ANTIGENIC PROPERTIES OF INDIVIDUAL LAYERS OF THE HUMAN BLOOD VESSEL WALL

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Besides antigens common to all layers of the vessel wall, each separate layer has one or two specific antigens. No difference between analogous layers of intact and atherosclerotic vessels could be detected by means of antisera against individual walls of unchanged blood vessels.

In some diseases of the vascular system (atherosclerosis, endarteritis obliterans, etc.) antivascular antibodies are found in the patients' blood serum [1-4]. This indicates a possible role of immunochemical mechanisms in the development of these diseases. The fact that lesions of the intima, media, and adventitia differ in severity in different diseases of the vascular system suggests that besides other factors, changes in the antigenic structure of the vessel wall arising both in the course of life and also at various stages of development of the pathological process may also play an important role in their genesis. Evidence of this is given by the discovery of antibodies in the blood serum against individual layers of vessel walls in patients with atherosclerosis and with endarteritis obliterans [5, 6].

The object of the investigation described below was to study antigenic properties of individual layers (intima, media, and adventitia) of the wall of unchanged blood vessels and of blood vessels from persons with atherosclerosis.

EXPERIMENTAL METHOD

Blood vessels (aorta, carotid and iliac arteries) of persons dying from atherosclerosis at the age of 55, 58, and 63 years, and of persons aged 21 years dying from accidental causes, were used in the investigation.

The blood vessels were washed to remove blood and then carefully separated into intima, media, and adventitia, then again washed several times with cold physiological saline until the washing fluid was completely colorless. Each layer of the vessel wall was homogenized separately in a porcelain mortar with the addition of cold distilled water (9 ml/g tissue); the pH of the homogenate was adjusted to 9.4 by addition of 1% NaOH solution. After centrifugation at 1500 rpm for 30 min, the supernatant was treated with NaCl to give an 0.85% solution, and 1% acetic acid was then added to bring the pH of the fluid to 7.2. This fluid was then used as antigen.

Antisera against each of these layers of vascular tissue were obtained by immunizing rabbits with antigen mixed with Freund's complete adjuvant, injected subcutaneously into all the limbs. Immunization was carried out in three cycles at intervals of 30 days; each cycle of immunization included three injections at intervals of 3 days. The antigen was given in increasing doses measured as protein (from 6 mg at the first injection to 15 mg at the last). Protein was determined by Lowry's method.

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TABLE 1. Results of CFT and Agar Diffusion Test

Antiserum		Antigens: intima (titer of antivascular antibodies in CFT)	media (titer)	adventitia (titer)	human serum (titer)	intima (number of precipitation bands in agar)	media (number)	adventitia (number)
AIY	Whole	1:1000 / 1:1000	1:200 / 1:200	1:200 / 1:300	1:100 / 1:100	3 / 2—3	4—5 / 4—5	2 / 2
	Absorbed	1:400 / 1:400	1:50 / 1:55	1:50 / 1:30	—	1 / 1	0 / 0	0 / 0—1
AMY	Whole	1:200 / 1:300	1:600 / 1:700	1:100 / 1:150	1:200 / 1:200	1—2 / 1—2	2—4 / 2—3	0 / 0
	Absorbed	1:500 / 1:75	1:200 / 1:200	1:10 / 1:30	1:10 / 1:10	0—1 / 0—1	2 / 2	0 / 0
AAY	Whole	1:100 / 1:75	1:100 / 1:75	1:400 / 1:300	1:100 / 1:100	2—3 / 2—3	2—4 / 2—3	3 / 2—3
	Absorbed	1:10 / 1:5	1:10 / 1:25	1:200 / 1:150	1:10	0 / 0	0 / 0	1 / 1

Legend: AIY — antiserum against intima, AMY — against media, AAY — against adventitia of unchanged blood vessels. Reactions with antigens from unchanged vessels in numerator; with antigens from atherosclerotic vessels in denominator.

The antigenic composition of the individual layers of the unchanged vessels was studied by Ouchterlony's agar diffusion method, by immunoelectrophoresis in 1.5% Difco agar, and by the complement fixation test (CFT) in the cold. All tests were carried out with both whole and absorbed antisera. The antisera were absorbed by titrated doses of antigens from other, neighboring layers of the blood vessel wall and from healthy human serum. Altogether six series of antisera were obtained and the investigation was carried out with them.

RESULTS

Antiserum against the intima from unchanged blood vessels of young persons (AIY) formed three precipitation bands with homologous intima (IY), four bands with media from young subjects (MY), and two bands with adventitia of young subjects (AA) in agar gel, corresponding to the number of antigens in them. Some antigens gave a reaction of identity, particularly with media.

After absorption, AIY formed one precipitation band with IY only. Disappearance of the other bands was evidently caused by the presence of similar proteins, antigenically identical (for example, soluble connective-tissue proteins), in preparations from different layers of the vessel wall, and also by removal of antibodies against serum proteins from the antiserum after exhaustion (Fig. 1A).

