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CCR7 Modulates Pulmonary and Lymph Node Inflammatory Responses in Cigarette Smoke-Exposed Mice

Tine Demoor,* Ken R. Bracke,* Karim Y. Vermaelen,† Lisa Dupont,* Guy F. Joos,* and Guy G. Brusselle2*3

Peribronchial lymphoid follicles have recently been identified as one of the hallmark features of (severe) chronic obstructive pulmonary disease (COPD). However, little is known about the relative contribution of peribronchial lymphoid follicles vs mediastinal lymph nodes in inflammatory responses in COPD patients and animal models. In a murine model of COPD, we studied inflammatory responses in airways, lungs, and mediastinal lymph nodes of wild-type (WT) vs CCR7 knockout (CCR7−/−) mice upon subacute or chronic exposure to cigarette smoke (CS). Although crucial for the organization of the secondary lymphoid organs, CCR7 was not required for the development of chronic CS-induced pulmonary lymphoid follicles. Moreover, T cell numbers were significantly increased in airways and lungs of air-exposed CCR7−/− mice, and they continued to increase upon chronic CS exposure. Unexpectedly, subacute CS-induced inflammation in airways and lungs, including airway neutrophilia and the recruitment of inflammatory-type CD11b+ dendritic cells, depended greatly on CCR7. In the draining lymph nodes, chronic CS exposure induced CCR7-dependent recruitment of airway-derived dendritic cells, accompanied by increases in CD4+ and CD8+ T cells. Correspondingly, CS exposure up-regulated mRNA expression of CCR7 ligands CCL19 and CCL21 in lymph nodes of WT mice, but not CCR7−/− mice. In the lungs of WT mice, chronic CS exposure significantly increased CCL19 mRNA and protein. Furthermore, double staining for CCL19 and pro-surfactant protein C showed that alveolar type II cells express high levels of CCL19. These data unveil a so far unappreciated role for CCR7 in modulating inflammatory responses in airways and lungs. The Journal of Immunology, 2009, 183: 8186–8194.

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3 Abbreviations used in this paper: COPD, chronic obstructive pulmonary disease; BAL, bronchoalveolar lavage; CS, cigarette smoke; DC, dendritic cell; Iac, mean linear intercept; WT, wild type.

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lymphoid follicles upon CS exposure. Next, we were interested in the contribution of CCR7 to the CS-induced recruitment of inflammatory cells in the lungs and airways, as well as the cellular trafficking to the draining mediastinal lymph nodes, and how this relates to the tissue-specific expression of the corresponding ligands. Finally, we examined the impact of CCR7 on the CS-driven development of emphysema.

Materials and Methods

Animals

Homozygous male C57BL/6 CCR7<sup>−/−</sup> (CCR7<sup>tm1Dgen</sup>) mice and C57BL/6 WT mice (8 wk old) were obtained from The Jackson Laboratory (16). All in vivo manipulations were approved by the local Animal Experimentation Ethics Committee of the faculty of Medicine and Health Sciences (Ghent, Belgium).

Smoke exposure

Mice (n = 8 per group) were exposed to CS, as described previously (17). Briefly, groups of eight mice were exposed whole body to the tobacco smoke of five cigarettes (reference cigarette 3R4F without filter; University of Kentucky, Lexington, KY) four times a day with 30-min smoke-free intervals, 5 days a week for 4 wk (subacute exposure) or 24 wk (chronic exposure). During the exposure an optimal smoke-to-air ratio of 1:6 was obtained. The control groups were exposed to air. Carboxyhemoglobin in serum of smoke-exposed mice reached a nontoxic level of 8.7 ± 0.31% (compared with 0.65 ± 0.25% in air-exposed mice; n = 4 for both groups), similar to carboxyhemoglobin blood concentrations of human smokers (18).

