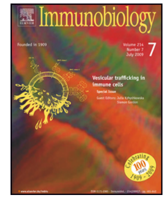
Immunobiology 220 (2015) 1284–1291

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio



# Treg depletion attenuates irradiation-induced pulmonary fibrosis by reducing fibrocyte accumulation, inducing Th17 response, and shifting IFN-γ, IL-12/IL-4, IL-5 balance

Shanshan Xiong[a], Renfeng Guo[b], Zhihua Yang[a], Long Xu[a], Li Du[a], Ruoxi Li[a], Fengjun Xiao[a], Qianjun Wang[a], Maoxiang Zhu[a,*], Xiujie Pan[a,*]

[a] Beijing Institute of Radiation Medicine, Beijing 100850, China
[b] Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109-0602,USA

ARTICLE INFO

*Article history:*
Received 14 September 2014
Received in revised form 17 March 2015
Accepted 7 July 2015
Available online 17 July 2015

*Keywords:*
Irradiation-induced pulmonary fibrosis
Regulatory T cells
Fibrocyte
Th17 cells
Th1/Th2 cytokines

ABSTRACT

Irradiation-induced pulmonary fibrosis results from thoracic radiotherapy and severely limits radiotherapy approaches. $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells (Tregs) are involved in experimentally induced murine lung fibrosis. However, the precise contribution of Tregs to irradiation-induced pulmonary fibrosis still remains unclear.

We have previously established the mouse model of irradiation-induced pulmonary fibrosis and observed an increased frequency of Tregs during the process. This study aimed to investigate the effects of Treg depletion on irradiation-induced pulmonary fibrosis and on fibrocyte, Th17 cell response and production of multiple cytokines in mice. Treg-depleted mice were generated by intraperitoneal injection with anti-CD25 mAb 2 h after 20 Gy $^{60}$CO γ-ray thoracic irradiation and every 7 days thereafter. Pulmonary fibrosis was semi-quantitatively assessed using Masson's trichrome staining. The proportions of Tregs, fibrocyte and Th17 cells were detected by flow cytometry. Th1/Th2 cytokines were assessed by Luminex assays. We found that Treg depletion decelerated the process of irradiation-induced pulmonary fibrosis and hindered fibrocyte recruitment to the lung. In response to Treg depletion, the number of $CD4^+$ T lymphocytes and Th17 cells increased. Moreover, Th1/Th2 cytokine balance was disturbed into Th1 dominance upon Treg depletion.

Our study demonstrates that Tregs are involved in irradiation-induced pulmonary fibrosis by promoting fibrocyte accumulation, attenuating Th17 response and regulating Th1/Th2 cytokine balance in the lung tissues, which suggests that Tregs may be therapeutically manipulated to decelerate the progression of irradiation-induced pulmonary fibrosis.

© 2015 Published by Elsevier GmbH.

## 1. Introduction

Thoracic radiotherapy currently plays an indispensable role in the management of cancer of the lungs, breast, and esophagus, as well as lymphatic system cancer (Kaya et al., 2014). However, its use is hampered by the high sensitivity of the lungs to irradiation, with resultant irradiation-induced acute pneumonitis and lung fibrosis (Brickey et al., 2012). It has been reported that the incidence rate of irradiation-induced pulmonary injury is between 5% and 24% in patients who have undergone thoracic irradiation (Sun et al., 2014).

$CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells (Tregs) play an important role in the suppression of autoimmune responses by inhibiting self-reactive T cells (Sakaguchi 2005; Sakaguchi et al., 1995, 2001). Moreover, these cells are also implicated in immunological tolerance and immune homeostasis (Piccirillo and Shevach 2001; Suvas et al., 2003; Takahashi et al., 2000). It has been demonstrated that Tregs are less sensitive to irradiation compared to other $CD4^+$ T cells (Qu et al., 2010). We have previously observed an increased frequency of Tregs in the lungs of mice with irradiation-induced fibrosis (Xu et al., 2014). Other investigators also reported the role of Tregs in other types of pulmonary fibrosis such as bleomycin-induced pulmonary fibrosis and silicosis (D'Alessio et al., 2009; Trujillo et al., 2010; Liu et al., 2010). However, the mechanisms whereby Tregs modulate irradiation-induced pulmonary fibrosis remain largely unelucidated.

* Corresponding authors.
*E-mail addresses:* zhumx@nic.bmi.ac.cn (M. Zhu), aaronpancn@126.com (X. Pan).

http://dx.doi.org/10.1016/j.imbio.2015.07.001
0171-2985/© 2015 Published by Elsevier GmbH.

Another piece of evidence implicating Tregs in pulmonary fibrosis demonstrated that Tregs regulate the differentiation of fibrocyte through TGF-β or IL-10 (Hong et al., 2007; Kingsley et al., 2002; Sun et al., 2011). Fibrocyte cells are bone marrow-derived mesenchymal stem cells and express hematopoietic stem cell markers CD34, leukocyte markers CD45 and fibroblast product collagen I (Bucala et al., 1994) and they have been shown to play an important role in lung repair and pulmonary fibrosis (Niedermeier et al., 2009; Phillips et al., 2004; Abe et al., 2001; Metz 2003). Our recent findings also implicate that the number of fibrocyte increased in the lung tissues of mice with irradiation-induced pulmonary fibrosis (Xiong et al., 2013).

These findings prompt us to hypothesize that Tregs contributed to the development of irradiation-induced lung fibrosis. In the present study, we depleted Tregs with monoclonal anti-CD25 antibodies in C57BL/6 mice receiving thoracic irradiation to investigate the contribution of Tregs to irradiation-induced pulmonary fibrosis, as well as its effects on fibrocyte, Th17 response and Th1/Th2 cytokine balance.

