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Genetic deletion of IL-17A reduces cigarette smoke-induced inflammation and alveolar type II cell apoptosis

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Submitted 29 April 2013; accepted in final form 3 October 2013

Chronic obstructive pulmonary disease; interleukin-17

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is defined as "a disease state characterized by airflow limitation that is not fully reversible, and that is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" (13). Caused predominantly by cigarette smoking, COPD is one of the leading causes of mortality globally (37). Reduced responsiveness to the anti-inflammatory effects of corticosteroids is a major barrier to effective management of inflammation in the majority of COPD patients (3). The mechanism by which cigarette smoke (CS) causes COPD in susceptible individuals is complex, but involves both aberrant, chronic inflammation coupled with the loss of lung structural cells due to heightened apoptosis. The inflammatory response in the lungs of COPD patients is strongly linked to tissue destruction and alveolar airspace enlargement, characteristic of the emphysematous component of COPD.

The inhalation of noxious particles such as those in CS leads to the influx of inflammatory cells, including macrophages, neutrophils, and CD8+ T lymphocytes, in the airways and lungs, leading to chronic inflammation (15). Apoptosis is critical for the maintenance of normal tissue homeostasis and is in equilibrium with proliferation and differentiation. There is increasing evidence that disturbance of the balance between apoptosis and proliferation in lung tissue contributes to the pathogenesis of COPD (22, 25, 43). In the lungs of COPD patients, the abundance of inflammatory cells may interact with apoptosis. For example, alveolar macrophages from patients with COPD are less effective in phagocytosing apoptotic airway epithelial cells compared with controls (23). This might be mediated by the presence of activated numbers of neutrophils in COPD (29). Similarly, cytotoxic CD8+ T cells could cause apoptosis of alveolar epithelial cells through the release of perforins, granzyme-B, and tumor necrosis factor (TNF)-α (5, 31).

The interleukin (IL)-17 cytokine superfamily triggers the production of numerous chemokines, resulting in neutrophil and macrophage recruitment and subsequent pathogen clearance. IL-17 is key to the defense against bacteria and fungi, mediating cross talk between the adaptive and innate immune systems (28). It is of interest that IL-17 expression has been associated with diminished steroid responsiveness (33), which
in the context of a steroid-resistant disease like COPD, raises the possibility that IL-17 and related cytokines may contribute to this disorder. IL-17A can influence the expression of mucin (MUC5AC), a hallmark of chronic airway diseases, including COPD, in human bronchial epithelial cells (21). Furthermore, transgenic overexpression of IL-17A in the alveoli of murine lung induces inflammation with a COPD-like phenotype (36). Elevated IL-17A secretion has been reported to be present in the bronchial mucosa of COPD patients (17). In our previous study, we have demonstrated the upregulated expression of IL-17A/F in the airways of COPD patients (12), supporting the notion that IL-17 could play an important role in the pathogenesis of COPD.

Although prevention of most cases of COPD is possible by avoidance of exposure to environmental agents such as CS, there is currently no satisfactory therapy to help treat individuals once the disease is established, making the need for novel approaches essential. In this study, we aimed to clarify the potential important roles of IL-17 in COPD by using the mouse subacute CS exposure model. Here, we showed that the genetic deletion of IL-17 ameliorates the inflammatory response in the lung and reduces the apoptosis of alveolar epithelial cells induced by CS exposure.

MATERIALS AND METHODS

Mice. C57BL/6 female mice (10–12 wk) were purchased from Charles River (Montreal, Canada). IL-17−/− mice were prepared as previously described (35). All animals were treated and maintained under a protocol approved by the Animal Care Committee of McGill University, in accordance with guidelines set by the Canadian Council on Animal Use and Care.

Smoke exposure in vivo. Wild-type mice and IL-17−/− mice were exposed nose only to the mainstream smoke from Kentucky 3R4F cigarettes (University of Kentucky) without filter (4 cigarettes/day, 6 days/wk for 4 wk) using a CS exposure system (Cigarette Laboratory Lexington, KY). Control mice were exposed to room air. Each group contained 8–10 mice. The mice were killed at 24 h after the last exposure.

