

AGE-ASSOCIATED CHANGES IN ELASTIN AND COLLAGEN CONTENT AND THE PROPORTION OF TYPES I AND III COLLAGEN IN THE LUNGS OF MICE

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Abstract

We investigated the effect of aging on the extracellular matrix of the lungs by examining age-associated changes in the elastin and collagen content, and the proportion of types I and III collagen, in the whole lungs of BALB/c and SAMR1 male mice between the ages of 3 and 24 months. The elastin content was determined by the hot alkali method. The hydroxyproline content was measured and assessed to be the collagen content. The relative proportion of types I and III collagen was assessed by cyanogen bromide digestion, followed by separation of the resultant peptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The elastin content did not change significantly with age in either strain of mice. The total collagen content of the whole lung was significantly higher at 24 months of age, although there were no significant changes with aging in the hydroxyproline content per dry lung weight nor in the proportion of type III to type I collagen. We conclude that in terms of the extracellular matrix, the lungs of aged mice are not very different in feature from the lungs of younger mice, and this is probably the simple consequence of growth of the lungs of young mice.

INTRODUCTION

Morphologic and physiologic studies on the effect of aging on the lungs have been performed (Kawakami *et al.*, 1984; Kurozumi *et al.*, 1994; Teramoto *et al.*, 1994; Hirai *et al.*, 1995), and it is likely that the age-associated changes in lung structure and function reflect alterations in the extracellular matrix (ECM) of the lung (Mead *et al.*, 1961).

Elastin and collagen are the major ECM proteins which make up the framework of the alveolar structure. Although age-related changes in the elastin and collagen content of human and animal lungs have been reported (Pierce *et al.*, 1960; Ranga *et al.*, 1979; Bradley *et al.*, 1974; Kato *et al.*, 1992; Mays *et al.*, 1988), the results have varied widely and are inconclusive. This is likely due to the variety of materials and methods used. In particular, in human lungs, various environmental factors including pollutants such as cigarette smoke, ozone, nitrous oxide and sulfur dioxide may affect the aging process. In order to study age-associated changes in the lungs, it is therefore necessary to use a proper animal model to control these environmental factors.

Mice have been widely used in the fields of cellular and molecular biology, and are a useful animal model to study aging because of their short life span. However, the lungs of mice are so tiny that it is difficult to obtain accurate measurements of lung mechanics and the amount of ECM proteins such as elastin and collagen. To our knowledge, only one previous report by Ranga *et*

al. (1979), examined age-related changes in the ECM proteins of the murine lung. They used BALB/c mice, and reported that the static compliance (Cst) increased whereas the elastin content decreased with aging, and that these two findings were compatible with each other.

We recently developed a new method for precisely measuring the lung mechanics of small animals, and reported that the specific compliance, which is the Cst corrected by the lung volume, did not change with aging in the accelerated senescence-resistant strain of mice, SAMR1 (Hirai *et al.*, 1995). Therefore, studying the age-associated changes in the elastin and collagen content of the lungs of SAMR1 mice is important from the point of view of aging in the lung.

In the present study, we used two strains of male mice undergoing normal aging; BALB/c mice, which were used by Ranga *et al.*, and SAMR1 mice, which we used in our previous study on the morphologic and physiological changes of the lungs as a result of aging. The aims of the present study were to: (I) investigate the effects of aging on the elastin and collagen content of the whole lungs of mice, and (II) determine whether these results are compatible with age-associated changes in lung mechanics.

MATERIALS AND METHODS

Animals

A total of 123 male mice of SAMR1 mice (Takeda *et al.*, 1991) and BALB/cCrSlc mice were used in the present study. The mice were reared under conventional conditions at a temperature of $24 \pm 2^{\circ}\text{C}$, humidity of $45 \pm 5\%$, and a 12 hr light-dark cycle. They were housed in groups in cages, and were supplied with pellet food (CE2, Nihon CLEA, Tokyo, Japan) and tap water ad libitum. Eleven to twenty mice of each strain were sacrificed at ages 3 months (mo), 6 mo, 12 mo, and 24 mo (Table 1). The mice within each age group were randomly divided into three groups: the lungs of the first group of mice were used for quantification of the elastin content, the lungs of the second group were used for quantification of the collagen content, and the lungs of the third group of mice were used for measurement of the relative amount of types I and III collagen.

Preparation of the lung samples

The mice were anesthetized by an intraperitoneal injection of sodium pentobarbital ($50 \mu\text{g/g}$ body weight), and were exsanguinated by cutting the major abdominal vessels. The lungs were excised by sectioning the hilum from the extrapulmonary bronchus and pulmonary vessels. The excised lungs were rinsed with distilled water. The lungs of the mice of each study group were

pooled in 100 ml of acetone for 3 days at room temperature for delipidation.. Each delipidated lung was then lyophilized at -80°C for 24 hr, and the dry weight of the lung tissue was measured on a Mettler AE240 analytic balance (Mettler, Zurich, Switzerland).

Measurement of elastin content

Elastin was purified from the homogenate of the whole lung by the hot alkali method (Lansing *et al.*, 1952). Briefly, whole lung samples were homogenized in 10 ml of 0.1M NaOH, and then boiled for 50 min. After the pellets were washed with 10 ml of cold 0.1 M NaOH and then with 10 ml of cold deionized distilled water, the pellets were lyophilized. The elastin content was measured by quantifying the amount of protein in the elastin residues using Kjeldahl's semi-micro method (Thompson and Morrison, 1951). Briefly, the elastin pellets were boiled with 0.3 ml of 50% H₂SO₄ for 2 hr. at 350°C, 3 to 4 drops of H₂O was added, and this was boiled for an additional 45 min. After deionized distilled water was added, the mixture was cooled. Then, 1 ml of Nessler's reagent was added and the solution was kept at 25°C for 20 min. The solution was then analyzed densitometrically at 430 nm to quantify the amount of protein in the elastin residues.

