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Mice overexpressing latent matrix metalloproteinase-2 develop lung emphysema after short-term exposure to cigarette smoke extract

Masahiro Onishi [a], Tetsu Kobayashi [a], Corina N. D'Alessandro-Gabazza [b], Hajime Fujimoto [a], Ayshwarya-Lakshmi Chelakkot-Govindalayathil [b], Yoshinori Takahashi [a], Taro Yasuma [b], Kota Nishihama [b], Masaaki Toda [b], Yoshiyuki Takei [c], Osamu Taguchi [d], Esteban C. Gabazza [b, *]

[a] Department of Pulmonary and Critical Care Medicine, Japan
[b] Department of Immunology, Japan
[c] Department of Gastroenterology and Hepatology, Japan
[d] Mie University Center for Physical and Mental Health, Mie University Graduate School of Medicine, Edobashi 2-174, Tsu, Mie, 514-8507, Japan

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ABSTRACT

Chronic obstructive pulmonary disease is the major growing cause of mortality and morbidity worldwide, and it is going to become the third most common cause of death by 2020. Chronic obstructive pulmonary disease is pathologically characterized by lung emphysema and small airway inflammation. Animal models are very important to get insights into the disease pathogenesis but current models of chronic obstructive pulmonary disease take a long time to develop. The need of a new model is compelling. In the present study we focus on the role of matrix metalloproteinases in the pathogenesis of chronic obstructive pulmonary disease and hypothesized that lung overexpression of latent matrix metalloproteinases-2 would allow the development of emphysema after short-term exposure to cigarette smoke extract inhalation. Human latent matrix metalloproteinases-2 transgenic mouse expressing high level of the protein in the lungs and wild type mouse were exposed to aerosolized cigarette smoke extract for two weeks. Transgenic mice showed significant lung emphysematous changes, increased infiltration of inflammatory cells and enhanced lung concentrations of inflammatory cytokines in the lungs compared to their wild type counterparts after inhalation of cigarette smoke extract. This novel mouse model will be a very useful tool for evaluating the mechanistic pathways and for development of novel therapies in cigarette smoke-associated lung emphysema.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by small airway disease, lung emphysematous changes and progressive airflow limitation. It is one of the leading causes of disability, morbidity and mortality worldwide [1]. Presently, there are about 200 million people suffering from some degree of COPD in the entire world [2]. COPD is now the fourth leading cause of death globally, and it is going to be the third leading cause of death by 2020 [1–3]. The major risk factor of COPD is cigarette smoke exposure followed by air pollution and occupational exposures [4,5]. Susceptibility to cigarette smoke varies among individuals but 50% of the elderly smokers develop COPD [6,7]. Important events in the pathogenesis of COPD are chronic inflammation, an increased oxidant burden, and a protease/anti-protease imbalance in the lungs [8]. Long-term exposure to cigarette smoke causes increased infiltration of neutrophils, macrophages, lymphocytes and the release of chemokines, cytokines and proteinases including matrix metalloproteinases resulting in chronic small airway inflammation and alveolar destruction [9,10].

Matrix metalloproteinase (MMP)-2, a 72 kDa type IV collagenase also known as gelatinase A, is a member of the MMP family of extracellular matrix degrading enzymes with important roles in many physiological and pathological conditions [11]. Similar to other MMPs, MMP-2 is expressed in a catalytically inactive state under inflammatory conditions or in tissues undergoing

* Corresponding author. Department of Immunology, Mie University School of Medicine, Edobashi 2-174, Tsu, Mie, 514-8507, Japan.
E-mail address: gabazza@doc.medic.mie-u.ac.jp (E.C. Gabazza).

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remodelling where it is activated by proteolysis [11]. MMP-2 can be produced in the lungs by bronchial epithelial cells, clara (club) cells, alveolar type II cells, smooth muscle cells and fibroblasts [12]. MMP-2 also plays an important role in inflammatory responses by activating various cytokines and chemokines [12,13]. A large number of investigations in patients and in animal models have implicated MMPs in the pathogenesis of cigarette smoke-associated COPD [14,15]. However, the contribution of MMP-2 to cigarette smoke-induced COPD remains elusive.

In the present study, we hypothesized that lung overexpression of the latent form of human MMP-2 will predispose the mouse to cigarette smoke-associated emphysema.

2. Materials and methods

2.1. Experimental animals

The homozygous human proMMP-2 transgenic (TG) mice backcrossed with C57BL/6 mice have been previously characterized [16]. Briefly, the full-length human proMMP2 was sub-cloned into a vector containing the CAG-promoter (cytomegalovirus enhancer plus chicken β-actin promoter) and rabbit β-globin polyadenylation sites. All organ tissues including the lungs express the human proMMP-2 RNA and its reported plasma concentration is 67.63 ± 5.68 ng/ml [16]. The Committee for Animal Investigation from Mie University approved the experimental protocol of this study (Approval No 24-1-1-1) and all procedures were performed according to the institutional guidelines. Mice received humane care following the “Guide for the Care and Use of Laboratory Animals” issued by the National Academy of Sciences and published by the National Institutes of Health. The research followed the ARRIVE Guidelines for animal investigation.

