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The Role of ChemR23 in the Induction and Resolution of Cigarette Smoke-Induced Inflammation

Tine Demoor,*§† Ken R. Bracke,* Lisa L. Dupont,* Maud Plantinga,§ Benjamin Bondue,§ Marie-Odile Roy,§ Vincent Lannoy,§ Bart N. Lambrecht,§ Guy G. Brusselle,* and Guy F. Joos*

Chronic obstructive pulmonary disease is mainly triggered by cigarette smoke (CS) and progresses even after smoking cessation. CS induces an exaggerated influx of inflammatory cells to the bronchoalveolar space and lung parenchyma, likely resulting from a complex interplay between chemoattractants and their respective receptors. In a murine CS model of chronic obstructive pulmonary disease, we studied the importance of chemokine-like receptor ChemR23 for the induction and resolution of inflammation in CS-exposed lungs. Subacute and chronic CS exposure increased protein levels of the ChemR23 ligand and chemoattractant, chemerin, in bronchoalveolar lavage fluid (BAL) of wild-type (WT) mice. Moreover, the proinflammatory chemokines CXCL1, CCL2, and CCL20 were increased in the airways of CS-exposed WT mice, accompanied by a massive accumulation of inflammatory neutrophils and monocytes, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells. The lung parenchyma of WT mice was infiltrated with inflammatory neutrophils, CD11bhiCD103− and CD11bhiCD103+ dendritic cells (DCs), and CD4+ and CD8+ T cells.
inflammatory lipid resolvin E1, derived from the ω-3 fatty acid eicosapentaenoic acid, also binds to ChemR23 (16). Interestingly, resolvin E1–ChemR23 interaction could favor inflammation resolution by inhibiting the activation of NF-κB, a transcription factor that can be activated by CS components in both inflammatory and structural cells of the lung (17, 18).

These findings prompted us to study the involvement of ChemR23 in CS-induced COPD. cDCs and monocytes/macrophages (ChemR23+ cells) and neutrophils (chemerin processors) are increased in lungs of patients with COPD and CS-exposed mice. Moreover, the irreversible nature of COPD points toward impaired resolution of inflammation. Therefore, we hypothesized that an imbalance between pro- and anti-inflammatory ChemR23 signaling directs the CS-induced response toward inflammation and the suppression of resolution.

Using a murine model of COPD, we revealed the role of ChemR23 in CS-induced inflammation. First, we characterized the expression of chemerin and ChemR23 in lungs of wild-type (WT) mice after subacute (4 wk) or chronic (24 wk) exposure to air or CS. Second, we investigated the inflammatory response upon subacute CS exposure in WT versus ChemR23 knockout (ChemR23−/−) mice in different anatomic compartments (airway lumen, lung tissue, and mediastinal lymph nodes). Third, we monitored the resolution of CS-induced inflammation in these three compartments on days 1, 14, and 56 after the final CS exposure.

Materials and Methods

Animals

Male C57BL/6 WT controls (6–8 wk old) and homozygous C57BL/6 ChemR23−/− mice (9) (6–8 wk old), originally developed by Deltagen, were obtained via Charles River Laboratories. All mice were housed in sterilized cages with filter tops and received food and water ad libitum. The local Ethics Committee for animal experimentation of the Faculty of Medicine and Health Sciences (Ghent University, Ghent, Belgium) approved all in vivo manipulations.

Smoke exposure

Male mice (n = 8 per group) were exposed to CS, as described previously (19). 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 d/wk for 4 wk (subacute exposure) or 24 wk (chronic exposure). During the exposure, an optimal smoke/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 (20).

