

# Age-Related Changes of Anti-Elastin Antibodies in Senescence-Accelerated Mice

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## Key Words

Anti-elastin antibodies · Anti-tropoelastin antibodies · Elastin · Senescence-accelerated mice

## Abstract

**Background:** Antibodies recognizing the elastin precursor tropoelastin (ATEAb) or degradation products  $\alpha$ -elastin (AEAb) are found in the serum of healthy human subjects, as a part of a homeostatic mechanism which assembles new or clears altered elastin structures. Serum ATEAb (reflecting elastin synthesis) and AEAb (reflecting elastin destruction) appear to correlate with the production and breakdown of the elastic tissue, respectively. **Objective:** The aim of this study was to investigate plasma levels of AEAb and ATEAb in senescence-accelerated prone (SAMP8) and senescence-accelerated resistant (SAMR1) mice, compared with imprinting control region (ICR) mice in order to evaluate their age-related changes. **Methods:** The levels of AEAb and ATEAb were measured by home-made ELISA in plasma of SAMP8, SAMR1, and ICR mice, grouped according to their age (3 and 9 months) and sex. The specificity of AEAb and ATEAb activity in mouse plasma, and elastin-derived peptides (EDP) in sera

of ICR mice at 3 and 9 months of age were tested by competitive ELISA. **Results:** The specificity of AEAb and ATEAb in mouse plasma was confirmed by the competitive investigations. The levels of AEAb in the plasma of SAMR1 and SAMP8 were increased compared to the levels measured in ICR on the matched ages ( $p < 0.001$ ). Age-related increase of the levels of AEAb and ATEAb was established in the 3 strains ( $p < 0.001$ ). Significantly higher levels of AEAb were established in female 9-month-old mice compared to males in all strains. The ATEAb:AEAb ratio was significantly lower in the SAM compared to the ICR strain. Positive correlation was established between the levels of serum AEAb and EDP in mouse sera of ICR mice. **Conclusion:** Variations with age in the plasma levels of AEAb and ATEAb were established in SAM compared with ICR, and in SAMP8 compared with SAMR1. Our findings suggest that increased anti-elastin IgG autoantibodies could be used as a marker of aging in SAM and possibly contribute to the processes of aging. The absence of a difference between SAMP8 and SAMR1 regarding the ATEAb:AEAb ratio raises the question if SAMR1 are an appropriate control of SAMP8 in terms of the senescence of the elastic tissues.

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## Introduction

The elasticity of distensible tissues such as arterial wall, skin and lung is associated with the presence of elastic fibers in the extracellular matrix. Mature elastic fibers consist of 2 morphologically distinct components: a core comprised of amorphous material containing elastin (90%) and, interspersed in the periphery, a microfibrillar component (10%) [1]. Elastin, the main provider of tissue elasticity, is one of the long-lived connective tissue proteins with a very slow turnover. It is a polymer of linear polypeptide chains, the precursor is tropoelastin, stabilized by lysine-derived cross-links, such as desmosine, isodesmosine, 5-dehydrolysylnorleucine or lysylnorleucine under the action of lysyloxidase [2]. Elastin is mainly synthesized during late gestation and infancy, although expression at a very low rate persists in adulthood, and is degraded during individual life. Degradation of elastic fibers is mediated by elastolytic enzymes which are liberated from different types of cells including granulocytes, monocytes, lymphocytes, skin fibroblasts, cancer cells and others. These enzymes include: elastase from neutrophils and platelets, cathepsin G, metalloproteinases and the macrophage metalloelastase-12 [3]. Although only small amounts of elastin are normally degraded, increased degradation and fragmentation of elastic fibers may play a significant role in the progression of disease and age-related alterations [4].

Autoantibodies (IgG) to  $\alpha$ -elastin (elastin breakdown product; AEAb) and tropoelastin (elastin precursor; ATEAb) are found in the sera of healthy human subjects [5, 6]. Relatively high levels AEAb were established in the serum of children, which reach even higher values in the serum of 18–20 years old subjects. Their levels then stabilize in the serum of 30- to 60-year-old persons and gradually decrease thereafter [5, 7]. AEAb seem to be a part of a homeostatic mechanism that cleans altered elastin structures via in situ destruction or via opsonization of the products of degradation. An increase in circulating AEAb has been registered in patients with diabetes [8], atherosclerosis [9], autoimmune diseases (systemic lupus erythematosus [10], scleroderma [11, 12], polyarteritis nodosa [13]), and systemic diseases (polymyalgia rheumatica [14] and fibromyalgia syndrome [15]). Serum antibodies to tropoelastin, reflecting elastin synthesis, and  $\alpha$ -elastin, reflecting elastin degradation, appear to correlate with the production and breakdown of elastic tissue, respectively [10]. Several studies established that abnormal variations in elastin metabolism may be detected by measuring the ATEAb/AEAb as respective marker of elastin synthesis and degradation [11, 13, 14].

