Nicotine Strongly Activates Dendritic Cell–Mediated Adaptive Immunity: Potential Role for Progression of Atherosclerotic Lesions

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Nicotine Strongly Activates Dendritic Cell–Mediated Adaptive Immunity

Potential Role for Progression of Atherosclerotic Lesions

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Background—Antigen-presenting cells (APCs) such as monocytes and dendritic cells (DCs) stimulate T-cell proliferation and activation in the course of adaptive immunity. This cellular interaction plays a role in the growth of atherosclerotic plaques. Nicotine has been shown to increase the growth of atherosclerotic lesions. Therefore, we investigated whether nicotine can stimulate APCs and their T cell–stimulatory capacity using human monocyte–derived DCs and murine bone marrow–derived DCs as APCs.

Methods and Results—Nicotine dose-dependently (10^{-8} to 10^{-4} mol/L) induced DC expression of costimulatory molecules (ie, CD86, CD40), MHC class II, and adhesion molecules (ie, LFA-1, CD54). Moreover, nicotine induced a 7.0-fold increase in secretion of the proinflammatory T_H1 cytokine interleukin-12 by human DCs. These effects were abrogated by the nicotinic receptor antagonist α-bungarotoxin and mecamylamine, respectively. The effects of nicotine were mediated in part by the phosphorylation of the PI3 kinase downstream target Akt and the mitogen-activated kinases ERK and p38 MAPK. Nicotine-stimulated APCs had a greater capacity to stimulate T-cell proliferation and cytokine secretion, as documented by mixed lymphocyte reactions and ovalbumin-specific assays with ovalbumin-transgenic DO10.11 mice. In a murine model of atherosclerosis, nicotine significantly enhanced the recruitment of DCs to atherosclerotic lesions in vivo.

Conclusions—Nicotine activates DCs and augments their capacity to stimulate T-cell proliferation and cytokine secretion. These effects of nicotine may contribute to its influence on the progression of atherosclerotic lesions. **(*Circulation*. 2003;107:604-611.)**

Key Words: cells ■ immunity ■ atherosclerosis

Nicotine is a major component of cigarette smoke, a widely accepted risk factor for atherosclerosis. It is not known how and to what extent nicotine contributes to the adverse effects of chronic tobacco use, apart from its psychoactive actions and addictive properties. Nicotine activates nicotinic acetylcholine (nACh) receptors that belong to a family of ionotropic receptors and consist of 5 transmembrane subunits building up ion channels. The nACh receptors are widely distributed throughout the central and peripheral nervous system and are involved in signal transmission at the skeletal neuromuscular junction, in autonomic ganglia, and in the brain. In addition, nonneuronal cells such as monocytes, endothelial cells, and epithelial cells also express nACh receptors.[1–4] However, the effect of nicotine on immune cells is incompletely characterized and controversial. Some investigators have provided evidence that nicotine promotes inflammation.[5,6] By contrast, other studies indicate that nicotine may have immunosuppressive effects, although these results were achieved with relatively high doses of nicotine.[7] Moreover, nicotine was recently demonstrated to promote progression of advanced atherosclerotic plaques.[8] This may result in ischemia and infarction of the heart. Innate and adaptive immune responses play a crucial role for the growth and instability of atherosclerotic plaques.[9] Recently, dendritic cells (DCs) were detected in the arterial wall.[10,11] DCs are potent antigen-presenting cells required for the initiation of adaptive immune responses.[12] The number of DCs is increased in atherosclerotic lesions, suggesting that DCs might contribute to T-cell activation in atherosclerosis.[13] Proinflammatory factors can induce maturation of DCs corresponding to a switch from a phagocytic stage to a stage of strong T cell–stimulatory capacity. Accordingly, we examined the effect of nicotine in activation of DCs

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and on their ability to activate T-cell proliferation and cytokine secretion.