Measurement of collagen content

The total collagen content of the lung was measured using hydroxyproline which is a relatively specific marker of collagen, based on a modification of the method of Kivirikko *et al.* (1967). Essentially, whole lung samples were homogenized in 3 ml of 6 M HCl, and then autoclaved for 3 hr. at 125°C. After the addition of Dowe-X and charcoal, the pellets were then centrifuged. The solution was neutralized with KOH, and oxygenated with chloramine T for 25 min. Sodium thiosulphate was then added. The solution was then extracted with toluene, boiled for 30 min at 100°C, and again extracted with toluene. Ehrlich's reagent was then added to the solution, and after 30 min the solution was analyzed densitometrically at 560 nm to quantify the amount of hydroxyproline.

Measurement of the proportion of types I and III collagen

The relative amount of type I and type III collagen in the whole lung was measured based on the method of Laurent *et al.* (1981). The initial purification step involved homogenization with sodium dodecyl sulfate to remove most of the non-collagenous proteins from the homogenate of the whole lung. The residue was acetone-dried and then treated with cyanogen bromide, which cleaves the collagen into smaller peptides, prior to lyophilization. These

peptides were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (a running gel of 12.5 % acrylamide). Following is the staining procedure which we used: the gel was first stained with Coomassie brilliant blue solution (0.1%, v/v, Sigma, St. Louis, USA) for 1 hr, and destained with aqueous acetic acid (8%, v/v) for 24 hr. Quantification was performed by scanning the gel densitometrically into a Macintosh computer, and then measuring the area under the relevant peaks using Image 1.55 software (Research Service Branch, NIH). The peptides chosen for quantification were $\alpha 1(\text{III})$ -CB-5 for type III collagen and $\alpha 1(\text{I})$ -CB-8 for type I collagen, which denoted in Figure 1.

The mean value of two samples obtained from each mouse was used as the amount of elastin and hydroxyproline, and the relative ratio of types I and III collagen.

Validity of the measurement

We validated the linearity and reproducibility of our methods using commercially available pure elastin (Sigma, St. Louis, USA), hydroxyproline (Nacalai Tesque, Kyoto, Japan), and types I and III collagen (Sigma, St. Louis, USA). In establishing the standard curves for the amounts of elastin and hydroxyproline, the relationship between the measured and loaded amounts was quite linear. The standard curves were $y = 0.993x + 0.052$ ($r^2=0.973$) for 0.05

mg to 1 mg of elastin, and $y = 0.969x + 0.043$ ($r^2=0.993$) for 0.5 μ g to 10 μ g of hydroxyproline.

Statistical analysis

Statistical analysis was performed using analysis of variance (Scheffe's test) for comparison of values between different age groups of the BALB/c mice and the SAMR1 mice. The unpaired t-test was used for comparing values between the same age group of the two strains. A p-value of less than 0.05 was considered to be statistically significant, and the results were expressed as mean $\pm$ S.D.

RESULTS

Age-associated changes in the dry weight of the lungs

The dry lung weight of each age group of mice within each strain did not differ significantly. However, the dry lung weight of the SAMR1 mice was significantly larger than that of the BALB/c mice in each age group (Table 1).

Age-associated changes in the amount of elastin and collagen

The elastin content of the whole lung did not change significantly with age in either the BALB/c mice (3 mo: 658.6 ± 31.5 ; 24 mo: 702.5 ± 37 μg ; $p = 0.806$), or in the SAMR1 mice (3 mo: 947.8 ± 82.6 ; 24 mo: 941.7 ± 95.3 μg ; $p = 0.981$) (Figure 2).

The hydroxyproline content of the whole lung was significantly higher at 24 months of age than at 3 months or 6 months in both the BALB/c mice (3 mo: 102.7 ± 6.6 ; 24 mo: 122.8 ± 15.3 μg ; $p < 0.05$), and the SAMR1 mice (3 mo: 147.4 ± 14.0 ; 24 mo: 172.6 ± 12.4 μg ; $p < 0.01$) (Figure 3).

To standardize for differences in body size between the two strains, the elastin and hydroxyproline contents were normalized to the dry lung weight. The elastin content per dry lung weight did not change significantly with aging in either strain of mice (BALB/c: 3 mo 26.7 ± 1.0 , 24 mo 24.6 ± 4.1 $\mu\text{g}/\text{mg}$, $p = 0.709$; SAMR1: 3 mo 29.2 ± 3.0 , 24 mo 26.3 ± 0.3 $\mu\text{g}/\text{mg}$, $p = 0.291$), and there were no significant differences in the corrected content at each age between the

two strains ($p = 0.740$) (Figure 4). In the SAMR1 mice, the hydroxyproline content per dry lung weight showed no significant change with aging (3 mo: $4.64 \pm 0.70 \mu\text{g/mg}$; 24 mo: $4.95 \pm 0.42 \mu\text{g/mg}$; $p = 0.308$). In the BALB/c mice, the hydroxyproline content per dry lung weight at 3 months of age was the lowest among the age groups (3 mo: $4.06 \pm 0.33 \mu\text{g/mg}$; 6 mo: 4.24 ± 0.61 ; 24 mo: $5.06 \pm 0.13 \mu\text{g/mg}$; $p < 0.05$), and was also significantly lower than that in the SAMR1 mice at 3 months of age ($p < 0.05$). In contrast, the hydroxyproline content per dry lung weight of the 6 month, 12 month, and 24 month-old mice did not differ significantly within each strain ($p = 0.11$). At each of these ages, there were also no significant differences between the two strains ($p = 0.554$) (Figure 5).