The senescence-accelerated mice (SAM) are a model of accelerated aging that was selected phenotypically from the AKR/J mouse strain [16]. This mouse model includes 9 substrains prone to accelerated senescence (SAMP) and 3 substrains resistant to accelerated senescence (SAMR), each one of these substrains being characterized by different abnormalities. Most SAMP8 mice die shortly after 1 year of age, and they are considered already old when they reach 9 months [17]. In our investigation 3- and 9-month-old SAMP8 and SAMR1 were used. The main characteristics of SAMP8 are age-related learning and memory deficits, anxiety, impaired immune system and age-dependent deposition of amyloid  $\beta$ -peptide. Regarding the cardiovascular system, SAMP8 mice have a high degree of oxidative stress [18], hypertrophied hearts [19], a dysfunctional endothelium [20] and a susceptibility to atherosclerosis [21]. SAMR1 are considered as a control group of SAMP8 and characterized by normal aging, although undergoing non-thymic lymphoma, histiocytic sarcoma and ovarian cysts [17].

Until now, elastin turnover and anti-elastin autoantibodies have not been the subject of investigation in SAM. The aim of this study was to investigate elastin metabolism in the plasma of SAMP8 and SAMR1 mice at 3 and 9 months of age by determination of the ratio of anti-tropoelastin to anti- $\alpha$ -elastin antibodies. The comparison was also extended to the more classical imprinting control region (ICR) mice of matching ages. Our study suggests that increased anti-elastin IgG autoantibodies could be used as a marker of aging in these animal models and possibly contribute to the processes of aging.

## Materials and Methods

### Animals

Male and female SAMP8 and SAMR1 were obtained from Harland (Blackthorn, UK), and were bred and cared for according to the aims of the project in the animal facility of the CEA-Grenoble, France. Plasma samples were collected from 3- and 9-month-old SAMP8 and SAMR1, shipped frozen on dry ice from Grenoble, France to the Center of Immunology of the University Hospital of Pleven, Bulgaria. Plasma and sera samples from 3- and 9-month-old ICR mice were collected at the biology department of the Medical University of Pleven, Bulgaria, and kept frozen until performing the assay. The number of animals in each group is shown in table 1.

### ELISA for Determination of AEAb and ATEAb

Alpha-elastin was obtained from aortas of ICR mice of different ages by the method of Sauvage et al. [22]. Porcine aortic tropoelastin was generously provided by Prof. Sandberg, Loma Linda University, Calif., USA.

**Table 1.** The number of animals in each investigated group

Strain	3 months old		9 months old	
	female	male	female	male
ICR	10	10	9	5
SAMR1	7	8	7	5
SAMP8	8	7	11	10
ICR <sup>a</sup>	5	5	8	5

<sup>a</sup> Animals used for investigation of correlation of serum EDP with AEAb.

AEAb and ATEAb were assessed on the principles of direct ELISA [23]. The assay was performed as follows: microtiter 96-well plates (Microton U-bottom, high binding; Greiner Bio One, Frickenhausen, Germany) were coated with 100  $\mu$ l per well of a solution of 10  $\mu$ g/ml antigen ( $\alpha$ -elastin or tropoelastin) in carbonate buffer (pH 9.6). The plates were incubated for 2 h at 37°C and then overnight at 4°C to complete the binding. The plates were then washed 3 times with PBS with 0.05% Tween 20 (PBS-Tween), before blocking with 0.1% BSA and incubating for 1 h at 37°C. The next step consisted of the addition of 100  $\mu$ l of mouse plasma diluted at a 1:5 ratio with PBS-Tween. Bound antibodies were incubated with anti-mouse IgG peroxidase conjugate (dilution 1:3,200) for 1 h at 37°C (Bul Bio, Sofia, Bulgaria). O-phenylenediamine was used as colorimetric substrate. Wells were washed 3 times with PBS-Tween 20 after each step. The reaction was terminated by addition of 50  $\mu$ l H<sub>2</sub>SO<sub>4</sub> (8 N) and the absorbance was read at 492 nm on an automatic micro-ELISA plate reader. The following controls of the reaction were used: (1) substrate control: assay buffer only, washing solutions and substrate added to the polystyrene wells coated with antigen ( $\alpha$ -elastin or tropoelastin); (2) detection antibody control: detection antibody added directly to the wells coated with antigen; (3) negative control to assess the specificity of the reaction: antigen replaced by human albumin solution and plasma samples, and (4) positive control: the tested sample replaced by antibodies to mouse  $\alpha$ -elastin or tropoelastin in assay buffer. All the samples were analyzed in triplicates and the average established.