Methods

Reagents

Nicotine [1-methyl-2-(3-pyridyl)pyrrolidine] was obtained from Sigma and tested for potential endotoxin contamination by use of the Endotox assay (Sigma). In addition, to further exclude a contribution of endotoxin to our results, we performed some experiments in the presence of 5 μg/mL polymyxin B (Sigma) that binds and neutralizes lipid A, the active moiety of lipopolysaccharide (LPS). LPS from *Salmonella minnesota* Re595 as well as the nicotinic receptor antagonists α-bungarotoxin and mecamylamine were purchased from Sigma. The p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 was obtained from Alexis, the MEK inhibitor PD98059 from Biomol, and the phosphatidylinositol-3-kinase (PI3K) inhibitor LY294002 from Calbiochem. Human granulocyte-macrophage colony stimulating factor (GM-CSF; Leucomax) was from Novartis, and interleukin-4 (IL-4) was bought from R&D. Murine GM-CSF was obtained from supernatants of NIH-3T3 cells transfected with murine GM-CSF (provided by Thomas Blankenstein). FITC-dextran (40 000 kDa) and carboxyfluorescein diacetate succinimidyl ester (CFSE) were purchased from Molecular Probes. Synthetic ovalbumin (OVA) peptide representing amino acids 323 to 339 of chicken OVA was obtained from Biosynthan.

Animals

OVA-specific DO11.10 transgenic mice (BALB/c background, I-A^d) to obtain OVA-specific T lymphocytes were purchased from Charles River (Sulzbach, Germany). Murine bone marrow–derived DCs as stimulator cells were prepared from sex-matched BALB/c mice (Charles River). Annexin V, 7-aminoactinomycin D (AAD), and CD3-FITC were purchased from BD Pharmingen. CD40L (CD154) was from Beckman Coulter.

Preparation of Human Monocytes and Monocyte-Derived Human DCs

Human monocytes and DCs were prepared as previously described[14] and were stimulated with nicotine for 12 hours.

Bone Marrow–Derived Murine DCs

Bone marrow–derived DCs were prepared as previously decribed.[15]

Mixed Lymphocyte Reactions

For the mixed lymphocyte reactions (MLRs), allogeneic CD3$^+$ T lymphocytes were isolated from peripheral blood by immunomagnetic beads (Milteny Biotech). To measure their proliferation, CD3$^+$ T cells were labeled with the fluorescent dye CFSE. With each cell division, the fluorescence intensity of the cells is reduced by half.[16]

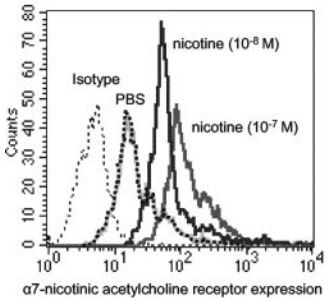

Figure 1. α_7-nACh receptor is expressed by human DCs and upregulated by nicotine. Human DCs (10^6 DCs/mL) were starved in RPMI 1640 medium + 0.5% FCS for at least 6 hours and then exposed to nicotine (10^{-8} or 10^{-7} mol/L) for 12 hours before FACS analysis. A representative FACS analysis out of 3 is shown.

