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Respiratory tract dendritic cells (DCs) are juxtaposed to directly sample inhaled environmental particles. Processing and presentation of these airborne Ags could result in either the development of immunity or tolerance. The purpose of this study was to determine the consequences of cigarette smoke exposure on DC function in mice. We demonstrate that while cigarette smoke exposure decreased the number of DCs in the lungs, Ag-induced DC migration to the regional thoracic lymph nodes was unaffected. However, cigarette smoking suppressed DC maturation within the lymph nodes as demonstrated by reduced cell surface expression of MHC class II and the costimulatory molecules CD80 and CD86. Consequently, DCs from cigarette smoke-exposed animals had a diminished capacity to induce IL-2 production by T cells that was associated with diminished Ag-specific T cell proliferation in vivo. Smoke-induced defects in DC function leading to impaired CD4+ T cell function could inhibit tumor surveillance and predispose patients with chronic obstructive pulmonary disease to infections and exacerbations. The Journal of Immunology, 2008, 180: 6623–6628.

Cigarette smoke has been shown to alter both innate and adaptive immune responses (5, 6) and some cigarette smoke constituents, such as oxygen radicals and endotoxin, can activate and exert a direct immunomodulatory effect on respiratory DCs. In vitro studies using bone marrow and monocyte-derived DCs exposed to varying doses of nicotine (7, 8) and cigarette smoke extract (9) have yielded contrasting results with respect to their effect on DC function. Because of limitations in interpreting the biological relevance of exposure of individual cigarette smoke components as well as whole cigarette smoke extract on cells in culture, we assessed the effect of mainstream cigarette smoke exposure in vivo on lung DC number, migration to lymph nodes, and Ag presentation within the nodes. Despite the limitations of the classical maturation paradigm linked to the complexity and heterogeneity of the DC network (1, 10), we believe this approach provides a useful model to dissect the effects of cigarette smoke on lung DCs. DCs have a central role in controlling immune responses during infection, allergy, and cancer, therefore knowledge of how smoking affects this system of cells is likely key in elucidating the mechanisms by which smoking affects the immune system during disease processes.

Materials and Methods

Mice

C57BL/6 and BALB/c female mice 8 wk of age were obtained from Charles River Laboratories. Same age and gender nonsmoking control mice were housed in the same conditions as the smoke-exposed animals for the duration of the study. OVA-TCR-transgenic mice (DO11.10) on a BALB/c background were obtained from The Jackson Laboratory. All mice were housed in sterile microisolator cages. The Harvard Standing Committee for Animal Research at Harvard University School of Public Health approved all animal protocols.

Cigarette smoke exposure

Mice were exposed to cigarette smoke from four unfiltered cigarettes (two administered in the morning and two in the afternoon; University of Kentucky) 5 days/week for 1 mo using a smoking apparatus with the chamber adapted for mice, as previously described (11). Instillation of macromolecule solutions into the trachea

Mice were anesthetized by i.p. injection of 2.5% avertin and then intubated as described (12) and 600 μg of FITC-conjugated OVA (OVA-FITC, screened for low endotoxin content; Molecular Probes) was administered...
intratracheally (i.t.) in a total volume of 60 μl of sterile PBS (Invitrogen Life Technologies) to both smoke-exposed and control mice. In some experiments, 600 μg of OVA (chromatographically purified, screened for low endotoxin content; Worthington Biochemical) was administered in a volume of 60 μl of sterile PBS to both smoke-exposed and control mice.

Preparation of lung single-cell suspensions

Mice were euthanized by CO₂ narcosis. A thoracotomy was performed, followed by right heart catheterization with a 21-G (3/4) siliconized needle and the pulmonary circulation was perfused with 20 ml of sterile PBS to remove the intravascular pool of cells. A total of 2 ml of digestion medium (RPMI 1640 (obtained from Invitrogen Life Technologies), supplemented with 1 mg/ml collagenase type IV and 0.5 mg/ml DNase from bovine pancreas (both obtained from Sigma-Aldrich), was then injected i.t. using a 22-G catheter and the trachea was quickly sealed with a silk suture. The trachea and lungs were then removed. Lungs were carefully separated from the heart, thymus, and trachea and incubated at 37 °C in an additional 3 ml of digestion medium for 30 min. Incubation was then prolonged for additional 30 min, with vigorous pipetting of the samples at 10-min intervals.