Bronchoalveolar lavage

One, 14, or 56 d after the last exposure, mice were weighed and euthanized with an overdose of pentobarbital (Sanofi, Libourne, France), and a cannula was inserted in the trachea. A total of 3 × 300 µl, followed by 3 × 1 ml HBSS, free of ionized calcium and magnesium, but supplemented with 0.05 mM sodium EDTA, was instilled via the tracheal cannula and recovered by gentle manual aspiration. The six lavage fractions were pooled and centrifuged, and the cell pellet was washed twice and finally resuspended in 1 ml HBSS. The 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 bronchoalveolar lavage (BAL) cells was performed to enumerate monocytes, neutrophils, DC subsets, and T lymphocytes.

Preparation of lung and lymph node single-cell suspensions

After rinsing of pulmonary and systemic circulation, the left lung was used for histology and the right lung for the preparation of a single-cell suspension (19). Mediastinal lymph nodes were removed and digested, as described previously (21). Briefly, lungs and lymph nodes were thoroughly minced, digested, subjected to RBC lysis, and finally passed through a 50-µm cell strainer. Cell counting was performed with a Z2 Beckman Coulter particle counter (Beckman Coulter, Ghent, Belgium).

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

Cells were preincubated with FcR-blocking Ab (anti-CD16/CD32, clone 2.4G2) to reduce nonspecific binding. The following mAbs were used to identify mouse DC populations: anti-CD11c (HL3), anti-I-Ab (AF6-120.1), anti-CD11b (M1/70), anti-CD103 (M290), and anti-pDC Ag-1 (PDCA-1; JF05-1C2.4.1). We discriminated between macrophages and DCs using the methodology described by Vermaelen and Pauwels (22). After gating on the CD11c-bright population, two peaks of autofluorescence can be distinguished. The macrophages were identified as the CD11c-bright, high-autofluorescent population, with a low to absent expression of MHC class II (MHCIIB). DCs were identified as CD11c-bright, low-autofluorescent cells, which strongly express MHCI. DCs enumerated by these criteria correspond with cDCs, encompassing two major populations in the lung: CD11b+CD103+ and CD11b+CD103− DCs. In the lymph nodes, we distinguished the CD11c+MHCIIB airway-derived DCs from the CD11c+MHCIIB non–airway-derived DCs. pDCs were characterized as...
PDCA-1+ cells within the low-autofluorescent CD11c<sup>med</sup>MHCII<sup>med/lo</sup> population (23).

Monocyte and neutrophil populations were studied with anti-CD11c, anti-CD11b, anti-Ly6G (1A8), and anti-Ly6C (AL-21). Inflammatory monocytes were defined as CD11c<sup>hi</sup>CD11b<sup>hi</sup>Ly6C<sup>med</sup>Ly6G<sup>lo</sup> and inflammatory neutrophils as CD11c<sup>lo</sup>CD11b<sup>hi</sup>Ly6ChighLy6G<sup>lo</sup>. The following mAbs were used to stain mouse T cell subpopulations: anti-CD4 (GK1.5), anti-CD8 (53-6.7), and anti-CD3 (145-2C11). Anti-CD69 (H1.2F3) was included as a marker for early T cell activation.

With the exception of anti-PDCA-1 (Miltenyi Biotec) and anti-CD11b (Biolegend), all Abs were obtained from BD Pharmingen. In a last step before analysis, cells were incubated with 7-aminoactinomycin D (BD Pharmingen) to check cell viability. All labeling reactions were performed on ice in FACS-EDTA buffer. Flow cytometry data acquisition was performed on an FACScalibur and a BD LSRII running CellQuest and FACSDiva software (BD Biosciences). FlowJo software (Tree Star) was used for data analysis.

**Separation of pulmonary cell subsets**

Following incubation with anti-CD11c microbeads (Miltenyi Biotec), CD11c<sup>+</sup> cells were purified from the total lung single-cell suspension by passage through a VarioMACS magnetic cell separator (Miltenyi Biotec). Subsequently, cDCs versus macrophages were sorted using anti-CD11c, anti-MHCII, and the gating strategy detailed above on an FACSVantage with a Sort Enhancement Module (24). Using this strategy, we obtained for the cDCs a cell purity of 90.1 and 92.9% in air- and CS-exposed lungs, respectively. For the macrophage population, a purity of 96.1 and 97.6% was reached in air- and CS-exposed lungs, respectively.