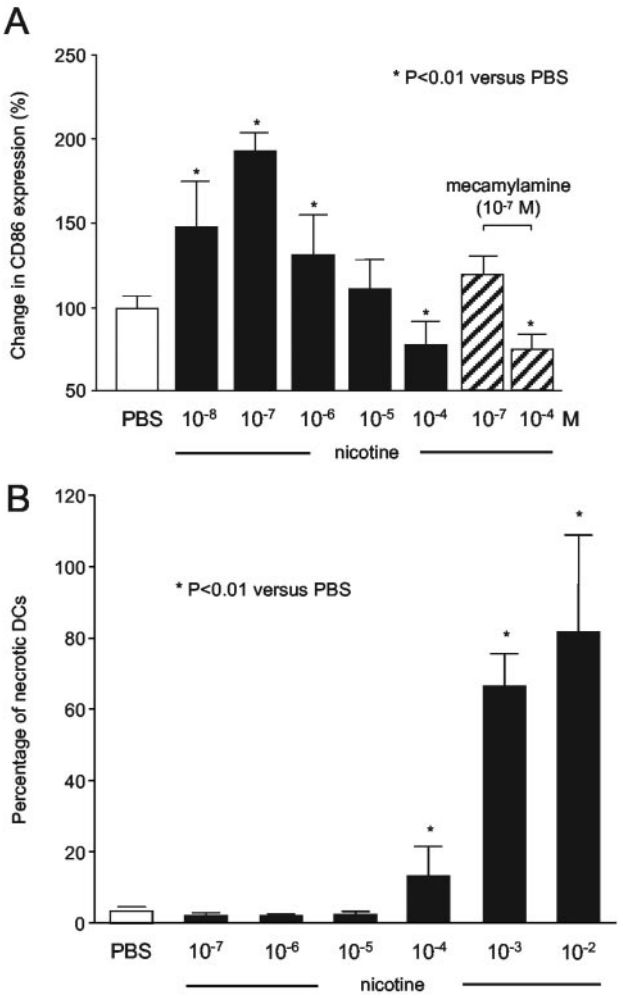

Figure 2. Nicotine induces expression of CD86 on human DCs (A). FACS analysis revealed that nicotine induced a dose-dependent increase in mean fluorescence intensity of costimulatory molecule CD86 by human DCs. Effect of nicotine was blocked by nicotinic receptor antagonist mecamylamine. Data are given as mean±SEM (n=3; $P<0.01$). Nicotine-dependent cytotoxicity (B). In presence of graded doses of nicotine, percentage of dead cells as annexin V$^+$/7-AAD$^+$ cells was determined by FACS analysis. Data are given as mean±SEM (n=4; $P<0.01$).

After thorough washing to remove unbound fluorescent dye, 200 000 CD3$^+$ T cells were mixed with human DCs and monocytes as stimulators (to ratios of 1:10 and 1:50, DCs/monocytes:T cells). Antigen-presenting cells were prestimulated with nicotine (10^{-7} mol/L) for 12 hours before use in MLRs. Experiments were performed in 96-well plates in triplicate. After 3 days of coculture of DCs or monocytes together with T cells in 200 μL RPMI containing 5% FCS, supernatants were collected, stored at −70°C, and analyzed by ELISA for production of human IL-2, a marker of T-cell activation. Cell pellets were washed with PBS and stained with mouse anti-human CD4-allophycocyanin (BD Pharmingen). Finally, costaining of CD4-allophycocyanin with CFSE labeling was determined by FACS analysis.

OVA-Specific Proliferation Assay

Lymph nodes from OVA-specific DO11.10 transgenic BALB/c (H-2^d) mice were homogenized to obtain single-cell suspensions. Lymphocytes from total lymph nodes contained more than 50% CD4$^+$ T cells and were labeled with CFSE dye. Bone marrow–derived DCs from BALB/c mice were prestimulated with nicotine