Subsequently, samples were passed over a 70-μm nylon cell strainer and RBC were lysed. The cell suspension was then incubated in calcium- and magnesium-free PBS containing 10 mM EDTA for 5 min at room temperature on a shaker, resuspended in staining buffer (PBS without Ca²⁺/Mg²⁺, 0.5% FBS, 10 mM EDTA) and kept on ice until immunofluorescent labeling. Sodium azide was not added to the staining buffer when the cells were going to be used in functional studies.

Preparation of lymph node single-cell suspensions

For studies involving migration and costimulatory molecules, mice were euthanized by CO₂ narcosis 24 h after i.t. injection of OVA-FITC or OVA, respectively. For T cell proliferation studies, mice were euthanized by CO₂ narcosis 6 h after injection of OVA. Following thoracotomy, paratracheal and parathymic intrathoracic lymph nodes were removed under a stereomicroscope (Olympus SZ 60) and incubated at 37°C in 3 ml of lymph node digestion medium (1X HBSS (Cellgro Mediatech), with 2% 10 mM EDTA-treated FBS (HyClone), supplemented with 2.5 mg/ml collagenase type IV). After a 10-min incubation, lymph nodes were minced with 20-G HBSS (Cellgro Mediatech, with 2% 10 mM EDTA) and the cell suspension was enriched for DC by magnetic bead separation under sterile conditions using a mixture of anti-CD11c-PE-Cy5.5 hamster anti-mouse CD11c mAb (Caltag Laboratories) and data acquisition was performed using the FACScan running CellQuest software (BD Biosciences). FlowJo software (Tree Star) was used for data analysis. For lung and migration studies, 50,000 total events were acquired for each sample. For CCR7 expression and T cell proliferation studies, 500,000 total events were acquired for each sample. Dead cells were gated out based on light scatter properties.

Data acquisition for experiments assessing costimulatory molecule expression in lymph nodes was performed on a DakoCytoMation High Speed MoFlo Sorter running Summit 3.1 software, acquiring 500,000 events for each sample.

In vivo T cell proliferation studies

CD4⁺ T cells were enriched from spleens of DO11.10 OVA-transgenic mice by magnetic bead separation under sterile conditions using a mixture of biotin-conjugated mAbs against CD8α (Ly-2) (rat IgG2a), CD11b (Mac-1) (rat IgG2b), CD45R (B220) (rat IgG2a), DX5 (rat IgM) and Ter-119 (rat IgG2b), followed by anti-biotin MicroBeads (colloidal superparamagnetic MicroBeads conjugated to a monoclonal anti-biotin Ab, clone: pos-3-I8E7.2; mouse IgG1 (Miltenyi Biotec). CD4⁺ DO11.10 T cells were subsequently labeled with 10 μM CFSE (Sigma-Aldrich) at 37°C for 10 min, as previously described (14) and then resuscipated in sterile PBS. Mice received an i.v. injection of 10 × 10⁶ CFSE-labeled DO11.10 T cells 24 h before i.t. injection of 600 μg of OVA in a volume of 60 μl of PBS.

In vitro T cell activation studies

To assess the ability of lung DCs to activate T cells, smoke-exposed mice and controls received an i.t. injection of 600 μg of OVA and 15 h later lungs were digested and the lung cell suspension was enriched for DC under sterile conditions by CD11c magnetic bead separation (clone N418; Miltenyi Biotec). The enriched cells were subsequently stained with PE hamster anti-mouse CD11c mAb (clone HL3; BD Pharmingen). Within the PE-CD11c⁺ population, lung DCs were identified as the low AF Gating strategy was used to identify CD11c⁺ DCs that were CD11b⁻/CD11c⁺. The induction of T cell activation by lung DCs derived from smoke-exposed and controls was determined by detection of IL-2 in the coculture supernatants by ELISA as per manufacturer’s instructions (eBioscience).