Preparation of bronchoalveolar lavage fluid. After exsanguination, the lungs were lavaged using a cannula inserted in the trachea and the lungs were instilled with 0.5 ml PBS. Cytospins were prepared at a density of 0.5 × 10^6 cells/ml. Differential cell counts were performed using standard morphological criteria on Hema-Gurr-stained cytopsins (500 cells/sample) (Merck, Darmstadt, Germany).

Bronchoalveolar lavage cytokine analysis. Aliquots of cell-free bronchoalveolar lavage fluid (BALF) were frozen in liquid N2 and stored at −80°C. BALF was analyzed for IL-17A (Biolegend, San Diego, CA) and IL-17F (R&D Systems, Minneapolis, MN) by ELISA. The level of IL-18, IL-6, TNF-α, transforming growth factor (TGF)-β1, growth-related oncogene-α, macrophage inflammatory protein (MIP)-2, monocyte chemotactic protein (MCP)-1, regulated on activation, normal T cell expressed and secreted cytokines and of chemokines was normalized to GAPDH and compared by using the ΔΔct method. All results were expressed as relative quantity compared with wild-type air-only control.

Western blot. Lung tissue was homogenized in 0.5 ml of ice-cold lysis buffer [50 mmol/l Tris-HCl, 1 mmol/l ethyleneglycol-bis-[β-aminoethylether]-N,N,N’,N’-tetraacetic acid, 1 mmol/l EDTA, 1% Triton X-100, 1 mmol/l sodium orthovanadate, 50 mmol/l sodium fluoride, 5 mmol/l sodium pyrophosphate decahydrate, 0.27 mol/l sucrose, and protease inhibitor mixture (Roche, Mannheim, Germany)]. Protein lysate (10 µg) was loaded on 10% acrylamide SDS-PAGE NEXT GEL (Amresco) followed by transfer to nitrocellulose membranes (Bio-Rad). The membranes were then blocked with Odyssey blocking buffer (LI-COR Biosciences) for 1 h at room temperature and then incubated overnight at 4°C with antibodies specific for IL-17A (Santa Cruz Biotechnology, Santa Cruz, CA), matrix metalloproteinase (MMP)-9 (Abcam, Cambridge, MA), MMP12 (Abcam), cleaved caspase-3 (Cell Signaling Technology, Danvers, MA), glyceraldehyde-3-phosphate dehydrogenase (Millipore, Temecula, CA), and β-actin (Millipore). Membranes were then incubated with a 1:15,000 dilution of IRDye 680 goat anti-mouse IgG, IRDye 800 goat anti-rabbit IgG, and IRDye 680 donkey anti-goat IgG (Rockland) in blocking buffer. Membranes were scanned and analyzed with an Odyssey IR scanner using Odyssey imaging software 3.0 (LI-COR Biosciences).

Immunohistochemistry. The immunohistochemistry staining for IL-17A (Santa Cruz) and for neutrophils (Santa Cruz) was performed on paraffin-embedded mouse lung tissue sections as previously described (12).

Immunofluorescence double staining. The apoptosis of type II alveolar epithelial cells (AEC II) was observed by immunofluorescence double staining on mouse lung sections with antibodies for surfactant protein (SP)-C (Abcam) and terminal deoxynucleotidyl-

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### Table 1. The sequence of primers for real-time PCR

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**IL**, interleukin; TNF-α, tumor necrosis factor-α; GRO-α, growth-related oncogene-α; MIP-2, macrophage inflammatory protein-1; MCP-1, monocyte chemotactic protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; MMP, matrix metalloproteinase.
transferase dUTP nick end labeling (TUNEL; Millipore) according to the manufacturer’s protocol. With the use of laser scanning confocal microscopy (LSM780; Carl Zeiss Microscopy), stained lung sections were viewed at a magnification of ×200, and images were analyzed using the Image-J program (National Institutes of Health, Bethesda, MD).