2.2. Polymerase chain reaction (PCR)

Total RNA was isolated from tissue samples using the Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RNA samples were reverse transcribed with Superscript (Invitrogen) using Oligo-dT. PCR reactions were performed using 28 or 38 cycles of 94 °C 10s, 60 °C for 20s and 72 °C for 40s, followed by a final extension of 5 min at 72 °C with a PTC-100 Thermal Controller (MJ Research). Control reactions without reverse transcriptase (RT) were also performed. PCR products were run on 2% agarose gel and visualized using ethidium bromide staining and ultraviolet *trans*-illumination. The Supplementary Table 1 describes the sequence of primers.

2.3. Induction of emphysema with cigarette smoke extract (CSE) solution

We used homozygous human proMMP2 TG mice and wild type C57BL/6 mice (Nihon SLC -Hamamatsu, Japan) of 25–30 weeks and weighing 27–30 g in the study. Mice were randomised, allocated to two groups and exposed to aerosolized saline or CSE solution 60min every day for 2 weeks in an exposure chamber (MIPS, Osaka, Japan). All mice were sacrificed on day 15 after exposure by an overdose of sodium pentobarbital. Tidal volume was measured non-invasively with a whole body plethysmograph system (Buxco, Sharon, CT, USA).

2.4. X-ray imaging

A micro-computed tomography (CT) (Latheta LCT-200, Hitachi Aloka Medical, Tokyo, Japan) was used to assess emphysematous changes in the mouse model. Data were acquired from mice in prone position and under isoflurane\inhalation anaesthesia as previously described [17].

2.5. Histological and morphometric analysis

Lungs were removed after flushing off the pulmonary circulation with saline. The tissue was embedded in paraffin after 24h of fixation in 10% neutral buffered formalin. Sections of 5 μm-thick were cut and mounted on slides to stain with hematoxyline and eosin (H&E). Olympus BX50 microscope with a plain objective combined with an Olympus DP70 digital camera (Tokyo, Japan) was used to evaluate the sections. Histological slides from each sample stained with H&E was used for morphometric analysis. Randomly selected ten microscopic fields form the central and peripheral regions of the lungs were photographed, enlarged uniformly and overlaid with horizontal and vertical lines with equal intervals. Mean linear intercept (Lm), which determines the alveolar septal distance or alveolar destruction, was calculated by counting the number of intersections with the alveolar walls and dividing it by the total length of the line. The number of alveoli in a defined area was also recorded, and was normalized per unit area (100 μm).

2.6. Sampling of bronchoalveoalr lavage fluid (BALF) and peripheral blood

BALF was collected under profound anaesthesia with sodium pentobarbital as previously described [23]. Nucleocounter from ChemoMetec (Allerod, Denmark) was used for counting the total number of cells. BALF supernatant was separated by centrifugation (1000 g, 10min, 4 °C) and stored at −80 °C until use. BALF cells were separated by cytospin and stained with May-Grünwald Giemsa stain (Merk, Darmstadt, Germany) [18]. The cells were counted in 20 fields of 40× magnification in each of the slide and differential cell count was calculated.

2.7. Isolation of spleen cells

Mice were sacrificed by anaesthesia overdose and the spleen was dissected with scissors into 2–3 mm pieces, incubated for 30 min at 37 °C in 0.5 mg/ml collagenase solution, further homogenized between the frosted-end of two glass slides and filtered through a mesh as described [19]. We used the following antibodies for flow cytometry analysis: fluorescein isothiocyanate (FITC)-labeled anti-mouse Ly-6G/Ly-6C (Gr-1; clone RB6-8C5) rat IgG2bκ, phycoerythrin (PE)-labeled anti-mouse F4/80 (clone ClA3-1) rat IgG2bκ, PE/Cy5-labeled anti-mouse CD11c (clone N418) hamster IgG, FITC-labeled anti-mouse CD3ε (clone 145-2C11) hamster IgG, PE/Cy5-labeled anti-mouse CD45R/B220 (clone RA3-6B2) rat IgG2aκ, FITC-labeled anti-mouse CD25 (clone PC61) rat IgG1λ, PE-labeled anti-mouse CD8a (clone 53–6.7) rat IgG2aκ, and PE/Cy5-labeled anti-mouse CD4 (clone GK1.5) rat IgG2bκ were from Bio-Legend, Inc. (San Diego, CA). PE-labeled anti-mouse NK1.1 (clone PK136) mouse IgG2aκ, biotin-FasL (clone MFL3) Armenian hamster IgG2, FITC-streptavidin and FITC-annexin V were from BD Pharmingen. Monocytes/macrophages were defined as SSChiF4/80hiGr-1lo population, granulocytes as SSChiGr-1hi cells and dendritic cells as SSCloF4/80- cells. B cells, T cells, and NK cells were defined as SSClo CD45R+, SSCloCD3ε+NK1.1-, SSCloCD3ε+NK1.1+, respectively. CD4 T cells ($CD4^+CD8$-), CD8 T cells (CD4-$CD8^+$), and regulatory T cells ($CD4^+CD8$-$CD25^+$) were also counted.

2.8. Biochemical analysis

The concentrations of mouse interferon (IFN)-γ and mouse tumor necrosis factor (TNF)-α (eBioscience, Santa Diego, CA) were

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