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Cigarette Smoke-Induced Oxidative Stress Suppresses Generation of Dendritic Cell IL-12 and IL-23 through ERK-Dependent Pathways

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IL-12p70, a heterodimer composed of p35 and p40 subunits, is a key polarizing cytokine produced by maturing dendritic cells (DCs). We report that cigarette smoke extract (CSE), an extract of soluble cigarette smoke components, suppresses both p35 and p40 production by LPS or CD40L-matured DCs. Suppression of IL-12p70 production from maturing DCs was not observed in the presence of nicotine concentrations achievable in CSE or in the circulation of smokers. The suppressed IL-12p70 protein production by CSE-conditioned DCs was restored by pretreatment of DCs or CSE with the antioxidants N-acetylcysteine and catalase. Inhibition of DC IL-12p70 by CSE required activation of ERK-dependent pathways, since inhibition of ERK abrogated the suppressive effect of CSE on IL-12 secretion. Oxidative stress and sustained ERK phosphorylation by CSE enhanced nuclear levels of the p40 transcriptional repressor c-fos in both immature and maturing DCs. Suppression of the p40 subunit by CSE also resulted in diminished production of IL-23 protein by maturing DCs. Using a murine model of chronic cigarette smoke exposure, we observed that systemic and lung DCs from mice “smokers” produced significantly less IL-12p70 and p40 protein upon maturation. This inhibitory effect was selective, since production of TNF-α during DC maturation was enhanced in the smokers. These data imply that oxidative stress generated by cigarette smoke exposure suppresses the generation of key cytokines by maturing DCs through the activation of ERK-dependent pathways. Some of the cigarette smoke-induced inhibitory effects on DC function may be mitigated by antioxidants.

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obust dendritic cell (DC)4 responses are essential for the development of protective host immune responses during vaccination, clearance of many infectious pathogens, and are also essential for the control and elimination of cancer (1). Although many factors control the quality of the host immune response to endogenous or exogenous Ags, the generation of the Th-1 cytokine IL-12 (IL-12p70), a heterodimer composed of p35 and p40 subunits, by maturing DCs, is critical for the development of appropriate host responses that enable elimination of certain infectious pathogens and malignancies (2). IL-23 is a related member of the IL-12 family composed of the p40 subunit coupled with a distinct second subunit referred to as p19 (3). Like IL-12p70, IL-23 also influences host immune responses to pathogens, is an important regulator of IL-17-secreting T cells, and plays important roles in host responses during certain bacterial infections such as Klebsiella pneumonia (4, 5).

IL-12 and IL-23 have the capacity to induce immunological pathways with distinct and also complementary functions. In murine models deficient of the IL-12p40 subunit (which results in functional deficiency of both IL-12 and IL-23), protective immune responses to mycobacteria are impaired, resulting in increased bacterial growth and decreased Ag-specific inflammation (6). The enhanced susceptibility of IL-12p40-deficient mice to mycobacteria is primarily a consequence of IL-12p70 deficiency, as in IL-23p19-deficient mice mycobacterial growth is controlled and there is no diminution in Ag-specific IFN-γ-producing CD4 T cells (6). The importance of IL-12 in human responses to mycobacterial pathogens is also highlighted by the observation that humans with mutations in the IL-12B1 receptor resulting in functional IL-12 deficiency are markedly susceptible to develop disseminated mycobacterial infections (7). IL-12 also provides critical functions in the context of antitumor responses. IL-12 activates NK and T cells to generate efficient Th1 responses, facilitates DC maturation and Ag presentation, suppresses IL-10 production, and may prevent or reverse the development of anergy to tumor peptide (8, 9). IL-23 has some overlapping functions with IL-12, but is distinctive in its capacity to drive the expansion of memory T cells (10), and promotes the development of a novel CD4+ T cell subset that is distinguished from Th1 and Th2 cells by its capacity to secrete IL-17, a cytokine believed to have important roles in host responses toward certain extracellular pathogens (5) and chronic
inflammatory diseases (11). An essential role for IL-23 in host immunity to extracellular bacterial pathogens was recently provided by Aujla et al. (12), who described an essential role for IL-23 in the generation of IL-22-secreting T cells that are mandatory for adequate clearance of pulmonary infection by *K. pneumoniae* (12). In animal models of cancer, IL-23 suppressed tumor growth by vaccine-induced T cells, enhanced tumor-specific T cell levels, and enhanced the effector function of intratumoral T cells (13).

Cigarette smoking (CS) is an important cause of lung and other cancers and also predisposes to tuberculosis (14–16) and invasive pneumococcal infection (17). Although considerable epidemiologic data links CS with increased predisposition to certain lung infections (14–16, 18), the mechanisms by which smoking impairs host responses to these pathogens are not fully understood. A number of studies have implicated nicotine as an in vitro suppressor of macrophage, dendritic, and T cell functions, and suggested that this may explain some of the immunosuppressive properties of smoking in the context of infection (19–21). However, studies that explored nicotine concentrations in the range of 0.01–1.0 μg/ml, which seem to be physiologically relevant (22, reported either a lack of suppression (23) or even augmentation of certain immune cell functions (24). Recently, we reported that DCs conditioned with cigarette smoke extract (CSE-DCs) demonstrated significant functional defects upon maturation and produced considerably less IL-12 than control DCs (25). This reduction in IL-12-secreting capacity of CSE-DCs was not a marker of diminished viability or generalized suppression of cellular functions, since the production of cytokines like IL-6 were unaffected by CSE while IL-10 production was actually enhanced (25). In the current study, we investigated in detail mechanisms and potential mediators present in tobacco smoke that suppress the generation of cytokines during DC maturation. Due to the prominent role of IL-12 and IL-23 in mediating immune responses during cancer and numerous infections, conditions that smokers are at increased risk of developing, we designed the current study to investigate in detail the effect of CSE and nicotine on the generation of IL-12p70 and p40 by maturing DCs. We also sought to define cigarette smoke components other than nicotine that may be responsible for suppression of IL-12p70 and p40 by maturing DCs. We examined the effect of CSE on the activation of different MAPK in the generation of IL-12p70 by maturing DCs. Furthermore, we used a murine model of chronic tobacco exposure to confirm the effect of smoking on lung and systemic DC generation of IL-12p70 and the p40 subunit that is shared by both IL-12p70 and IL-23.