Mouse lung epithelial cell culture. Mouse lung epithelial cells (MLE-12 cells), a distal bronchiolar and alveolar epithelial cell line (ATCC, Manassas, VA) (38), were cultured in HITES medium (50:50, DMEM-Ham’s F-12) supplemented with 2% FBS, 2 mM l-glutamine, 10 mM HEPES, 1:100 insulin/transferrin/selenium supplement (Invitrogen), and antibiotics. After 24 h of starvation with HITES medium containing 0.1% FBS, cells were treated with 2.5 or 5% of cigarette smoke extract (CSE) or medium alone (control) with or without 20 ng/ml of mouse recombinant IL-17A protein (R&D Systems) for 6 h. The cell protein lysate was collected for Western blot.

Mouse IL-17A small-interfering RNA knockdown studies. MLE-12 cells were seeded at a density of 1 × 10^5 cells in a six-well plate and transiently transfected with 40 nM small-interfering RNA (siRNA) against mouse IL-17A (catalog no. sc-39650; Santa Cruz) or nontargeting control siRNA (catalog no. sc-37007; Santa Cruz). Transfections were performed according to the manufacturer’s instructions. After the transfection, the cells were switched to starving HITES medium (0.1% FBS) for 24 h and treated with CSE for 6 h. Verification of target knockdown was done by Western blot.

Preparation of CSE. CSE was generated as previously described (1, 2, 9, 40). Research-grade cigarettes were obtained from Kentucky 3R4F cigarettes (Lexington, KT). An optical density of 0.65 (320 nm) was considered to represent 100% CSE. This CSE preparation was diluted to the appropriate concentration in HITES medium containing 0.1% FBS.

Hoechst fluorescence. Hoechst stain is a cell-permeant dye that fluoresces upon binding to DNA. An increase in fluorescent intensity is associated with chromatin condensation, a characteristic feature of an apoptotic cell (15). To therefore assess the apoptosis of MLE-12 cells, the cells were seeded on eight-well chamber slides (BD Biosciences) and left undisturbed for 24 h. Cells were then treated with 2.5 and 5% CSE or media alone for 6 h. Cells were then fixed in 4% paraformaldehyde, incubated with the Hoechst stain for 10 min, cover slipped, and viewed as described above. Viable and apoptotic cells were counted, and results were quantitatively expressed as the percentage of apoptotic cells compared with the total number of cells.

Fig. 1. Interleukin (IL)-17A expression in lung tissue of mice exposed to cigarette smoke (CS) is significantly increased. A: the level of IL-17A and IL-17F in bronchoalveolar lavage fluid (BALF) of mice with CS exposure; n = 8–10 mice/group, *P < 0.05. B: Western blot for IL-17A expression in lung tissue of mice with CS exposure; n = 4–5 mice/group, *P < 0.05. C: immunohistochemistry staining for IL-17A in lung tissue of mice with CS exposure. Brown color represents the positive staining of IL-17A (arrows). One representative section from each group is shown. Magnification ×100. D: IL-17A and -F mRNA expression in lung tissue of mice exposed to CS; n = 8–10 mice/group, *P < 0.05.
**Statistical analysis.** Statistical analysis was performed using the GraphPad Instat 3 software (GraphPad Software, La Jolla, CA). An analysis of variance was used to assess differences between treatment groups of more than two. Results are expressed as means ± SE. In all cases, a *P* value <0.05 is considered statistically significant.

**RESULTS**

*IL-17A expression is increased in the lung of mice exposed to CS.* Because IL-17 mediates cross talk between the adaptive and innate immune systems and consequently allows for orchestration of an effective immune response (28), we chose to investigate the effect of subacute cigarette exposure. To first determine if there was a dose- and time-dependent increase in pulmonary IL-17 production, preliminary experiments were performed by exposing mice to 0, 2, and 4 cigarettes/day for 1, 2, and 4 wk, respectively. Results for these experiments showed that 4 cigarettes/day with duration of 4 wk led to the highest IL-17A expression (data not shown). To determine the time course, the mice were killed at 6, 24, and 48 h after the last exposure. The results indicated that the time point 24 h is associated with the highest IL-17A expression (data not shown). These findings suggest that CS-induced IL-17A expression may require a constant exposure. Thus, in all subsequent experiments, mice
were exposed to 4 cigarettes/day for 4 wk and killed at 24 h after the last exposure.

