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LETTER

Increased T-regulatory cells in lungs and draining lymph nodes in a murine model of COPD

To the Editors:

The characterisation of regulatory T (T_{reg}) cells in chronic obstructive pulmonary disease (COPD) has become of particular interest considering the recent reports on adaptive immune responses in COPD. More specifically, peribronchial and parenchymal lymphoid follicles (*i.e.* tertiary lymphoid organs) correlate in number with disease severity in COPD patients [1] and murine models of COPD [2], but their developing mechanisms and function have yet to be revealed [3].

In COPD patients, pulmonary lymphoid follicles mainly appear as a B-cell core surrounded by a predominant cluster of differentiation (CD)4^+ T-cell population [3]. T_{reg} cells situated within these structures are ideally located to exert cell contact-dependent immunosuppression. The observation on increased T_{reg} cells in lymphoid follicles of moderate COPD patients by PLUMB *et al.* [4], is therefore particularly intriguing.

The different reports on T_{reg} cells in COPD vary greatly, depending on the pulmonary anatomical compartment of interest and the phenotype used to define T_{reg} cells. Moreover, the molecular mechanisms underlying T_{reg} cell recruitment to the lung remain largely unknown. In addition to the relevant study on T_{reg} cells in lymphoid follicles of COPD patients [4], it would be interesting to determine T_{reg} cell numbers in the draining lymph nodes (*i.e.* secondary lymphoid organs) of these patients.

We developed a murine model manifesting hallmarks of COPD upon chronic cigarette smoke (CS) exposure, including pulmonary inflammation, emphysema and lymphoid follicles [5, 6]. In this model, we set out to determine the T_{reg} cell numbers within the lung (including lymphoid follicles) and draining lymph nodes after subacute or chronic exposure to air or CS. Additionally, we investigated the need for C-C chemokine receptor (CCR)7, a T_{reg} cell homing receptor, in T_{reg} cell migration to secondary lymphoid organs *versus* lung tissue containing tertiary lymphoid follicles.

Homozygous male C57Bl/6 CCR7 knockout ($CCR7^{-/-}$) mice and C57Bl/6 wild-type (WT) mice (8 weeks old) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). As described previously [6], mice (n=8 per group) were exposed to air or CS for 4 weeks (subacute exposure) or 24 weeks (chronic exposure). 24 hours after the last exposure, the right lung and mediastinal lymph nodes were removed and digested [5]. T_{reg} cells were characterized using anti-CD3-APC, anti-CD4-PerCP, anti-CD25-fluorescein isothiocyanate antibodies (BD Pharmingen, San Diego, CA, USA) and intracellular staining with FOXP3-phycoerythrin (PE) (eBioscience, San Diego, CA, USA) *versus* PE-conjugated rat immunoglobulin (Ig)G2a isotype control (eBioscience). T_{reg} cells were identified as $CD4^+CD25^+FOXP3^+$ cells (fig. 1a). Flow cytometry data acquisition was performed on a FACScalibur™ running CellQuest™ software (BD Biosciences). Statistical analysis was performed with Sigma Stat software (SPSS Inc., Chicago, IL, USA) using nonparametric tests (Kruskall–Wallis; Mann–Whitney U).

Subacute CS exposure was sufficient to increase T_{reg} cells in the lung, but not in the lymph nodes, and only in WT mice (fig. 1b and c). Chronic CS exposure induced T_{reg} cell accumulation in lungs and lymph nodes of both WT and $CCR7^{-/-}$ mice, accompanied by the development of pulmonary