For in vitro assessment of ability of lung-derived DCs to stimulate T cells, smoke-exposed mice and controls were euthanized by CO₂ narcosis 24 h after i.t. injection of 600 μg of OVA-FITC and TLNs were extracted as described. Single-cell suspensions were obtained under sterile conditions and labeled with PE-Cy5.5 hamster anti-mouse CD11c mAb (Caltag Laboratories) as described. OVA-FITC⁺ CD11c⁺ PE-Cy5.5⁺ cells were sorted by flow cytometry and cocultured with purified CD4⁺ DO11.10 TCR-transgenic T cells at a 1:100 ratio for 24 h at 37°C in tissue culture medium (RPMI 1640 supplemented with 10% FBS, 0.1% 2-ΜE (Invitrogen), 200 μM L-glutamine (Invitrogen), 1% penicillin/streptomycin (Invitrogen), and 1% HEPES (Sigma-Aldrich)). The induction of T cell activation by lung DCs derived from smoke-exposed and controls was determined by detection of IL-2 in the coculture supernatants by ELISA.

TLN cell culture

TLN cells were cultured in RPMI 1640 alone or with 40 μg of OVA/well at 8 × 10⁵ cells/well in a flat-bottom, 96-well plate (BD Biosciences). After 4 days of culture, supernatants were harvested for cytokine measurement by commercial ELISA.

Data analysis

Data are expressed as means ± SEM. Statistical interpretation of the results is indicated in the figure legends. All statistical analysis was performed using SigmaStat statistical software (version 2.0; SPSS). Differences were considered statistically significant at p < 0.05.

Results

Cigarette smoke exposure decreases the number of lung DCs

We began by investigating the effect of cigarette smoke exposure on the number of lung DCs. Mice were exposed to cigarette smoke for 4 wk. Lung cells were isolated and flow cytometry was used to identify CD11c⁺ cells. Within the CD11c⁺ cell population, DCs were identified within the low AF gate as previously described (13) (Fig. 1A). Smoke-exposed mice showed a significant reduction in lung DCs compared with non-smoke-exposed controls. DCs represented 1.03% (±0.1) of the total lung cells in smoke-exposed mice.
Cigarette smoke exposure decreases DC numbers in the lung. A. Lung cells were isolated from smoke-exposed and non-smoke-exposed control mice and flow cytometric analysis was used to identify DCs (CD11c+tum). Macrophages (MAC) were identified as (CD11c-). Absolute numbers of lung DCs from A. Data represent means ± SEM; n = 8. Statistical analysis was performed using the Student t test; p < 0.05. C, CD11c+tum lung DCs were isolated from smoke-exposed and control mice 15 h following OVA administration and cocultured with DO11.10 CD4+ T cells. IL-2 was measured in cell culture supernatants by ELISA.

To determine whether the reduction in DC number induced by smoke exposure had functional consequences, we tested the ability of residual lung DCs to act as stimulators of T cell proliferation in vitro. Lung DCs (CD11c+tum) were sorted from smoke-exposed and control mice 15 h following i.t. injection of OVA and cocultured for 24 h with CD4+ DO11.10 cells (1:100). IL-2 levels were measured in cell culture supernatants by ELISA. On a per cell basis, IL-2 production by T cells did not differ significantly between smoke-exposed and control mice (data not shown).

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Cigarette smoke exposure does not inhibit migration of lung DCs to TLNs

We next determined whether cigarette smoke influences lung DC migration to the regional TLNs. TLN cells from smokers and controls were isolated 24 h after intratracheal injection of OVA-FITC and stained with an anti-CD11c mAb. Lung DCs were identified as described, and recently migrating, lung-derived DCs were defined as the FITC+ population (shown gated in Fig. 2A). Similar percentages of lung-derived nodal CD11c+ cells were observed in both smoke-exposed and control mice (16 ± 2% vs 14 ± 1.5%, respectively). Calculation of absolute numbers of recently lung-derived DCs in the TLNs demonstrated that the mean number of CD11c+FITC+ cells in the TLNs did not differ significantly between smokers and controls (14,541 ± 4,590 vs 10,535 ± 1,774, respectively) (Fig. 2B). Furthermore, the total number of TLN cells isolated following OVA-FITC administration was similar between the two groups (data not shown).