**Histology**

The left lung was fixed by intratracheal infusion of fixative (4% paraformaldehyde), as described previously (19). After excision, the lung was immersed in fresh fixative during 2 h. The lung lobe was embedded in paraffin and cut into 3-µm transversal sections. Photomicrographs were captured using a Zeiss KS400 image analyzer platform (KS400; Zeiss, Oberkochen, Germany).

**Immunohistochemistry and ELISA for chemerin**

Sections obtained from formalin-fixed, paraffin-embedded lung lobes were stained for chemerin. Following treatment with Ultra V Block (Labvision, Fremont, CA), sections were incubated with polyclonal anti-mouse chemerin. Following treatment with Ultra V Block (Labvision, Fremont, CA) and diaminobenzidine (DakoCytomation, Glostrup, Denmark) were used for detection. Prochemerin staining was quantified within the airway epithelium in a marked area between the airway lumen and the basement membrane, using KS400 software (Zeiss). The area with positive staining for prochemerin was normalized to the length of the basement membrane (Pbm). All airways with a Pbm <2000 µm and cut in a reasonable cross sections were included.

Total levels of secreted chemerin (active and inactive forms) were determined in BAL fluid, using a commercially available ELISA kit (R&D Systems).

**Measurement of inflammatory chemokines**

Protein levels of CXCL1 (keratinocyte-derived chemokine), CCL2 (monocyte chemotactic protein 1), and CCL20 (macrophage inflammatory protein 3α) were determined in BAL fluid using commercially available ELISA kits (R&D Systems).

**RT-PCR analysis**

RNA was extracted from total lung and pulmonary cell subsets with the RNeasy Mini Kit (Qiagen, Hilden, Germany). RT-PCR results were obtained via absolute quantification, relating the PCR signal to a standard curve. Expression of preprochemerin and ChemR23 mRNA was determined relative to GAPDH mRNA, using previously described primer sequences. RT-PCR was performed on a LightCycler 480 Instrument (Roche Diagnostics, Basel, Switzerland) with murine leukemia virus RTase (Applied Biosystems). RT-PCR was performed at 42°C for 30 min, followed by 10 min incubation at 95°C for denaturation of RNA-DNA heteroduplexes, and 45 cycles of 95°C for 15 s and 62°C for 1 min. Monitoring of the RT-PCR occurred in real time using FAM/DABSYL probe. All reactions were performed starting from 10 ng total RNA.

**Statistical analysis**

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

**Results**

CS decreases proprochemerin and ChemR23 mRNA expression in the lung

We first investigated whether CS exposure affects mRNA expression of chemerin and ChemR23 in total lung tissue (Fig. 1).
Both preprochermelin (Fig. 1A) and ChemR23 (Fig. 1B) mRNA were significantly downregulated in total lung upon subacute and chronic CS exposure.

Aiming to further characterize the cells expressing ChemR23, we determined ChemR23 mRNA expression in different pulmonary cell subsets, including CD11c<sup>+</sup> cells, cDCs, and macrophages, sorted by flow cytometry (Fig. 1C). Unexpectedly, the CD11c<sup>−</sup> cell population, depleted from cDCs and macrophages, emerged as a major source of ChemR23, whereas ChemR23 mRNA was minimal in macrophages. Moreover, CD11c<sup>−</sup> cells approximately contained 6-fold and 100-fold more ChemR23 mRNA than cDCs and macrophages, respectively. Corresponding with total lung, ChemR23 mRNA expression tended to decrease in cDCs and CD11c<sup>−</sup> cells upon subacute CS exposure.