**Materials and Methods**

**General reagents**

Mecamylamine hydrochloride, N-acetylcysteine (NAC), and bovine catalase were purchased from Sigma Biochemicals. CD11c^+^ magnetic beads were purchased from Miltenyi Biotec. Human recombinant CD40L was purchased from Axxora Platform Biochemicals. Purified hamster anti-mouse CD40 agonist Ab (clone HM40) was purchased from BD Biosciences. Recombinant human IFN-γ and IL-4 were obtained from R&D Systems, while recombinant human GM-CSF was obtained from Genzyme CXTT; Sigma-Aldrich or 1 μg/ml soluble recombinant human CD40L (Axxora Platform Biochemicals). Where indicated, IFN-γ was added at a concentration of 5–50 ng/ml at the time of maturation (when LPS was added to the DC culture).

**Measurement of cytokines**

Human IL-12p35, p40, p70, and total IL-23 (p19/p40) levels were measured using commercially available ELISA according to the manufacturer’s instructions (eBioscience). Human IL-23 p19/40 levels were also measured in supernatants using a commercially available ELISA from Bender Systems.

**Determination of cellular ERK and p38 protein levels by immunoblotting**

Semiquantitative determination of cellular ERK 1/2 and p38 proteins were obtained by immunoblotting. Human monocyte-derived DCs (days 6 and 7) were plated at a concentration of 1 × 10^6/10 ml in complete medium (RPMI, 1640 and 10% FBS) and GM-CSF/IL-4 as described above. CSE and LPS were added to the cells at the time points indicated. Protein lysates were prepared using radioimmunoprecipitation assay buffer (150 mM NaCl, 1.0% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris). Protein concentrations in respective extracts were determined by Bradford assay referenced against an albumin standard. Equal amounts of protein lysates were separated on 10–12% polyacrylamide gels and transferred electrophoretically to nitrocellulose membranes. The membranes were blocked with 5% milk or 5% BSA in TBS and incubated with relevant secondary polyclonal Abs for 1–2 h or overnight at 4°C. In the final step, membranes were washed and incubated with HRP-conjugated secondary Ab and detected by a chemiluminescence detection system ECL (Amerham Biosciences). An polyclonal rabbit anti-human phospho-p44/42 MAPK (Thr202/Tyr204) Ab was used to detect levels of p42 and p44 MAPK (ERK1 and ERK2, catalog no. 9101; Cell Signaling Technology). Endogenous total ERK (p44/p42) levels were determined using a polyclonal rabbit anti-human Ab (catalog no. 9102; Cell Signaling Technology). A mouse mAb to phosphorylated p38 MAPK was used to detect endogenous levels of phosphorylated p38 (catalog no. 9217; Cell Signaling Technology).

**Preparation of nuclear protein lysate fractions**

Nuclear protein fractions were prepared from human monocyte-derived DCs (1 × 10^6 cells/ml) incubated in the presence or absence of CSE (1–2%) or LPS (100 ng/ml) or combinations of both. At specified times, the cells were placed on ice and cytosolic and nuclear lysates were prepared. DCs were collected at specific time points, washed in ice-cold PBS supplemented with phosphatase inhibitors (Active Motif), and cytoplasmic protein fractions were extracted by incubating 5–10 × 10^6 DCs with 500 μl of a commercially available hypotonic buffer (Active Motif) on ice for 15 min. Nuclear fractions were prepared following extraction of cytosolic protein by resuspending nuclear pellets in a commercially available complete nuclear lysis buffer (Active Motif) according to the manufacturer’s instructions. Cytosolic and nuclear fractions were stored at −20 to −70°C until assayed. Protein concentrations in respective extracts were determined by the Bradford assay referenced against an albumin standard. Immunoblotting for c-fos protein in nuclear lysates was performed with an anti-human phospho-p44/42 MAPK (Thr202/Tyr204) Ab (clone 2520; Cell Signaling Technology) at 4°C. Chemiluminescent detection of bound anti-c-fos Ab was performed as described above.
Quantitative measurement of cellular ERK and nuclear c-fos protein levels

Since immunoblotting provides semiquantitative measurement of cellular protein levels, we also determined changes in whole-cell phospho-ERK levels and nuclear c-fos protein using commercially available ELISA kits according to the manufacturer’s instructions (Assay Design ERK 1/2 kit and Activ Motif TransAM AP-1 c-fos kit).

c-fos knockdown

To determine directly the role of c-fos as a mediator of CSE-induced suppression of IL-12 production, small interfering RNA (siRNA) was used to silence c-fos in a murine macrophage cell line before stimulation with CSE and LPS. In a 6-well tissue culture plate, 2 × 10^5 RAW cells (murine macrophage-like cell line, RAW 264.7 purchased from American Type Culture Collection) were inserted per well in 2 ml of antibiotic-free normal growth medium supplemented with FBS. Subconfluent cells were transfected with 60 pmol of c-fos or control-scrambled siRNA (control siRNA, catalog no. SC-37007, and c-Fos siRNA (mouse), SC-29222, both reagents from Santa Cruz Biotechnology) in 100 μl of siRNA Santa Cruz Biotechnology Transfection Medium (SC-36868). Cells were incubated for 5 h at 37°C, followed by the addition of normal growth medium containing 10% FCS, 2% FBS, 1 × 10^5 H9262 cells and LPS (100 ng/ml) or IL-12p70 (55.6 ng/ml, based on four measurements) for an additional 18 h. Cytokine levels were subsequently measured in the supernatants using ELISA. Knockdown of c-fos was confirmed using Western blotting.

RT-PCR for semiquantitative gene expression analysis

Monocyte-derived DCs were matured with LPS (100 ng/ml) and IFN-γ (50 ng/ml, unless otherwise stated) in the presence or absence of CSE for a period of 4–6 h. Total RNA was isolated with an RNasey mini kit according to the manufacturer’s instructions (Qiagen). All samples were digested with DNase I to remove contaminating genomic DNA. A 20-μl first-strand cDNA synthesis reaction was performed using SuperScript III (Life Technologies) and 1.0 μl of resulting cDNA was used in a 25-μl PCR. The following primers were used in amplification: human IL-12 p35 sense primer, ACCAGAGAGTTGTCCTGAC and antisense primer, TCTGT CAAACACCTGGGTATC; and human IL-12 p40 sense primer, GCGACGGTCTTAAGCC and antisense primer, TTTGGGGCATG ACCGTG: human p19 sense primer and antisense primer. As a loading control, the housekeeping gene β-actin was amplified: sense primer, GTG GGGGCCCAAGACA and antisense primer, CTCGCTAAGTCAC GCCAGATTTC. For cDNA amplifications, conditions consisted of an initial 2-min hot start at 94°C, followed by 30 cycles at 94°C for 60 s, 55°C for 60 s, and 72°C for 60 s. A final 10-min extension at 72°C PCR amplification was run on a 1.4–2% agarose gel and photographed. To determine the expression of the IL-12p35 gene in maturing lung DCs extracted from mice exposed to cigarette smoke, lung DCs (procedure of extraction and murine model described above) were incubated for 6 h with LPS (100 ng/ml). RNA was extracted as described above, and RT-PCR was performed using a commercially available murine p35 primer pair from Invitrogen.

