkable that whereas imprinted animals responded to a second course of malathion by maintenance of a stable relative number of cells of the plasma-cell series at the normal level, the number of monocytes, macrophages, eosinophils, and mast cells continued to rise appreciably, whereas poisoning with malathion in a dose of 4 ARD led

to the appearance even of erythrocytes in the lymphoid parenchyma of the medullary cords, evidence either of intensification of diapedesis of these cells through the endothelial lining [9, 10], or of damage to that structure [1]. Clearly, under conditions of imprinting, quite different cell responses are observed in the popliteal lymph nodes.

Enteral administration of malathion in doses of 2 and 4 ARD thus leads to a marked plasma-cell reaction in the mesenteric lymph nodes, to a marked macrophagal reaction in the hepatic lymph nodes, and to a marked granulocytic reaction in the popliteal lymph nodes. Moreover, whereas preliminary imprinting has been shown to have a favorable effect on the mesenteric and hepatic lymph nodes, its effects on the popliteal lymph nodes is characterized by responses in opposite directions: restoration of the normal plasma cell population accompanied by a rapid increase in the number of granulocytes.

LITERATURE CITED

1. Yu. I. Borodin, Arkh. Anat. Gistol. Embriol., **96**, No. 6, 5 (1989).
2. N. V. Vasil'ev, Cytologic Characteristics of Reactions of Lymphoid Tissue in Antibody Formation [in Russian], Tomsk (1975), pp, 128-153.
3. G. V. Kovalevskii, Outlines of Immunomorphology [in Russian], Novosibirsk (1976), p. 264.
4. V. V. Kupriyanov, Yu. I. Borodin, Ya. L. Karaganov, et al., Microlymphology [in Russian], Moscow (1983).
5. V. V. Serov and A. B. Shekhter, Connective Tissue [in Russian], Moscow (1981).
6. T. Tasche, Introduction to Quantitative Cytohistologic Morphology [in Russian], Bucharest (1980).
7. N. A. Yurina and A. I. Radostina, The Macrophagal System [in Russian], Moscow (1978).
8. J. R. Casley-Smith, Microvasc Res., **19**, No. 2, 244 (1980).
9. S. C. Luk, C. Nopajaroonsri, and G. T. Simon, Lab. Invest., **29**, No. 2, 258 (1973).