EFFECT OF CYCLOPHOSPHAMIDE ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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In adult rabbits receiving an atherogenic diet combined with an immunodepressant (cyclophosphamide) atherosclerosis either did not develop or was extremely mild. At the same time, no deposition of β-lipoproteins was observed in the aortic wall. Cyclophosphamide did not depress total protein synthesis, for the experimental animals developed a marked hyper-β-lipoproteinemia.

Recent investigations have shown that immune processes probably play a role in the development of atherosclerosis. For example, in atherosclerosis in man and experimental animals specific immunological changes have been found: antibodies against antigens of the vessel wall [2, 3, 5, 8, 18] or to cholesterol [19], and the presence of a circulating immune complex consisting of β-lipoproteins and the corresponding antibody [11-13]. In other investigations the development of atherosclerotic lesions has been successfully inhibited by active immunization of rabbits with protein–cholesterol conjugates [10]. Similar results have been obtained by immunization of rabbits and cocks with heterologous lipoproteins [4, 6, 7, 14-16].

In previous experiments the writers obtained resistance to the development of experimental atherosclerosis when they injected atherogenic lipoproteins into newborn rabbits [1].

In the present investigation the effect of the immunodepressant cyclophosphamide on the genesis of experimental atherosclerosis was studied. An inhibitory action would confirm the hypothesis that immunopathological processes play a role in the formation of atherosclerotic plaques.

EXPERIMENTAL METHOD

Experiments were carried out on 19 rabbits weighing 2.5 kg, of which 12 animals (experimental group) received cyclophosphamide before and during administration of the atherogenic diet, while seven rabbits of the control group received cholesterol only.

To suppress the immunocomponent system the rabbits of the experimental group received preliminary injections of cyclophosphamide intravenously in doses of 20 mg/kg body weight daily, starting two weeks before the beginning of cholesterol feeding. The effectiveness of the dose was verified previously on the basis of inhibition of hemolysin production during immunization of rabbits with sheep's red cells. The high-cholesterol diet consisted of 0.5 g cholesterol dissolved in oil, administered through a tube for 17 weeks. Throughout this period the experimental rabbits continued to receive cyclophosphamide in the above mentioned dose twice a week. Each experimental rabbit thus received 44 injections of cyclophosphamide in a total dose of 2.2 g.

Every month the cholesterol and β-lipoprotein concentrations were determined in the blood serum of both groups of rabbits. By the end of the experiment the weight of the rabbits receiving cyclophosphamide was greater than the weight of the controls: 4 and 3.6 kg, respectively. At the end of the experiment the

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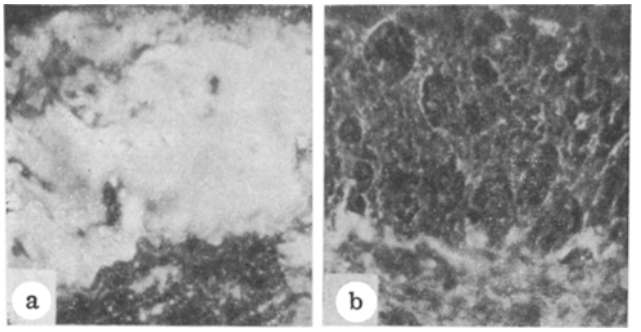

Fig. 1. Localization of lipids and β-lipoproteins in aortic wall of a rabbit with experimental atherosclerosis: a) fluorescence of lipids throughout the thickness of an atherosclerotic plaque when stained with 3,4-benzpyrene and photographed in ultraviolet light; b) specific fluorescence of β-lipoproteins in a parallel section stained by Coons' method, 68 ×.

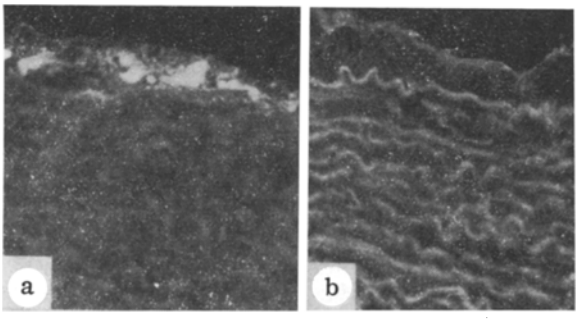

Fig. 2. Localization of lipids and β-lipoproteins in aortic wall of a rabbit receiving cyclophosphamide together with an atherogenic diet: a) fluorescence of total lipids in lipid stain when stained with 3,4-benzpyrene; b) absence of specific fluorescence of β-lipoproteins in parallel section stained by Coons' method, 68 ×.

rabbits were exsanguinated and the area of the atherosclerotic plaques in the aorta was measured by G. G. Avtandilov's planimetric method.

For histological investigation pieces were cut from different segments of the rabbits' aortas and sections were cut from them in a cryostat and stained for total lipids with 3,4-benzpyrene and for β-lipoproteins by the indirect Coons' method, using the corresponding antiserum containing antibodies in a titer of 1:10,000.

EXPERIMENTAL RESULTS

Postmortem examination of the experimental rabbits killed at the end of the experiment revealed growth adiposity of the animals with appreciable enlargement of the spleen and adrenals. The remaining internal organs showed no visible changes. On macroscopic and microscopic examination of the aortas of the control rabbits, marked atherosclerotic changes, such as are usually observed under these experimental conditions and are well known from investigations by other authors, were found. Immunoluminescence investigation of β-lipoproteins in sections from the atherosclerotic plaques showed bright specific fluorescence of lipoproteins where lipids were deposited throughout the thickness of the plaques (Fig. 1).

By contrast, in the experimental rabbits receiving the immunodepressant before and during administration of the atherogenic diet, atherosclerosis either did not develop or was very slight. The atherosclerotic index (in per cent), based on the area and intensity of the process, was significantly lower in the experimental rabbits (5.0 ± 3.4) than in the controls (52.3 ± 24.6).

Immunoluminescence investigations showed that the lipid stains and atherosclerotic plaques of the experimental animals contained no β-lipoproteins, whereas total lipids were revealed by staining with 3,4-benzpyrene (Fig. 2).

When the results of this experiment are discussed it must be remembered that the blood levels of β-lipoproteins and cholesterol in the animals of the experimental and control groups were not significantly different: β-lipoproteins in the experimental group 20.6 ± 12.7 and in the control 32 ± 1.8; cholesterol in the experimental group 16.2 ± 1.9 and in the control 18.5 ± 5.6. This is against the view that the immunodepressant causes significant inhibition of total protein synthesis, including the synthesis of proteins included among the β-globulins. On the other hand, sharp differences in deposition of β-lipoproteins in the aortic wall were found between the experimental and control rabbits: in rabbits receiving immunodepressant no fluorescence of β-lipoproteins was observed in the vessel wall. In the last case conditions or factors responsible for the deposition of lipoproteins in the vessel wall with the formation of atherosclerotic plaques were evidently absent. One such factor could probably be antibodies against β-lipoproteins whose production is inhibited by the immunodepressant. In this way, the formation of the antigen–antibody complex, which in other immunopathological states can be localized in blood vessel walls, is prevented. The possibility cannot be ruled out that the immunodepressant exerts some action on the cellular factors of allergic reactions, which have been inadequately studied in atherosclerosis.

Future investigations will shed further light on the role of immunological mechanisms in the pathogenesis of experimental atherosclerosis.

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