IMMUNOLOGICAL CHARACTERISTICS OF EXPERIMENTAL ATHEROSCLEROSIS

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The role of the following immunological factors, acting in a certain sequence, in the development of experimental atherosclerosis in rabbits was demonstrated: formation of antibodies against atherogenic lipoproteins; formation of the corresponding circulating immune complexes inducing the initial lesion of the vessel wall; progression of the atherosclerotic lesions with the formation of secondary autoantigens of the vessel walls and the response to them, aggravating the existing immunopathological processes.

Previous investigations [1-3, 5] have shown that selective depression of the immunological response of rabbits to homologous atherogenic lipoproteins prevented the development of experimental atherosclerosis in the animals during prolonged cholesterol feeding by N. N. Anichkov's method. This suggested a role of immunological factors in the development of atherosclerosis, acting in a certain sequence: the formation of (auto-) antibodies against atherogenic lipoproteins; the formation of corresponding circulating immune complexes inducing the initial lesion of the vessel wall; progression of the lesions with the formation of atherosclerotic plaques and deeper destruction of the vessel walls; the appearance of secondary autoantigens (vascular antigens) and the response to them, aggravating the existing immunopathological process.

A few reports have been published [6, 7] of the isolation (admittedly rarely) of immune complexes among the lipoproteins and autoantibodies in the sera of patients with atherosclerosis. There is no corresponding evidence as regards experimental atherosclerosis.

The next step to be taken was, accordingly, to determine the more detailed immunological characteristics of experimental atherosclerosis, viz.: to determine the curve of free antibodies against homologous lipoproteins; to study the dynamics of accumulation of the immune complex (atherogenic lipoprotein – autoantibodies) and its circulation and deposition in the wall of the aorta; to determine the level of complement as an indicator of the formation of immune complexes; to investigate local allergic skin reactions to atherogenic lipoproteins and to vascular antigens.

EXPERIMENTAL METHOD

Experiments were carried out on 89 male rabbits weighing 2.5-3 kg. Experimental atherosclerosis was induced by N. N. Anichkov's method, giving cholesterol in a daily dose of 0.5 g. The immunological tests were carried out at weekly intervals starting from the first day after the beginning of cholesterol feeding and continuing until the 26th week.

Free antibodies (8 rabbits) were determined by the complement fixation test (CFT at 4°C) with atherogenic lipoproteins (total fraction of β- and pre-β-lipoproteins) isolated by the method of Klimov et al. [4] from the sera of "hypercholesterol" rabbits and frozen to −70°C.

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TABLE 1. Immunological Characteristics of Experimental Atherosclerosis

Immunological reaction	Duration of cholesterol feeding (in weeks)																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14—21	22	23	24	25	26
Determination of anti-lipoprotein antibodies in blood serum	1:2	1:32	1:8	1:2	0	0		0	0			0	0	0					0
Determination of anti-lipoprotein antibodies isolated from circulating immune bodies	0	0	1:2	1:4	0	1:2	1:4		1:8		1:32	1:64		1:8	1:4	0			0
Skin tests for immune complex	0	+	2+	3+	0	+	3+	3+	3+										
Fixation of components of immune complex in aortic walls:																			
lipoprotein	0	0	+	2+	3+	3+	3+	3+	3+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
γ-globulin	0	0	+	+	2+	2+	2+	3+	3+	3+	3+	2+	2+	2+	2+	2+	2+	2+	2+
Determination of complement level in blood serum	1:16	1:32	1:32	0	0	1:16	1:4	1:4	1:8	1:2	1:8	1:16	1:8						
Skin tests with atherogenic lipoproteins	0	+	2+	2+	2+	3+	3+	3+		0	0	0	0	0	0	0	0	0	0
Skin tests with vascular antigen	0	0	0	0	0	0	+	2+		3+	3+	3+	3+	3+	3+	2+	2+	+	+

Note. Mean values of antibody titers, complement level, and area of skin reactions are given. 0) Negative results.

The result was taken as positive if hemolysis was completely inhibited with a double dose of complement.

Immune complexes were isolated in two ways: 1) directly from the blood serum (44 rabbits) and 2) by intradermal tests (11 rabbits). The immune complexes were prepared and tested by the following scheme: precipitation of the immune complex by fractionation of the serum with a current of CO_2 (by Ioffe's method); resuspension of the precipitate in Michaelis buffer at pH 2.5 and precipitation of the antigen (lipoproteins) with $BaSO_4$ (by the method of Burstein et al. [8]); isolation of antibodies from the supernatant and their concentration with ammonium sulfate; determination of their specificity in the CFT with atherogenic lipoproteins. The use of skin tests is based on the established ability of immune complexes (in a small excess of antigen) to induce a local reaction when injected intradermally into an animal not only of another, but also of the same species, or even into the same animal. To reproduce local reactions native sera were injected intradermally in doses of 0.05-0.1 ml into healthy rabbits and also into cholesterol-fed rabbits, including the donors of the sera. The reactions were read after 18-20 h in accordance with the diameter of the inflammatory zone: + (0.5-1 cm), ++ (1-1.5 cm), +++ (1.5-2 cm). The same system was used to evaluate local allergic reactions in the experimental and control animals to atherogenic lipoproteins (prepared by Witebsky's method from aortas of normal rabbits). Antigens were injected in doses of 0.06 and 0.6 mg protein in a volume of 0.1 ml. These doses did not cause reactions in the control animals.

