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Preliminary Note

Age-related changes in anti-elastin antibodies in serum from normal and atherosclerotic subjects

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Summary

A modified version of the enzyme-linked immunosorbent assay (ELISA), utilising human insoluble aortic elastin, was used for determination of anti-elastin antibodies in serum from normal and atherosclerotic subjects. The age-related changes in their level among healthy persons were investigated.

Anti-elastin antibodies were found in all the tested human sera, showing the highest level at the age of 18–20 and the lowest at the age over 60 and especially among atherosclerotic patients. The possible role of the immune system in the turnover of elastin is discussed.

Key words: Anti-elastin antibodies; Atherosclerosis; Elastin; ELISA; Turnover of elastin

Introduction

Elastin is the extracellular component mainly responsible for the elasticity of the human aorta and the connective tissue as a whole [1]. It was previously considered that there was no turnover of elastin in the mature organism [2]. However, some investigations showed the existence of biosynthesis of elastin not only in the growing, but also in the mature organism [3–6]. Recently we

reported that during this turnover some elastin-derived peptides may appear in human sera [7].

Bearing in mind the investigations of [8], who found anti-elastin antibodies in normal and pathological human sera, we investigated in more detail the eventual role of the immune system in the human organism. The main purpose of our work was the introduction of some immunological techniques, based on the main principles of the enzyme-linked immunosorbent assay (ELISA), in order to find out whether it is possible by such immunological methods to evaluate the condition of elastic structures in the human organism, mainly those in the arterial wall. In this study we discuss the possible role of the immune system in the turnover of elastin.

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Materials and methods

Human sera

Human sera were obtained from healthy subjects of different age and from patients with clinically manifested atherosclerosis. The sera to be tested were grouped as follows: I – sera from 1–7-year-old children, 40 cases; II – sera from 18–20-year-old healthy subjects, 102 cases; III – sera from 30–40-year-old healthy subjects, 40 cases; IV – sera from 41–50-year-old healthy subjects, 40 cases; V – sera from 51–60-year-old healthy subjects, 40 cases; VI – sera from 61–75-year-old healthy subjects. The persons included in these 6 groups had no clinical or laboratory signs of atherosclerosis. The next group (VII) consisted of sera from patients with clinically manifest atherosclerosis (88 cases). All of them showed signs of heart and brain ischaemia, and marked dyslipidaemia (cholesterol over 9.0 mmol/l, LDL over 6.0 g/l and HDL less than 1.5 g/l), and the main diagnosis in the hospital records was ‘atherosclerosis generalisata’.

Antigen

The material for the preparation of elastin was taken from the aorta of a 30-year-old healthy person (killed by accident), with no macroscopically detectable alterations of the intima. Insoluble elastin was prepared according to [9].

Enzyme-linked immunosorbent assay (ELISA)

This was carried out according to [10]. We modified ELISA for utilisation of insoluble antigens in an assay for detection of specific antibodies. The following reagents were used: human insoluble elastin, obtained from the aorta of a 30-year-old healthy person, killed by accident; immunoconjugate: rabbit-anti-human IgG, conjugated with horse-radish peroxidase (HRP type VI, Sigma Chemical Co., St. Louis, U.S.A.) according to [11]. The immunoconjugate was diluted 1:600 in diluting buffer (0.15 M phosphate-buffered saline, pH 7.2, containing 1% bovine serum albumin and 0.05% Tween 20); substrate solution: *o*-phenylenediamin (0.4 mg/ml in 0.05 M citrate buffer, pH 5.0) and 0.01% H₂O₂.

Before starting the immuno-enzyme assay 1 ml of each serum to be tested was incubated with 2

mg insoluble human aortic elastin for 1 h at 37°C. The samples were then washed 6 times with ice-cold saline and centrifuged at 5000 × g for 15 min at 4°C. The immune complex of insoluble elastin and anti-elastin antibodies was resuspended in 0.5 ml 2% solution of NaCl by incubation for 10 min at 60°C. Then the samples were centrifuged again (at the above-mentioned conditions) and the supernatants were tested for the detection of anti-elastin antibodies.