Age-associated changes in the ratio of type III to type I collagen

Figure 6 shows the age-related change in the ratio of type III to type I collagen. There were no significant changes in the ratio of type III to type I collagen with aging in the BALB/c mice (3 mo: 0.32 ± 0.07 ; 24 mo: 0.32 ± 0.05 ; $p = 0.47$), nor in the SAMR1 mice (3 mo: 0.27 ± 0.08 ; 24 mo: 0.34 ± 0.06 , $p = 0.11$).

DISCUSSION

We found that the amount of elastin and collagen corrected by the dry lung weight, as well as the ratio of collagen type I and III, remained constant with aging in both SAMR1 and BALB/c mice.

In this study, the amount of elastin and collagen was measured using the whole lung in order to minimize errors in sampling. Thus, the lung samples included non-parenchymal tissues such as the bronchus, pulmonary vasculature and pleura, as it is nearly impossible to exclude these structures without destroying the lung. In addition, the amount of elastin and collagen in the parenchyma was much greater than in the other structures. Elastin and collagen are found not only in the parenchymal tissues, but also in non-parenchymal tissues such as the bronchus, pulmonary vasculature and pleura of the lung. To assess the ratio of the elastin and collagen content of the tracheobronchial tree plus pulmonary vasculature to the elastin and collagen content of the whole lung, we measured the amount of elastin and collagen in the trachea and aorta of the middle-aged mice (Table 2). The dry weight of the intrapulmonary bronchus and vasculature is only several percent of the weight of the alveolar structures (Sandberg *et al.*, 1981). If we assume that the dry weight of the intrapulmonary bronchus and vasculature accounts for 3 % of the dry weight of the whole lung, then the net weight of elastin and collagen (hydroxyproline) is approximately 18 μg and 6 μg , respectively, in the intrapulmonary bronchus,

and approximately 54 μg and 7 μg , respectively, in the intrapulmonary vasculature. The intrapulmonary bronchus contains 2% and 4% of the net weight of elastin and collagen (hydroxyproline), respectively, of the whole lung, and the intrapulmonary vasculature contains about 6% and 4% of the net weight of elastin and collagen, respectively, of the whole lung. Moreover, based on the morphological structure of the airway tree and pulmonary vasculature, the elastin and collagen content per dry weight of the intrapulmonary bronchus and vasculature should be less than the respective value in the trachea and aorta. Thus, the amount of collagen and elastin in the bronchial tree and vasculature should be much lower than that in the parenchyma. The amount of collagen and elastin in the pleura was not investigated in this study because the murine pleura is too tiny to measure using our method. However, the amount of collagen and elastin in the pleura has been reported to be almost equal to that in the trachea (Schellenberg *et al.*, 1987). Therefore, the amount of collagen and elastin in the pleura should be much less than in the parenchyma. Therefore, the age-associated changes in the amount of elastin and collagen in the whole lung are most likely due to changes in the parenchyma.

A significantly lower level of collagen per dry lung weight was observed at 3 months of age than at 24 months of age in the BALB/c mice. This may be due to the immature stage of development of the lungs at 3 months of age in this strain.

Elastin and collagen fibers are major elements of the connective tissue network within the lungs. Based on the elastic properties of the elastin fibers and the rigid, supportive characteristics of the collagen fibers, it has been suggested that the elastin fibers are responsible for changes in lung compliance during normal breathing, whereas collagen fibers limit the lung volume (the Mead-Setnikar theory) (Mead *et al.*, 1961; Setnikar *et al.*, 1955; Mercer *et al.*, 1990). Kurozumi *et al.* (1994), reported that the main, morphological, age-associated change in SAMR1 mice is enlargement of airspace size without alveolar wall destruction, and proposed that this strain of mice was an ideal animal model for senile hyperinflation of the lung.

Hirai *et al.* (1995) previously reported that lung elasticity normalized by lung volume remains constant with age, and that the effects of aging on pulmonary mechanics are due solely to this increase in lung volume. Our finding that the amount of elastin per dry lung weight did not decrease with aging may suggest that the elastic properties per unit volume also remain constant. The constant amount of collagen per dry lung weight with aging may be the sole consequence of the increase in lung volume. Thus, the constant amount of elastin and collagen observed through life in the present study are consistent with the mechanical changes which Hirai *et al.* (1995) previously reported.

Qualitative changes, as well as quantitative changes, of the elastin and collagen might also contribute to pulmonary properties. Types I and III

collagen which are located in the interstitium of the lung parenchyma, are the most abundant among the collagen subtypes in the lungs. In the normal lung, type I collagen is three to six times more abundant than type III collagen, and the ratio of type III to type I collagen is higher in the fibrotic and emphysematous lung (Laurent *et al.*, 1981; Crystal *et al.*, 1991). The main function of type I collagen is to add rigidity to the framework of the lung, whereas type III collagen mainly works in conjunction with the elastin fibers (Laurent *et al.*, 1981; Crystal *et al.*, 1991. Mays et al. 1988), also reported age-associated changes in the relative amounts of collagen subtypes I and III in the rat lung. According to their report, the amount of collagen and the proportion of type III collagen increased with age. However, the rats in their study gained body weight and size even after the animal had reached maturity. Therefore, this continuous gain in body weight may influence the synthesis and degradation of ECM proteins in rats. We found that the relative amount of collagen subtypes I and III did not change with aging in mice.