**FIGURE 3.** Cell differentiation in the BAL fluid of WT and ChemR23<sup>−/−</sup> mice on days 1, 14, and 56 after subacute exposure to air or CS. Cell subsets enumerated by flow cytometry include: total cDCs (A), CD11b<sup>+</sup>CD103<sup>−</sup> DCs (B), CD11b<sup>+</sup>CD103<sup>+</sup> DCs (C), inflammatory neutrophils (CD11c<sup>−</sup>CD11b<sup>+</sup>Ly6C<sup><med</sup>Ly6G<sup><hi</sup>)) (D), inflammatory monocytes (CD11c<sup>−</sup>CD11b<sup>+</sup>Ly6C<sup><med</sup>Ly6G<sup><med</sup>) (E), CD4<sup>+</sup> T cells (F), and CD8<sup>+</sup> T cells (G). Results are expressed as absolute cell numbers, mean ± SEM. n = 8 animals/group. Data on day 1 postexposure are representative of two independent experiments. *p < 0.05, **p < 0.01.
CS induces prochemerin release from the bronchial epithelium into the airway lumen

Next, we localized and measured chemerin protein in lungs of air-versus CS-exposed WT mice (Fig. 2). Using immunohistochemistry, we revealed the airway epithelium as the predominant source of chemerin in the lung (Fig. 2A–C). Prochemerin was abundantly present on the luminal side of the airway epithelium, but not in the endothelium. Using imaging software, we quantified the prochemerin-positive staining in the airways (Fig. 2D). Subacute CS exposure did not change the amount of prochemerin in the airway epithelium (data not shown). After chronic CS exposure, however, prochemerin protein was clearly decreased within the epithelium (Fig. 2B, 2C).

Interestingly, both subacute and chronic CS exposure increased the levels of total secreted chemerin in the BAL fluid of smoking WT mice, as measured by ELISA (Fig. 2E).

CS-induced inflammation is attenuated in ChemR23−/− mice

Because ChemR23 is implicated in the chemotaxis of monocytes/macrophages and immature DCs to inflamed chemerin-expressing sites (8, 11), we hypothesized a role for the chemerin-ChemR23 axis in the CS-induced recruitment of inflammatory cells to the...
airways and lungs. Therefore, we analyzed CS-induced inflammation in BAL fluid and lungs of WT versus ChemR23−/− mice, 1 d after the final exposure (Figs. 3, 4, left panels) using the gating strategy in Figs. 5 and 6.

In BAL fluid of WT mice, subacute CS exposure increased inflammatory neutrophils and monocytes, total cDCs (including the CD11bhiCD103− and CD11bhiCD103+ subset), and CD4+ and CD8+ T cells (Fig. 3). In contrast, this inflammatory response was severely attenuated in the BAL fluid of ChemR23−/− mice.

Similarly, subacute CS exposure increased inflammatory neutrophils, total cDCs (including the CD11bhiCD103− subset), and activated CD4+ T cells in lungs of WT mice, but not in ChemR23−/− mice (Fig. 4A, 4B, 4E, 4F).

Reportedly, ChemR23 also directs chemotaxis of pDCs to inflamed skin in response to chemerin. Therefore, we defined and determined pDCs in the lung as CD11cmedMHCIImed/loPDCA-1+ cells (23). After subacute CS exposure, we observed a decrease in pulmonary pDCs in lungs of both WT and ChemR23−/− mice (Fig. 4D).

**Dampened inflammatory chemokine response in CS-exposed ChemR23−/− mice**

To better understand the apparent hampered recruitment of inflammatory cells to the airways of CS-exposed ChemR23−/− mice, we measured protein levels of inflammatory chemokines, previously shown to be increased in the BAL fluid of smoking mice (Fig. 7).

CCL 2 (CCL2), CXCL 1 (CXCL1), and CCL 20 (CCL20), respectively attracting mainly monocytes, neutrophils, and cDCs/T cells, were significantly increased in BAL fluid of CS-exposed WT mice compared with air-exposed controls. In contrast, the CS-induced chemokine response was severely attenuated in ChemR23−/− mice.