Polystyrene plates were first coated with 100 µl of the ‘eluted’ anti-elastin-antibodies (diluted 1:2 in distilled water) by incubation for 3 h at 37°C and overnight at 4°C. Immediately before use plates were washed 3 times to remove unbound protein. The wells were then filled with 100 µl immunoconjugate and were incubated for 30 min at 37°C. After washing the plates thoroughly with diluting buffer the last step was carried out – incubation with substrate solution for 1 h at room temperature (20°C) in a dark chamber. The reaction was then stopped by adding 50 µl 8 N H₂SO₄. The extent of the reaction was measured spectrophotometrically at 492 nm after transferring the reaction mixture into a test-tube, containing 850 µl distilled water. The results were evaluated statistically by analysis of variance and the *t*-test [12].

Results

The mean extinction values (± SEM) obtained after the testing of the investigated human sera are presented in Table 1. We compared the mean extinction values for the sera of all the healthy subjects (from group I to group VI) in order to estimate the age-related changes in the level of the anti-elastin antibodies (AEAB) in their sera. Group I (the healthy children) showed statistically significant difference with all the other groups. When comparing group II with the rest, the smallest difference was established with group I, while in comparison with the others, group II showed markedly higher levels of AEAB. There was no statistically reliable difference among groups III, IV and V, but between these groups and the other groups statistically significant differences were established. In the serum from the 61–75-year-old healthy subjects (group VI) we found statistically

TABLE 1
EXTINCTION VALUES, OBTAINED AFTER TESTING OF HUMAN SERA BY THE MODIFIED VERSION OF ELISA.
Values are mean \pm SEM. The age range of the tested groups is given in brackets.

Tested groups	Extinction	Comparison with the other groups (value of <i>P</i> less than:)						
		I	II	III	IV	V	VI	VII
I (1-7)	0.697 \pm 0.050	-	0.05	0.02	0.02	0.01	0.001	0.001
II (18-20)	0.732 \pm 0.136	0.05	-	0.001	0.001	0.001	0.001	0.001
III (30-40)	0.655 \pm 0.090	0.02	0.001	-	-	-	0.001	0.001
IV (41-50)	0.660 \pm 0.080	0.02	0.001	-	-	-	0.001	0.001
V (51-60)	0.640 \pm 0.120	0.01	0.001	-	-	-	0.001	0.001
VI (61-75)	0.560 \pm 0.098	0.001	0.001	0.001	0.001	0.001	-	0.001
VII (50-75- athero- sclero- tic)	0.366 \pm 0.122	0.001	0.001	0.001	0.001	0.001	0.001	-

significant lower level of AEAB as compared with that in the serum of the younger.

The following tendency in the age-related changes of the level of AEAB in the serum of healthy subjects was established. The children show relatively high levels of AEAB and in the serum of the 18-20-year-old subjects the levels are even higher. After that age the level stabilises at lower values up to the age of sixty. Then it slowly decreases.

The lowest level of AEAB was found in the serum from atherosclerotic patients (group VII) in comparison with that in sera from healthy subjects.

Discussion

Our results showed that AEAB were present in all the sera tested. Such AEAB were found in sera from normal and atherosclerotic persons by using the passive haemagglutination assay with heterologous antigen - bovine kappa-elastin [8]. Those results were confirmed by [13,14] by using their

method of measurement of the consumption of the autocomplement. In our investigation we introduced a modified version of ELISA, utilising insoluble human aortic elastin, in order to improve the specificity of the assay, as the insoluble elastin is considered to have more intact molecular and antigenic structure than its soluble derivatives.

The existence of AEAB in all sera suggests that they are not autoaggressive. It is possible that the intact elastic fibre is inaccessible for these AEAB by virtue of its structure. Electron-microscopic investigations [15] have shown that the elastic fibres are covered by structural microfibrils, composed of glycoproteins. Some authors [16] observed in their ultrastructural immuno-histochemical investigations cross-reactions between these structural microfibrils and some other glycoprotein components of the connective tissue. Some antigenic similarity between the structural glycoproteins of the blood vessel wall and some glycoprotein components of the serum were found by [3,17]. The immune system may be tolerant to the

structural microfibrils due to their cross-reactivity with other glycoproteins in the human organism. Thus the intact elastin of the elastic fibre could be protected by these structural microfibrils against the 'aggression' of the AEAB.