As to qualitative changes in collagen, collagen crosslinks cannot be forgotten. Reiser *et al.* (1987) previously reported age-associated changes in the collagen crosslinks in the skin and lung of monkeys and rats. Changes in collagen crosslinks might also contribute to pulmonary properties. Age-associated changes in the collagen crosslinks in the lungs of mice require further exploration.

Our findings that elastin and collagen content, and the relative amount of collagen subtypes I and III remain constant, suggest that this might be the simple consequence of maintaining a nearly similar structure of the lungs of young mice throughout life. This result is also consistent with the mechanical findings which we previously reported (1995).

Our result stands in contrast to those of Ranga *et al.* (1979), even though we used the same strain of mice. They reported that Cst increased with aging, and that the elastin and collagen content decreased significantly with aging. They also reported that the elastin content per dry lung weight decreases with aging, whereas the collagen per dry weight does not change. Therefore, they concluded that Cst increased with aging due to a decrease in the elastin content. On the other hand, several biochemical studies on age-associated changes of the lungs of humans (Pierce *et al.*, 1960), rats (Mays *et al.*, 1989), and hamsters (Kato *et al.*, 1992) showed no decrease in elastin and collagen content with aging, as observed in this study. Ranga *et al.*, used young and middle-aged male mice, and elderly female mice which had a significantly lower body weight. They extracted elastin using the hot alkali method, and then quantified the elastin using the method of Kivirikko, *et al.* (1967). However, elastin is not very soluble in 6M HCl at 98°C, which they used in their experimental protocol. Ranga *et al.*, reported Cst values which were taken from the mean slope of the linear portion of the deflation line of their pressure-volume (P-V) curves.

However, P-V relationships below the functional residual capacity do not accurately reflect the intrinsic elastic properties of the lung because of airway closure. Differences in the materials and methods used may have resulted in the discrepancies between these studies.

Many organs of SAMR1 mice, including the bone (Matsushita *et al.*, 1986), eyes (Hosokawa *et al.*, 1988) and brain (Miyamoto *et al.*, 1986), show normal aging. However, aging of the extracellular matrix of the lungs from a biochemical aspect has not been investigated. We found that the age-associated changes in the composition of extracellular matrix proteins of the lungs of SAMR1 mice are similar to those of BALB/c mice; this suggests that the lungs of SAMR1 mice show normal aging in this respect. The genetic background and physiological and pathological features of SAMR1 mice have been thoroughly investigated. Therefore, SAMR1 mice are a useful animal model for studying aging of the lungs.

In conclusion, we found that the elastin and collagen content, corrected by the dry lung weight, as well as the relative amount of collagen subtypes I and III remain constant with aging in both BALB/c and SAMR1 mice. Based on these results, we speculate that the lungs of aged mice are not very different in feature from the lungs of younger mice, and this would be a simple consequence of normal lung growth, according to our results. Therefore, our report provides fundamental information on aging of the lungs.

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TABLE 1. AGE-ASSOCIATED CHANGES IN BODY WEIGHT AND DRY LUNG WEIGHT IN BALB/C AND SAMR1 MICE

		3 mo	6 mo	12 mo	24 mo
n	BALB/c	16	17	15	12
	SAMR1	13	19	19	13
body weight* (g)	BALB/c	24.9±1.6	26.2±2.6	23.4±2.7	22.6±1.2
	SAMR1	29.1±2.1	31.1±1.7	31.7±1.5	32.2±2.1
dry lung weight* (mg)	BALB/c	24.5±1.9	26.2±1.7	27.4±2.3	25.7±2.7
	SAMR1	32.1±2.3	32.7±1.1	32.4±2.2	34.4±3.3

*Values are expressed as mean± S.D.

TABLE 2. THE AMOUNT OF ELASTIN AND COLLAGEN IN THE TRACHEA, AORTA AND WHOLE LUNG OF SAMR1 MICE OF 6 MONTHS OF AGE

	Hyp* (μ g/mg)	elastin* (μ g/mg)	dry weight (mg)	n
trachea	6.99 ± 1.1	22.7 ± 1.8	3.1 ± 0.2	5
aorta	8.44 ± 1.2	60.1 ± 2.1	4.8 ± 0.3	4
lung	4.85 ± 0.5	27.3 ± 2.0	32.0 ± 1.0	12

* Values are normalized to dry weight of the respective organ, and are expressed as mean $\pm$ S.D.

Abbreviations: Hyp: hydroxyproline

Figure Legends

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cyanogen bromide cleaved peptides of standards and lung samples: The peptides chosen for quantification were $\alpha 1(\text{III})\text{-CB-5}$ for type III collagen and $\alpha 1(\text{I})\text{-CB-8}$ for type I collagen. For the standards, commercially available type I collagen (Sigma, St. Louis, USA) and type III collagen (Sigma, St. Louis, USA) were used.

The standards contain type I collagen (10 μg) and type III collagen in a 3 : 1 ratio based on the method of Mays *et al.* (1988).