**CS-induced increase in airway-derived cDCs is absent in mediastinal lymph nodes of ChemR23−/− mice**

The results above indicate a probable involvement of ChemR23 in CS-induced recruitment of cDCs to the airways and lungs. In addition, we monitored cDC migration from the airways to the draining (mediastinal) lymph nodes in WT versus ChemR23−/− mice (Fig. 8), because this event occurs secondary to myeloid DC recruitment to the lung and is greatly amplified upon CS exposure (23). We distinguished between airway-derived and non–airway-derived cDCs using a previously described method (21) (Fig. 9).

Subacute CS exposure significantly increased airway-derived cDCs in lymph nodes of WT mice for up to 14 d postexposure (Fig. 8A). In contrast, subacute CS exposure did not change the percentage of airway-derived cDCs in ChemR23−/− mice (Fig. 8B). Importantly, non–airway-derived cDC numbers were not af-

**FIGURE 5.** Flow cytometric analysis of cell populations in the BAL fluid. *A*, Gating strategy for the characterization of cDCs upon exposure to air or CS: cDCs are identified as CD11c+low, low-autofluorescent cells that strongly express MHCII (CD11c+high, high-autofluorescent cells are marked as macrophages [mφ]). cDCs in BAL fluid mainly encompass CD11bhiCD103− and CD11bhiCD103+ cDCs. *B*, Gating strategy for the characterization of inflammatory monocytes and neutrophils upon exposure to air or CS: within the CD11b+ population with low side scatter, inflammatory monocytes were defined as CD11c+Ly6C+Ly6G− cells and inflammatory neutrophils as CD11c−Ly6C−Ly6G+ cells.
lected by CS exposure in lymph nodes of both WT and ChemR23−/− mice (Fig. 8C, 8D).

Because ChemR23 has the potential to promote lymph node entry of immature pDCs via chemerin-expressing high endothelial venules (11), we determined pDCs in the mediastinal lymph nodes of WT and ChemR23−/− mice (Fig. 8E, 8F). pDC numbers were not impaired in the absence of ChemR23. Similar to the lung, subacute CS exposure reduced pDCs in lymph nodes of both WT and ChemR23−/− mice; however, pDC numbers were normalized in mice of both genotypes by day 14 after the final CS exposure.

Resolution of CS-induced inflammation

To determine a possible contribution of ChemR23 to the resolution of CS-induced inflammation, we also set out to analyze inflammatory cell accumulation in BAL fluid, lungs, and mediastinal lymph nodes of WT and ChemR23−/− mice on days 14 and 56 following subacute exposure to air or CS (Figs. 3, 4, 8).

CS-induced inflammation was significantly resolved in BAL fluid of WT mice on day 14 postexposure, except for a notable persistence in neutrophils and CD8+ T cells (Fig. 3D, 3G), which was accompanied by elevated levels of CXCL1 and CCL20 (Fig. 7B, 7C). Whereas pulmonary inflammatory neutrophils persisted (Fig. 4E), cDC and CD4+CD69+ T cell numbers were normalized in lungs of WT mice by day 14 after the final CS exposure (Fig. 5A, 5F). By day 56 postexposure, there was complete clearance of inflammatory cells in the airways and lungs of CS-exposed mice. BAL fluid levels of the inflammatory chemokines CCL2, CXCL1, and CCL20 declined accordingly and were fully restored to normal by day 56 postexposure (Fig. 7).

Whereas the inflammatory response was minimal in ChemR23−/− mice on day 1 after the final CS exposure, there was a remarkable delayed accumulation of T cells in the BAL fluid of ChemR23−/− mice on day 14 post-CS exposure (Fig. 3F, 3G).

