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A Novel Mouse Model of Spontaneous Pulmonary Emphysema: Mayumi-Emphysema Mouse

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Mayumi-Emphysema（ME）mice, which develop spontaneous pulmonary emphysema, were established from wild type C57BL/6J mice. ME mice showed air space enlargement from 2 weeks of age and rapidly developed severe emphysema. The mean linear intercept of the lungs in ME mice was higher than that in control mice at the age of 2 weeks and increased progressively with age in ME mice. However, there was no infiltration of inflammatory cells into the interstitium or alveolar air spaces in the lungs of ME mice. The serum α1-antitrypsin protein level was slightly decreased in ME mice at 4 weeks of age. In ME mice, lung destruction was not associated with an inflammatory reaction, and a slight decrease in serum α1-antitrypsin may be insufficient to induce severe emphysema in ME mice. Hereditary impairment of the normal developmental mechanism for alveoli in the early stage and an abnormal lung structure may induce spontaneous pulmonary emphysema in ME mice.

Key words: naturally occurring emphysema, mice model, C57BL/6 background

INTRODUCTION

Chronic obstructive pulmonary disease（COPD）is a major cause of morbidity and mortality worldwide and is characterized by persistent and non-reversible airflow obstruction, parenchyma destruction, and emphysema［1］. Although the precise pathophysiological mechanism for COPD is not fully understood, environmental factors are involved, and cigarette smoking is the most important risk factor for COPD. However, only approximately 15% of cigarette smokers develop clinically significant COPD［2］, and the prevalence of COPD is higher among first-degree relatives of subjects with COPD compared to relatives of control subjects［3］. These results suggest that a combination of various environmental factors and genetic factors affect the development of COPD. To date, α1-antitripsin deficiency is the most well-known genetic risk factor for COPD［4］. α1-antitripsin inhibits proteolytic enzymes, such as neutrophil elastase, and the depletion of α1-antitripsin leads to excessive tissue destruction at inflammatory site. However, only 1 to 5% of COPD patients have been shown to have α1-antitripsin deficiency［5, 6］. Therefore, it is important to identify candidate genes for COPD other than α1-antitripsin. Recent genome-wide association studies have been powerful for the identification various common variants responsible for COPD susceptibility［7, 8］. However, each identified common variant may confer weak susceptibility to COPD, and other methods are required to detect rare variants that show strong effects on COPD susceptibility.

Several mice strains with naturally occurring genetic mutations, such as tight-skin（Tsk）mice［9］, pallid mice［10］, beige mice［11］, and blotchy mice［12］ are known to spontaneously develop COPD. These mice strains are useful for investigating the effect of specific genes on the pathogenesis

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of COPD. Pallid mice have severe deficiency in serum α1-antitripsin, and emphysematous change with destruction of the alveolar septa from 12 months of age has been observed in these mice [10]. Pallid mice are considered an animal model of COPD in human patients with a genetic deficiency in α1-antitripsin. However, the effects of genetic mutations in pallid mice and other mice that spontaneously develop emphysema are not restricted to the development of pathophysiological abnormalities in lung, and these strains develop phenotypic disorders that are multisystemic. Therefore, it is important to find other mouse strains that develop emphysema with other minor phenotypic disorders. In the present study, we established a new mouse strain that spontaneously develop severe emphysema. Here, we present the basic features of this new mouse strain and the time-course of morphological changes in the lung of mice of this strain.

MATERIALS AND METHODS

Animal preparation

Wild type C57/BL6J mice were obtained from CLEA Japan (Osaka, Japan) and housed under conventional animal laboratory conditions with access to standard chow and tap water ad libitum. Phenotypically deviant mice with severe pulmonary emphysema were originally found in a small breeding colony of wild type C57/BL6J mice maintained by Mayumi Takechi at the Department of Experimental Animals of Shimane University. An inbred strain was established as an emphysema model by repeating brother-sister mating for 20 generations, and the animals obtained were named Mayumi Emphysema (ME) mice. At the age of 1, 2, 4, 8 and 56 weeks, ME mice (n = 6 for each time-point) were killed using a lethal dose of sodium pentobarbital (100 mg/kg), and lung lobes were collected for histological analysis. Age-matched normal wild type C57/BL6J mice (n = 6) served as normal controls. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Shimane University School of Medicine, and the protocols for this study were reviewed and approved by the University's Committee on the Care and Use of Laboratory Animals.