Lanes 1 - 2, standard mixed type I and type III collagen in a 3 : 1 ratio

Lanes 3 - 8, sample from the lungs of mice

Lane 3: the lungs of SAMR1 mice at 3 months of age

Lane 4: the lungs of SAMR1 mice at 6 months of age

Lane 5: the lungs of SAMR1 mice at 12 months of age

Lane 6: the lungs of SAMR1 mice at 12 months of age

Lane 7: the lungs of SAMR1 mice at 24 months of age

Lane 8: the lungs of BALB/c mice at 3 months of age

Figure 2. Age-associated changes in elastin content of the lungs of the BALB/c and SAMR1 mice

Error bars: SD

There was no significant change in elastin content with aging in either strain.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=4), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=5), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

Figure 3. Age-associated changes in hydroxyproline content of the whole lung of the BALB/c and SAMR1 mice

Statistical significance of differences between age groups:

* $p < 0.05$ 24 mo vs. 3 mo, 6 mo in SAMR1 mice.

$p < 0.05$ 24 mo vs. 3 mo, 6 mo in BALB/c mice.

Error bars: SD

The hydroxyproline content of the whole lung increased significantly at 24 months of age in the SAMR1 and BALB/c mice

Number of animals in each age group and strain: BALB/c: 3-month-old (n=4), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=5), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

Figure 4. Age-associated changes in elastin content per dry lung weight of the BALB/c and SAMR1 mice

Error bars: SD

There was no significant change with aging in either strain.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=7), 6-month-old (n=7), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=4), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

Figure 5. Age-associated changes in hydroxyproline content per dry lung weight of the BALB/c and SAMR1 mice

Statistical significance of differences between age groups:

$p < 0.05$ 3 mo vs. 24 mo in BALB/c mice.

Error bars: SD

There was no significant change in the hydroxyproline content per dry lung weight in the SAMR1 mice with aging. In the BALB/c mice, the hydroxyproline content per dry lung weight at 3 months of age was significantly lower than that at 24 months of age.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=7), 6-month-old (n=7), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=4), 6-month-old (n=7), 12-month-old (n=7), 24-

month-old (n=4).

Figure 6. Age-associated changes in the proportion of types I and III collagen (expressed as type III / type I) of the BALB/c and SAMR1 mice.

Error bars: SD

There was no significant change with aging in either strain.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=5), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=4), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4).

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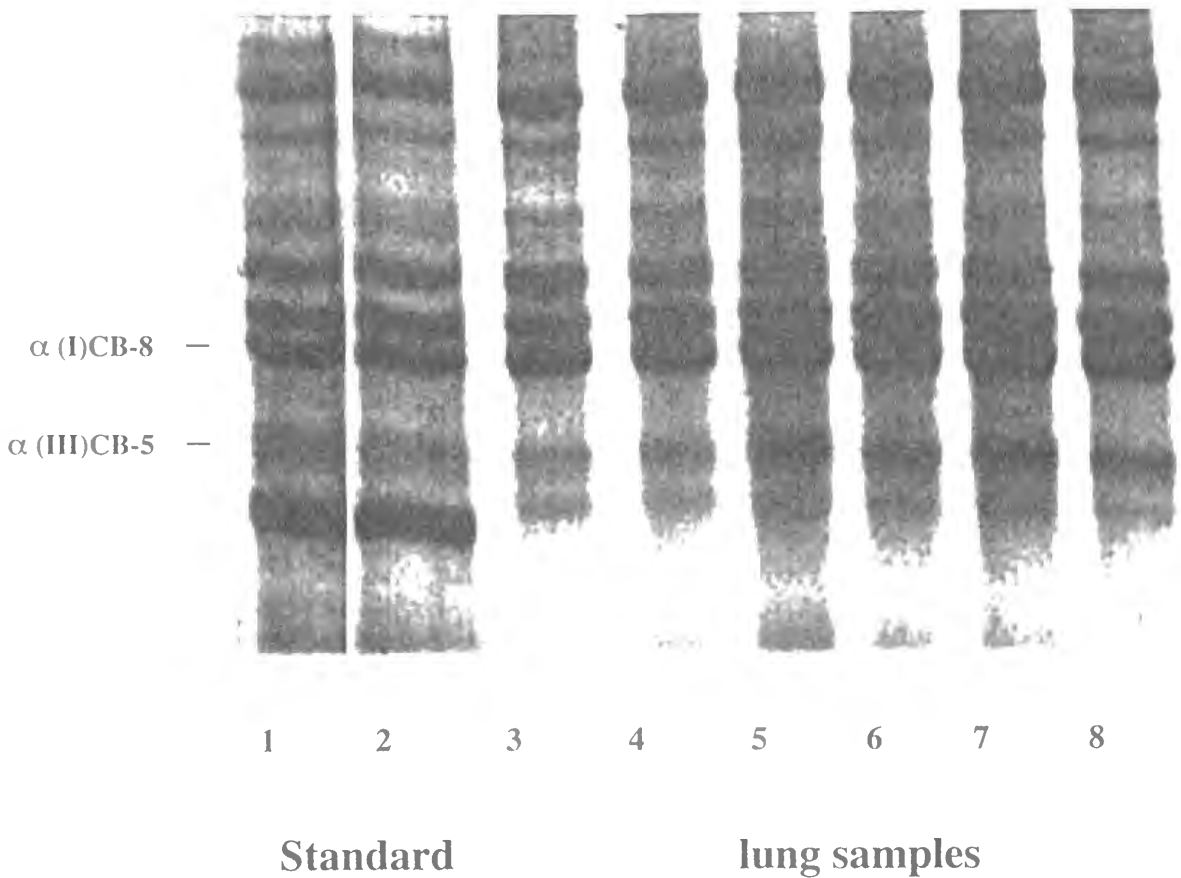


Figure 1

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cyanogen bromide cleaved peptides of standards and lung samples. The peptides chosen for quantification were $\alpha 1(III)$ -CB-5 for type III collagen and $\alpha 1(I)$ -CB-8 for type I collagen. For the standards, commercially available type I collagen (Sigma, St. Louis, USA) and type III collagen (Sigma, St. Louis, USA) were used.