In vivo exposure of mice to cigarette smoke

To further expand on our in vitro studies, we conducted in vivo studies to determine the effect of chronic CS on lung and systemic DC cytokone production. We used the Teague TE-2 System, (Teague Enterprises, Woodland, CA), a manually controlled cell machine that produces a combination of side-stream and mainstream cigarette smoke in a chamber, which is then transported to a collecting and mixing chamber where varying amounts of air are added to the smoke mixture at 72°C PCR amplification was run on a 1.4–2% agarose gel and photographed. To determine the expression of the IL-12p35 gene in maturing lung DCs extracted from mice exposed to cigarette smoke, lung DCs (procedure of extraction and murine model described above) were incubated for 6 h with LPS (100 ng/ml). RNA was extracted as described above, and RT-PCR was performed using a commercially available murine p35 primer pair from Invitrogen.

Cigarette smoke extract suppresses IL-12 and IL-23 production by maturing DCs

We recently reported that CSE, a preparation of soluble factors generated from mainstream cigarette smoke, potently inhibits IL-12p70 production by LPS-matured human myeloid DCs (25). An alternate mechanism by which DCs are induced to generate IL-12p70 is by activation of the CD40 receptor, following recognition of its ligand CD154 (CD40L). To determine whether CSE conditioning suppresses IL-12p70 production by CD40-activated DCs, human monocytes-derived DCs were incubated for 30–60 min with CSE at concentrations that do not alter cell viability (up to 2% CSE) before the addition of either LPS or CD40L and IFN-γ for an additional 18 h. Cytokine levels were subsequently measured in the supernatants using ELISA. Fig. 1A illustrates that CSE suppresses IL-12p70 release not only from LPS-matured but also CD40L-matured DCs. This inhibition occurs in a dose-dependent fashion, with maximal suppression observed with 2% CSE (final nicotine content of 2% CSE = 429.2 ± 55.6 ng/ml, based on four measurements), which consistently suppressed 75% or more of the maximal secreted IL-12p70 protein (Fig. 1A). Since the p35 and p40 subunits may be regulated independently, we determined whether the inhibitory effect of CSE on IL-12p70 was mediated through inhibition of either or both subunits. Fig. 1B demonstrates that the production of the p40 subunit by LPS-matured DCs is markedly inhibited by CSE. In addition, we tested whether the addition of IFN-γ priming (50 ng/ml) with LPS could abrogate the inhibitory effect of CSE on IL-12 production by LPS-matured DCs. Although IFN-γ priming substantially increased the capacity of DCs to generate IL-12p40, it failed to overcome the inhibitory effect of CSE conditioning on p40 production (Fig. 1B). In addition to IL-12p70, the p40 subunit also forms part of the IL-23p40/p19 heterodimer by binding to p19. The suppressed generation of p40 by CSE-conditioned DCs implies that CSE may also inhibit IL-23p40/p19 production by maturing DCs. To further determine the effect of CSE on p35 and p19, additional subunits of IL-12 and IL-23 heterodimers, respectively, we investigated the effect of CSE on the expression of p35 and p19 gene transcription in LPS and IFN-γ matured DCs conditioned for 60 min with 1% CSE. The addition of IFN-γ to LPS during DC maturation increased the quantity of IL-12 generated but did not alter the ability of CSE to suppress either IL-12p40 protein (as shown in Fig. 1B) or p19, p35, or p40 gene expression as determined using semiquantitative RT-PCR in Fig. 1C. In keeping with the observed inhibitory effect on p19 gene expression, production of IL-23p40/p19 heterodimer by DCs matured by LPS as a sole maturational factor was similarly impaired by CSE (Fig. 1D). Taken together these data demonstrate that CSE potently suppresses the generation of both IL-12.
Suppression of IL-12 production by CSE conditioning is not exclusively mediated by the nicotine component. A, Human DCs were matured with 100 ng/ml LPS alone in the presence or absence of increasing concentrations of nicotine or CSE. Following an 18-h period, IL-12p70 levels in the supernatant were measured by ELISA. ***, p < 0.001; ANOVA and Tukey’s multiple comparison test. iDC, Immature DC; mDC, mature DC. One representative of three independent experiments is shown.

B, Human DCs were matured with 100 ng/ml LPS and 50 ng/ml IFN-γ in the presence of 0, 1, or 10 μM of the nicotinic antagonist mecamylamine hydrochloride (added 30 min before the LPS/IFN), along with freshly generated CSE where indicated. Secreted IL-12p70 levels were measured in supernatants following an 18-h period of culture. The filled bars illustrate IL-12p70 production by maturing CSE-conditioned DCs pretreated with identical concentrations of the nicotinic antagonist. The data shown are representative of two independent experiments.

Nicotine is an alkaloid found in cigarette smoke and CSE (25). A number of studies have alluded to nicotine as an important immune modifier by virtue of its effects on both APC and T cell activation (20, 24). To determine whether the nicotine present in CSE plays a role in the observed suppression of cytokine production by DCs, we conducted experiments to determine the effect of nicotine at varying concentrations on LPS-induced production of IL-12p70. The concentrations of nicotine chosen included those reported to occur in the circulation of active cigarette smokers (range of serum nicotine levels reported to be 20–50 ng/ml) (22), as well as higher concentrations that may theoretically occur in local areas in the lungs or oral cavity. As demonstrated in Fig. 2A, a broad range of nicotine concentrations not only suppressed IL-12 but also IL-23 from maturing DCs, irrespective of whether IFN-γ is present during the process of DC maturation.

**FIGURE 2.** Suppression of IL-12 generation by CSE is not mediated by the nicotine component. A, Human DCs were matured with 100 ng/ml LPS alone in the presence or absence of increasing concentrations of nicotine or CSE. Following an 18-h period, IL-12p70 levels in the supernatant were measured by ELISA. ***, p < 0.001; ANOVA and Tukey’s multiple comparison test. iDC, Immature DC; mDC, mature DC. One representative of three independent experiments is shown.