Histological analysis

The inferior lobe of the right lung was carefully inflated with 10% buffered formalin at a constant pressure of 200 mm H_2O for 5 min and then fixed with 10% buffered formalin. The lung tissues were embedded in paraffin, and 5-μm sections were subjected to hematoxylin and eosin (H & E) staining. The mean liner intercept (Lm) was calculated according to an established method [13]. Briefly, H & E stained sections were examined in a blinded fashion at 400x. Twenty random fields of the superior and inferior lobes of the right lung were examined by means of a 500 μm x 500 μm grid scale in the eyepiece of a microscope. The number of alveoli intersected by the line was counted, and the average alveolar size was calculated.

Analysis of inflammatory cells in BALF

BALF was collected from the left lung by lavage with 1.0 mL of PBS (5 washes of 0.2 mL each) at 4 weeks of age and with 1.5 mL of PBS (5 washes of 0.3 mL each) at 56 weeks of age. The numbers of total cells, macrophages, lymphocytes, and neutrophils in BALF were counted as described previously [14].

Liver α1-antitrypsin mRNA level and serum α1-antitrypsin protein level

Liver and serum were collected from ME mice and normal mice at 4 weeks of age. The liver α1-antitrypsin mRNA level was measured by quantitative RT-PCR as described previously [14]. Briefly, total RNA was isolated from the liver. The PCR primers for α1-antitrypsin were selected according to the α1-antitrypsin cDNA sequence (sense primer: 5'-GATCCTGAGAACACTGAGGAAGC-3'; antisense primer: 5'-AGTCTGGGGAAGTGGATCTGG-3'). Each competitor DNA was prepared by inserting an external DNA fragment into a target cDNA at a unique restriction enzyme site. The serum α1-antitrypsin protein level was measured using an enzyme-linked immunoassay (ELISA) kit (Immunology Consultants Laboratories, OR, USA) according to the manufacturer's instructions.

Statistical analysis

All data are expressed as the means ± S.E.M.

Comparisons between two groups were performed using Student's *t*-test for unpaired data. For multiple comparisons, data were examined by analysis of variance (ANOVA) followed by the Bonferroni / Dunn's test. Differences were considered statistically significant when *P* values were less than 0.05.

RESULTS

Gross physical appearance of ME mice

The coat color of ME mice was black, and no abnormalities were visible on the skin of ME mice (Fig. 1). The mean body weight of the male ME mice was significantly less than that of the male normal mice at both 4 weeks of age (10.2 ± 1.1 g vs 15.9 ± 0.4 g; $p < 0.05$) and 56 weeks of age (21.5 ± 1.5 g vs 35.0 ± 0.6 g; $p < 0.05$). Although the growth rate of ME mice was slower than that of normal mice, both the male and female ME mice developed normally and were fertile. Bullous changes were identified macroscopically on the lung surfaces of ME mice from 4 weeks of age, and ME mice developed extensively diffuse bullous emphysema at 56 weeks of age (Fig. 2B). Some of the ME mice had other phenotypic abnormalities, such as a short face, malocclusion, and microphthalmia.

