様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２９　年　　６　月　　７　日現在

機関番号：　１４４０１
研究種目：　基盤研究(C)（一般）
研究期間：　2013～2016
課題番号：　２５４３０１８２

研究課題名（和文）喫煙によって引き起こされる慢性炎症の発症メカニズムの解明

研究課題名（英文）Identification of the mechanisms driving the onset of chronic inflammation by cigarette smoke

研究代表者

ディエス　ディエゴ（Diez, Diego）

大阪大学・免疫学フロンティア研究センター・准教授

研究者番号：９０５９７７４１

交付決定額（研究期間全体）：（直接経費）　　4,200,000円

研究成果の概要（和文）：タバコおよびシリカへの暴露によって、慢性的な炎症と病態進行を特徴とする慢性閉塞性肺疾患および珪肺症をそれぞれ発症する。 本研究課題の目的は、珪肺症モデルにおける慢性炎症の発症機構の解明である。我々は、1ヶ月間、異なるシリカ用量にマウスを曝露し、肺疾患の進行状況を1年間にわたって観察し、暴露後1、3および12ヶ月目の免疫細胞数と遺伝子発現を測定した。 最初の1ヶ月間に高シリカ用量に曝露されたマウスは、1年後の炎症性ケモカインおよびT細胞動員のレベルが高いことが示された。 トランスクリプトーム解析では、別々に発現し、抗原処理および提示経路における顕著な役割を担う82個の遺伝子が同定された。

研究成果の概要（英文）：Exposure to tobacco and silica leads to Chronic Obstructive Pulmonary Disease and silicosis respectively, diseases marked by chronic inflammation and progression. This project aims to understand the mechanisms leading to chronic inflammation in a silicosis model. We expose mice to different silica doses for one month, then monitor disease progression in the lungs over one year. We measure immune cells number and gene expression 1, 3 and 12 months after exposure. Mice exposed to high silica doses during the first month show higher levels of inflammatory chemokines and recruitment of T cells after one year. Transcriptomic analysis identified 82 genes differentially expressed, with a prominent role of the antigen processing and presentation pathway.

研究分野：　総合生物

キーワード：　Systems biology　Immunology　Bioinformatics

１．研究開始当初の背景

Chronic inflammatory diseases like Chronic Obstructive Pulmonary Disease (COPD) and silicosis are caused by exposure to environmental substances like tobacco smoke (COPD) and silica dust (silicosis). Disease progression remains after stopping exposure to the environmental trigger. Mortality is high, and COPD is predicted to be the third cause of death in industrialized countries. How the activation of a chronic inflammatory response is produced and maintained is not well understood. A working hypothesis is that these noxious substances induce changes in the transcriptional program of cells in a way that promotes the development of chronic inflammation. However, the changes in transcriptional regulatory networks associated with this process are largely unknown.

２．研究の目的

The goal of this project is to gain insight into the mechanisms regulating the development of chronic inflammation in silicosis. The hypothesis is that changes in transcriptional regulatory networks drive this process. To test this hypothesis a mouse model of silicosis induced chronic inflammation will be used. Measurements of transcript levels and transcription factor binding will be performed to reconstruct transcriptional regulatory networks during disease progression. Changes in transcriptional regulatory networks will be assessed using systems biology approaches.

３．研究の方法

Experimental methods CB57BL/6 mice were administered an intranasal silica suspension (50 microL of 30 mg/mL). Three groups of mice were tested: unexposed (control, 0x), treated once (1 week, 1x) and treated four times (4 weeks, 4x). Lung tissue was collected from each group at 1 month (1m), 3 month (3m) and 1 year (1y) after administration. FACS analysis was used to identify subsets of immune cells. RNA was extracted to measure gene expression. qPCR was used to measure the expression of target genes. High-throughput gene expression measurements were performed using next generation sequencing (NGS).

Bioinformatics methods Reads from NGS were aligned to the mouse reference genome (mm10) with Bowtie2, and counts per transcript estimated. All analyses were performed using using R and packages from the Bioconductor project (http://bioconductor.org). Linear models were used to identify changes in cell populations and gene expression associated with treatment, including age (number of months since start of experiment) and treatment as co-variates. Changes were considered significant at FDR < 0.01. Sets of differentially expressed genes (DEGs) were tested for enrichment of pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg).

４．研究成果

38 mice were exposed to an intranasal silica suspension (0x, 1x and 4x weeks) for 1 month, then sacrificed at 1, 3 and 12 months. Lung tissue was dissected and subjected to FACS, qPCR and NGS analysis.

FACS was used to measure the number of immune cell populations in the lungs. Difference in cell numbers were assessed using a linear model including age and treatment for each cell type independently. We found an age-related increase in B cells, macrophages, dendritic cells and neutrophils, independent of treatment. We also found an increase in T cells in the 4x group at 1y, in an age-independent manner. This suggests silica exposure induces accumulation of T cells in mice (Fig. 1).