Bronchoalveolar lavage (BAL)

Twenty-four hours after the last exposure, mice were euthanized with an overdose of pentobarbital and BAL fluid was collected, as described previously (17). A total cell count was performed in a Bürker chamber, and differential cell counts (on at least 400 cells) were performed on cytocentrifuge preparations using standard morphologic criteria after May-Grünwald-Giemsa staining. Flow cytometric analysis of BAL cells was performed to enumerate macrophages, DCs, and T cells.

Preparation of single-cell suspensions

After rinsing of pulmonary and systemic circulation, the left lung was used for histology, and the right lung was used for the preparation of a single-cell suspension, as detailed previously (17). Mediastinal lymph nodes were removed and digested (as described in Ref. 19). Cell counting was performed with a Z2 Beckman Coulter particle counter.

Labeling of BAL cells and single cell-suspensions for flow cytometry

The following mAbs were used to identify mouse DC populations: anti-CD11c-allophycocyanin (HL3), anti-CD11b-PE (M1/70), and anti-MHC II-biotin (AF6-120.1), followed by incubation with streptavidin-PerCP. We discriminated between macrophages and conventional DCs using the methodology described earlier (20). Additionally, we distinguished in the lymph nodes the CD11c<sup>int-high/MHC II<sup>high</sup></sup> airway-derived DCs from the CD11c<sup>high/h</sup>MHC II<sup>int</sup> non-airway-derived DCs. We previously demonstrated that, upon intratracheal instillation of FITC-conjugated OVA, the FITC signal is exclusively detected in CD11c<sup>int-high/MHC II<sup>high</sup></sup> cells with stellate morphology in the T cell zone of the mediastinal lymph nodes. Additionally, passive “leakage” of intraluminal macromolecules to the lymph nodes is minimal to absent, and depletion of the CD11c<sup>int-high/MHC II<sup>high</sup></sup> subset dramatically reduces airway Ag transport to the lymph nodes (19). The following mAbs were used to stain mouse T cell subpopulations: anti-CD4-FITC (GK1.5), anti-CD4-PerCP (RM4-5), anti-CD8-FITC (53-6.7), anti-CD3-allophycocyanin (145-2C11), and anti-CD69-PE (H1.2F3). Using anti-CD19-PE (1D3) and anti-CD11c, B lymphocytes were identified as the CD11c<sup>int</sup> and CD19<sup>+</sup> population. CCR7 expression was studied with anti-CCR7-PE (4B12; eBioscience) and PE-conjugated rat IgG<sub>2a</sub>, κ isotype control. In a last step before analysis, cells were incubated with 7-aminocoumarin cyanine D (Via-Probe) to check cell viability. All mAbs and reagents were obtained from BD Pharmingen unless stated otherwise. Flow cytometry data acquisition was performed on a FACS-Calibur running CellQuest software (BD Biosciences). FlowJo software was used for data analysis (Tree Star).

Histology

The left lung was fixated by intratracheal infusion of fixative (4% paraformaldehyde), as described previously (17). The lung lobe was embedded in paraffin and cut into 3-μm transversal sections. Photomicrographs were captured using a Zeiss KS400 image analyzer platform.

Quantification of pulmonary emphysema

To evaluate pulmonary emphysema, we determined the enlargement of the alveolar spaces by measuring the mean linear intercept (Lm), as described previously (17, 21). Using image analysis software (ImageJ 1.34a) a 100 × 100-μm grid was placed over images of H&E-stained lung sections, acquired, and scored in a blinded fashion. The total length of each line of the grid divided by the number of alveolar intercepts gives the average distance between alveolated surfaces, the Lm.

Morphometric quantification of lymphoid follicles

To evaluate the presence of lymphoid infiltrates in lung tissues, sections obtained from formalin-fixed, paraffin-embedded lung lobes were subjected to an immunohistological CD3/B220 double-staining (21). Infiltrates in the proximity of airways and blood vessels were counted. Dense accumulations of at least 50 cells were defined as lymphoid follicles. Counts were normalized for the number of bronchovascular bundles per lung section.
Lymphoid follicles, absent in the bronchovascular lung regions of
Using a CD3/B220 double staining, we were able to study CS-
require CCR7
Chronic CS-induced lymphoid neogenesis in the lung does not
Results
Reported values are expressed as mean ± SEM. n = 8 animals/group. *, p <
0.05; **, p < 0.01; ***, p < 0.001.