#### *Competitive ELISA for Testing the Specificity of AEAb and ATEAb*

The specificity of AEAb and ATEAb was tested via competitive ELISA, using  $\alpha$ -elastin and porcine tropoelastin as experimental competitors. Samples of several investigated mouse plasma (ICR and SAMP8) were preincubated with 50  $\mu$ g/ml solutions of each inhibitor for 2 h at 37°C and overnight at 4°C. The reactivity of such treated plasma samples was tested using ELISA for determination of AEAb and ATEAb and compared to the reactivity of untreated plasma, which was assumed to be 100%.

#### *Competitive ELISA for Determination of Elastin-Derived Peptides in Mouse Sera*

The assay was performed as follows: microtiter plates were coated with 100  $\mu$ l per well of a solution of 10  $\mu$ g/ml mouse aortic  $\alpha$ -elastin in carbonate buffer (pH 9.6). The plates were incubated

for 2 h at 37°C and then overnight at 4°C to complete the binding. The plates were then washed 3 times with PBS-Tween before blocking with 0.1% BSA and incubation for 1 h at 37°C. A standard curve was generated using dilutions of mouse aortic  $\alpha$ -elastin. The next step consisted of the addition of 100  $\mu$ l polyclonal immune serum, raised against mouse aortic  $\alpha$ -elastin in rabbit, diluted at a 1:1,200 with PBS-Tween. The immune serum was preincubated with serial dilutions of mouse aortic  $\alpha$ -elastin (0–100 ng/ml) or tested mouse serum in PBS-Tween overnight at 4°C. The preincubated solutions of antibody were then transferred to the coated plate and incubated for 1 h at 37°C. The preincubated period before addition to  $\alpha$ -elastin-coated plates creates the competitive mode of the ELISA. Bound antibodies were incubated with anti-rabbit IgG peroxidase conjugate (dilution 1:3,200) for 1 h at 37°C (Bul Bio). The following steps are described in ELISA for determination of AEAb and ATEAb. Standards and serum samples were analyzed in triplicates and the average established. The serum elastin-derived peptides (EDP) concentrations were calculated from the standard curve and expressed as nanograms per milliliter.

#### *Determination of Elastin Synthesis:Degradation Ratio*

The elastin synthesis:degradation ratio for each plasma sample was counted by dividing the mean plasma levels of anti-tropoelastin IgG antibody by the mean plasma levels of anti- $\alpha$ -elastin IgG antibody.

#### *Statistical Analysis*

The data are presented as means  $\pm$  standard deviations and analyzed using multifactor analysis of variance (MANOVA). The factors 'strain', 'age' and 'sex' were studied. Differences in anti- $\alpha$ -elastin and anti-tropoelastin IgG autoantibodies as well as in the elastin synthesis:degradation ratio between the groups were analyzed for statistical significance ( $p < 0.05$ ) with a multiple range test, the least significant difference. The correlations between the investigated parameters were tested by the method of Pearson. The statistical package used was SPSS v.15.