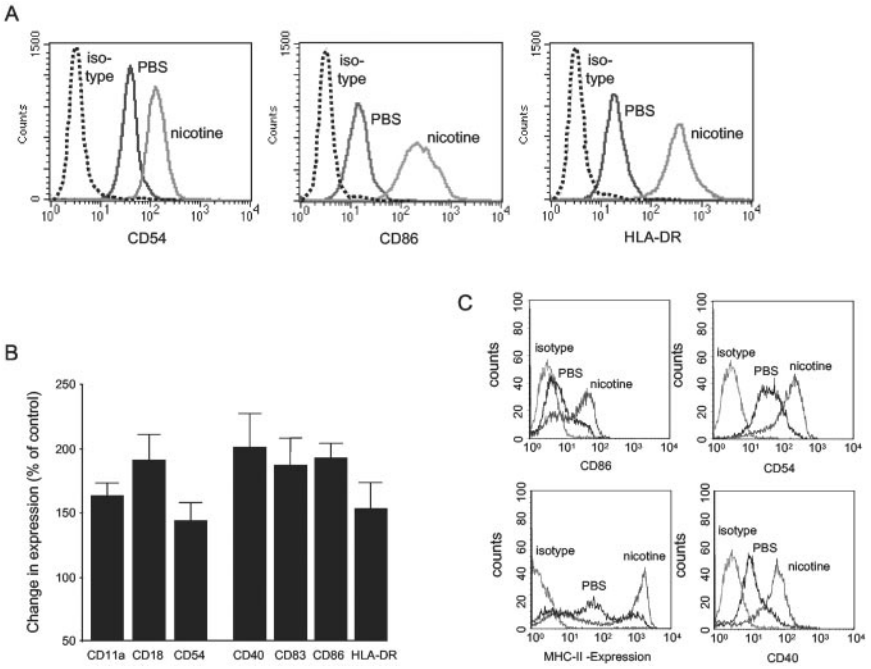

Figure 3. Nicotine enhances expression of surface molecules involved in inflammation. A, Costimulatory (CD86), adhesion (CD54), and MHC class II molecules (HLA-DR) are strongly upregulated in human DCs after stimulation with nicotine as revealed by FACS analysis. B, Histographic presentation of FACS analyses on expression of surface molecules involved in inflammation. C, Similar effects of nicotine are observed in DCs from BALB/c mice, with upregulation of CD54, CD86, CD40, and MHC class II molecules (I-A^d). This figure is representative of 3 similar experiments performed on different donors or mice.

(10^{-7} mol/L) for 12 hours. To assess their T cell–stimulatory capacity, 10^4 DCs were first loaded with 0.1 and 1 μg/mL OVA for 2 hours, washed twice with PBS, and then mixed with 10^5 $CD4^+$ DO11.10 transgenic T cells. After 3 days of coculture, supernatants were examined for murine IL-2 secretion. Cell pellets were washed, stained with CD4-allopyocyanine, and prepared for FACS analysis of CFSE fluorescence.

Flow Cytometric Measurement

Human DCs were blocked with 5% human hyperimmune globulin (Flebogamma, Grifols) for human cells and rat anti-mouse CD32 $F(ab')_2$ antibody (BD Pharmingen) at 5 μg/mL in PBS for murine cells at room temperature for 10 minutes. Then, cells were incubated with mouse anti-human CD11a-FITC, CD18-FITC, CD54-FITC, CD83-FITC, CD86-FITC, HLA-DR-FITC (all from BD Pharmingen), and CD40-FITC (Serotec). Surface expression of the nicotinic receptor was detected with mouse anti-human nicotinic receptor (Sigma) followed by goat anti-mouse $F(ab')_2$ antibody (Dako). To characterize murine DCs, rat anti-mouse CD40-FITC, CD54-FITC, CD86-FITC, and I-A^d-FITC (MHC class II) were used (all from BD Pharmingen). Finally, cells were fixed in 1% formaldehyde/PBS and analyzed by FACSCalibur (Becton Dickinson) using CellQuest software (BD Pharmingen). Cell fluorescence intensity obtained with a specific antibody was in each case compared with that obtained with isotype-matched control antibodies purchased from BD Pharmingen. All FACS data were analyzed on 10 000 cells per condition.

Endocytosis Assays

To assess endocytosis of the DC preparations, FITC-dextran was used according to a method described previously.[17] Briefly, cells stimulated with nicotine or unstimulated were incubated in complete RPMI medium with FITC-dextran at a final concentration of 1 mg/mL at 37°C for 30 minutes. Background staining at 4°C was used as reference. After the cells had been washed, FITC-dextran uptake of immature DCs was determined by FACS.

Quantification of Cytokine Production

Human IL-12p40 and human and murine IL-2 were measured by ELISA (OptEIA Set, BD Pharmingen). Human IL-10 was measured with a high-sensitivity ELISA (R&D). To detect cytokines, supernatants of 10^6 APCs/mL were used.