The standards contain type I collagen (10 μ g) and type III collagen in a 3 : 1 ratio based on the method of Laurent *et al.* (1981).

Lanes 1 - 2, standard mixed type I and type III collagen in a 3 : 1 ratio

Lanes 3 - 8, sample from the lungs of mice

Lane 3: the lungs of SAMR1 mice at 3 months of age

Lane 4: the lungs of SAMR1 mice at 6 months of age

Lane 5: the lungs of SAMR1 mice at 12 months of age

Lane 6: the lungs of SAMR1 mice at 12 months of age

Lane 7: the lungs of SAMR1 mice at 24 months of age

Lane 8: the lungs of BALB/c mice at 3 months of age

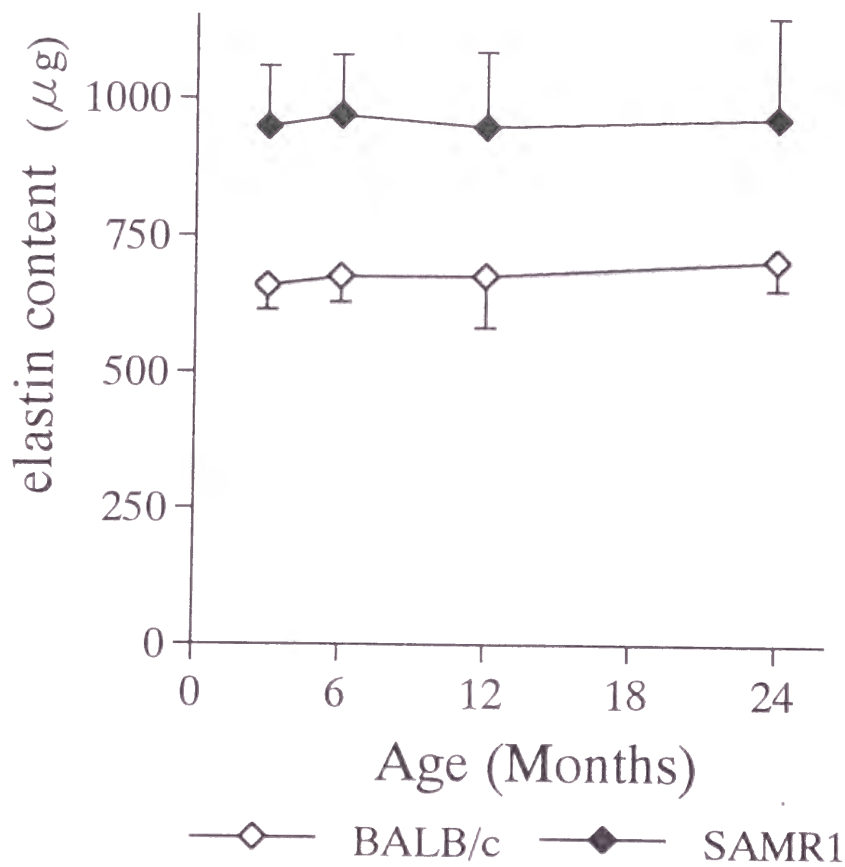


Figure 2

Age-associated changes in elastin content of the lungs of the BALB/c and SAMR1 mice

Error bars: SD

There was no significant change in elastin content with aging in either strain.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=4), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=5), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