B, Human DCs were matured with 100 ng/ml LPS and 50 ng/ml IFN-γ in the presence of 0, 1, or 10 μM of the nicotinic antagonist mecamylamine hydrochloride (added 30 min before the LPS/IFN), along with freshly generated CSE where indicated. Secreted IL-12p70 levels were measured in supernatants following an 18-h period of culture. The filled bars illustrate IL-12p70 production by maturing CSE-conditioned DCs pretreated with identical concentrations of the nicotinic antagonist. The data shown are representative of two independent experiments.

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**FIGURE 1.** CSE suppresses IL-12 and IL-23 generation by maturing DCs. A, Human monocyte-derived DCs were incubated for 30–60 min with freshly generated CSE (0, 1, or 2%) and subsequently cultured for an additional 18 h in the presence or absence of either 100 ng/ml LPS or a combination of 1 μg/ml recombinant human CD40L and 50 ng/ml rIFN-γ. IL-12p70 levels in the supernatants were measured using ELISA. Experiment shown is representative of four independent experiments. One-way ANOVA, p < 0.001; ***, p < 0.001 with Tukey’s multiple comparison test comparing IL-12 production by CSE-conditioned DCs with DCs matured without CSE.

B, Human monocyte-derived DCs were incubated for 30–60 min with freshly generated CSE (0, 1, or 2%) and subsequently cultured for an additional 18 h in the presence or absence of either 100 ng/ml LPS as a sole maturational agent or a combination of 100 ng/ml LPS supplemented with 50 ng/ml rIFN-γ. IL-12p40 levels in the supernatants were measured using ELISA. One-way ANOVA, p < 0.0001; ***, p < 0.001 with posttest Tukey comparison test. Experiment shown is representative of three independent experiments. C, Total RNA was isolated from 5 × 10⁶ DCs incubated with freshly generated CSE (0 or 1%) for 30 min before addition of 100 ng/ml *Escherichia coli* LPS and 50 ng/ml IFN-γ for an additional 4 h. RT-PCR was performed to measure p19, p35, and p40 mRNA expression. β-Actin mRNA was analyzed as a loading control. The experiment shown is representative of three independent experiments. D, Human DCs were incubated for 30–60 min with CSE before and IL-23 from maturing DCs, irrespective of whether IFN-γ is present during the process of DC maturation.

**Suppression of IL-12 production by CSE conditioning is not exclusively mediated by the nicotine component**

Nicotine is an alkaloid found in cigarette smoke and CSE (25). A number of studies have alluded to nicotine as an important immune modifier by virtue of its effects on both APC and T cell activation (20, 24). To determine whether the nicotine present in CSE plays a role in the observed suppression of cytokine production by DCs, we conducted experiments to determine the effect of nicotine at varying concentrations on LPS-induced production of IL-12p70. The concentrations of nicotine chosen included those reported to occur in the circulation of active cigarette smokers (range of serum nicotine levels reported to be 20–50 ng/ml) (22), as well as higher concentrations that may theoretically occur in local areas in the lungs or oral cavity. As demonstrated in Fig. 2A, a broad range of nicotine concentrations not only suppressed IL-12 but also IL-23 from maturing DCs, irrespective of whether IFN-γ is present during the process of DC maturation.
nicotine concentrations below 50 μg/ml failed to suppress IL-12p70 secretion by maturing DCs, whereas 1% CSE (which contains a nicotine content of ~200 ng/ml) caused marked suppression of IL-12p70 secretion (Fig. 2A). In control experiments, IL-12p70 was not induced in DCs incubated with nicotine concentrations ranging from 0.1 to 50 μg/ml (data not shown). To rule out the possibility that the lack of effect may be donor DC-specific or potentially due to the quality of the commercially available nicotine preparation, we repeated the experiment using DCs generated from three different donors and tested two different commercially available nicotine preparations (Sigma-Aldrich and Valeant Pharmaceuticals; see Materials and Methods) with identical results (data not shown). We did, however, observe that nicotine concentrations ≥100 μg/ml caused a statistically significant reduction in IL-12p70 secretion by maturing DCs by ~35% compared with controls (data not shown), consistent with what was reported by Nouri et al. (20). This was not a result of cell death, since cellular viability, determined by the XTT assay, of DCs incubated with up to 100 μg/ml nicotine was similar to that of controls. To further rule out a potential role for nicotine as an effector of CSE-induced suppression of Th1 cytokine production, DCs were pretreated with the nicotinic receptor antagonist mecamylamine hydrochloride (1–10 μM) for 30 min before the addition of CSE and LPS for an additional 18 h. The addition of mecamylamine hydrochloride failed to even partially restore IL-12 production, indicating that despite antagonism of nicotinic receptor signaling, CSE still induced marked suppression of IL-12 production (Fig. 2B). Taken together, these data indicate that nicotinic stimulation alters DC production of IL-12 only at relatively high concentrations (>50 μg/ml) and is not responsible for the suppression of IL-12 generation by CSE-conditioned maturing DCs.

Antioxidants prevent CSE-induced suppression of IL-12 and IL-23

Cigarette smoke contains many reactive oxygen species, themselves capable of altering immune responses (28). To determine whether reactive oxygen species play a role in the Th1 cytokine suppression observed in CSE-conditioned DCs, we determined IL-12p70 production by CSE-conditioned maturing DCs that were pretreated with the antioxidant NAC added to the culture medium 30–60 min before the addition of CSE and subsequent maturation with LPS. The addition of NAC (0.025–2.5 mM) resulted in a dose-dependent restoration of the IL-12-secreting capacity in DCs and prevented the inhibitory effect of CSE on IL-12p70 generation by LPS-matured DCs (Fig. 3A). DCs conditioned with 1% CSE produced only 4.4 ± 0.035 pg/ml IL-12p70 compared with 92.4 ± 1.15 pg/ml by the positive control DCs matured in the absence of CSE (Fig. 3A). Preincubation with increasing concentrations of NAC (0.025–2.5 mM) resulted in a dose-dependent and statistically significant augmentation of IL-12p70-secreting capacity (Fig. 3A; p < 0.001 with two-way ANOVA followed by Bonferroni posttest for NAC treatment effect). To further ascertain the direct effect of NAC as an inhibitor of CSE-induced suppression of maturation-associated DC IL-12p70 and p19 production, human DCs were preincubated with either CSE, 2.5 mM NAC, or both 60 min before the addition of both LPS and IFN-γ as robust inducers of maturation. Six hours after the addition of the LPS/IFN-γ maturation stimulus, RNA was extracted and RT-PCR for p19, 35, and 40 was performed. As demonstrated in Fig. 3B, preincubation of maturing DCs with NAC prevented CSE-induced suppression of IL-12 and IL-23 gene subunit transcripts during LPS/IFN-γ-induced maturation.