Antiserum against young media (AMY) formed 4 precipitation bands with MY, 2 bands with IY, and one with AY. After absorption of AMY with antigens IY, AY, and healthy human serum, two bands remained with MY and one with IY (Fig. 1B).

Antiserum against young human adventitia (AAY) formed three precipitation bands with the homologous antigen AY, and 2-4 bands each with IY and MY, one of them common to all antigens.

Antiadventitial serum absorbed with antigens IY and MY and with human blood serum formed only one precipitation band with AY (Fig. 1C).

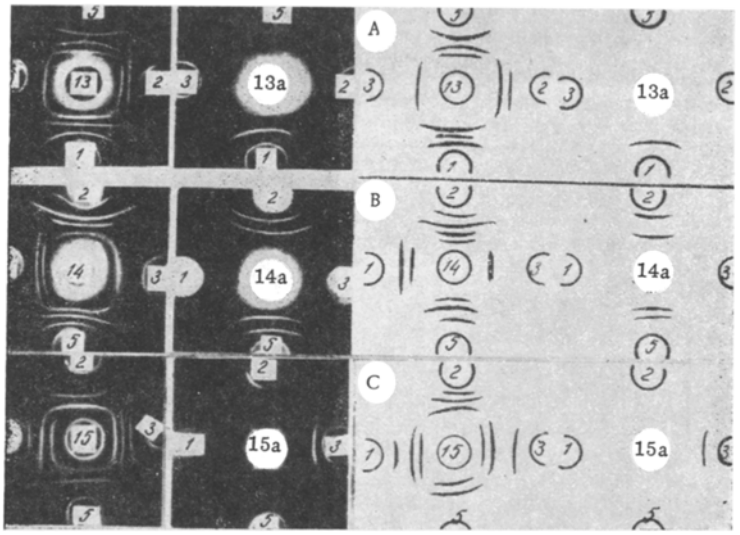

Fig. 1. Agar diffusion reaction between antisera against individual layers of wall of unchanged human blood vessels and antigens from layers of wall of unchanged human blood vessels. Antisera: 13) native, against intima; 14) against media; 15) against adventitia of unchanged vessels; 13a, 14a, and 15a) absorbed by neighboring layers of vessel wall, respectively. Antigens: 1) intima, 2) media, 3) adventitia of unchanged vessels, 5) media of atherosclerotically changed vessels.

The results of the agar diffusion reactions thus showed that individual layers of the blood vessel wall in young human subjects (unchanged vessels) each contains three or four antigens. The adventitia and intima each contain at least one specific antigen, while the media contains two specific antigens. In the precipitation test, clear results were not always obtained between AMY and MY.

The remaining antigens were related for all layers or were serum antigens (Table 1). Similar results were also obtained by immunoelectrophoresis in agar gel: absorbed antisera each formed one or two precipitation arcs with the corresponding antigens, and these were located in the zone of β and α globulins of the blood serum.

It is clear from Table 1 that in the CFT, AIY contained antibodies against IY in high titer (1:1000); the antibodies still remained in a fairly high titer after absorption of the antiserum (1:400), whereas antibodies against MY and AY remained in much lower titers – down to 1:50.

Whole antisera AMY and AAY contained antibodies against the corresponding antigen in titers up to 1:600 and 1:400. After their absorption, the titer of antibodies against the homologous antigen was lowered (to 1:200). They reacted in low titers (down to 1:10) with antigens from the other layers after absorption.

Comparative analysis of the antigenic structure of normal and atherosclerotically changed blood vessels by means of antisera against the layers of intact vessels revealed no special difference in antigenic structure on account of atherosclerosis (Table 1). Antiserum AIY revealed two to three antigens in the intima and adventitia of atherosclerotically changed vessels, while after absorption it revealed two antigens in MY and one (inconstant) antigen in IY. In the CFT no significant differences were found.

All three series of antisera contained antibodies against serum proteins, especially AMY, due to the impossibility of removing serum proteins from the tissue extracts used for immunization and in the reactions. Antisera absorbed by antigens IY, MY, and AY and by healthy human blood serum gave negative results, whereas their absorption by blood serum alone did not give negative results or cause disappearance of the precipitation bands.

The results thus show that besides antigens common all layers of the blood vessel wall, each layer also contains one or two specific antigens, detectable by the agar diffusion test and by immunoelectrophoretic analysis. The remaining proteins of the group are identical for these layers and may be either

extractable connective-tissue proteins of the intima, and adventitia, or serum proteins remaining in the tissue extracts. Finally, no antigenic differences could be detected by these experiments between the intima, media, and adventitia of the atherosclerotically changed vessels by the use of antisera against the separate layers of intact blood vessels. For this purpose, it is essential to use antisera obtained against layers of atherosclerotically changed vessels.

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