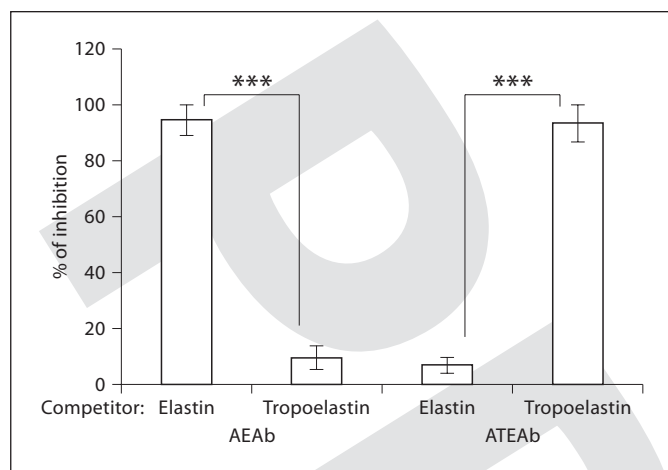
## **Results**

#### *Competitive ELISA for Testing the Specificity of the Measured AEAb and ATEAb*

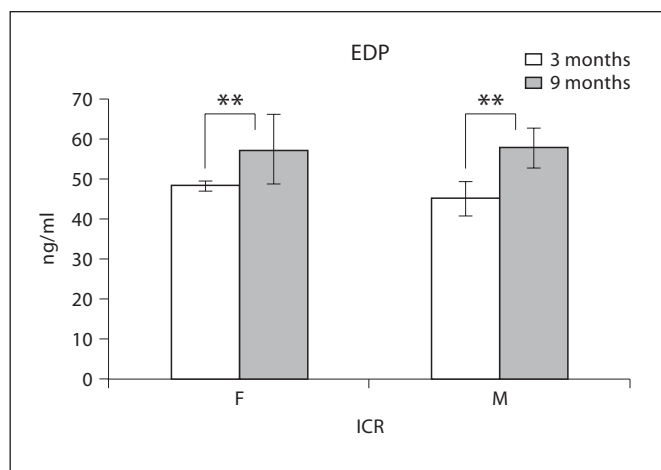
The specificity of the measured AEAb and ATEAb in mouse plasma was tested via competitive ELISA, using  $\alpha$ -elastin and porcine tropoelastin as experimental competitors. The results are presented in figure 1. Alpha-elastin inhibited 95% of anti-elastin activity of the mouse plasma, while tropoelastin inhibited by only 10% and vice versa, elastin inhibited only 7% of anti-tropoelastin activity of the mouse plasma, while the inhibition by tropoelastin was 94%.

#### *Correlation of Serum EDP with AEAb in ICR Mice*

The limited quantities of available plasma restricted determination of EDP in SAM (P8 and R1). It was there-



**Fig. 1.** Competitive ELISA for determination of the inhibitory effect of  $\alpha$ -elastin and porcine tropoelastin on the anti- $\alpha$ -elastin and anti-tropoelastin activity of the mouse plasma. \*\*\*  $p < 0.001$ .



**Fig. 2.** EDP in serum of ICR mice grouped according to age and sex. \*\*  $p < 0.01$ .

fore determined in the sera of ICR mice only. The largest quantity of EDP was measured in the sera of 9-month-old mice (57 ng/ml). The 3-month-old mice had significantly ( $p < 0.01$ ) lower quantity of elastin peptides (fig. 2). The levels of serum EDP of control ICR mice positively correlated with serum AEAb ( $r = 0.612$ ,  $p = 0.001$ ).

#### *Antibodies to $\alpha$ -Elastin (Elastin Breakdown Product): AEAb*

The optical density values (OD) representative of plasma AEAb levels significantly increased ( $p < 0.001$ ) with age in both SAMR1 and SAMP8 mice (fig. 3a). The same increase was observed in the female groups in all 3 strains (fig. 3b), while in the males only the values in the SAMP8 group showed significant increase with age (fig. 3c). Also a strong correlation between the levels of plasma AEAb and animal age was observed (table 2).

At 9 months of age, the AEAb levels in the plasma of female SAMR1 and SAMP8 of both sexes were higher relative to the levels measured in the ICR strain of the same age ( $p < 0.001$ ; fig. 3c). A strong correlation between the levels of plasma AEAb and mouse strain was established (table 3).

Nine-month-old female animals had significantly higher levels of plasma AEAb compared with corresponding male groups in all 3 strains. At 3 months of age, this difference was only observed in SAMR1. A significant correlation between the levels of AEAb and mouse sex was also found (table 2). Strain, age and sex were all

found to have a significant effect on the level of plasma AEAb (3-way ANOVA,  $p < 0.05$ ; table 3).

#### *Antibodies to Tropoelastin (Elastin Precursor): ATEAb*

Similar to AEAb, ATEAb levels were also influenced by age and sex (fig. 4). ATEAb significantly increased ( $p < 0.001$ ) with age in all 3 strains (fig. 4a). The only exception was observed in the ATEAb level in male SAMR1 (fig. 4c). In both female SAMR1 and SAMP8 mice (except 9-month-old SAMR1), ATEAb levels were elevated compared to the levels measured in ICR strain at the same age ( $p < 0.001$ ; fig. 4b). ATEAb plasma levels in male SAMR1 and SAMP8 did not differ significantly compared to the age-matched ICR groups (fig. 4c).

Significant differences in SAMP8 versus SAMR1 were observed only in 9-month-old male mice (fig. 4c). The levels of ATEAb correlated significantly with age and sex, but not with the strain of the investigated animals (table 2).

#### *Synthesis:Degradation Ratio*

The ATEAb:AEAb ratio is  $>1$  in the control ICR strain for both sexes at all ages (fig. 5). It is significantly lower in all the SAM animals, compared to the age-matched ICR controls. The only difference between SAMP8 and SAMR1 considering their ATEAb:AEAb ratio was observed in 9-month-old female mice, where SAMP8 animals had significantly higher ATEAb:AEAb ratios than their SAMR1 counterparts.