Discussion

Given our observation that CS exposure affects the pulmonary expression of chemerin and ChemR23 in WT mice, we set out to determine the role of ChemR23 signaling in both CS-induced pulmonary inflammation as well as the resolution of inflammation upon smoking cessation. We observed a remarkably reduced accumulation of inflammatory cells and chemokines in lungs of CS-exposed ChemR23−/− mice compared with WT mice. In contrast, steady-state and CS-induced numbers of pDCs in the lungs and mediastinal lymph nodes were not affected by the ChemR23 deficiency. Upon smoking cessation, neutrophils and CD8+ T cells notably persisted in the airways of WT mice for at
ROLE OF ChemR23 IN CIGARETTE SMOKE-INDUCED INFLAMMATION

The release of proinflammatory mediators by the airway epithelium is one of the earliest events directing the recruitment of innate immune cells to CS-exposed airways (25). We observed in the BAL fluid of CS-exposed WT mice an increase in secreted chemerin, a known chemoattractant for monocytes/macrophages and immature DCs. Immunohistochemistry revealed the airway epithelium, but not the endothelium, as a major source of chemerin in the lung. Prochemerin was predominantly localized on the luminal side of the epithelium, available for secretion. Elevated levels of secreted chemerin in the airway lumen together with a downregulation of preprochemerin mRNA in the lung could explain the apparent depletion of prochemerin protein in CS-exposed airway epithelium. CS exposure may thus induce the release of prochemerin in the airways and favor its proteolytic activation by inflammatory cell-derived proteases such as neutrophil elastase and cathepsin G, both proven activators of chemerin (15).

After proteolytic activation, chemerin can attract innate immune cells to the airway lumen via the chemotactic receptor ChemR23, which has been identified on monocytes and immature cDCs. Both inflammatory monocytes and cDCs accumulated strongly in the Airways of CS-exposed WT mice. Conversely, ChemR23−/− mice were largely impaired in their recruitment of cDCs to the airways and lung parenchyma upon CS exposure. First, cDCs may not have increased due to a lack of available DC precursors/monocytes. Indeed, the attenuated recruitment of CD11bhiCD103− DCs in ChemR23−/− mice could be partially attributed to lower numbers of inflammatory monocytes, the precursor cells for a proportion of the CD11bhi DC subset. Second, the significant expression of ChemR23 mRNA in pulmonary cDCs suggests the receptor can also be directly involved in the CS-induced recruitment of lung DCs to the airway lumen. In the mediastinal lymph nodes of CS-exposed mice, airway-derived cDCs increase secondary to the CS-induced recruitment of cDCs or their precursors from the circulation into the lung. Accordingly, CS-exposed ChemR23−/− mice did not have increased numbers of airway-derived cDCs in their lung draining lymph nodes.

In addition, the absence of ChemR23 unexpectedly interfered with the CS-induced recruitment of neutrophils and T cells, cell types that do not express ChemR23. Therefore, the dampened CS-induced pulmonary inflammation in ChemR23−/− mice may also be explained by the impaired expression of the proinflammatory chemokines CXCL1, CCL2, and CCL20, which mainly attract neutrophils, monocytes, and cDCs/T cells, respectively. Pulmonary CD11bhi DCs, important producers of the above-mentioned chemokines (26), were indeed poorly increased in the airways and lungs of CS-exposed ChemR23−/− mice. However, the airway epithelium should not be overlooked as additional source of chemokines (26), were indeed poorly increased in the airways and lungs of CS-exposed ChemR23−/− mice. Moreover, the airway epithelium should be not overlooked as additional source of chemokines (26), were indeed poorly increased in the airways and lungs of CS-exposed ChemR23−/− mice. Alternatively, ChemR23 signaling could be required for the CS-induced upregulation of epithelium-derived chemokines. The pronounced ChemR23 mRNA expression in lungs depleted from cDCs and macrophages is suggestive of ChemR23-expressing structural lung cells.