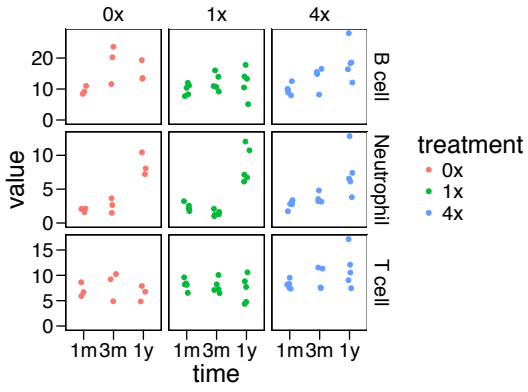

Fig. 1 Number of Neutrophils and T cells in lung.

Next, qPCR was used to measure the expression level of inflammatory markers (Cxcl1, Cxcl3, Cxcl5, Cxcl10, Ccl17, Il1b, Il6 and Tnf), and Hmox1, a gene known to be upregulated in silicosis. In contrast to control and 1x treated mice, 4x mice showed increased expression of chemokines Cxcl1, Cxcl3 and Cxcl5. Similar significant upregulation was detected for Hmox1. This suggests that silica induces changes in the expression of immune genes (Fig. 2).

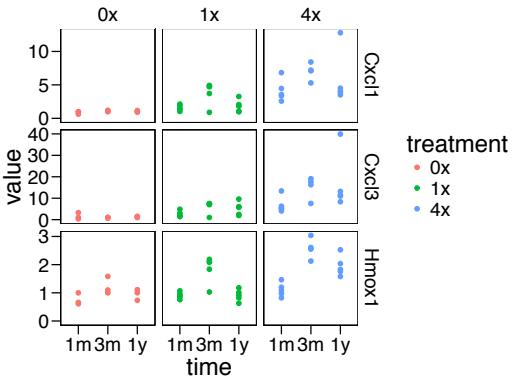

Fig. 2 qPCR based expression of chemokines Cxcl1, Cxcl3 and Cxcl5, and Hmox1.

Next, we searched for genes regulated by silica exposure by performing whole genome transcriptome analysis using NGS. Statistical analysis identified 461 DEGs associated with age, 47 associated with x4 silica exposure and 35 genes with changes due to both age and silica (4x) exposure. No significant changes were found in the 1x group (Fig. 3).

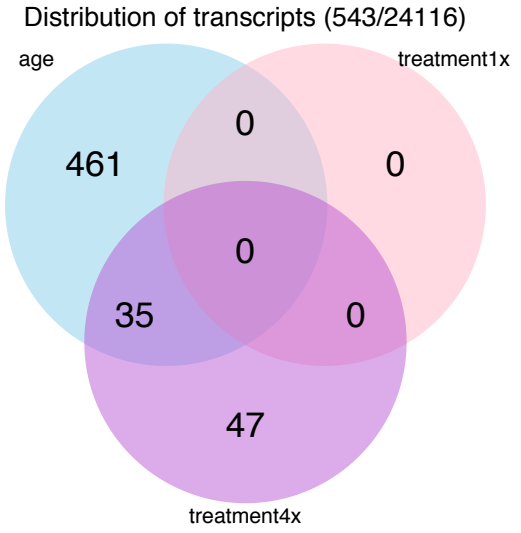

Fig. 3 Number of differentially expressed genes.

KEGG enrichment analysis was performed on the lists of DEGs to identify pathways up- or down-regulated due to silica exposure. We found that pathways associated with antigen processing and presentation, and phagosome, were the most significantly affected in the 4x group. In particular, many genes associated with MHC-I presentation, including beta2-microglobulin, HA-K1 and HA-D1 were upregulated in the 4x group, suggesting a role of CD8 T cells in the pathogenesis of silicosis (Fig. 4).

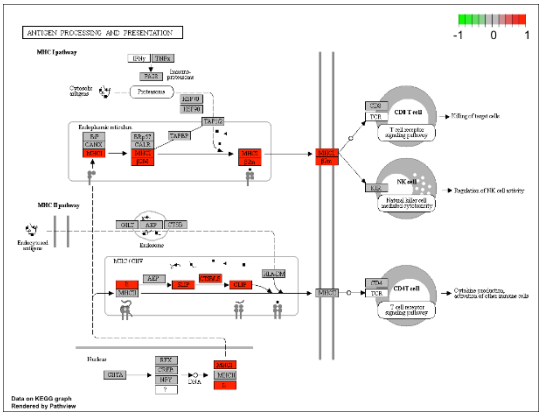

Fig. 4 Genes altered by silica in antigen processing and presentation pathway

Our data supports a role of silica dust in inducing chronic inflammation by promoting accumulation of T cells in the lung, and up-regulating pathways associated with antigen processing and presentation.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 0 件）
(Papers)
〔学会発表〕（計 0 件）
(Conferences)
〔図書〕（計 0 件）
(Books)
〔産業財産権〕
(Patents)
○出願状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

６．研究組織
(1)研究代表者

ディエス　ディエゴ（DIEZ, Diego）
大阪大学・免疫学フロンティア研究センター・准教授
研究者番号：90597741

(2)研究分担者
熊谷雄太郎（KUMAGAI, Yutaro）
大阪大学・免疫学フロンティア研究センター・特任助教（常勤）
研究者番号：00528408

(3)連携研究者
（　　）

研究者番号：

(4)研究協力者
（　　）