In subsequent experiments, there was an increased level of IL-17A but not IL-17F in the BALF from CS exposure (Fig. 1A). Elevated expression of IL-17A was confirmed by both Western blot (Fig. 1B) and immunohistochemistry (Fig. 1C). The expression of IL-17A mRNA in the lung was also significantly increased with CS exposure (Fig. 1D). Furthermore, the number of IL-17A-positive cells in lung tissue of the CS group was significantly higher than that of wild-type mice (CS vs. wild type: 1.77 ± 0.09 vs. 34.26 ± 9.16 cells/mm², P < 0.01, data not shown).

IL-17A contributes to CS-induced neutrophilia. Because increased expression of IL-17A was found in mice with the subacute CS exposure, we investigated the role of IL-17A on the inflammatory response using the IL-17A-deficient (IL-17A−/−) mice. Histologically, more inflammatory cells were found in wild-type mice with CS compared with IL-17−/− mice (Fig. 2A). In BALF, we found a significantly increased number of macrophages, neutrophils, and lymphocytes in wild-type mice with CS exposure compared with baseline (Fig. 2B). However, only neutrophils were significantly decreased in IL-17A−/− mice with CS compared with the wild-type mice (Fig. 2B), whereas macrophages and lymphocytes were not significantly decreased, although there was a trend to lower values. The reduced presence of neutrophils in the lungs of IL-17−/− mice exposed to CS was confirmed by immunocytochemistry staining of lung sections (Fig. 2C).

The production of IL-6, MIP-2, and MMP12 decreased in the lung of IL-17−/− mice with CS exposure. We next checked the expression of inflammatory mediators associated with COPD, including IL-1β, IL-6, TNF-α, TGF-β1, keratinocyte chemoattractant, MIP-2, MCP-2, RANTES, Eotaxin, MMP9, and MMP12. In the wild-type mice, CS significantly increased the expression of IL-6, MIP-2, and MMP12 in the lung (Fig. 3).
Although not statistically significant, there was a trend toward increased levels of IL-1β, TNF-α, RANTES, Eotaxin, and MMP9. In contrast, in IL-17A−/− mice, the expression of IL-6, MIP-2, and MMP12 was significantly less compared with the wild-type mice exposed to CS (Fig. 3). Changes in mRNA expression were consistent with that of the protein level except that of TGF-β1 and Eotaxin, where the mRNA expression was found significantly increased by CS exposure in wild-type mice (Fig. 4).

Ablation of IL-17 prevents CS-induced apoptosis of AEC II. Apoptosis of structural cells in the lung, including epithelial cells, is regarded as a critical event in the pathogenesis of COPD (15). IL-17 has been reported to contribute to intestinal epithelial cell apoptosis in a murine model of ischemia-reperfusion injury (30), but the contribution of IL-17 to lung epithelial cell apoptosis is not known. Therefore, we investigated apoptosis of AEC II in the lungs of CS-exposed wild-type and IL-17A−/− mice by immunofluorescence for SP-C, an epithelial cell marker and TUNEL, an indicator of apoptosis. There was a significant increase in the percentage of apoptotic AEC II cells in the lungs of wild-type mice exposed to CS compared with control (Fig. 5A). In contrast, there were significantly fewer apoptotic epithelial cells in the lungs of IL-17A−/− mice exposed to CS compared with wild-type mice. Apoptosis in the lungs was confirmed by Western blot analysis for cleaved caspase-3 (Fig. 5B). There was significantly more cleaved caspase-3 in the lungs of control mice exposed to CS compared with IL-17A−/− mice. These results support the concept that, in addition to promoting an inflammatory response, IL-17 also contributes to the apoptosis of AEC II cells in response to CS.

IL-17 contributes to MLE-12 cell apoptosis induced by CS exposure in vitro. In addition to Th17 cells, abundant IL-17A expression has been also found in structural cells. We explored this by measuring IL-17A expression in MLE-12 cells exposed to CSE. IL-17A expression was induced by 5% CSE exposure (2.7 ± 0.6-fold change, P < 0.05; Fig. 6), suggesting that AEC II cells could be also a source of IL-17A.