Cigarette smoke is known to contain chemicals capable of inducing oxidative stress, such as the potent oxidant hydrogen peroxide (H2O2) (29). The inability of CSE to suppress IL-12 generation by maturing DCs pretreated with NAC suggested that either NAC inhibited the activity of oxidants generated by CSE in the culture medium or potentially directly enhanced the capability of the DCs to withstand oxidative stress in vitro. To define which of these mechanisms prevails in our system, we tested the effect of incubating freshly generated CSE with NAC for 5 min before adding to the cell culture medium. NAC was added to freshly generated CSE to achieve a final concentration of 25 mM. The preparation was incubated for 5 min at room temperature before the addition to DCs in culture and its effect on IL-12 generation by LPS-matured DCs was compared with standard CSE (Fig. 3C). We observed that transient incubation of CSE with 25 mM NAC for only 5 min substantially abrogated its capacity to suppress DC IL-12p70 secretion, implying that substantial oxidative stress induced by the CSE is directly responsible for a substantial component of the IL-12 inhibition observed. This effect was most dramatic with the higher concentration of CSE: whereas DCs matured in the presence of 2% CSE produced only 1022 ± 243 pg/ml IL-12p70 (or <10% of IL-12p70 secreted by control maturing DCs without CSE), the DCs matured in the presence of 2% CSE that underwent pretreatment with 25 mM NAC produced 6668 ± 186.1 pg/ml IL-12p70 (Fig. 3C; ANOVA and Tukey comparison test, p < 0.001). This suggests that in addition to augmenting intracellular DC glutathione content, NAC may directly antagonize reactive oxidants generated in the CSE and partially reverse modulation of DC function and IL-12 secretion. To explore further the role of reactive oxidants like hydrogen peroxide (H2O2) in mediating IL-12 suppression in maturing DCs, we added catalase (500–1000 U/ml), a H2O2 scavenger, to the PBS used to generate CSE and then tested the effect of catalase pretreatment on the capacity of CSE to suppress IL-12 production by maturing DCs. A statistically significant increase in IL-12p70 generation was observed when catalase was added to freshly prepared CSE before addition to the cell culture (Fig. 3D; ANOVA and Tukey’s multiple comparison test, p < 0.001), implying that oxidants such as H2O2 generated in the CSE are responsible, to a substantial degree, for the observed inhibitory effect on maturation-associated IL-12p70 generation. These data imply that potent oxidants, including hydrogen peroxide, generated in CSE are responsible for the suppressive effect of CSE on IL-12 generation. Furthermore, these data suggest that DC IL-12 secretion may be restored by antioxidants like NAC and catalase that quench oxidative stress.

Suppression of IL-12 by CSE requires functional ERK

Activation of MAPK pathways regulates many LPS-induced genes and the relative phosphorylation of p38 in relation to ERK is now recognized as a critical regulator of IL-12 generation in both macrophages and DCs (30, 31). To determine the role of p38 and ERK MAPKs in regulating IL-12p70 generation by human DCs conditioned with CSE, we first sought to determine whether CSE induces phosphorylation of p38 or ERK. To determine this, immature DCs were incubated with CSE for varying periods of time, ranging from a few minutes to 2 h, and intracellular levels of phosphorylated p38 and ERK were determined with immunoblotting. Fig. 4A illustrates that CSE induces phosphorylation of both ERK and p38, although the magnitude of the early response was significantly less than that occurring with control DCs activated by LPS. We observed that LPS induces rapid phosphorylation of both ERK and p38 within 5–15 min of activation. CSE similarly induced phosphorylation of both ERK and p38 within 5–15 min of incubation (Fig. 4A). However, in contrast to the rapid transient ERK phosphorylation observed following stimulation with LPS, CSE induced sustained phosphorylation of ERK (Fig. 4B). Activation of immature DCs conditioned with CSE resulted in higher
levels of phosphorylated ERK than observed in DCs matured with LPS without CSE (Fig. 4C). Taken together, these data demonstrate that CSE activates both ERK and p38 pathways, induces sustained and prolonged phosphorylation of ERK, and augments cellular phospho-ERK levels in LPS-activated DCs.

Activation of different MAPKs results in diverse functional effects. To determine the relative functional role of CSE-induced p38 and ERK phosphorylation in LPS-induced IL-12p70 generation, DCs were pulsed for 30–60 min with the specific p38 inhibitor SB202190 or the specific ERK inhibitors U0126 or ERK Activation inhibitor peptide before the addition of CSE and LPS as a maturation stimulus (Fig. 4D). Fig. 4D illustrates that activation of ERK-dependent pathways is essential for the observed inhibitory effect of CSE on IL-12 generation. Preincubation of DCs with 0.1 μM ERK inhibitor U0126 or 10 μM ERK activation inhibitor peptide resulted in complete abrogation of the inhibitory effects of CSE (Fig. 4D). A statistically significant reduction in IL-12p70 secretion occurred when DCs were matured in the presence of 2% CSE when compared with control DCs, 512.6 ± 19.8 pg/ml vs 323.8 ± 5.3 pg/ml (Fig. 4C; p < 0.001, two-way ANOVA with Bonferroni posttest analysis). When either 0.1 μM U0126 or 10 μM ERK activation inhibitor peptide was added to the culture medium for 30 min before the addition of CSE and subsequent maturation with LPS, the inhibitory effect of CSE on IL-12p70 production was markedly attenuated (Fig. 4D). In contrast, inhibition of the p38 pathway by the selective p38 inhibitor SB202190 at concentrations of 1–10 μM resulted in complete suppression of IL-12p70 generation (Fig. 4D), implying that intact signaling through p38 is critical for generation of IL-12 by LPS-activated DCs. This effect was not a result of nonspecific toxicity because even at a 1 μM concentration (concentrations of SB202190 >10 μM have not been associated with diminished cell viability in in vitro studies (32, 33)), the p38 inhibitor caused marked suppression of IL-12p70 generation, even from control DCs (Fig. 4D).