The chemokine receptor CCR7 is thought to regulate migration of peripheral DCs to lymph nodes (15–17). Recently migrated (OVA-FITC+) DCs (CD11c+tum/MHC class II (MHCII)+) express higher levels of CCR7 than the semimature/steady-state nodal DCs (OVA-FITC− and CD11c+tum/MHCIIinterm) as previously described (18). To assess CCR7 expression by migrated lung DCs in response to cigarette smoking, TLNs were extracted from smoke-exposed and control mice 24 h after i.t. injection of OVA, and the cells were stained with anti-MHCII (I-Ek), anti-CCR7, and anti-CD11c mAb. CCR7 expression in the CD11c+tum/MHCIIhigh TLN DC population was not different between smoke-exposed and control cells (average mean fluorescence intensity 493 vs 512, respectively) (Fig. 2C).

Cigarette smoke exposure impairs DC maturation

We next determined whether cigarette smoke exposure altered the maturation of TLN DCs following OVA exposure by assessing cell surface costimulatory molecule expression. TLNs from smoke-exposed and control mice were collected 24 h after i.t. injection of OVA. DCs were analyzed for costimulatory markers by
Cigarette smoke exposure impairs DC maturation. A, TLN cells were isolated from smoke-exposed and control mice 24 h following intratracheal administration of FITC-labeled OVA and flow cytometry was used to identify costimulatory molecule expression on DCs. Cells were gated through CD11c and then evaluated for the distribution of CD80, CD86, MHCII, and CD40 with CD11c. A total of 5,730 events were collected from the TLN cell suspension for each costimulatory marker. No difference was observed for CD40 expression between smoke-exposed and control cells, whereas a lower percentage of CD11c and MHCII cells expressed CD80, CD86, or CD40 with CD11c. B, Absolute number of CD11c and MHCII hours after exposure to i.t. OVA than in control cells. The absolute number of CD11c and MHCII was statistically significant in smokers as compared with controls (Fig. 3B). The observed differences were not due to an effect of smoking on the trafficking of adoptively transferred cells to the TLNs because in the absence of OVA exposure, similar numbers of DO11.10 T cells migrated to the TLNs in both smokers and control animals (data not shown).

Table I. In vitro OVA-specific cytokine production

<table>
<thead>
<tr>
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<th>Control</th>
<th>Smoke</th>
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<tbody>
<tr>
<td>IL-5</td>
<td>23.3 ± 17.5</td>
<td>39.7 ± 23.2</td>
</tr>
<tr>
<td>OVA</td>
<td>746.7 ± 69.8 b</td>
<td>549.1 ± 95.1 b</td>
</tr>
<tr>
<td>IL-10</td>
<td>41.7 ± 16.0</td>
<td>51.0 ± 16.0</td>
</tr>
<tr>
<td>OVA</td>
<td>723.6 ± 96.9 b</td>
<td>643.9 ± 69.3 b</td>
</tr>
<tr>
<td>IL-13</td>
<td>277.6 ± 147.4</td>
<td>392.7 ± 109.8</td>
</tr>
<tr>
<td>OVA</td>
<td>8880.7 ± 1058.5 b</td>
<td>9483.5 ± 1425.5</td>
</tr>
</tbody>
</table>

*a* Smoke-exposed and control mice were injected i.v. with DO11.10 T cells during the last week of exposure, followed 24 h later by intratracheal administration of 600 μg of OVA. Four days later, TLN cells were isolated and placed in culture. *b* Cytokine production was assessed in cell culture supernatants by ELISA. Data are expressed as means ± SEM (n = 9–11). Statistical analysis was performed using one-way ANOVA; p < 0.05.

Cigarette smoke exposure impairs T cell proliferation

To investigate the impact of cigarette smoke-induced impairment of DC maturation on T cell responses, we next assessed T cell-derived cytokine production by TLN cells in vitro. Mice were exposed to cigarette smoke for 4 wk. During the last week of exposure, mice received a single i.v. delivery of DO11.10 T cells, followed 24 h later by intratracheal administration of 600 μg of OVA. Four days later, TLN cells were isolated and placed in culture. OVA-specific production of IL-5, IL-10, IL-13, and IFN-γ was assessed in cell culture supernatants by ELISA. We observed similar levels of OVA-specific IL-5, IL-10, and IL-13 in both control and cigarette smoke-exposed mice (Table I). IFN-γ was not detected in any of the culture conditions (data not shown).

