HISTOLOGICAL AND RADIOMETRIC STUDIES OF THE IMMUNOCOMPETENT SYSTEM IN RABBITS WITH ATHEROSCLEROSIS

P. V. Pigarevskii, V. A. Nagornev, and Yu. N. Zubzhitskii

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Changes in the immunocompetent organs during the development of atherosclerosis in rabbits were investigated histologically and radiometrically. An increase in the number of cells of the plasma-cell series in the sinuses of the medullary layer of the mesenteric lymph nodes was accompanied by a decrease in their over-all proliferative activity. It is suggested that cells of the immune response in atherosclerosis are formed not by active proliferation, but by differentiation of original small lymphocytes. The results broaden the view of the immune genesis of atherosclerosis.

KEY WORDS: atherosclerosis; immunocompetent system; mesenteric lymph nodes.

Recent investigations have shown the important role of immunological factors in the morphogenesis and pathogenesis of experimental atherosclerosis. In particular, antibodies have been found against β- and pre-β-lipoproteins, and an autoimmune complex (atherogenic lipoprotein – IgG) capable of being fixed in the vessel wall in experimental animals [3, 4], has been isolated and studied. The dynamics of circulation of antibodies against vessel wall antigens and relations between fixed antibodies and the character of morphological manifestation of atherosclerosis have been studied [1, 2]. However, there have been no investigations of the organs of immunity, so that it is impossible to draw a complete picture of the place of immune reactions in the pathogenesis of experimental atherosclerosis.

The object of this investigation was to study changes taking place in the immunocompetent organs during the development of atherosclerosis in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 44 noninbred rabbits weighing 2.5-3.0 kg. Experimental atherosclerosis was induced by Academician N. N. Anichkov's method by injecting cholesterol dissolved in sunflower oil, in a dose of 0.5 g/kg body weight daily through a gastric tube.

Nine animals were studied 1 week, six rabbits 2 weeks, six 3 weeks, 17 4 weeks, and six 6 weeks after the beginning of the experiments. Twelve normal rabbits also were investigated. The mesenteric lymph nodes, spleen, bone marrow, thymus, Peyer's patches, and the lymphoid diverticulum were taken for histological and radiometric analysis.

During the radiometric investigation 1 h before the rabbits were sacrificed they were given an intravenous injection of methyl-^{3}H-thymidine in a dose of 1 μCi/g body weight (specific activity 14-17 μCi/ml). Pieces of tissue were weighed and dissolved without residue in a Beckman tissue solubilizer (BTS). The samples were poured into measuring flasks and treated with 10 ml scintillation fluid. They were then counted on an Isocap/300 liquid scintillation counter. The results were expressed as the number of counts per minute per milligram weight of the organ.

The histological material was fixed in Carnoy's fluid and embedded in paraffin wax. Sections 3 μ thick were stained with methyl green–pyronine by Brachet's method, with hematoxylin-eosin, and with azure-eosin.

Laboratory of Morphology of the Cardiovascular System, N. N. Anichkov Department of Atherosclerosis and Lipid Biochemistry, and Laboratory of Immunomorphology, Department of Microbiology and Immunology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 7, pp. 101-103, July, 1978. Original article submitted January 20, 1978.

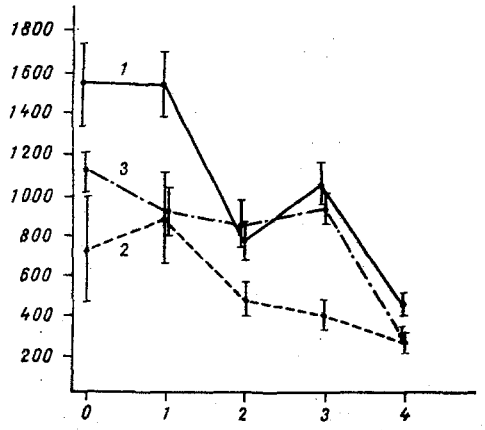

Fig. 1. Changes in specific proliferative activity in secondary lymphoid organs. 1) Mesenteric lymph nodes; 2) spleen; 3) lymphoid diverticulum. Abscissa, times of cholesterol feeding (in weeks); ordinate, specific activity (in cpm/mg tissue).

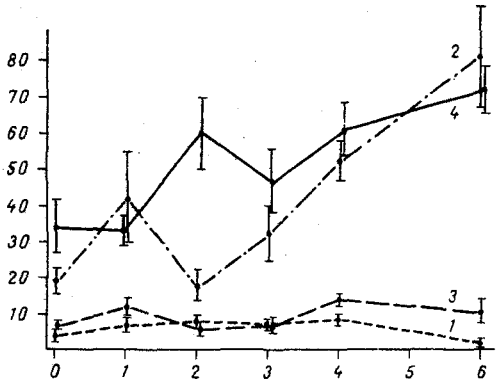

Fig. 2. Quantitative changes in cell composition in medulla of mesenteric lymph nodes: 1) blast cells; 2) plasmablasts; 3) immature plasma cells; 4) small lymphocytes. Abscissa, times of cholesterol feeding (in weeks); ordinate, mean number of cells per 10 fields of vision.