CSE induces nuclear translocation of c-fos through ERK-dependent pathways

The requirement of ERK phosphorylation in CSE-induced suppression of IL-12 generation by maturing DCs led us to search for negative regulators of IL-12 transcription regulated by ERK. One such ERK-dependent transcription factor is the c-fos protein, a direct inhibitor of IL-12 p40 gene transcription (34). To determine
and densitometry was performed (ratio of p-ERK (1:2): actin determined). ERK 1/2 Abs. Actin protein levels were determined as an internal control using phospho-specific Abs. lysates were separated on 12% gels, transferred to nitrocellulose, and detected using phospho-ERK 1/2 Abs. Total ERK protein levels were determined as an internal control (CNTRL) to ensure equal protein loading. Experiment shown is representative of three independent experiments. B. Equal amounts of protein from whole-cell protein lysates prepared from 5×10^6 DCs incubated with either 1% CSE or 100 ng/ml LPS were separated on 12% gels, transferred to nitrocellulose, and detected using phospho-ERK 1/2 Abs. Total ERK protein levels were determined as an internal control to ensure equal protein loading. Experiment shown is representative of three independent experiments. C. Human DCs were incubated with either 1% CSE, LPS, or both 1% CSE and LPS. The CSE was added to the cells 30 min before the addition of LPS and cell lysates were prepared 150 min after the addition of CSE. Equal amounts of protein from 5×10^6 DCs were separated and detected using phospho-ERK 1/2 Abs. Actin protein levels were determined as an internal control and densitometry was performed (ratio of p-ERK (1:2): actin determined) using Image J software. D. Human DCs were incubated with 0.1 μM ERK inhibitor (U0126), 10 μM ERK inhibitor (inh) peptide, 1 μM p38 inhibitor (SB202190), or no inhibitor (no Inhib) for 30 min before the addition of freshly prepared 2% CSE (H) or equivalent volume of PBS (H). Thirty minutes following the addition of CSE or PBS, DCs were matured for an 18-h period with 100 ng/ml LPS and 50 ng/ml IFN-γ. IL-12p70 was measured by ELISA. Data are representative of four independent experiments.

We extended in vitro observations by testing the effect of smoking on the production of IL-12 and IL-23 by maturing lung DCs. To test this, we used a manual smoking machine that allows exposure of mice to high concentrations of mixed environmental and mainstream cigarette smoke. Following 4–8 wk in the smoking chamber, mice were sacrificed and CD11c^+ DCs were isolated from lungs or spleens. At the time of sacrifice, serum nicotine levels were measured in whole blood using the same assay performed in our clinical laboratories for measurement of serum nicotine in human blood samples. Using protocol of 3 h of daily exposure to cigarette smoke, mean serum nicotine levels attained were 125.5 ± 57.4 ng/ml (n = 8 separate determinations; number of mice in each experimental group was four; nicotine levels in controls was zero).

Equal numbers of lung CD11c^+ DCs (placed in medium at 0.5×10^6/ml) extracted from cigarette smoke (Cig. Sm., Fig. 6) and control wild-type mice were then incubated with LPS (100 ng/ml) or agonistic CD40 Ab (HM40, 1 μg/ml) for an additional 14–18 h, and cytokine levels were measured in the supernatants. In accordance with previous observations, lung DCs extracted from mice that had been smoking for 4 wk or more demonstrated suppressed IL-12p40 production following maturation by LPS (Fig. 6A). An identical response was observed when lung DCs were matured with agonistic CD40 Abs (Fig. 6B); whereas wild-type lung DCs produced 3115 ± 21 pg/ml IL-12p40 following overnight maturation with agonistic CD40 Ab, lung DCs from cigarette smoker mice produced only 1708 ± 90 pg/ml, p = 0.035 (t test comparison). Since IL-12 is composed of p35 and p40 subunits, we also tested the effect of smoking on the induction of the murine p35 gene and production of IL-12p70 protein. Lung DCs from control and cigarette smoke-exposed mice incubated with or without LPS for 6 h, RNA extracted, and RT-PCR was performed to

whether CSE regulates c-fos expression in DCs, we measured nuclear c-fos levels in DCs conditioned with CSE by preparing nuclear or whole-cell protein lysates of DCs incubated with CSE and/or LPS. We observed that CSE concentrations ≥1%, which profoundly suppress IL-12 generation from DCs, also induce robust increases in nuclear c-fos levels in both immature and LPS-matured DCs (Fig. 5A). Using a quantitative ELISA-based method to measure nuclear total c-fos protein content, we observed that nuclear levels of c-fos significantly increase following activation with CSE and peak 2 h following stimulation with CSE (Fig. 5B). Consistent with the observation that activation of ERK is required for CSE to exert an inhibitory effect on IL-12 generation, preincubation of DCs with the specific ERK inhibitor U0126-abrogated CSE-induced nuclear accumulation of c-fos protein (Fig. 5C). To determine this, immature DCs were incubated for 60 min with 1 μM of the ERK inhibitor U0126 before the addition of 2% CSE. After 60 min of incubation with CSE, nuclear lysates were prepared and quantitative determination of total nuclear c-fos protein was performed using ELISA. Whereas the nuclear c-fos content in DCs stimulated with 2% CSE increased >3-fold (Fig. 5C), pretreatment of DCs with U0126 blocked subsequent nuclear accumulation of c-fos following stimulation with CSE.

To demonstrate direct involvement of c-fos in CSE-mediated suppression of IL-12, we used commercially available siRNA to silence murine c-fos in the RAW 264.7 macrophage cell line and subsequently challenged c-fos siRNA-transfected and control siRNA-transfected cells with either LPS (100 ng/ml) or a combination of 1% CSE followed by LPS (100 ng/ml). Consistent with our hypothesis that CSE induces ERK-dependent accumulation of nuclear c-fos and transcriptional suppression of IL-12, we observed that knockdown of c-fos in the RAW macrophage cell line rescued IL-12p40 production in LPS-stimulated cells pretreated with CSE (Fig. 5D).
determine mRNA levels of the p35 gene. In accordance with the observed suppression of IL-12p40 production by lung DCs from mice exposed to cigarette smoke, we observed diminished p35 gene expression in maturing lung DCs from “smoking” mice (Fig. 6C) and correspondingly diminished secreted IL-12p70 protein (Fig. 6D). The observed suppression of IL-12 production was not a result of nonspecific toxicity. As a primary surrogate of viability, we determined the capacity of the lung DCs from CS mice to generate other key cytokines. As demonstrated in Fig. 6E, the production of TNF-α by lung DCs from cigarette smoker mice was not only intact, but actually enhanced when compared with the control DCs from wild-type mice. Lung DCs from cigarette smoker mice produced a statistically significant greater amount of TNF-α following maturation with LPS (6783 ± 181 vs 4865 ± 141 pg/ml by the wild-type lung DCs; Fig. 6E, p = 0.006, t test comparison). Since others have reported apoptosis of certain cell types following exposure to cigarette smoke components (35–37), we also directly validated cellular viability of the lung DCs using an XTT bioassay to determine mitochondrial viability and observed no decrease in cellular viability of lung DCs from CS mice (data not shown), indicating that premature cellular death from tobacco-related toxicity does not explain the observed suppression in IL-12 protein production by lung DCs from CS mice. These data suggest that CS alters lung DC function in specific ways, suppressing the generation of key Th1 cytokines while enhancing the production of proinflammatory cytokines like TNF-α.