To confirm if IL-17A is an important inducer for apoptosis of AEC II cells, we exposed MLE-12 cells to CSE with or without addition of IL-17A. CSE exposure significantly induced apoptosis of MLE-12 cells (medium vs. 5% CSE: 8.2 ± 3.3 vs. 17.2 ± 1.8%, respectively, P < 0.05) (Fig. 7A). Addition of IL-17A in conjunction with CSE resulted in a significant increase in the apoptosis of MLE-12 cells (medium: 16.9 ± 5.1%; 5% CSE: 31.3 ± 8.1%, P < 0.05 compared with the culture without IL-17A).

To confirm the role of IL-17 in promoting epithelial cell apoptosis, the expression of IL-17A in MLE-12 cells was reduced with transfection of siRNA for mIL-17A (knockdown efficiency: 61.7 ± 16.0%, P < 0.01, data not shown). IL-17A knockdown significantly decreased the percentage of apoptotic cells compared with the control cells receiving scrambled siRNA (control siRNA vs. mIL-17A siRNA: 24.8 ± 3.8 vs. 17.2 ± 2.9%, respectively, P < 0.05). These results were confirmed by the detection of cleaved caspase-3 expression of cells (Fig. 7B). The addition of IL-17A significantly increased the cleaved caspase-3 expression in both medium alone and 2.5% CSE-exposed cells (1.6 ± 0.2-fold increase for medium with IL-17A, P < 0.01; 2.5% CSE vs. 2.5% CSE with IL-17A: 1.1 ± 0.1-fold increase vs. 1.8 ± 0.3-fold increase, P < 0.05).

Fig. 4. mRNA expression of proinflammatory factors in lung tissue of mice with CS exposure. Real-time PCR was performed to detect the mRNA expression of proinflammatory factors in lung tissue. Results were presented as the relative quantity compared with wild-type control; n = 8–10 mice/group. *P < 0.05, **P < 0.01, and ***P < 0.001.
In contrast, IL-17A knockdown reduced the cleaved caspase-3 expression induced by 2.5% CSE exposure (control siRNA vs. mIL-17A siRNA: 1.3 \pm 0.2- vs. 0.8 \pm 0.1-fold increase, \( P < 0.05 \)).

We investigated possible mechanisms underlying IL-17-enhanced CSE-induced MLE-12 apoptosis by evaluating the expression of key factors of different apoptosis pathways, including the extrinsic pathway (Bax, Survivin) and the intrinsic pathway (caspase-12, cleaved caspase-9). The results indicated that only cleaved caspase-9 was found significantly increased in MLE-12 cells cultured with IL-17A alone or IL-17A plus 5% of CSE (Fig. 8) (IL-17A: 1.31 \pm 0.15-fold change compared with medium control, \( P < 0.05 \); IL-17A plus 5% CSE: 1.53 \pm 0.18-fold change compared with medium control, \( P < 0.05 \)). These findings suggest that the mitochondrial pathway may be involved in IL-17-enhanced CSE-induced MLE-12 apoptosis.

**DISCUSSION**

Th17 cells are a critical component of the adaptive immune response and have also been implicated in chronic inflammatory diseases and steroid resistance (10), raising the possibility that Th17 cells may play important roles in COPD, a chronic inflammatory lung disease mainly induced by cigarette smoking and resistant to steroids (3). In the present study, we investigated the involvement of IL-17 in the CS-induced inflammatory response and apoptosis of AEC II.