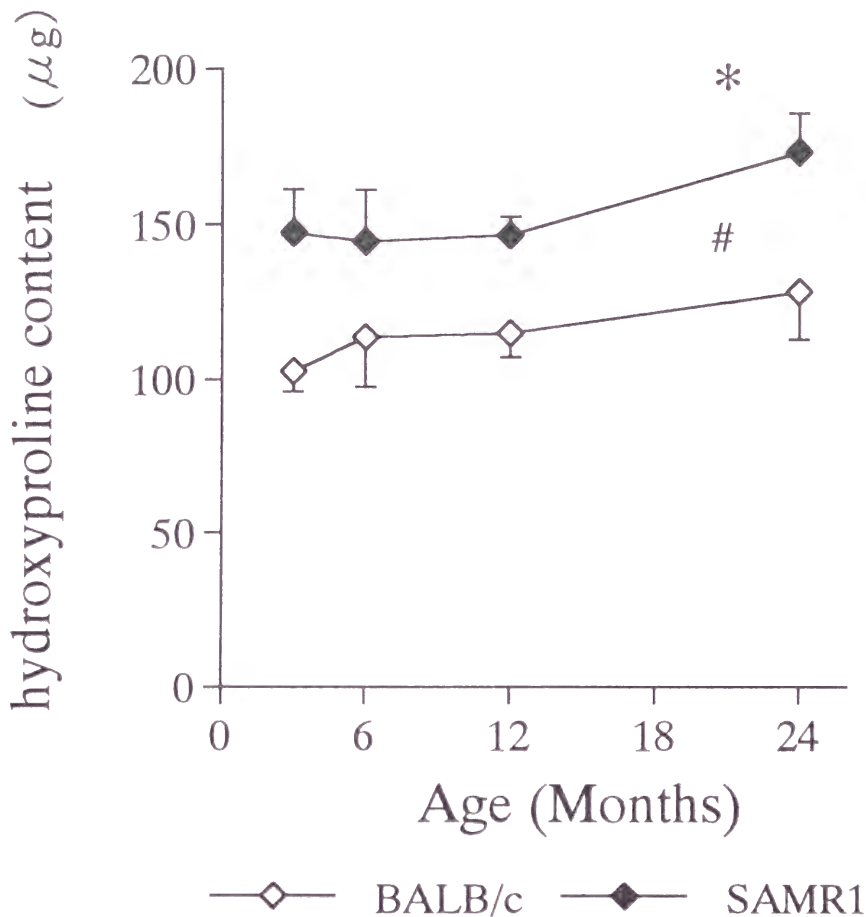


Figure 3

Age-associated changes in hydroxyproline content of the whole lung of the BALB/c and SAMR1 mice

Statistical significance of differences between age groups:

* $p < 0.05$ 24 mo vs. 3 mo, 6 mo in SAMR1 mice.

$p < 0.05$ 24 mo vs. 3 mo, 6 mo in BALB/c mice.

Error bars: SD

The hydroxyproline content of the whole lung increased significantly at 24 months of age in the SAMR1 and BALB/c mice.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=4), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=5), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

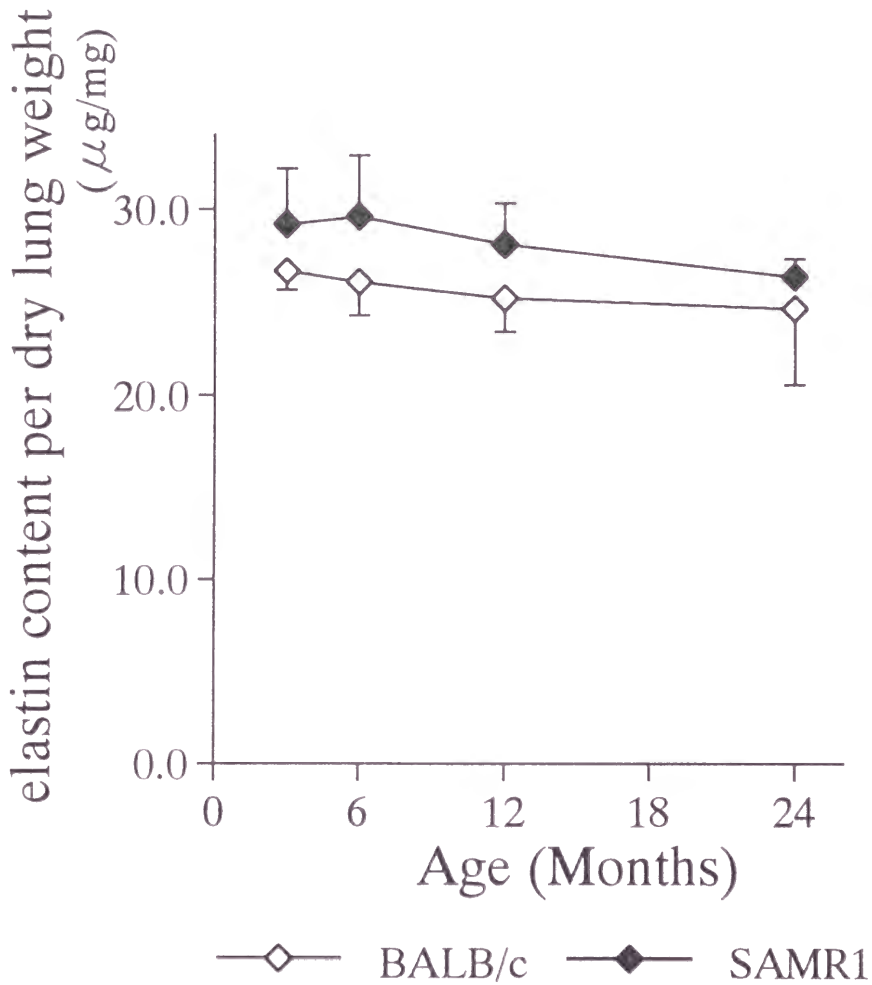


Figure 4

Age-associated changes in elastin content per dry lung weight of the BALB/c and SAMR1 mice

Error bars: SD

There was no significant change with aging in either strain.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=7), 6-month-old (n=7), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=4), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