Immunohistochemistry and ELISA for CCL19, CXCL1/KC
Paraffin sections were incubated with primary anti-CCL19 Ab (R&D Sys-
tems). Ab binding was detected using an anti-goat IgG AP-polymer kit
(Biocare Medical), followed by incubation with fuchsins (Dako). Pulmo-
mary CCL19 expression was determined semiquantitatively in a blinded
fashion by scores between 0 and 4 (0, no staining; 4, very strong positive
staining).
Following incubation with anti-CCL19 Ab, paraffin sections were incu-
bated with anti-pro-SPC Ab (Chemicon International) for CCL19/pro-SPC
double staining. Ab binding was visualized using the PowerVision anti-
rabbit IgG poly-AP detection system (Immunologic; Klinipath) combined
with the Vector Blue AP substrate kit (Vector Laboratories).

RT-PCR analysis
Total lung and lymph node RNA was extracted with the RNeasy Mini kit
(Qiagen). Expression of CCL19 relative to HPRT mRNA was analyzed
with the Assays-on-Demand gene expression products (Applied Biosys-
tems). RT-PCR was performed on a LightCycler 480 Instrument (Roche
Diagnostics) with murine leukemia virus RTase (Applied Biosystems) un-
der previously described conditions (21). Real-time PCR for CCL21-Leu
and CCL21-Ser was started from 25 ng of cDNA. Primers and FAM/
TAMRA probes were synthesized on demand (Sigma-Proligo). Primer/
probe sequences and PCR conditions were described previously (22).

Statistical analysis
Reported values are expressed as mean ± SEM. Statistical analysis was
performed with Sigma Stat software (SPSS 15.0) using nonparametric tests
(Kruskal-Wallis; Mann-Whitney U). A p value of ≤0.05 was considered
significant.

Results
Chronic CS-induced lymphoid neogenesis in the lung does not
require CCR7

Using a CD3/B220 double staining, we were able to study CS-
induced lymphoid follicles in WT vs CCR7<sup>−/−</sup> mice (Fig. 1).
Lymphoid follicles, absent in the bronchovascular lung regions of
air-exposed WT mice (Fig. 1, A and B), were strongly induced
upon chronic CS exposure (Fig. 1, A and C). In accordance with
earlier reports (15), CCR7<sup>−/−</sup> animals already showed abundant
perivascular and bronchial infiltration with lymphocytes upon air
exposure (Fig. 1, A and D); however, the number of follicles in-
creased further after chronic exposure to CS (Fig. 1, A and E).

Delayed development of CS-induced pulmonary inflammation in
CCR7<sup>−/−</sup> mice
To further characterize the inflammatory response upon CS expo-
sure, we enumerated in lung tissue several cell types, typically
constituting CS-induced lymphoid follicles. In WT mice, both sub-
acute and chronic CS exposure induced infiltration of the lung
parenchyma with DCs and T cells, including activated
CD4<sup>+</sup>CD69<sup>+</sup> and CD8<sup>+</sup>CD69<sup>+</sup> T cells (Fig. 2, A and C–E).
However, in lungs of CCR7<sup>−/−</sup> mice, these cell types were only in-
creased after prolonged, not subacute, CS exposure (Fig. 1,
A and E). In accordance with
earlier reports (15), we observed abundant
perivascular and bronchial infiltration with lymphocytes upon air
exposure (Fig. 1, A and D); however, the number of follicles in-
creased further after chronic exposure to CS (Fig. 1, A and E).