## 2. Materials and methods

### 2.1. Mice and mice treatment

Healthy female C57BL/6 mice with 6- to 8-week old (Vital River Laboratory Animal Co., Beijing, China) were housed in environmentally controlled conditions (22 °C, a 12 h light/dark cycle with the light cycle from 6:00 to 18:00 and the dark cycle from 18:00 to 6:00) with ad libitum access to standard laboratory chow and water. The animals were randomly divided into four groups ($n = 15$) as follows: non-irradiation control group, irradiation plus normal saline group, irradiation plus isotype monoclonal antibody group and irradiation plus monoclonal anti-CD25 antibody group.

For thoracic irradiation, dose and uniformity of distribution were determined before initiating the study as described earlier (Xu et al., 2014). Mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg body weight), and then received a single dose of thoracic irradiation (20 Gy) with a $^{60}$Co γ-ray source at a dose rate of 232.06 cGy/min. Organs above and below the thorax were shielded with 10 cm thick lead bricks. For depletion of Tregs, mice were injected intraperitoneally with 100 μg monoclonal anti-CD25 antibody (PC61) or isotype antibody (rat IgG1) (both from Sungene Biotech, Tianjin, China) 2 h after irradiation and every 7 days thereafter until the end of the experiment (Liu et al., 2011). The animals were sacrificed at the indicated time points up to 24 weeks post irradiation.

The study protocol was approved by the Animal Use Committee of Beijing Institute of Radiation Medicine and all experiments were performed in accordance with the USA National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

### 2.2. Hematoxylin/eosin (H&E) and Masson's trichrome staining

The right lung was harvested and fixed in 4% neutral paraformaldehyde and paraffin-embedded. The tissue specimens were sectioned at a thickness of 5 μm and conventionally stained with hematoxylin/eosin (H&E) for histological examination. Fibrosis was semiquantitatively assessed using Masson staining with Image-Pro plus analysis system (Szapiel et al., 1979).

### 2.3. Flow cytometry

For detection of Tregs in blood, heparinized murine blood was stained with mouse monoclonal APC-conjugated anti-CD4 antibody (GSK1.5, eBioscience, San Diego, CA) and PerCP-Cy5.5-conjugated anti-CD25 antibody (3C7, BD Pharmingen, San Diego, CA). Subsequently, red blood cells were lysed and the remaining cells were permeabilized using transcription factor fixation/permeabilization concentrate and diluent (eBioscience) followed by staining with monoclonal FITC-conjugated anti-Foxp3 antibody (FJK-16s, eBioscience).

Lung single-cell suspensions were prepared by collagenase dispersion as previously described (Sun et al., 2009) and stained with monoclonal V500-conjugated anti-mouse CD4 antibody, APC-conjugated anti-mouse CD25 antibody and APC-Cy7-conjugated anti-mouse CD45 antibodies (30-F11, all from BD Pharmingen). After fixation and permeabilization, cells were incubated with monoclonal PE-cy7-conjugated anti-mouse Foxp3 antibody (FJK-16s, eBioscience) and PerCP-conjugated anti-Col I antibody (Rockland, PA) or isotype control antibody. The FACSAriaII (BD, NJ) system was used for 5-color flow cytometric analysis. Single-color staining was performed synchronously for fluorescence compensation.

For staining of CD4$^+$ IL-17A$^+$ T helper (Th17) cells, lung single cell suspensions were stimulated with a cell stimulation cocktail (plus protein transport inhibitors) (eBioscience) in complete RPMI-1640 for 5 h. The cells were then stained with monoclonal FITC-conjugated anti-mouse CD4 antibody (RM4-5, eBioscience). After fixation and permeabilization, cells were incubated with monoclonal PE-conjugated anti-mouse IL-17A antibody (eBio17B7, eBioscience).

### 2.4. Multicytokine analysis

The isolated left lungs were homogenized in 1 mL T-PER Tissue Protein Extraction Reagent (Pierce, Rockford, IL) using a tissue homogenizer (Ikea, Germany). IFN-γ, IL-12, IL-4, and IL-5 levels were determined by Luminex assays using a Th1/Th2 Mouse 6-Plex Panel (Life Technologies) according to the manufacturer's protocols. In brief, mixed antibody-coupled microspheres (50 μL) and the lung homogenate (100 μL) were added into each well of 96-well filter plates. After an overnight incubation with agitation on a plate shaker at 4 °C, the supernatants were washed by phosphate buffered saline (PBS) containing 1% bovine serum albumin and aspirated by the vacuum manifold. Then, mixed anti-cytokine antibodies (50 μL) were added, and after 1 h incubation at room temperature, streptavidin–phycoerythrin reporter (50 μL) was added into each well. The microspheres were quantified using a BioPlex 100 platform (BioRad, Hercules, CA). IFN-γ, IL-12, IL-4, and IL-5 were assayed in triplicate to generate the standard curve and the correlation coefficient ($R^2$) was calculated in each experiment to see the linearity of the standard curve.

### 2.5. Statistical analysis

Normally distributed continuous variables were expressed as mean ± SD. Differences were analyzed by a one-way ANOVA followed by the Fisher's least significant difference (LSD) test to determine differences between groups for normally distributed continuous variables or by Kruskal–Wallis H test followed by Mann–Whitney U test to determine differences between groups for non-normally distributed continuous variables. $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Anti-CD25 antibodies attenuate thoracic irradiation-induced increase in the proportion of Tregs in the lung tissues of mice

We were interested in investigating the effect of thoracic irradiation on Tregs given their role in the suppression of autoimmune responses by inhibiting self-reactive T cells. Our flow cytometric

Download English Version:

https://daneshyari.com/en/article/2182808

Download Persian Version:

https://daneshyari.com/article/2182808

Daneshyari.com