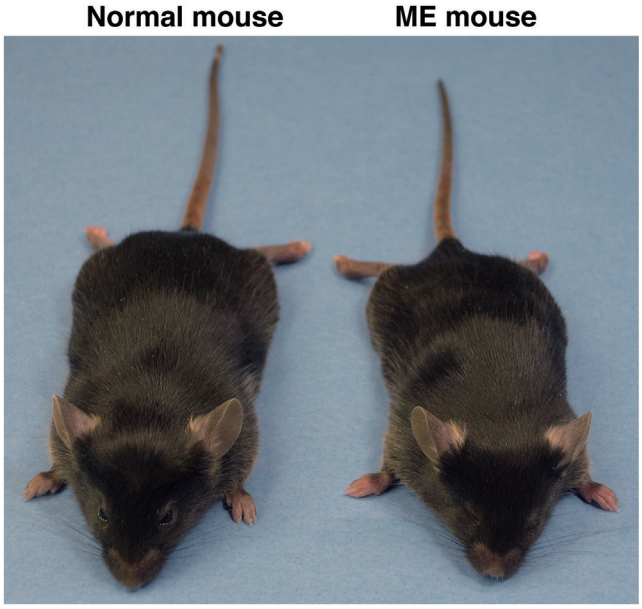

Fig. 1. Gross appearance of normal mouse (left) and ME mouse (right) at 56 weeks of age. ME mouse is smaller than wild type C57BL6/J mouse.

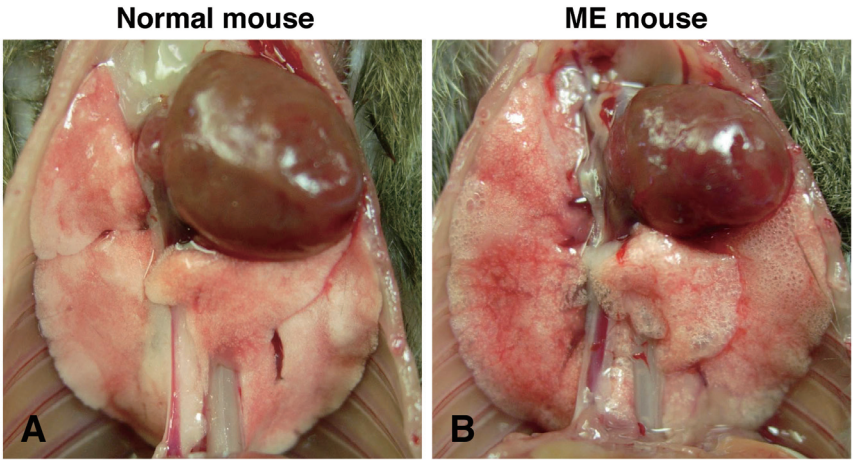

Fig. 2. Gross pathology of normal mouse (A) and ME mouse (B) at 56 weeks of age. Lung of ME mouse shows diffuse bullous emphysema.

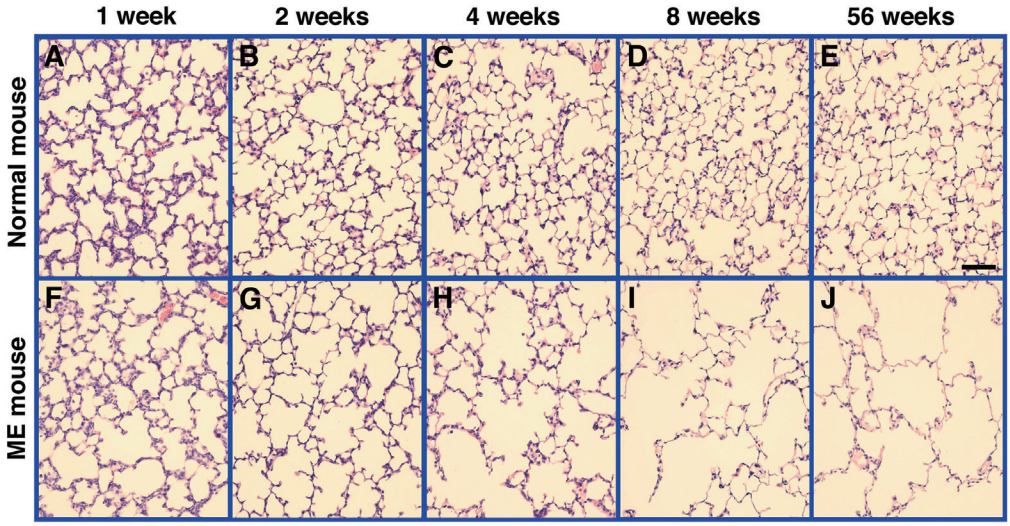

Fig. 3. Representative photographs of H & E stained lung tissue cross sections from normal mice (A-E) and ME mice (F-J) at 1, 2, 4, 8 and 56 weeks of age. Lung tissues of ME mice showed air space enlargement with alveolar septa destruction from 2 weeks of age, and ME mice progressively developed severe emphysema with marked enlargement of alveoli after 4 weeks of age. Scale bar = 50 μm.