IL-2 is instrumental in promoting the clonal expansion of Ag-specific T cells. To better understand the effect of smoking on DC-induced T cell proliferative responses, mice were exposed to cigarette smoke for 4 wk, followed by a single i.t. administration of FITC-labeled OVA. Twenty-four hours later, CD11c+/OVA+ DCs were isolated from the TLNs and cocultured with CD4+/DO11.10 T cells. IL-2 production was measured in culture supernatants by ELISA. DCs from smoke-exposed mice induced significantly less IL-2 production from CD4 T cells than controls (434 ± 23 pg/ml vs 590 ± 19 pg/ml, respectively) (Fig. 4A).

To assess the effect of smoking on T cell proliferation in vivo, mice were exposed to cigarette smoke for 4 wk. During the last week of exposure mice received a single i.v. delivery of CFSE-labeled DO11.10 T cells, followed 24 h later by intratracheal administration of 600 μg of OVA. Four days later, TLN cells were isolated and flow cytometry was used to assess the CFSE staining profile of proliferating T cells. Shown in Fig. 4B is a representative histogram (from one of three experiments, all with similar results, six mice per experimental group) illustrating CFSE division profiles of T cells from smoke-exposed and control animals. We demonstrate that compared with controls, cigarette smoke exposure attenuated proliferation of DO11.10 T cells (Fig. 4B). Specifically, smoking resulted in a significantly lower percentage of DO11.10 T cells undergoing six or more cycles of cell division compared with controls (Fig. 4C). To determine the consequence of impaired T cell proliferation, we quantified the absolute number of OVA-specific T cells in these animals and show that compared with controls, smoking was associated with the expansion of significantly fewer DO11.10 T cells in the TLNs (Fig. 4D). The observed differences were not due to an effect of smoking on the trafficking of adoptively transferred cells to the TLNs because in the absence of OVA exposure, similar numbers of DO11.10 T cells migrated to the TLNs in both smokers and control animals (data not shown).
Discussion

The objective of this study was to determine the effects of cigarette smoke exposure on DC function in vivo. We demonstrate that while cigarette smoke exposure decreased the number of DCs in the lungs of mice, Ag-induced DC migration to the regional TLNs was unaffected. However, cigarette smoking suppressed DC maturation within the lymph nodes as demonstrated by reduced cell surface expression of MHC and the costimulatory molecules CD80 and CD86. Consequently, DCs from cigarette smoke-exposed animals had a diminished capacity to induce IL-2 production by T cells that was associated with diminished Ag-specific T cell proliferation in vivo.

Previous studies have shown that constituents within cigarette smoke impact DC function in vitro. Exposure of human monocyte-derived DCs and bone marrow-derived murine DCs to different doses of nicotine, for example, have led to conflicting reports; DC activation in one instance (8), but suppressed DC function in another (7). Other compounds within cigarette smoke may also have immunomodulatory potential. Indeed, cigarette smoke extract which, in addition to nicotine, contains many of the other substances that are found in cigarette smoke, inhibits DC function in a manner that is only partly dependent on nicotine (9). By exposing mice to mainstream tobacco smoke, we collectively assessed the impact of the >4000 constituents on the immune system. Furthermore, in vivo modeling of the DC response to OVA afforded the opportunity to recapitulate the complex cellular interactions between DCs and the surrounding tissue environment that accompanies the induction of immune responses.

We demonstrate that cigarette smoke exposure decreased the number of DCs in the lung. Our study is in agreement with a previous report demonstrating that 2–4 mo of smoke exposure significantly decreased the number of pulmonary CD11c<sup>high</sup> MHCII<sup>+</sup> DCs (19). In contrast, D’Hulst and colleagues (20) showed that cigarette smoke induced acute lung inflammation that was associated with increased numbers of DCs as early as 3 days following exposure that persisted for up to 6 mo. In the former study, a nose-only exposure system was used where the mainstream cigarette smoke from 2 cigarettes was delivered daily while in the latter mice were exposed whole body to the smoke from 20 cigarettes per day. Hence, differences between the two studies may be related to the amount of cigarette smoke delivered. Indeed, serum carboxyhemoglobin levels were ~3.6-fold increased in smokers over controls in the nose-only exposure system and ~8.3-fold increased in smokers in the whole body exposure system (21, 22). Furthermore, high-dose smoke exposure in the D’Hulst study (20) was associated with an inflammatory response suggestive of acute lung injury, a phenomenon less akin to the effects of chronic cigarette smoke exposure on humans.