Because IL-17 mediates cross talk between the adaptive and innate immune systems (28), we performed our investigation into the role of IL-17 in CS-induced lung damage using subacute (i.e., 2 or 4 wk) exposures. With 4 wk CS exposure, the expression of IL-17A in the lung was significantly increased (Fig. 1), whereas IL-17F expression did not change. This was surprising, since our previous study demonstrated the elevated expression of both IL-17A and -F in airways of COPD patients (12). Besides the possible explanation of a species-specific effect or the difference between acute exposure and long-term adaptation, another possibility is that IL-17F may not be expressed concurrently with IL-17A during the early phase of an inflammatory response. This may be because of the predominance of newly defined naturally occurring IL-17-producing T cells (nTh17 cells), which express substantial amounts of IL-17A, but not IL-17F. It is believed that nTh17 cells facilitate the early phase of antigen-induced airway re-
sponses and host defense against pathogen invasion before the establishment of acquired immunity (47). Here, nTh17 cells regulate the infiltration of neutrophils to the BALF (16). It is interesting to speculate that nTh17 cells are recruited to the lung during initial exposure to CS and may represent a unique T cell subset mediating neutrophilia in COPD. It is also noteworthy that, in addition to Th17 cells, abundant IL-17A expression was also found in structural cells, including both airway and alveolar epithelial cells, which we have also found in ex vivo human lung tissue explants exposed to CS (unpublished observations). In addition, a study showed that IL-17 is expressed by tubular epithelial cells in renal transplant recipients (32).

Because elevated IL-17A expression was found in CS-exposed mice, we next investigated the role of IL-17A in contributing to lung inflammation using IL-17A-deficient mice. Genetic deletion of IL-17A resulted in significantly less neutrophil infiltration to lung compared with the wild-type mice (Fig. 2), consistent with the notion that IL-17 is a major factor involved in neutrophil recruitment (36, 41, 51). We also examined the inflammatory profile in the lungs of CS-exposed wild-type and IL-17A−/− mice by evaluating proinflammatory factors involved in the pathogenesis of COPD. Our main conclusion is that IL-17A is involved in the elevated expression of IL-6, MIP-2, and MMP12 induced by CS (Fig. 3). IL-6 often works in concert with other cytokines and provides a link between innate and acquired immunity (4). IL-6 is increased in sputum, exhaled breath, and plasma of patients with COPD, particularly during exacerbations (7, 8). IL-6 is also a key factor in the differentiation and immunity of Th17 cells (27, 28). IL-17 induces IL-6 release from multiple cell types (20, 26, 50). Together with the findings in the present study, this suggests that a positive loop may exist between these two cytokines. MIP-2 has been shown to be significantly elevated in the bronchoalveolar lavage of smoke-exposed mice (48), where it is a neutrophilic CXC chemokine (49). Our results are consistent with the previous study where endogenous IL-17 acts in part to promote inflammation by inducing the local release of neutrophil chemoattractants such as IL-6 and MIP-2, thereby contributing to the recruitment of neutrophils in the airways (34). MMP-12 (macrophage elastase) has been implicated in the tissue-destructive processes associated with chronic lung diseases, including COPD and asthma (14, 18). IL-17A has been shown to enhance secretion of MMP-12 from lung macrophages (44). It is also noteworthy that TGF-β1 levels were decreased in IL-17A−/− mice compared with the wild-type mice even in the absence of CS exposure (Fig. 3), indicating that IL-17 may have a regulatory role on TGF-β1.

![Image of double-immunofluorescence staining for SP-C and IL-17A in MLE-12 cells exposed to cigarette smoke extract (CSE).](http://ajplung.physiology.org/)

**A**

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**Fig. 6.** IL-17A expression on MLE-12 cells exposed to cigarette smoke extract (CSE). A: double-immunofluorescence staining for SP-C and IL-17A. MLE-12 cells were cultured with 2.5 or 5% of CSE for 6 h. Hoechst was used for the counterstaining of nuclear. One representative experiment is shown. Magnification ×600. B: Western blot for IL-17A. One representative experiment is shown. *P < 0.05 compared with medium control; n = 4 independent experiments.
production. However, because IL-17 is not known to regulate TGF-β1 production, this may be an indirect effect. In addition, the level of RANTES was significantly increased in IL-17 mice, suggesting IL-17 may inhibit RANTES expression, which is supported by an in vitro study showing that IL-17 inhibits TNF-induced RANTES expression in human synovial fibroblasts and mouse lung fibroblasts (42). Taken together, our results demonstrated that IL-17A acts as an important upstream mediator to promote the production of IL-6, MIP-2, and MMP12 implicated in inflammation and tissue destruction in COPD.

