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EFFECT OF IMMUNIZATION WITH SMALL DOSES OF ANTIGEN ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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Some authorities consider that sensitization of man and animals under the influence of foreign information potentiates the development of atherosclerosis [9, 14]. Investigations [12, 13] have shown, for instance, that keeping animals on a high-cholesterol diet, accompanied by immunization with various antigens, leads to more severe changes in the aorta than the high-cholesterol diet alone for the same duration. However, it should be pointed out that other workers [8, 10] have found that injection of heterologous proteins have a protective effect against the development of atherosclerosis.

In the experiments described above antigens of different types were injected in different doses before the beginning of cholesterol feeding or during feeding, so that the results were not at all comparable. Nevertheless the study of the character of the antigen, its dose, and the times of its injection is an essential factor for the understanding of the role of immunization in the development of experimental atherosclerosis.

The object of this investigation was to study the effect of immunization on the development of atherosclerosis in rabbits with established experimental hyperlipidemia, on the grounds that in atherosclerosis the affected individual is frequently exposed to antigen stimulation, and its role in the course of the disease has not yet been explained.

EXPERIMENTAL METHOD

Experiments were carred out on male rabbits weighing 2.5 kg. Atherosclerosis was induced by feeding the animals on a diet containing 500 mg cholesterol daily [2] for 13-15 weeks.

Both autologous γ-globulin obtained by the salting out method [7] and heterologous (human) therapeutic standard γ-globulin were used for immunization. Heterologous γ-globulin was injected subcutaneously during the 6th or 9th week of cholesterol feeding on three successive days (20, 25, and 30 mg protein, respectively). The course of injections was repeated after an interval of 7 days (total dose of antigen injected 150 mg). Since autologous protein is much less antigenic than heterologous, the course of immunization was longer and was not interrupted [4]: Autologous γ-globulin (total dose 163 mg) was injected in the 11th week of cholesterol feeding on ten consecutive days, intravenously in increasing concentrations (from 5 to 30 mg protein), together with mechanically disintegrated yeast cells (*Candida albicans;* from 1.2 to 12 mg protein, total dose of yeast protein 62 mg). The last injection in both series of experiments contained γ-globulin labeled with ^{125}I [11], and it was given intravenously. A culture of *C. albicans* was used because it has common antigens with vascular tissue structures [6], so that cross-reacting antibodies injuring the vessel

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TABLE 1. Lipid Content and Atherosclerotic Index of Aortas of Rabbits Immunized with Autologous γ-Globulin and with Disintegrated *C. albicans* Cells (M ± m)

Group of animals	Number of animals	Antigen injected	Time of determination	Component detected, mg%			Atherosclerotic index, percent
				β- and pre-β-lipoprotein	total cholesterol	triglycerides	
1	5	—	Before immunization	1005±422	383±125	71±41	24±15
			On 28th day after beginning of immunization	2070±596	748±156	186±116	
			Increase compared with initial values	2,06±0,33	1,95±0,33	2,62±0,96	
2	4	Autologous γ-globulin + disintegrated C. albicans	Before immunization	881±478	317±123	88±30	29±25
			On 28th day after beginning of immunization	1200±154	504±118	110±51	
			Increase compared with initial values	1,36±0,80*	1,59±0,80	1,25±0,49*	
3	5	Disintegrated C. albicans	Before immunization	725±100	248±83	65±33	23±9
			On 28th day after beginning of immunization	925±310	432±93	61±35	
			Increase compared with initial values	1,28±0,14*	1,74±0,48	0,94±0,46*	

*P < 0.05 compared with values in group 1.

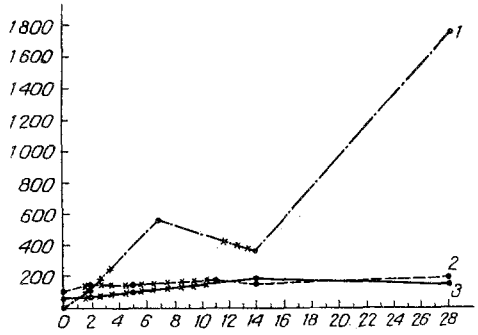

Fig. 1. Skin tests after injection of various antigens into rabbits (area of hyperemia). 1) human γ-globulin; 2) disintegrated *C. albicans* cells; 3) autologous γ-globulin. Abscissa, time after beginning of immunization (in days); ordinate, area of hyperemia (in mm²); injection of antigen indicated by asterisks.

wall can be induced. The level of sensitization was judged from disappearance of labeled antigen from the blood after 1, 2, 3, 4, 6, 8, 15, and 18 days, and also from the area of hyperemia 14 h after a skin test (1.5-2 mg protein injected intradermally on the 2nd, 7th, 14th, and 28th days after the beginning of immunization).