Upon C-terminal cleavage of prochemerin, the nature of the executing protease is determining for the function of the chemerin isoform (29). Neutrophils, for instance, have the ability to generate both activate and inactivate forms of chemerin via the secretion of, respectively, cathepsin G, elastase (15), and proteinase 3 (30), whereas activated macrophages could release proteases that generate anti-inflammatory chemerin (31). Such proteolytic regulation of chemerin activity is consistent with recent studies attributing an anti-inflammatory role to chemerin and chemerin-derived peptides in mouse models of acute lung injury (9) and peritonitis (32), respectively. In our smoke model, we observed an attenuated recruitment of inflammatory cells in CS-exposed ChemR23−/− mice compared with WT mice, which is consistent with chemoattractive and proinflammatory chemerin–ChemR23 signaling. Nevertheless, we cannot rule out the generation of inactivated or anti-inflammatory chemerin upon prolonged CS exposure.

The resolution of CS-induced inflammation in mice is understudied. Neutrophils and lymphocytes have been reported to persist in lungs of chronic CS-exposed mice after smoking cessation (33, 34). Remarkably, our data in WT mice show that subacute CS exposure sufficed to sustain neutrophilic inflammation and elevated levels of the neutrophil attractant CXCL1 in the lung, for at least 2 wk after smoking cessation. According to a recent report, chronic CS exposure itself can cause a persistent adaptive T cell immune response (35). In our model, the initial CS-induced increase in pulmonary T cells probably reflects the nonspecific in-
The inflammatory recruitment of effector memory T cells via CCL20–CCR6 signaling. However, for at least 2 wk into the resolution phase, airway-derived DCs persisted in the draining lymph nodes of CS-exposed WT mice, notably accompanied by a strong accumulation of CD8+ T cells in the airway lumen. At this point, effector T cells, generated in the draining lymph nodes upon CS exposure, were recruited to the airways.

**FIGURE 8.** DCs in mediastinal lymph nodes of WT and ChemR23−/− mice after subacute exposure to air or CS. Airway-derived cDCs (A, B), non–airway-derived cDCs (C, D), and pDCs (E, F) on days 1, 14, and 56 postexposure, enumerated by flow cytometry. Results are expressed as percentage of total cells, mean ± SEM. n = 8 animals/group. Data on day 1 postexposure are representative of two independent experiments. *p < 0.05, **p < 0.01.

**FIGURE 9.** Flow cytometric analysis of DC populations in the mediastinal lymph nodes. Gating strategy for the characterization of conventional and pDCs upon exposure to air or CS: within the low-autofluorescent population, we distinguished the CD11cmed/MHCIImed airway-derived DCs (AW-DCs) from the CD11cmed/MHCIImed non–airway-derived DCs (NAW-DCs). pDCs were characterized as PDCA-1+ cells within the low-autofluorescent CD11cmed/MHCIImed population.


Disclosures

The authors have no financial conflicts of interest.

References


4. ChemR23 is of the few chemotactic receptors directing the migration of pDCs to the lymph nodes and inflamed peripheral sites (11, 12). However, pulmonary and lymph node pDC numbers did not differ between WT and ChemR23−/− mice in the steady state or after CS exposure. Studies on the expression of ChemR23 on murine pDCs have generated conflicting results (9, 37). Furthermore, in addition to ChemR23, CXCR4 has been shown to direct the migration of pDCs (38). Alternatively, the decrease in pDCs may not represent altered recruitment, but could be the result of CS-induced maturation and differentiation of this immature DC subset into cDCs (39). Finally, the CS-induced reduction of pDCs could also result from a defect in available circulating pDCs or their precursors (40).

In summary, targeting of ChemR23 may be of therapeutic interest, considering its apparent involvement in the initial recruitment of inflammatory cells and the expression of proinflammatory mediators in CS-exposed lungs, most likely in response to the enhanced local activation and release of its ligand chemerin.

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