The AEAB may be directed against the elastin peptides, derived during the degradation of the elastic fibres along with their turnover [8]. There is evidence that such elastin peptides are released during the turnover of the elastic fibres [7,18,19]. Probably, these peptides carry some antigenic determinants that are otherwise hidden inside the macromolecular elastin complex. Along with the degradation of elastin by elastases these hidden antigenic sites may be exposed and recognised by the immunocompetent cells as 'foreign'.

One of the possible functions of these 'physiological' AEAB may be the binding of the circulating elastin-derived peptides and the enhancing of their final degradation and elimination. Another possible function may be the recognition and 'marking' of the old or somehow altered elastic structures, which have exposed their 'hidden' antigenic determinants. The AEAB would bind to those sites and attract cells (monocytes for example) that would secrete elastase [20] and would hydrolyse the altered elastic structures. But during this degradation more elastin peptides would be released and they would attract more monocytes via their chemotactic activity [21]. This positive feed-back may lead to uncontrollable destruction of the elastic structures in the region. And here the AEAB may play an important role by binding the elastin peptides and blocking their chemotactic activity for monocytes, as it was established by [21].

This hypothesis is relevant for the mature organism with stable turnover of elastin. But there are some age-related changes in this 'balance' between the activity of the immune response towards elastin and the activity of the turnover of the latter.

Children show relatively high levels of AEAB and this may be related to the active remodeling of the elastic structures during growth of the organism. This relatively high turnover of elastin is confirmed by the results of [22]. But the level of AEAB in the serum of children is lower than that in the serum of the 18–20-year-old subjects. This

may be due to the relative immaturity of the immune system in children.

Recent investigations [1,4] show that the activity of elastin turnover increases at advanced age (over 60). It is logical to suppose that the immune system should react to this increased stimulation with increased production of AEAB. But their level in the serum of the old subjects is low.

In fact the production of AEAB may be activated but they can be consumed by the higher level of elastin peptides, as well as by the increased amount of altered elastic structures. The deposition of AEAB in the region of atherosclerotic lesions was shown by [23,24]. Though only persons with no clinical or laboratory signs of atherosclerosis were included in the group of 'old healthy subjects' (group VI), we cannot deny the possibility of the existence of some age-related minor lesions of the elastic structures in the arterial wall, or the elastic fibres in the skin or in lung, and they may consume AEAB.

Another possibility is that the production of AEAB may really decrease in old subjects due to aging of the immune system or to regulatory mechanisms, which do not allow the activation of the immune response towards elastin peptides to go over certain limits. The hyperactivity of the immune system against elastin may cause damage, as it may activate the elastolytic processes via the attraction of monocytes.

A similar explanation may be offered for the atherosclerotic patients, but as the above-mentioned processes are expressed to a greater degree, the decrease in the level of AEAB is more marked. We suppose that this decrease is mainly due to the restriction of the immune response towards elastin, as the uncontrolled activity may aggravate the atherosclerotic process. This conclusion is based on the investigations of [3,25–27], who caused experimental atherosclerosis in rabbits via immunisation with elastin antigens. On the other hand [28] reported that if an increased cholesterol diet is combined with immunosuppressive therapy, no atherosclerosis develops. Thus we conclude that the restriction of the immune response towards elastin may be a significant adaptive mechanism for limitation of the destruction of the elastic structures.

The immune system may play some physiologi-

cal role in the turnover of elastin by enhancing the final elimination of elastin peptides from the serum, by marking the altered elastic structures and by blocking the chemotactic activity of elastin peptides for elastase-producing cells and thus regulating the activity of the elastolytic process. The age-related changes in the level of the AEAB mirror the changes in the activity of the turnover of elastin (to a certain extent) up to the age of 60. We confirm the results of [8,13,14] that the level of AEAB in the serum of atherosclerotic patients is considerably lower than that in the serum of healthy persons and we suggest that this decrease may be an adaptive mechanism for limitations of the destruction of elastic structures.

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