**Table 2.** Correlations between the factors 'age', 'strain' and 'sex' and AEAb, ATEAb and the synthesis:degradation ratio

	Age	Strain	Sex
AEAb			
r	0.492**	-0.307**	-0.432**
p	0.000	0.002	0.000
ATEAb			
r	0.556**	-0.303**	-0.159
p	0.000	0.003	0.125
Ratio			
r	0.062	0.000	0.409**
p	0.548	0.999	0.000

r = Pearson correlation. \*\* p < 0.01 for correlation (2-tailed). Data analyzed using SPSS v. 15.

**Table 3.** Multifactor analysis of variance for AEAb: type III sums of squares. The factors 'strain', 'age' and 'sex' are studied

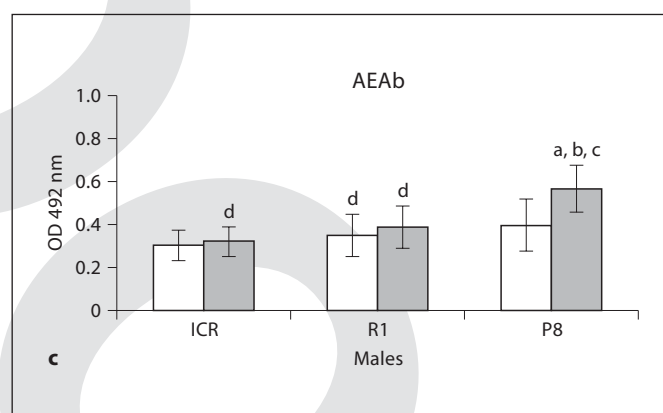
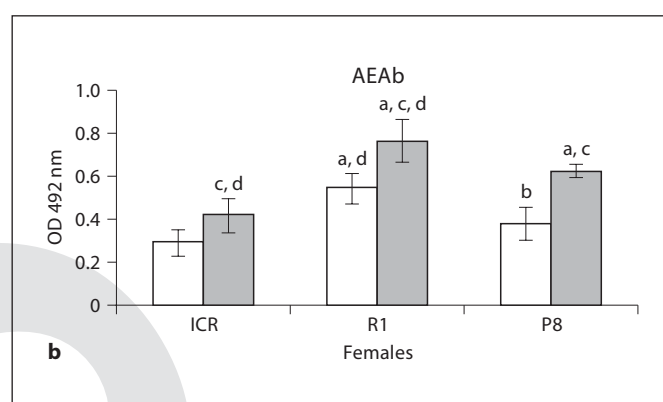
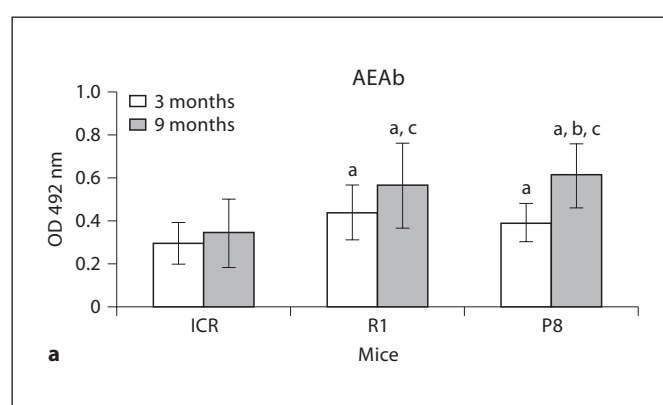
Main effects	Sum of squares	d.f.	Mean square	F-ratio	p value
Age	0.168046	2	0.084023	7.19	0.0012
Sex	0.051039	1	0.051039	4.37	0.0393
Strain	0.348127	2	0.174064	14.89	0.00001
Residual	1.12206	96	0.011688		
Total (corrected)	3.42728	102			

All F-ratios are based on the residual mean square error.