The observation that chronic exposure to cigarette smoke causes suppression of lung DC Th1 cytokine production led us to investigate whether defective DC activation occurred also systemically. To address this issue, we isolated CD11c+ DCs from the spleens of mice exposed for 4–8 wk to cigarette smoke and controls. As observed with lung DCs, activation of CS mice splenic DCs by LPS resulted in significantly less generation of IL-12p40 than controls (Fig. 6F). Identical data were obtained when splenic DCs were activated with agonistic CD40 Abs (data not shown). These responses imply that smoking affects systemic DC function. It is unlikely that nicotine is responsible for the suppressive effect on IL-12 generation by either lung or splenic DCs, because the concentrations of nicotine generated in these mice is considerably lower than that required to suppress IL-12 generation in vitro.

To directly determine the role of cigarette smoke-induced oxidative stress on DC IL-12 production in vivo, mice were injected daily i.p. with 150 mg/kg per day NAC dissolved in PBS throughout the duration of the cigarette smoke exposure (NAC dose selected based on published literature (38–40). As controls, age-matched mice received an equivalent volume of PBS i.p. throughout the cigarette smoke-exposure period. Following 4 wk of exposure to cigarette smoke and either NAC or PBS treatment, mice were sacrificed, spleens were removed, and CD11c+ DCs were isolated and matured overnight in 100 ng/ml LPS. As expected, DCs extracted from mice chronically exposed to cigarette smoke and treated with PBS demonstrated diminished IL-12p40
production compared with control mice (Fig. 6G, 4196 ± 52.8 vs 2189 ± 138.9 pg/ml). In accordance with the in vitro studies performed on human DCs, treatment of cigarette smoke-exposed mice with NAC resulted in almost complete reversal of the inhibitory effect of smoking on DC IL-12p40 production by maturing systemic DCs (Fig. 6G). DCs from cigarette smoke-exposed mice treated with PBS produced 2189 ± 138 pg/ml IL-12p40 compared with 4196 ± 52 pg/ml from control DCs from mice treated with PBS ($p < 0.001$, $t$ test comparison of means). In contrast, DCs from NAC-treated mice exposed to cigarette smoke produced 3817 ± 60 pg/ml IL-12p40 compared with 3801 ± 65 pg/ml by control DCs treated with NAC only ($p = 0.77$, $t$ test comparison).

**Discussion**

We recently reported that CSE causes specific defects in DC function, suppresses DC-mediated priming of T cells, inhibits the production of IL-12 by maturing DCs, and favors development of Th2 responses in vitro (25). In the current study, we demonstrate that oxidative stress induced by soluble cigarette smoke components potently inhibits the production of IL-12 and IL-23 by maturing DCs. This inhibition occurs through activation of ERK-dependent

**FIGURE 6.** CS suppresses p40 protein generation by maturing lung and systemic DCs. A, Lung DCs were isolated from murine lungs of control mice and CS mice (CS-mice) following collageanse digestion of whole lungs, selected using CD11c magnetic beads, and cultured overnight in the presence or absence of LPS (100 ng/ml). IL-12p40 was measured in the supernatants by ELISA. The figures represent one of four identical experiments ($n = 4$ mice in each group). B, Lung DCs were similarly isolated from control and CS mice and matured overnight with agonistic CD40 Ab (1 µg/ml) in the presence of 50 ng/ml IFN-γ. IL-12p40 was measured by ELISA. The figures represent one of two identical experiments ($n = 4$ mice in each group). C, Lung DCs isolated from control and cigarette smoke (Cig. Sm.)-exposed mice were stimulated with 100 ng/ml LPS for 6 h, RNA was extracted, and RT-PCR was performed using commercially available murine p35 primers as described in Materials and Methods. An anticipated 354-bp amplicon was amplified as demonstrated. D, Lung DCs isolated from CS mice and controls were matured overnight with 100 ng/ml LPS or left immature and IL-12p70 levels were determined using ELISA ($n = 4$ mice in each group). E, Lung DCs extracted from CS mice and controls were cultured overnight in the presence or absence of LPS (100 ng/ml) and TNF-α was measured in the supernatants using ELISA. The figures represent one of three identical experiments ($n = 4$ mice in each group). F, Spleen DCs were isolated from control and CS mice following collageanse digestion, selected using CD11c magnetic beads, and cultured overnight in the presence or absence of LPS (100 ng/ml). IL-12p40 was measured in the supernatants by ELISA. The figures represent one of four identical experiments ($n = 4$ mice in each group). *A, B, and D–F, $p < 0.05$ using $t$ test comparing mean values from control and CS mice. ***G, $p < 0.001$ using $t$ test comparing mean values from CS mice treated with PBS vs CS mice treated with NAC. iDC, Immature DC.
pathways that lead to enhanced activity of the transcriptional p40 repressor c-fos (41). These effects can be reversed by antioxidants, suggesting a novel pharmacologic strategy to augment certain impaired immune responses in smokers.

Cigarette smoke contains an extraordinarily complex mixture of chemicals. As a result, identifying cigarette smoke constituents responsible for certain biological effects is a daunting task, leading some to favor a reductionist approach over more complex systems such as the generation of extracts from cigarette smoke. Although CSE is arguably not fully representative of "true" cigarette smoke exposure, in vivo, cells are not exposed to cigarette smoke, but rather to cigarette smoke constituents that have been solubilized into biological fluids such as the epithelial and alveolar lining fluid in the lungs. Similarly, there are concerns with the use of single chemicals like nicotine or carbon monoxide as surrogates of tobacco toxicity, since the concentrations used are often significantly higher than those attained in vivo and the reductionist approach ignores the fact that many of the chemicals present in cigarette smoke may have additive, synergistic, or potentially even opposing effects on specific cellular functions. In recognition of these caveats, we used a number of parallel and complimentary approaches to define the effect of soluble and whole cigarette smoke on DC IL-12 and 23 generation.