Three zones were studied in the lymph nodes, sections through which passed in strict succession through the hilus of the lymph node: the cortex, the paracortical zone, and the medulla. After staining by Brachet's method, the types of cells which are the most characteristic indicators of the immune response were counted in these zones: blast cells, plasmablasts, immature and mature plasma cells, and small lymphocytes. Ten fields of vision were counted in each of the three zones by means of a grid; the results were analyzed by Student's criterion. The arithmetic mean values were plotted graphically (the method of calculation of the standard error and confidence intervals of the arithmetic means was used).

EXPERIMENTAL RESULTS

In the secondary lymphoid organs (mesenteric lymph nodes, spleen) and the lymphoid diverticulum a reduction in proliferative activity, as measured by the radiometric method, was observed in all zones starting with the 2nd week of feeding the rabbits on an atherogenic diet (Fig. 1). After 4 weeks of the experiments the proliferative activity of the mesenteric lymph nodes was one-quarter that of the activity of normal tissues ($P < 0.05$), the activity of the spleen was reduced to two-sevenths ($P < 0.05$), and that of the lymphatic diverticulum to one-fifth ($P < 0.05$).

Despite the reduction in proliferative activity, histological quantitative analysis of the mesenteric lymph nodes revealed accumulation and an increase in the number of different types of cells belonging to the plasma-cell series, especially plasmablasts but also small lymphocytes, in the sinuses of the medulla. For instance, after feeding the rabbits with cholesterol for 1 week the number of plasmablasts in the medullary sinuses was 2.3 times greater than in the normal organs, and the number of blast cells and immature plasma cells was doubled ($P < 0.05$). Later, after feeding for 3, 4, and 6 weeks, a constant increase in the number of cells of the plasma-cell series was observed; for example, after 4 weeks of the experiment the number of plasmablasts was increased by 3 times ($P < 0.05$) and the number of blast cells and immature plasma cells was doubled ($P < 0.05$; Fig. 2). Histological analysis of the cortex of the same lymph nodes at the above-mentioned times of the experiment showed that well-developed germinal centers could be distinguished in half of the animals studied. All intermediate forms from small lymphocyte to plasmablast could be clearly made out in them, although as a rule there were no mitotic figures. Among small lymphocytes in the dense mantle zone cells of the plasma-cell series of different degrees of maturity could frequently be seen, probably as they left the germinal centers. The medullary layer of the mesenteric lymph nodes was evidently filled through the peripheral and intermediate sinuses. This is all the more likely since at the 2nd, 3rd, and 4th weeks of the experiment peripheral and intermediate sinuses filled mainly with plasmablasts could frequently be seen. The paracortical zone showed no histological changes at any time of the experiments. In the spleen a weak and inconstant cellular reaction was observed, and was manifested as some increase in the number of blast cells on the boundary between the white and red pulp.

Comparison of the results obtained by histological and radiometric methods of investigation of lymphoid tissue in the initial stages of experimental atherosclerosis thus points to an increase in the number of cells of the plasma-cell series accompanied by a decrease in their proliferative activity. It can tentatively be suggested that cells taking part in the immune response in atherosclerosis are formed in the germinal centers, not by active proliferation, but by differentiation of original small lymphocytes. It is difficult to accept that these cells enter the medullary sinuses from the primary organs of the lymphoid system, for during radiometric investigation the proliferative reaction in the bone marrow and thymus at the above-mentioned times of the experiment did not differ significantly from normal.

Even though the role of immunologic factors in the genesis of experimental atherosclerosis has been proved, the process as described above differs significantly from the immune reactions developing after injection of such foreign antigens as sheep's red blood cells [5]. This difference is expressed as a decrease in the over-all proliferative activity of the lymph nodes, whereas in most immune processes it increases. On the other hand, marked accumulation of immunoblasts was observed in the medulla despite the complete passivity of the paracortical and the relative passivity of the cortical zone.

From the standpoint of the autoimmune theory of atherosclerosis the results of these experiments can be compared with those of Glynn [6], who observed similar infiltration of the medulla of lymph nodes by plasma cells in systemic lupus erythematosus, but found no change in the paracortical zone, even when atrophy of the germinal centers took place. The appearance of many cells of the plasma-cell series in the sinuses may indicate that a β-type of immune response developed.

The data obtained at the various times supplement the results of investigations which showed the presence of circulating antibodies against β- and pre-β-lipoproteins, as well as the formation of the corresponding autoimmune complex during the 2nd and 3rd weeks that rabbits were kept on an atherogenic diet, although no macroscopically visible atherosclerotic lesions were present at that time.

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