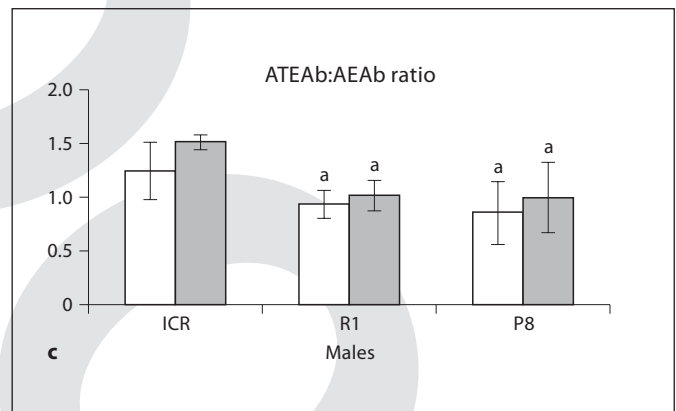
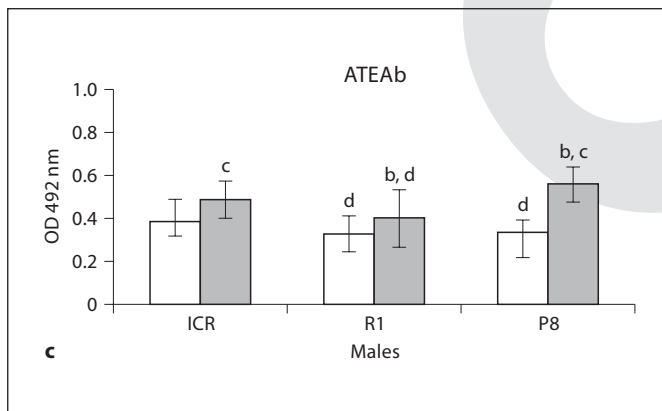
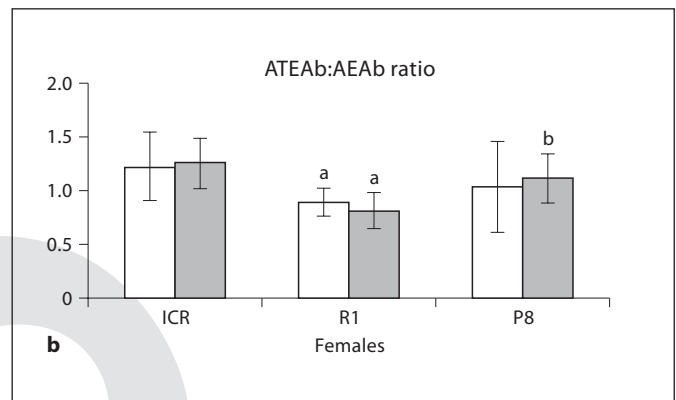
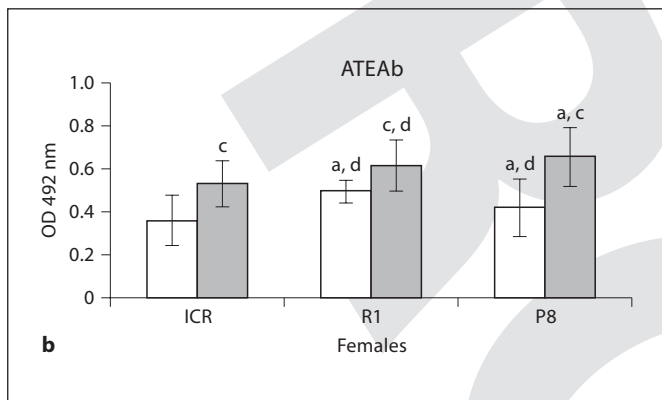
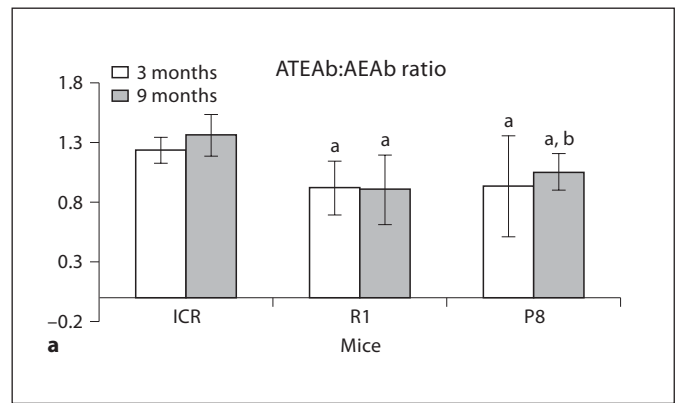
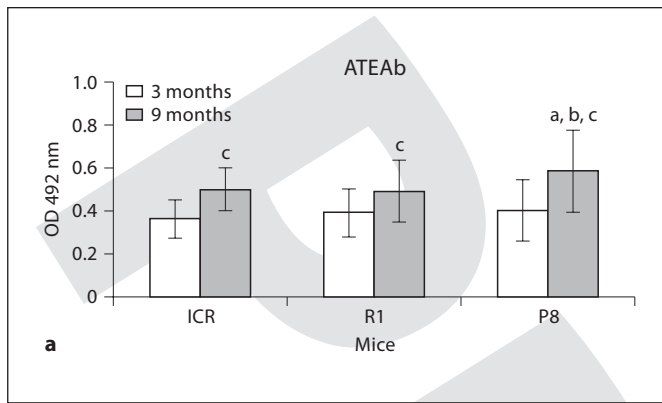
The synthesis:degradation ratio significantly correlated with the strain of the investigated animals but not with their age or sex (table 2).