Histological analysis

Lung tissues of ME mice were not different from those of normal mice at 1 week of age. However, ME mice showed air space enlargement with destruction of alveolar septa from 2 weeks of age and developed severe emphysema with marked enlargement of alveoli after 4 weeks of age (Fig. 3). The Lm of normal mice decreased markedly at 2 weeks of age during the early neonatal period, and then remained constant through 56 weeks of age. In contrast, the Lm of ME mice did not decrease at 2 weeks of age and continuously increased during the experimental period from 2 weeks to 56 weeks of age. The Lm of ME mice was not different from that of normal mice at 1 week of age but was larger than that of normal mice after 2 weeks of age (Fig. 4).

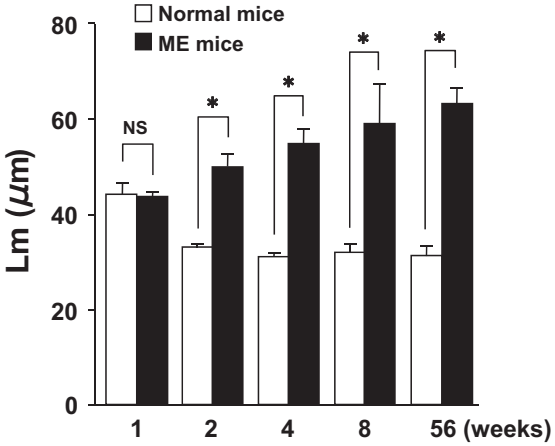

Fig. 4. Mean linear intercept (Lm) of normal mice and ME mice at 1, 2, 4, 8, and 56 weeks of age. Lm increased continuously with age in ME mice up to 56 weeks of age. The results are shown as the means ± S.E.M. *P < 0.05 for comparison between normal mice and ME mice. NS = not significant.

Inflammatory cells in BALF

The numbers of total cells, macrophages, lymphocytes, and neutrophils in BALF of ME mice were not different from those of normal mice at 4 weeks of age (Fig. 5A). Although the numbers of total cells, macrophages, lymphocytes, and neutrophils in BALF were higher in ME mice than in normal mice at 56 weeks of age, this difference was not statistically significant. (Fig. 5B).

α1-antitrypsin mRNA level in liver and α1-antitrypsin protein level in serum

The mean α1-antitripsin mRNA level in the livers of ME mice was not different from that in normal mice (Fig. 6A). However, the mean α1-antitripsin protein level of ME mice was slightly lower ($p < 0.05$) than that of normal mice (Fig. 6B).