Activity of complement in the sera from 12 experimental and four control animals was determined repeatedly at intervals of three days by the standard method. Immunoluminescent investigation of the aortic wall for the presence of components of the immune complex was carried out by the classical Coons' method using antisera against rabbit lipoproteins, γ-globulin, and fibrin in 44 rabbits tested serologically for the presence of circulating immune complex and exsanguinated at different times.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. Free antibodies against atherogenic lipoproteins were obtained in very low concentration towards the end of the first week of cholesterol feeding of the animals and the maximal titer (1 : 32) was obtained by the end of the second week of the experiment after which the concentration of antibodies fell gradually until they disappeared by the 5th week of cholesterol feeding and did not subsequently reappear.

Circulating immune complex (44 rabbits tested, four rabbits at each time) was first detected only in the third week after the beginning of the experiment, both by skin tests and isolation of antilipoprotein antibodies from the sera. The latter were found in the 3rd-7th weeks of the experiment in low concentration, although none could be found in the 5th week; at this period no immune complex could be found in the test sera by means of skin tests. Later, parallel with progression of the atherosclerotic lesions, the level of specific antibodies which could be isolated from the circulating immune complex rose sharply to reach a maximum (1 : 64) by the 11th-12th week from the beginning of the experiment. The immune complex could be detected regularly in sera taken in the 7th-9th weeks after their use in the skin test (no tests were carried out with the sera obtained later).

Immunomorphological investigations starting with the 3rd-4th week revealed fixation of the components of the immune complex (lipoproteins and autologous γ-globulin) in the aortic wall of the experimental rabbits in the same areas of the intima (Fig. 1). Definite fixation of the immune complex in the tissues was found at all subsequent times of observation, including in the 6th week, i.e., at the time when circulating complex first disappeared from the blood. This phenomenon can evidently be explained by its fixation in the tissues.

Under these circumstances the permeability of the endothelial barrier was evidently increased, as shown by fixation of fibrin in increasing amounts in the aortic wall.

The dynamics of the changes in the complement level corresponded to the observations on circulating and fixed immune complexes described. For instance, the first considerable decrease in the complement level also was observed in the 4th-5th week, corresponding to the initial morphological manifestations of atherosclerosis, possibly as a result of the fixation of complement by immune complex localized in the vessel wall. Later, a wave-like curve at a lowered level with distinct "troughs" was observed, for example, in the 7th-8th week, when fixation of γ-globulin on elastic fibers in the disintegrating atherosclerotic plaques could be observed simultaneously by immunomorphological methods.

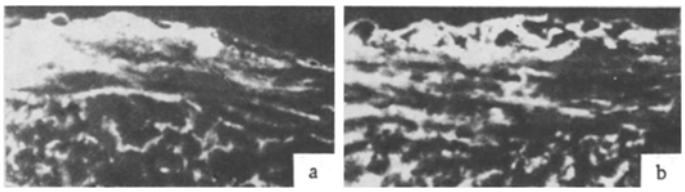

Fig. 1. Localization of lipoproteins and γ-globulin in the aortic wall of a rabbit: a) luminescence of lipoproteins; b) luminescence of γ-globulin in a parallel section. Duration of cholesterol feeding 4 weeks; treated by the Coon's method, objective 40, ocular, water immersion, 1.7.

Finally, attention is drawn to the characteristic dynamics of the allergic skin reactions reflecting sensitization to the atherogenic and vascular antigens. Positive reactions to atherogenic lipoproteins (the total fraction of β- and pre-β-lipoproteins) were observed in the experimental animals by the end of the 2nd week from the beginning of the experiment; they reached their maximal intensity in the 7th-8th week and disappeared in the 10th week of the experiment. Reactions to the vascular antigen appeared much later – in the 7th-8th week (the end of the period of greatest intensity of the skin reaction with lipoproteins). They remained positive at the 22nd week of the experiment and gradually disappeared by the 26th week.

The results described above thus confirmed the hypothesis of a role of immunological factors in the development of experimental atherosclerosis, of their character (an initiating factor, in the form of the immune response to atherogenic lipoproteins and autosensitization to vascular antigens in the later stages), and of the sequence of immunological events in the form in which it was described provisionally at the beginning of this paper. The immunological characteristics of experimental atherosclerosis described above cannot, of course, claim to be exhaustive, and much still requires close study. Nevertheless, the results so far obtained provide a sufficiently sound basis and reference point for the study of factors influencing experimental atherosclerosis.

They also permit some degree of extrapolation toward clinical problems, with an indication of ways and methods of investigation.

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