## Discussion

Anti-elastin antibodies together with anti-tropoelastin antibodies and elastin-derived peptides present in sera of healthy subjects [5, 6], are markers of elastin degradation and synthesis. Abnormal variations in elastin metabolism were established in a variety of autoimmune disorders [10, 12, 13] and by measuring ratios of anti-tropoelastin and anti- $\alpha$ -elastin IgG antibodies [13]. Anti-elastin antibodies may play a physiological role in the remodeling of injured or senescent elastic structures by binding and clearance of elastin degradation products.



**Fig. 3.** Plasma anti- $\alpha$ -elastin antibodies (AEAb) levels present as mean values of OD  $\pm$  standard deviation in ICR, SAMR1 and SAMP8 at 3 and 9 months of age. **a** Pooled data (males + females) showing the effect of age and strain regardless of the sex. **b, c** Different age effect in each sex and each strain, respectively. Different superscript letters denote statistically significant difference (p < 0.05). <sup>a</sup> SAM (R1 or P8) vs. age- and sex-matched control ICR group. <sup>b</sup> SAMP8 vs. age- and sex-matched SAMR1. <sup>c</sup> p < 0.05, 9-month-old mice vs. strain- and sex-matched 3-month-old mice. <sup>d</sup> Significant differences between females and males in the matched groups.



**Fig. 4.** Plasma anti-tropoelastin antibodies (ATEAb) levels present as mean values of OD  $\pm$  standard deviation in ICR, SAMR1 and SAMP8 at 3 and 9 months of age. **a** Pooled data (males + females) showing the effect of age and strain regardless of the sex. **b, c** Different age effect in each sex and each strain, respectively. Different superscript letters denote statistically significant difference ( $p < 0.05$ ); see legend to figure 3.

**Fig. 5.** ATEAb:AEAb ratio present as mean values  $\pm$  standard deviation in ICR, SAMR1 and SAMP8 at 3 and 9 months of age. **a** Pooled data (males + females) showing the effect of age and strain regardless of the sex. **b, c** Different age effect in each sex and each strain, respectively. Different superscript letters denote statistically significant difference ( $p < 0.05$ ); see legend to figure 3.

Wei et al. [24] established that monoclonal and polyclonal anti-human aorta elastin antibodies stained elastic fibers on tissue sections, suggesting that the recognized epitopes are available at least on the native fibers for reactions with the antibodies.

The role of the extracellular matrix protein elastin in animal models of aging is an object of many investigations. Takubo et al. [25] found that in male BALB/c and SAMR1 mice which do not undergo accelerated aging, lung elastin content does not significantly change with age. It has been shown that arterial elastin content does not significantly change with age in mice hemizygous for the elastin gene and in wild-type mice in the C57B16/J background [26]. Also, Yoshino and Komura [27] found increased content and altered supramolecular structure of elastin isolated from dorsal neck skin of 12- to 14-month-old male SAMP1 (which undergo accelerated senescence) compared with SAMR1 at matching age. However, AEAb and ATEAb in SAM strain have not been the subject of previous studies.

Here, we measured the plasma levels of anti-tropoelastin and anti- $\alpha$ -elastin antibodies and their ratio, in order to use these values as markers of elastin synthesis and degradation. First, to check the specificity of AEAb and ATEAb we used competitive ELISA. The competitive investigations confirmed the presence of 2 distinct populations of circulating autoantibodies, i.e. ATEAb and AEAb, in the investigated mouse plasma. We therefore excluded the possibility for interaction between AEAb from the mouse plasma and tropoelastin as sensiblized antigen and vice versa – ATEAb with  $\alpha$ -elastin as antigen.

To verify the correlation between the levels of anti- $\alpha$ -elastin antibodies and elastin-derived peptides in mice we tested AEAb and EDP in the sera of ICR at 3 and 9 months of age by indirect competitive ELISA. Age- and sex-related changes of AEAb levels in the sera of ICR mice reproduced the mode of changes in the AEAb in plasma of ICR mice (the results are not shown). EDP increased with age and strongly correlated with the levels of AEAb. An age-dependent increase in elastin degradation is in accordance with the work of Baydanoff et al. [28] and Shinohara et al. [29], which showed gradual increase of elastin peptide levels in human sera.