Cigarette smoke constituents capable of immune-modulating effects include nicotine (19–21, 23, 24), carbon monoxide (42), acrolein (43), reactive oxidant species (44), peroxynitrites (45), and possibly others. The term immunomodulatory seems more appropriate than immunosuppressive when describing the effect of possibly others. The term immunomodulatory seems more appropriate than immunosuppressive when describing the effect of cigarette smoke constituents that have been solubilized into biological fluids such as the epithelial and alveolar lining fluid in the lungs. Similarly, there are concerns with the use of single chemicals like nicotine or carbon monoxide as surrogates of tobacco toxicity, since the concentrations used are often significantly higher than those attained in vivo and the reductionist approach ignores the fact that many of the chemicals present in cigarette smoke may have additive, synergistic, or potentially even opposing effects on specific cellular functions. In recognition of these caveats, we used a number of parallel and complimentary approaches to define the effect of soluble and whole cigarette smoke on DC IL-12 and 23 generation.

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In addition to oxidative stress, other cigarette smoke constituents may be responsible for suppression of IL-12 and IL-23 generation. Although our data suggest that nicotine is not primarily responsible for suppression of IL-12 production by maturing DCs, it does not completely rule out the possibility that in some organ systems, such as the lung, local nicotine levels may be substantially higher than those described to occur in the blood of heavy smokers, and potentially might contribute to IL-12 or IL-23 suppression by DCs in the lung. However, it is unlikely that nicotine concentrations in lung tissue are log-fold orders of magnitude greater than the circulation, since the lung is an extraordinarily efficient absorptive organ. Nevertheless, it is conceivable that in the architecturally distorted emphysematous lung, nicotine may accumulate to sufficiently high local concentrations that cooperate with oxidative stressors to further suppress generation of IL-12 by maturing DCs. Carbon monoxide is another cigarette smoke constituent that was recently reported to suppress IL-12p40 generation in macrophages and to potently inhibit Th1-mediated colitis in a murine model (42). However, the restoration of IL-12 secretion by antioxidants argues against a prominent role for carbon monoxide as a suppressor of Th1 cytokine generation.

Oxidative stress is an important modulator of immune responses. For instance, oxidants augment macrophage inflammatory responses to LPS challenge by enhancing activation of NF-kB-dependent genes (47), promoting the generation of chemokines from macrophages and epithelial cells (48, 49), and have also been demonstrated to induce functional DC maturation (50). The current study suggests oxidative stress as a predominant mechanism responsible for the suppression of IL-12 and 23 generation by CSE-conditioned DCs, and our findings are parallel to the recently reported effect of oxidative stress induced by diesel exhaust particles which suppresses Th1 functions of murine DCs (51). In contrast, others have shown that in the presence of oxidants, DC responses are augmented with increased allostimulatory capacity and T cell priming as well as augmented Th- responses (secretion of IL-12) (52, 53). These seemingly contrasting observations illustrate the diverse nature of DC responses to oxidative stress, which are only partially understood. Oxidative stress may be induced in DCs by exogenous agents, but may also be induced during the course of activation of the DCs (endogenous oxidative stress). Thus, the subsequent DC phenotype that emerges may depend on the source and type of oxidative stress, the “dose” of oxidative stress, the state of maturation of the DC, and other cofactors (such as presence of Ag or Toll receptor ligand at the time of exposure to oxidative stress).

It is increasingly appreciated that MAPKs play an important role in regulating DC maturation and can profoundly influence the nature of the T cell response during Ag presentation (30, 31, 34). For instance, the generation of prominent Ag-specific Th1 responses in a model of vaccination required correspondingly robust activation of p38-dependent factors in APCs (31). In contrast, certain DC activators that promote a DC-2 phenotype achieve this by facilitating sustained induction of ERK-dependent pathways, implying that ERK regulates downstream responses that suppress the Th1 response and facilitate proallergic phenotypes (30). Furthermore, in vitro studies using specific pharmacologic inhibitors and in vivo studies using ERK-deficient mice convincingly demonstrate that immune responses to Ag in the absence of functional ERK are significantly Th1 biased, implying ERK as an important negative regulator of Th1 immunity (30). Our studies are consistent with the concept that ERK is a negative regulator of Th1 responses by inducing nuclear accumulation of c-fos. Although CSE induced phosphorylation of both p38 and ERK MARK in human DCs, the suppressive effect of CSE on IL-12 generation required ERK activation. Indeed, in the absence of functional ERK signaling, the generation of IL-12 by DCs was unaffected, even when exposed to 2% CSE, which typically causes almost complete suppression of IL-12p70 generation by maturing DCs. These observations imply that intact ERK signaling is essential for CSE to achieve its inhibitory effect on IL-12 and IL-23 generation by maturing DCs. Similar to LPS, CSE also enhanced cellular levels of phosphorylated ERK in DCs. In contrast to LPS, the increase in phosphorylated ERK was sustained rather than transient and was apparent for up to 2 h following stimulation with CSE, suggesting that sustained activation of ERK rather than rapid and transient activation is essential in regulating downstream factors that influence IL-12 protein expression. It is well established that the immediate early gene product c-Fos functions as a sensor for ERK 1/2 signal duration (54). When ERK activation is transient (such as following activation with LPS), its activity declines before nuclear c-Fos protein accumulates (54). However, when ERK signaling is sustained, c-Fos is phosphorylated by persistently active ERK (54). This phosphorylation of c-Fos results in its stabilization and primes additional phosphorylation by exposing a docking site for...
ERK (54). Our findings are consistent with the observations made by Dillon et al. (55), who reported that the paucity of IL-12 produced by DCs matured with the TLR2 ligand 2,3-bis(palmitoyloxy)-(2RS)-propyl-N-palmitoyl-(R)-Cys-(S)-Ser(S)-Lys(5)-trihydrochloride correlates with enhanced ERK activation and downstream c-Fos accumulation.

Th1 and Th17 immune pathways are important in host responses to infectious pathogens like Mycobacterium tuberculosis, K. pneumoniae, and certain enteric infections (2, 12, 56). Our studies provide potential mechanistic insight regarding the epidemiologic observations that cigarette smokers are more susceptible to develop both latent and active tuberculosis infection (14, 58, 59). IL-12 production is critical for the induction of IFN-γ-dependent host control of tuberculosis and sustained production of IL-12 is required for the maintenance of host resistance and the prevention of reactivation of latent disease (57). The current study suggests that the induction of repetitive oxidative stress on lung DCs of smokers may suppress adaptive pulmonary Th1 immune responses to mycobacterial pathogens. Through its capacity to reconstitute IL-12-secreting functions in DCs, antioxidants may provide utility as adjunctive therapy for the treatment of certain pulmonary infections in patients unable to quit smoking. The current study suggests that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria through the inhibition of IL-23 production by maturing DCs. It is now apparent that CS may promote infection by certain bacteria.