To our knowledge, this is the first study to investigate the effect of cigarette smoke exposure on DC trafficking from the lung to the regional lymph nodes where they exert their action on T lymphocytes. We demonstrate that OVA-containing DCs from smoke-exposed and control mice were equally able to migrate from the lungs to the TLNs (Fig. 2). Moreover, smoking had no effect on DC cell surface expression of CCR7, an important chemokine receptor involved in the migration of DCs into lymphoid tissues (15–17). Thus, it is unlikely the decreased number of lung DCs associated with smoking is the result of enhanced migration to regional lymph nodes. More likely the half-life of lung DCs is diminished with smoke exposure, however, reduced numbers of monocyte precursors or impaired monocyte–DC differentiation may also account for the decreased number of lung DCs associated with smoking.

Although cigarette smoke did not impact DC trafficking from the lung to the lymph nodes, DC maturation was affected. Twenty-four hours following OVA administration, the number of CD11c<sup>+</sup> cells expressing the maturation markers CD80 (B7-1), CD86 (B7-2), and MHCII were significantly reduced in the TLNs of smoke-exposed mice. Interestingly, CD40 expression was not modified by smoke exposure. The CD40-CD40L pathway has been implicated in the production of matrix metalloproteinases (23–25) therefore cigarette smoke could potentially impair DC maturation while promoting matrix metalloproteinase production and tissue destruction, important processes in the pathogenesis of COPD.

The diminished DC maturation status associated with smoking could be the result of increased numbers of T regulatory cells (Tregs) in the draining lymph nodes. Indeed, Tregs have been demonstrated to suppress the up-regulation of CD80 and CD86 on DCs in vitro (26). To address this, we assessed the number of Foxp3<sup>+</sup> Tregs in the TLNs of smoke-exposed animals. We did not observe any difference in the number of OVA-specific T regulatory cells in the TLNs of smoke-exposed mice vs controls (data not
shown). Therefore, it is unlikely that Tregs influenced the maturation status of DCs in our experimental system.

Cigarette smoke-induced inhibition of DC maturation should manifest itself in functional impairment of T cell activation. Within the TLNs, we observed a decreased ability of smoke-exposed DCs to induce Ag-specific T cell proliferation. Engagement of the TCR with the peptide/MHC complex and B7/CD28 co-stimulation is necessary to promote T cell survival, enhance production and stabilization of IL-2, and facilitate T cell-cycle progression (27). We compared the ability of TLN lung-derived DCs from smokers and controls to stimulate OVA-specific DO11.10 CD4+ T cell proliferation in vitro and in vivo. OVA-loaded DCs obtained from the lungs of both non-smoke-exposed and smokers displayed similarly modest ability to induce IL-2 production by DO11.10 CD4+ T cells. Upon transit to the regional lymph nodes, however, control DCs acquired a significantly greater capacity to stimulate proliferation of CD4+ T cells than smoke-exposed DCs.

We observed that on a per cell basis, smoking had no effect on the capacity of lung-derived TLN DCs to induce T cell cytokine production. DO11.10 CD4+ T cells from control and cigarette smoke-exposed mice produced similar levels of OVA-specific IL-5, IL-10, and IL-13 following restimulation in vitro. Collectively, our findings suggest a specific effect for smoking on T cell responses; execution of the T cell effector program remains intact, but diminished clonal expansion in the TLNs means that fewer cells are available to perform these duties.

Diminished CD4+ T cell proliferation that results from impaired lung DC maturation caused by smoking may have important consequences for CD4+ T cell function with respect to immunity to microbes, allergens, and in tumor surveillance. CD4+ T cells control virtually all adaptive immune responses to protein Ags and only a few cell types that express MHCII molecules can function to stimulate proliferation of CD4+ T cells than smoke-exposed DCs.

In conclusion, we provide evidence that cigarette smoke exposure induces specific defects in DC maturation and suppresses the proliferative capacity of CD4+ T cells in thoracic regional lymph nodes in mice. This study links the effects of cigarette smoke on DC function to the known consequences of cigarette smoke on immunity and lung disease.

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Disclosures
The authors have no financial conflict of interest.

References