Significant increase in AEAb and ATEAb with age was established in ICR and SAM mice. Studies in humans have shown elevated levels of AEAb in children and gradual decrease after 60 years of age [5, 6]. In the investigation of the correlation between AEAb and EDP in 3-, 9-, and 20-month-old ICR mice the same decrease was observed in the oldest mice (data not shown). The decrease

in older subjects could be due to the consumption of AEAb by the elastin peptides and by the increased amount of altered elastic structures. Another reason could be that the production of AEAb decreases in the old subjects due to the aging of the immune system [6]. Most SAMP8 mice die shortly after 12 months of age [17] and it was impossible to collect plasma from older mice. The control SAMR1 mice showed high mortality because of the high frequency of malignancies. That is why we could not test the levels of plasma AEAb in the older SAM animals.

Sera from SAMP1 were registered to contain elevated levels of auto-antibodies, including natural thymocytotoxic autoantibody, anti-nuclear antibodies, and IgG anti-single-stranded and anti-double-stranded DNA antibodies [30, 31]. In 9-month-old SAMP8 of both sexes we established significantly increased levels of anti- $\alpha$ -elastin autoantibodies compared to age-matched ICR controls. Even SAMR1, the controls for SAMP8, had elevated levels of AEAb, although only in female individuals. These results are in accordance with the Wallford's theory, which states that 2 types of changes in immunologic aging occur in humans and mice: decreased capacity of response to exogenous stimuli and increased autoimmunity with age [32].

Also, in all female groups of 9-month-old animals we measured significantly higher levels of plasma AEAb and ATEAb compared to age- and strain-matched male animals. Such elevated AEAb and ATEAb levels were observed in the 3-month-old female SAMR1, too. At 9 months of age, the wild, female mice had undergone 4–5 gestations. SAMP8 strains exhibit reduced fecundity and premature loss of fertility [33]. From the investigated female SAM animals, only three 9-month-old SAMP8 had given birth to a litter. The other SAMP8 and all SAMR1 had not reproduced, but regardless of this, the ATEAb plasma levels were elevated compared with ICR-matched animals. It was noticeable that the ATEAb but not AEAb plasma levels were the highest in the 3 SAMP8 which had undergone 1 pregnancy each. According to Woessner and Brewer [34], during pregnancy the wet weight of the human uterus increases at least 11-fold, its collagen content 7-fold and its elastin content 5- to 6-fold. After parturition, there is a rapid uterus involution which is 75% complete by 8–11 days post partum. Collagen and elastin undergo rapid breakdown during the process of massive remodeling in the reproductive organs through pregnancy and birth. In mice, the number of pregnancies is much greater than in humans. Growth and remodeling of elastin must involve highly coordinated interactions between

cells, cytokines, proteinases, proteinase activators and inhibitors, as well as the matrix itself [34]. Because there is elastin production then degradation within a short period of time, alterations in elastin metabolism during pregnancy could induce an increased production of either anti-elastin and/or anti-tropoelastin autoantibodies. This hypothesis is supported by the significant correlation between the levels of ATEAb and age and sex of the investigated animals (table 2).

The ATEAb:AEAb ratio was significantly higher in ICR controls. The lower ratio in SAM mice may reflect the increased destruction of elastin. However, SAMR1 (controls) had the same lower ratio as SAMP8 mice. This could be due to the fact that SAMR1 presented a higher incidence of malignancies, such as non-thymic lymphoma and sarcoma [35], since transformed leukocytes have elevated enzymatic activity and can digest elastin [36–38]. The circulating levels of elastin degradation products – i.e. elastin-derived peptides – correlate with the levels of anti-elastin antibodies in normal [14] and some pathological conditions [39]. The absence of difference

between SAMP8 and SAMR1 regarding the ATEAb:AEAb ratio raises the question of knowing whether SAMR1 are the appropriate control of SAMP8 in terms of the senescence of the elastic tissues.

In conclusion, the results of our study showed variations in plasma AEAb and ATEAb levels of senescence-accelerated mice compared to the levels measured in the normal aging ICR strain. Our findings suggest that increased anti-elastin IgG autoantibodies could contribute to the processes of aging and be used as a marker of aging in these animals. This hypothesis needs further confirmation by other methods, such as measurement of serum elastin-derived peptides in SAM, as well as metalloproteinase and lysyl oxidase-like 1 activities in SAM mice.

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