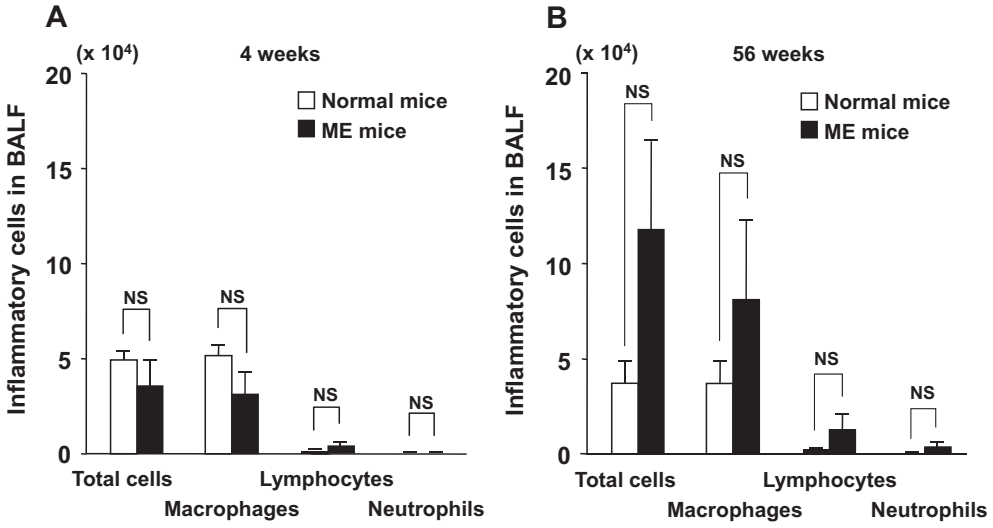

Fig. 5. Numbers of total cells, macrophages, lymphocytes, and neutrophils in BALF from normal mice and ME mice at 4 weeks of age (A) and 56 weeks of age (B). The results are shown as the means ± S.E.M. NS = not significant.

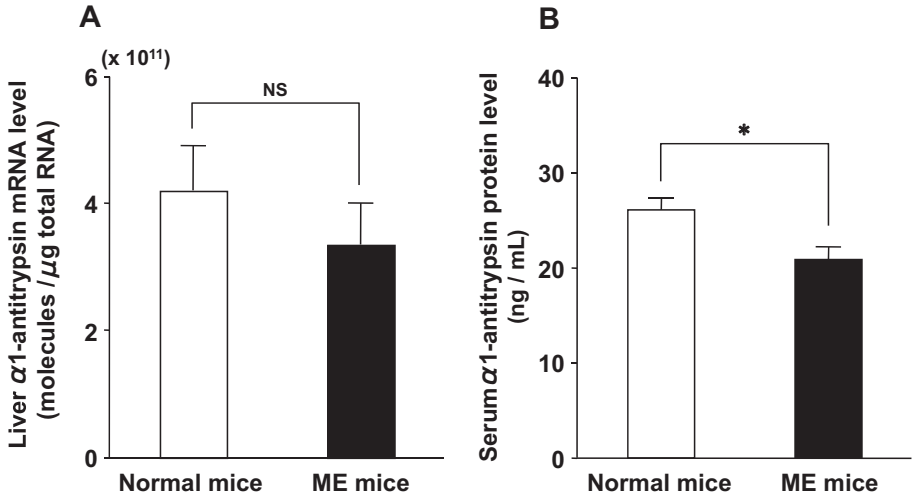

Fig. 6. Liver α1-antitrypsin mRNA level (A) and serum α1-antitrypsin protein level (B) of normal mice and ME mice at 4 weeks of age. The results are shown as the means ± S.E.M. *P < 0.05 for comparison between normal mice and ME mice. NS = not significant.

DISCUSSION

Four different strains of mice with the C57BL/6 background, Tsk, pallid, beige, and blotchy mice have been shown to spontaneously develop emphysema due to naturally occurring genetic abnormalities [15]. These genetic abnormalities not only induce defects in lung structure and homeostasis but also cause multisystemic abnormalities, including emphysema. Tsk mice have an autosomal dominant duplication mutation of the fibrillin gene that results in the synthesis of a larger than normal fibrillin 1 protein. Abnormal fibrillin 1 molecules cause systemic deposition of extracellular matrix, and heterozygous Tsk mice develop severe skin tightness [16]. In addition to connective tissue abnormalities, Tsk mice exhibit an early onset of emphysema between 4 to 15 days after birth, and it develops rapidly [17]. ME mice also show enlarged air spaces and alveolar septa destruction early in life at 2 weeks of age,

and rapidly develop severe emphysema. ME mice and Tsk mice have similar properties of emphysema, including early onset and rapid development, but ME mice do not exhibit skin structure abnormalities. Another important difference between Tsk mice and ME mice is that macrophages and neutrophils have been shown to accumulate in the lung tissues of Tsk mice at 3 weeks of age [9], whereas the histological analysis of the lungs of ME mice in this study demonstrated no accumulation of macrophages or neutrophils during the entire experimental period from 2 to 56 weeks of age. Bronchoalveolar lavage analysis also showed that macrophages and neutrophils were not increased in the lungs of ME mice at 4 and 56 weeks of age. In addition, Tsk mice have been shown to have a high level of elastase activity in neutrophil lysosomal extracts and a low level of α1-protease inhibitor in serum [18]. These results suggest that an imbalance between protease and antiprotease activities, which is induced by the accumulation of protease-secreting inflammatory cells, such as macrophages and neutrophils, is not the main pathogenetic mechanism for emphysema in ME mice.