Subacute CS-induced neutrophilia and DC increase in BAL fluid
are CCR7 modulated
We next evaluated CS-induced inflammation in the airway com-
partment of WT and CCR7<sup>−/−</sup> mice by studying innate and adap-
tive immune cells in the BAL fluid.
CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets were both increased in BAL
fluid of WT mice upon subacute and chronic CS exposure (Fig. 3B–D). Moreover, airways of WT mice were highly infiltrated
with DCs, macrophages, and neutrophils upon CS inhalation (Fig.
3E–G).
Both subacute and chronic CS exposure induced a massive T
lymphocyte accumulation in BAL fluid of CCR7<sup>−/−</sup> mice (Fig.
A

FIGURE 2. Cell differentiation in lungs of WT and CCR7<sup>−/−</sup> mice after subacute (4 wk) or chronic (24 wk) exposure to air or CS: A, dendritic cells
(DCs); B, B cells; C, T cells; D, CD4<sup>+</sup>CD69<sup>+</sup> T cells; and E, CD8<sup>+</sup>CD69<sup>+</sup> T cells. Results are expressed as mean ± SEM; n = 8 animals/group. *, p <
0.05; **, p < 0.01; ***, p < 0.001.

B

C

D

E

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More specifically, airways of air-exposed CCR7−/− mice, already infiltrated with CD4+ and CD8+ T cells, did show an additional increase in both T cell subsets upon CS exposure (Fig. 3, C and D).

Remarkably, subacute CS-induced neutrophilia was strongly attenuated in BAL fluid of CCR7−/− mice (Fig. 3G). Correspondingly, the CS-induced increase in protein levels of CXCL1 (KC), a neutrophil chemoattractant, was absent in BAL fluid of CCR7−/− mice (Fig. 3H). In contrast, chronic CS exposure elicited strong BAL neutrophilia, accompanied by elevated CXCL1 levels in BAL fluid of both WT and CCR7−/− mice (Fig. 3, G and H).

Further characterization showed that particularly (“inflammatory-type”) CD11bhigh DCs were increased upon subacute CS exposure (percentage CD11bhigh of total DCs: WT air, 37.10 ± 2.42% vs WT smoke, 74.55 ± 2.62%; CCR7−/− air, 31.68 ± 1.33% vs CCR7−/− smoke, 52.22 ± 5.70%).

CS-induced DC and T cell increases in mediastinal lymph nodes are CCR7-dependent

Since CCR7 is crucially involved in DC and T cell homing, we verified the need for CCR7 in potential CS-induced inflammation at the lymph node level. Using a method we published earlier (19), we discriminated between CD11cint-high/MHC IIhigh airway-derived DCs (AW-DCs) and CD11cint-high/MHC IIint non-airway-derived DCs (NAW-DCs) within the mediastinal lymph nodes of WT and CCR7−/− mice (Fig. 4A). Lymph nodes of air-exposed...
CCR7−/− mice were largely devoid of AW-DCs, whereas NAW-DCs were hardly affected by the CCR7 deficiency. Also, flow cytometry histograms revealed almost no CCR7 expression on NAW-DCs vs abundant presence of CCR7 on AW-DCs in WT mice (Fig. 4B). Chronic CS exposure did not further modulate CCR7 expression on lymph node DCs. Nevertheless, chronic CS exposure induced significant CCR7-dependent recruitment of DCs from the airways toward the mediastinal lymph nodes (Fig. 4C).

Air-exposed CCR7−/− mice had significantly lower numbers of lymph node T cells compared with air-exposed WT mice. Furthermore, chronic CS exposure induced an increase in CD4+ and CD8+ T cells, which was strongly attenuated in CCR7−/− mice compared with WT mice (Fig. 4, D and E).