Before immunization and on the 28th day after its beginning (the time of development of skin reactions of the Arthus phenomenon type) the concentrations of β- and pre-β-lipoproteins in the blood serum of the rabbits were determined by the method in [5]; cholesterol and triglycerides were estimated on the AA-2 automatic analyzer (Technicon).

At the end of the experiment the aorta was removed and stained with Sudan III-IV to calculate the area affected, by Avtandilov's method [1] — the atherosclerotic index.

EXPERIMENTAL RESULTS

The changes in the area of hyperemia in skin tests and in the rate of elimination of labeled antigen from the blood stream (Figs. 1 and 2) indicate the formation of antibodies in response to immunization with human γ-globulin and the absence of an immune response to injection of autologous γ-globulin and of *C. albicans* [15].

In experiments with autologous γ-globulin (Table 1), on the 28th day after the beginning of immunization the increase in the concentration of β- and pre-β-lipoproteins, total cholesterol, and triglycerides in the blood serum was not so sharp as in the unimmunized group, in all probability because of injection of the disintegrated *C. albicans* cells and not

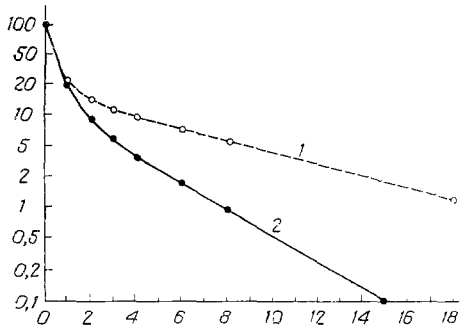

Fig. 2

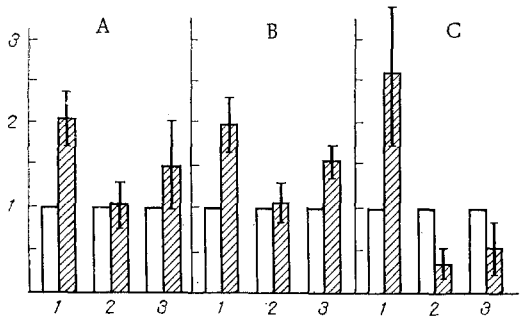

Fig. 3

Fig. 2. Concentration of ^{125}I-labeled γ-globulin in blood of rabbits after immunization (blood level of ^{125}I immediately after injection taken as 100%). 1) Autologous γ-globulin; 2) heterologous γ-globulin. Abscissa, time after injection of labeled antigen (in days); ordinate, percent of antigen in total blood volume.

Fig. 3. Changes in concentration of lipids in blood serum of rabbit fed with cholesterol on immunization with human γ-globulin. Unshaded columns — lipid concentration before immunization (taken as unity); shaded columns — lipid concentration on 28th day after beginning of immunization. A) β- and pre-β-lipoprotein; B) total cholesterol; C) triglycerides. 1) Cholesterol feeding without immunization (n = 5); 2) injection of antigen in 6th week of feeding (n = 4); 3) injection of antigen in 9th week of feeding (n = 8).

of autologous γ-globulin.

Immunization of the rabbits with human γ-globulin led to stabilization of the levels of β- and pre-β-lipoproteins and of total cholesterol and to a fall in the triglyceride level in the blood; this effect, moreover, was more marked when the animals were immunized in the 6th week and in the 9th week of cholesterol feeding (Fig. 3). After 13 weeks of cholesterol feeding the atherosclerotic index of the aortas of rabbits receiving injections in the 6th week (13 ± 9%) or in the 9th week (17 ± 7%) was lower than would be expected in the case of feeding by the same scheme but without immunization (about 40 ± 3%).

Injections of small doses of foreign protein into rabbits thus causes a definite protective effect against the development of experimental hyperlipidemia and atherosclerosis; this effect is more clearly defined in the case of earlier immunization relative to the beginning of onset of hyperlipidemia.

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