Pallid mice have a nonsense mutation at codon 69 of the pallidin gene. Pallidin interacts with syntaxin 13, which is important for vesicle docking and fusion. This mutation produces defects in subcellular organelles and prevents the secretion of α1-antitrypsin into the circulation, consequently decreasing the elastase inhibitory capacity in pallid mice [10, 18]. Although the serum αl-antitrypsin level in pallid mice has been shown to be 54% lower than that in normal C57/BL6 mice at 2 months of age, in pallid mice, alveolar septum disruption was first observed at 8 months of age, and air space enlargement was observed late in life from 12 months of age [10]. In contrast to Tsk and ME mice, emphysema in pallid mice develops slowly and late in life. In pallid mice, a time lag between the genetic deficiency that causes a decreased serum α1-antitrypsin level and the onset of emphysema has been observed. In this study, the serum α1-antitrypsin level in ME mice was also slightly decreased at 4 weeks of age. However, ME mice showed air space enlargement early at 2 weeks of age and rapidly developed severe emphysema. Therefore, the slight decrease in serum αl-antitrypsin level observed in ME mice may not be a crucial factor in the induction of emphysema in ME mice.

Beige mice have a 5 kb deletion mutation in the Lyst gene resulting in lysosomal missorting of proteins, such as elastase and cathepsin G [19]. Beige mice express various phenotypic disorders, such as dilution of coat color, giant lysosomes, and increased susceptibility to infection [15]. Beige mice also show enlarged alveolar spaces at 1 month of age [20]. Previous morphological analyses of normal mouse lung revealed that large size primary saccules were subdivided into small true alveoli at 3 to 4 days after birth, and the alveoli development process was completed by day 14 after birth [21]. Thus, in that study, the Lm of normal mice decreased markedly during the early neonatal period and reached a constant adult value prior to 20 days of age [11]. However, this early decrease in Lm has not been observed in Beige mice, which showed a constant Lm up to 24 months of age [20]. Furthermore, an ultrastructural analysis of lung matrix components in beige mice revealed neither elastin destruction nor collagen remodeling [22]. These findings suggest that deficient alveolar septation during the neonatal and infant periods induces an enlargement of the alveolar space in beige mice. In the current study, similar to beige mice, the Lm of ME mice did not decrease during the early neonatal period, but it increased progressively and reached its maximum value at 56 weeks of age. Postnatal alveolarization has been shown to be impaired in both beige and ME mice, but the alveolar walls of ME mice may be more susceptible to emphysema than those of beige mice.

Blotchy mice have a mutation in the gene that encodes copper-transporting ATPase [23, 24]. This mutation results in a general copper-ion deficiency and reduces the activity of several copper-requiring enzymes, such as lysyl oxidase, which is involved in the cross-linking of collagen and elastin [25]. Blotchy mice develop aortic aneurysms, osteoarthrosis, skin tensile weakness, and emphysema [26]. Although newborn blotchy mice have been shown to have larger lungs than those of normal littermates, the Lm of blotchy mice was shown to mildly increase from 8 to 12 weeks after birth [26]. In

blotchy mice, emphysema has been shown to progress more slowly than in Tsk and ME mice.

In conclusion, we established a new mouse model of spontaneous severe emphysema. Although some of the ME mice had phenotypic abnormalities, such as short face, malocclusion, and microphthalmica, ME mice did not have major systemic abnormalities. In contrast to the other naturally occurring genetic mutant mouse strains with multisystemic disorders, ME mice may be a unique model for the development of emphysema. Further genetic analyses and detailed molecular studies are required to determine the candidate genes and the precise mechanisms of the induction of emphysema in ME mice. ME mice are expected to be a highly useful mouse model for investigating the pathogenesis of emphysema.

FOOTNOTES

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