**CCR7 expression on DCs and lymphocytes in airways and lungs**

Because of the aberrant CS-induced inflammatory responses in CCR7−/− mice, we studied CCR7 expression on several cell types playing a key role in CS-induced inflammation and lymphoid neogenesis. As determined by measuring the mean fluorescence intensity, CS exposure did not significantly affect CCR7 expression.
The observed CCR7-independent pulmonary lymphoid neogenesis on the one hand, and CCR7-dependent DC and T cell increase in the lymph nodes on the other hand, prompted us to investigate the expression of the CCR7 ligands upon CS exposure in lungs vs lymph nodes. We characterized mRNA expression of CCL19 and CCL21 (both isoforms CCL21-Ser and CCL21-Leu) in lungs and mediastinal lymph nodes of WT vs CCR7–/– mice after exposure to air or CS (Fig. 6). In the lymph nodes, subacute CS exposure induced a moderate up-regulation on DCs in lung parenchyma and BAL fluid (Fig. 5, D and E). In contrast, both CCL21 isoforms were down-regulated in WT lung by subacute exposure to CS (Fig. 6, D and F). This response was not seen in CCR7–/– mice, which already had impaired pulmonary expression of CCL21-Ser and CCL21-Leu upon subacute air exposure. Upon chronic exposure, CS decreased pulmonary CCL21 expression in both WT and CCR7–/– mice.

CCL19 protein expression in lungs and airways is modulated by CCR7

Given the CS-induced increase in pulmonary mRNA expression of the lymphoid chemokine CCL19, we visualized the anatomical distribution of CCL19 protein expression within the lung using immunohistochemistry (Fig. 7). Double staining for CCL19 and pro-surfactant protein C (pro-SPC) revealed alveolar type II cells as an important source for pulmonary expression of this CCR7 ligand (Fig. 7A–C). Immunohistochemistry for CCL19 was scored semiquantitatively on sections of WT and CCR7–/– mice upon chronic exposure to air or CS (Fig. 7D). The immunohistochemistry scoring data matched the mRNA expression data closely (Fig. 7E): a CS-induced increase in pulmonary CCL19 expression in WT mice, which was less pronounced in CCR7–/– mice. Additionally, CCL19 levels were significantly higher in lungs of air-exposed CCR7–/– mice compared with WT mice. CCL19 protein levels, measured in BAL fluid, confirmed the immunohistochemical data (Fig. 7F).

Role of CCR7 in chronic CS-induced emphysema

An important hallmark of COPD is emphysema, which is airspace enlargement as a consequence of proteolytic destruction of alveolar walls following chronic CS exposure. Given the impact of CCR7 on the dynamics of inflammatory cells in this model, and based on the association between pulmonary lymphoid follicles, emphysema, and COPD severity in humans (3), we determined airspace size in long-term air- vs CS-exposed WT and CCR7–/– mice. Airspace size was determined by measuring Lm (Fig. 8A) on H&E-stained lung sections (Fig. 8B–E). Chronic CS exposure induced significant airspace enlargement in WT (Fig. 8A–C) (Lm air, 33.57 ± 0.71 μm vs Lm smoke, 37.65 ± 0.81 μm; 12.15% increase; p = 0.002), but not in CCR7–/– mice (Lm air, 36.70 ± 1.35 μm vs Lm smoke, 39.00 ± 2.06 μm; 6.29% increase; p = 0.487), the latter remarkably showing larger alveolar spaces upon air exposure (p = 0.049) (Fig. 8D).