In addition to chronic, heightened inflammation, COPD is associated with loss of alveolar structural integrity, a feature that is proposed to contribute to the development of emphy-
AEC II are a lung structural cell that secretes pulmonary surfactant, the effect of which is to lower the surface tension of water and allow the cell membrane to separate, thereby increasing its capability to exchange gases. AEC II cells also repair the endothelium of the alveolus when it becomes damaged (19). The impaired surfactant secretion by CS in the alveoli can contribute to alveolar wall rupture and the development of emphysema in COPD (16). CS has been shown to directly increase apoptosis of lung epithelial cells, in vitro and in mouse models (6, 39). In this study, we showed that CS-induced apoptosis of AEC II cells is lessened in the absence of IL-17A (Fig. 5), supporting a role for IL-17A in the progression from inflammatory response toward structural damage from CS exposure. AEC II apoptosis is the result of complex pathophysiological procedures that may involve both the direct and the indirect effects of IL-17A. Evidence from our study supports a direct role of IL-17A on AEC II apoptosis, as was demonstrated by in vitro supplementation of IL-17A in the CS exposure systems or knockdown of IL-17A cells (Fig. 7). The apoptotic effect of IL-17A has also been reported in vascular endothelial cells in human acute coronary syndrome (52). Interestingly, our previous study found evidence of an anti-apoptotic effect of IL-17A on airway smooth muscle cells (11). In rheumatoid arthritis, IL-17 contributes to disease chronicity by inhibiting synoviocyte apoptosis (24). Therefore IL-17A may have different effects on the apoptotic response, depending on the cell type. The mitochondrial pathway was found to be involved in IL-17-enhanced CS-induced apoptosis (Fig. 8). In the intrinsic pathway of apoptosis, which results from alterations at the level of the mitochondria and activation of the apoptosome, cleaved caspase-9 recruits and activates executioner caspases such as caspase-3 and caspase-7 (46). The mitochondrial pathway has previously been shown to be involved in IL-17-induced apoptosis of vascular endothelial cells (52). Recently Shan et al. (45) reported that chronic CS exposure induced IL-17A expression. The authors further demonstrated that IL-17A is required for CS-induced emphysema by show-

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<th>Medium</th>
<th>mIL-17A</th>
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<td><strong>Caspase-12/Actin</strong></td>
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*Fig. 8. Expression of proteins involved in IL-17-enhanced CS-induced apoptosis of MLE-12 cells. MLE-12 cells were cultured with 2.5 or 5% of CSE with or without 20 ng/ml mIL-17A for 6 h. The expression of extrinsic pathway factors (Bax, Survivin) and intrinsic pathway factors (caspase-12, cleaved caspase-9) was evaluated by Western blot. *P < 0.05; n = 5 independent experiments.*
ing enhanced inflammation and emphysema in IL-17A transgenic mice and reduced disorders in IL-17A−/− mice. In addition to supporting the notion that inflammatory responses are decreased in IL-17A−/− mice exposed to CS, our study also identified the important proapoptotic role of IL-17A on AEC II cells during CS exposure, which may be a key factor contributing to the loss of alveolar structural integrity.

In conclusion, the present study demonstrated that IL-17A plays important roles not only in the initial inflammatory response to CS exposure but also on alveolar epithelial cell damage, both of which are pathological processes associated with the development and progression of COPD. These findings support an important role for IL-17 in the pathogenesis of COPD and provide a basis for future studies aimed at determining whether this can be exploited as the basis for novel therapeutic targets.

GRANTS
This study was supported by a grant from the CIRF program, the Canada Foundation for Innovation Leaders Opportunity Fund; Department of Medicine, McGill University; the Research Institute of the McGill University Health Centre, and the Canadian Institutes of Health Research. C. J. Baglole was supported by a salary award from the Fonds de recherche du Quebec-Sante (FRQ-S).

DISCLOSURES
No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: Y.C., L.A.-A., Y.I., C.J.B., D.H.E., and F.R.Q-S. supported by a salary award from the Fonds de recherche du Quebec-Sante and the Canadian Institutes of Health Research. C. J. Baglole was supported by a salary award from the Fonds de recherche du Quebec-Sante (FRQ-S).

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