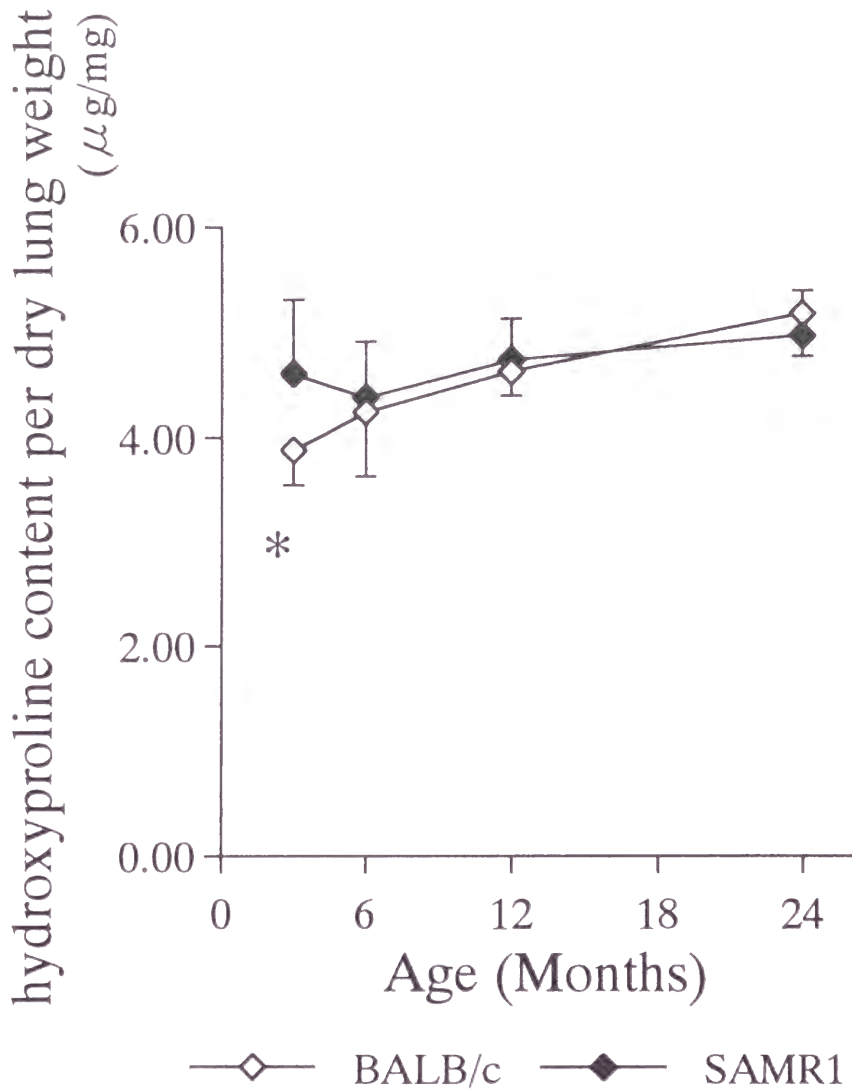


Figure 5

Age-associated changes in hydroxyproline content per dry lung weight of the BALB/c and SAMR1 mice

Statistical significance of differences between age groups:

$p < 0.05$ 3 mo vs. 24 mo in BALB/c mice.

Error bars: SD

There was no significant change in the hydroxyproline content per dry lung weight in the SAMR1 mice with aging. In the BALB/c mice, the hydroxyproline content per dry lung weight at 3 months of age was significantly lower than that at 24 months of age.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=7), 6-month-old (n=7), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=4), 6-month-old (n=7), 12-month-old (n=7), 24-month-old (n=4).

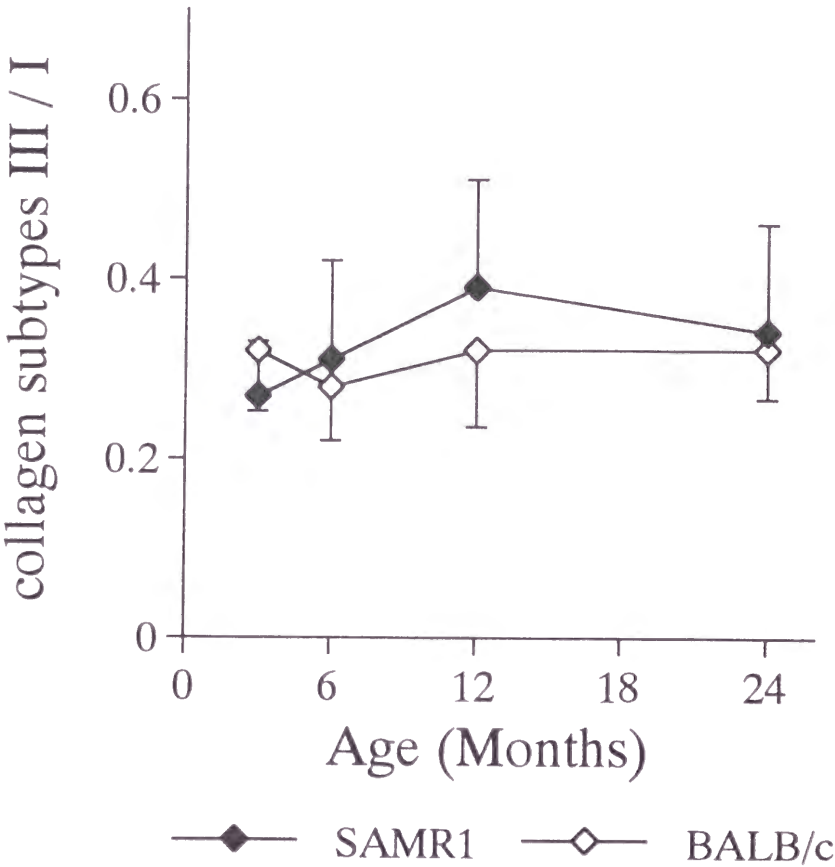


Figure 6

Age-associated changes in the proportion of types I and III collagen (expressed as type III / type I) of the BALB/c and SAMR1 mice
Error bars: SD

There was no significant change with aging in either strain.

Number of animals in each age group and strain: BALB/c: 3-month-old (n=5), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4). SAMR1: 3-month-old (n=4), 6-month-old (n=5), 12-month-old (n=5), 24-month-old (n=4).