Discussion

In COPD patients, the progressive pathological increase in lymphoid follicles around the airways correlates with disease severity (3). A similar observation is made in CS-exposed animals (4). However, the processes regulating this phenomenon at the cellular and molecular level are still obscure. CCR7 and its ligands play an important role in the induction and organization of secondary and tertiary lymphoid organs, both in homeostatic conditions as well as during adaptive immune responses. In this report we reveal an as yet unappreciated role of CCR7 in the dynamics of innate and adaptive pulmonary immune cells following short- and long-term CS exposure.
First, we confirm that the homing deficiency of CCR7−/− T cells into secondary lymphoid organs results in a “spillover” to the periphery, similar to lymphotoxin-α (LTα) knockout mice lacking all lymph nodes (23). Impaired homing of T lymphocytes in general and regulatory T cells in particular in CCR7−/− mice (24) results in peripheral T cell infiltration of major organs in the absence of inflammatory stimuli (15). Our results indeed show dramatically elevated T cell numbers in BAL fluid and lungs of air-exposed CCR7−/− mice (Figs. 1–3). In contrast, B cells appear to home independently of CCR7 (10, 25) and are not increased in the lung parenchyma of CCR7−/− mice. Moreover, neither CCR7 nor LTα signaling (14), essential for the organization of secondary lymphoid organs, is required for CS-induced lymphoid neogenesis. Lymphocytes of CCR7−/− mice have the ability to form organized lymphoid aggregates in the proximity of blood vessels and airways upon chronic CS exposure, very similar to those seen in WT animals (Fig. 1). Upon prolonged CS exposure, CCR7−/− mice show a strong increase in pulmonary CD4+ T cells, the predominant T cell population in CS-induced lymphoid follicles (4). In the absence of CCR7, chemokine responsiveness of DCs and lymphocytes may shift toward CXCL12 and CXCL13 via their respective receptors CXCR4 and CXCR5. CXCL12 is

![Figure 7](image_url)

**Figure 7.** Quantification and localization of CCL19 protein in lungs and airways. Photomicrographs of immunohistochemistry (IHC) for pro-surfactant protein C (pro-SPC) (A) or CCL19 (B) and pro-SPC/CCL19 double staining (C), showing colocalization of both components. Photomicrographs (D) and semiquantitative scoring (E) of IHC for CCL19 in lungs of WT and CCR7−/− animals chronically exposed to air or CS, showing CCL19+ alveolar type II cells. Magnification, ×200; scale bar = 50 μm; n = 8 animals/group. **, p < 0.01; ***, p < 0.001. F, Protein levels of CCL19 in BAL fluid of WT and CCR7−/− mice after chronic (24 wk) exposure to air or CS, as measured by ELISA. Results are expressed as pg/ml (mean ± SEM); n = 8 animals/group. *, p < 0.05.

![Figure 8](image_url)

**Figure 8.** Quantification of pulmonary emphysema. A, Airspace size expressed by Lm in WT and CCR7−/− mice after chronic (24 wk) exposure to air or CS. Results are expressed as mean ± SEM; n = 8 animals/group. *, p < 0.05; **, p < 0.01. B–E, Photomicrographs of H&E-stained lung sections at 24 wk (magnification, ×200): B, air-exposed WT mice; C, CS-exposed WT mice; D, air-exposed CCR7−/− mice; and E, CS-exposed CCR7−/− mice. Scale bars = 100 μm.
constitutively expressed in the lung, while pulmonary CXCL13 expression is increased upon CS exposure, colocalizing with lymphoid aggregates (14).

More detailed analysis of the pulmonary immune cell infiltration reveals a more complex picture, with differential effects of CCR7 deficiency depending on cell type, pulmonary anatomical compartment, and duration of CS exposure. The most surprising finding in our study is the dramatic reduction in neutrophil and inflammatory DC influx into the airways of CS-exposed CCR7−/− mice compared with WT mice (Fig. 3). This observation is clearly correlated with the lack of increase in CXCL1/KC, a neutrophil chemoattractant, in the lungs of CS-exposed CCR7−/− mice. The airway epithelium is a known important source of CXCL1 (26), and its physiology may be altered in CCR7−/− mice. An alternative hypothesis is that the CD11bhigh or “inflammatory-type” DCs (reviewed in Ref. 27), previously identified as potent producers of CXCL1 (28), are attracting neutrophils toward the airways. Accordingly, human immature and maturing DCs have been shown to exert neutrophil chemotactic activity in vitro upon stimulation with cigarette smoke extract (29).

Still, it is unclear why the inflammatory DC influx to the lung, which in several reports has been shown to involve CCR2 (30), CCR5 (31), and/or CCR6 (21), should be so strongly CCR7-dependent. The presence of CCR7 on DCs in the lung (Fig. 5) together with the pulmonary expression of CCR7 ligands (Fig. 6), conventionally associated with the organization of lymphoid organs, may provide a piece of the puzzle. For instance, our study reveals alveolar type II cells as important producers of CCL19 in the lung, and this CCL19 expression is up-regulated following chronic CS inhalation (Fig. 7) (14).