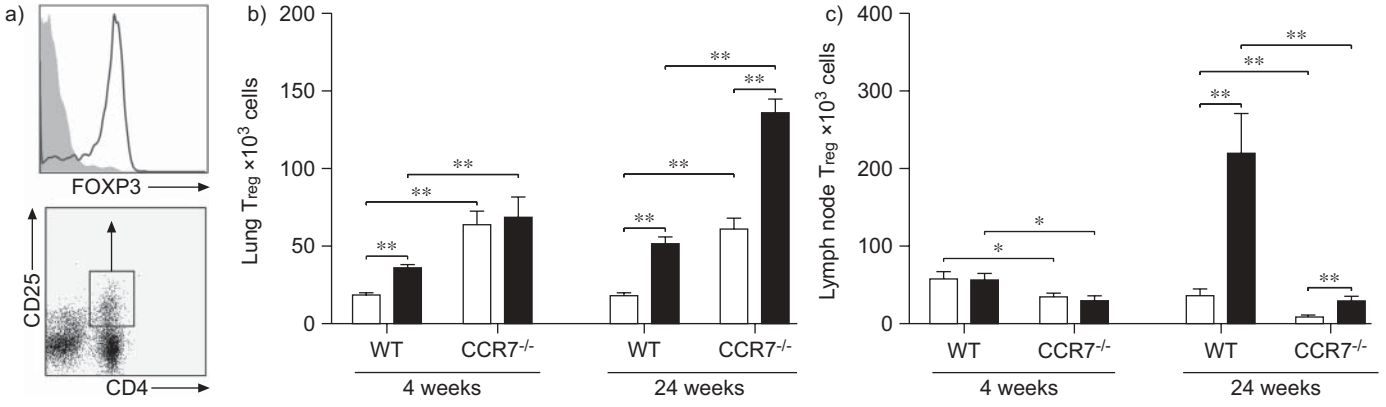

FIGURE 1. a) regulatory T (T_{reg}) cells were identified as cluster of differentiation (CD)$4^+CD25^+FOXP3^+$ cells. T_{reg} cells were enumerated by flow cytometry in b) lungs and c) mediastinal lymph nodes of wild-type (WT) and C-C chemokine receptor (CCR)$7^{-/-}$ mice after subacute (4 weeks) or chronic (24 weeks) exposure to air (□) or cigarette smoke (■). Data are presented as mean±SEM. n=8 animals per group; *: $p<0.05$; **: $p<0.01$.

lymphoid follicles [7]. Furthermore, the significantly higher pulmonary T_{reg} cell numbers in air- and CS-exposed CCR7$^{-/-}$ mice correlated with a stronger abundance of lymphoid follicles, compared with WT animals (fig. 1b and [7]). In contrast, baseline and chronic CS-induced T_{reg} cell accumulation were severely compromised in lymph nodes of CCR7$^{-/-}$ mice compared to WT controls (fig. 1c).

Our study provides evidence that CS exposure induces increased T_{reg} cell numbers, first in the lung and secondly in the lymph node compartment. Whereas CCR7 is crucial for the homing of T_{reg} cells to the lymph nodes, it is ultimately not required for the chronic CS-induced accumulation of these cells in the lung. However, we can not differentiate whether the observed CS-induced T_{reg} increase results from the recruitment of natural T_{reg} cells as opposed to the local induction of T_{reg} cells from precursors. The methylation status of the FOXP3 promoter is generally considered to discriminate between natural and induced T_{reg} cells.

Further translational research is needed to elucidate the functional role of lymphoid follicles and T_{reg} cells in the pathogenesis of COPD, for instance by determining the contribution of natural committed T_{reg} cells *versus* induced T_{reg} cells in pulmonary lymphoid follicles and lymph nodes.

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Diagnostic accuracy of digital chest radiography for pulmonary tuberculosis in a UK urban population

To the Editors:

Population screening for tuberculosis was discontinued in most Western countries, largely due to the decreasing prevalence of the disease. The use of chest radiograph examination for population screening has, therefore, appropriately declined and is now limited to the screening of high-risk groups, such as immigrants [1], prisoners or homeless persons in certain countries [2] and for disease prevalence surveys [3]. Chest radiography, however, remains a key tool for the clinical diagnosis of pulmonary tuberculosis. Previous studies suggest that chest radiograph abnormalities in tuberculosis are not specific and levels of intra- and inter-reader agreement are very variable [4–6]. Modern digital tuberculosis screening has been noted to have high levels of sensitivity where chest radiograph examination is standardised, quality assured and a simple coding system is used [7–9]. Levels of reader agreement are also reported to be higher among experienced readers [10].

In many low incidence countries, tuberculosis is concentrating in specific urban populations, such as homeless persons and drug users, with high prevalence rates reported [2, 11]. In the UK, this may be contributing to the general rise in tuberculosis [12]. Using a sample of digital chest radiographs from a hard to reach population at high risk of tuberculosis in London, we assessed some of the factors associated with greater diagnostic accuracy and determined levels of agreement between readers.

The study was carried out in London (UK), Amsterdam and Rotterdam (the Netherlands) between July and October 2008. Of 20 physicians invited, 13 participated: three radiologists, six respiratory physicians and one infectious disease physician from London and three public health tuberculosis physicians from Amsterdam and Rotterdam. 56 randomly selected chest radiographs were chosen: 18 confirmed cases of tuberculosis, 19 other abnormalities and 19 normal films from a screening programme which targeted homeless persons, problem drug users and prisoners. All cases had culture confirmed disease and eight were sputum smear positive. Study participants reviewed images using a DICOM (digital imaging and communications in medicine) viewer in a darkened room