As CCR7 is associated with adaptive immune responses, the observation that CCR7 deficiency profoundly affects the prototypical innate response toward CS inhalation was unexpected in this study. This finding potentially has important pathogenetic implications, as both neutrophils and DCs are important sources of matrix metalloproteinases (32), such that a persistent and amplified trafficking of these cells through the lung may contribute to the parenchymal destruction observed in COPD. It is not known whether the CCR7-dependent signaling axis is dysregulated in the chronically inflamed airways of COPD patients. A report by Bratke et al. found decreased CCR7 expression on BAL DCs from human smokers, and it was suggested that this may result in decreased homing of these cells to the draining lymph nodes. In our animal model, smoke exposure does not down-regulate CCR7 expression on airway DCs (Fig. 5). Moreover, chronic CS inhalation induces a CCR7-dependent massive increase in the migratory airway-derived DC subset in the draining mediastinal lymph nodes, accompanied by increases in CD4+ and CD8+ T cells (Fig. 4). Corresponding with enhanced CCR7-mediated DC recruitment, CCL exposure induces lymph node expression of CCL19 and CCL21-Ser in WT animals (Fig. 6). These data are consistent with a recent report from our group showing amplified DC-mediated transport of inhaled protein Ag to the lymph nodes during CS exposure (33). Conversely, Robbins et al. found no effect of CS on lung DC migration to the regional lymph nodes, using a different CS exposure protocol during a subacute period (34).

The complex picture emerging from this animal model of COPD raises the question whether local interference with CCR7-dependent signaling would be a rational therapeutic concept. Our data show widespread expression of CCR7 on diverse immune cells in direct contact with the airway lumen, that is, BAL DCs and T cells. On the one hand, CCR7 blockade may have a beneficial effect on acute and subacute inflammatory disease flare-ups, as indicated by our data on neutrophil and inflammatory-type DC influx. On the other hand, shutting off CCR7 signaling induces a build-up of pulmonary lymphoid tissue, which is further enhanced by CS exposure. Pulmonary lymphoid follicles may be harmful by sustaining immune responses and subsequent chronic inflammation (reviewed in Ref. 35) or protective against bacterial and viral infection (36). Notably, the CCL19-CCL21-CCR7 axis appears important for the function of pulmonary lymphoid tissue, and blockade at this level has been shown to negatively affect adaptive immune responses against respiratory pathogens (13).

Finally, we studied CS-induced emphysema in WT vs CCR7−/− mice (Fig. 8), since airspace enlargement has been shown to correlate with pulmonary lymphoid infiltrates in smoking mice (4). However, it remains to be elucidated whether this association is causal. Our results rather indicate a dissociation between emphysema and lymphoid follicles, since the modest CS-induced increase in Ls does not correlate with the massive CS-induced lymphoid neogenesis in CCR7−/− mice. The Ls values, however, were difficult to interpret in CCR7−/− mice because these mice already had a certain degree of baseline airspace enlargement.

In summary, this murine model of COPD unveils an unexpected role for CCR7 in modulating tobacco smoke-induced inflammatory responses in the lung. CCR7 and its corresponding ligands are conventionally associated with adaptive immune responses, focused at the level of specialized lymphoid organs. However, our study shows a striking CCR7 dependency of prototypical innate immune responses following (sub)acute CS inhalation, that is, airway neutrophilic and DC influx, and reveals the expression of CCR7 ligands within the lung parenchyma. Nevertheless, CS-driven pulmonary lymphoid neogenesis emerged as a CCR7-independent process.

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Disclosures

The authors have no financial conflicts of interest.

References


ROLE FOR CCR7 IN CS-INDUCED INFLAMMATION