Western Blot Analyses

For analysis of ERK 1/2, p38 MAPK, and Akt phosphorylation, proteins were obtained in lysis buffer as previously described.[18] Protein lysates (30 μg/mL) were electrophoresed on 10% SDS-PAGE gels, transferred to PVDF membranes (Millipore), and blotted with polyclonal phospho-ERK antibodies (New England BioLabs), followed by anti-rabbit horseradish peroxidase (Santa Cruz Biotechnology) and detection by chemiluminescence ECL (Amersham). Antibodies to phospho-p38 MAPK or phospho-Akt (Santa Cruz) were used to analyze phospho-p38 MAPK and phospho-Akt expression. As loading controls, antibodies against tubulin (Labvision Corp) were used.

DC Recruitment Assay

Apolipoprotein (apo)E–knockout mice were purchased from Jackson Laboratories as retired breeders (males with C57BL/6J background). Fifty-week-old animals fed a normal chow diet were used for experiments. Bone marrow–derived murine DCs from wild-type C57BL/6J were labeled with CSFE (1 μmol/L) for 10 minutes at 37°C. After 3 washing steps, 10^6 DCs were resuspended in 200 μL RPMI containing 0.5% FCS and injected intravenously via the tail vein. Starting 3 days before the injection of the DCs, animals were treated with vehicle or nicotine (1 μg per 10 g body weight twice daily) via subcutaneous injection. Forty-eight hours after the injection of the DCs, mice were killed and perfused with 4% formaldehyde with 20 μmol/L EDTA by injection through the apex of the left ventricle. The base of the heart and the ascending aorta were isolated and mounted in TissueTek (Sakura) freezing medium. We analyzed the atheroma of the aortic root using native cryostat sections (10 μm). Serial sections spanning 1.0 mm of the ascending aorta at the level of the sinus of Valsalva were examined under light and fluorescence microscopy. The number of CSFE-labeled DCs attached to the intimal surface or in atheromatous plaques was quantified for each aorta.[19,20]

Statistical Analysis

All results for continuous variables are expressed as median±SEM intervals. Comparisons between groups were analyzed by *t* test (2-sided) or ANOVA for experiments with more than 2 subgroups. Post hoc range tests and pairwise multiple comparisons were performed with the *t* test (2-sided) with Bonferroni adjustment. Comparison of categorical variables was generated by the Pearson χ^2

test. All analyses were performed with SPSS 11.0 (SPSS Inc). Probability values of $P<0.05$ were considered statistically significant.

Results

Expression of the Nicotinic Receptor on Human Monocyte–Derived DCs

To explore the role of nicotine and its receptors in DCs, we first tested whether nicotinic receptors are expressed on monocyte-derived DCs and their precursors, the monocytes. One of the most abundant nicotinic receptors is the α_7-nicotinic receptor, whose constitutive expression was demonstrated on both DCs (Figure 1) and monocytes (data not shown). Moreover, stimulation with its ligand nicotine upregulated the expression of the α_7-nicotinic receptor in a dose-dependent manner.

Nicotine-Induced Upregulation of Proinflammatory Surface Molecules and Cytokines in Human and Murine DCs

CD86 is an essential costimulatory molecule on DCs required for efficient T-cell stimulation. Without sufficient costimulation, stimulation of T cells will induce anergy and apoptosis of T cells. Using increasing doses of nicotine (10^{-8} to 10^{-6} mol/L), we observed an increase in CD86 expression that peaked at 10^{-7} mol/L (Figure 2A; also shown in Figure 3A). Higher concentrations (10^{-4} mol/L) resulted in decreased expression of CD86 compared with control cells. This reflects the increasing toxicity of nicotine concentrations higher than 10^{-5} mol/L, as shown in Figure 2B. In contrast, lower doses of nicotine tended to reduce the number of dead cells ($P=0.07$). To confirm that upregulation of CD86 is a result of stimulation of nACh receptors (using the optimal nicotine dose of 10^{-7} mol/L), we preincubated DCs with the broad-spectrum nicotinic antagonist mecamylamine (10^{-7} mol/L). Mecamylamine abrogated the nicotine-induced upregulation of CD86 (Figure 2A).

Moreover, nicotine (10^{-7} mol/L) increased the DC expression of adhesion molecules such as the β_2-integrin LFA-1 (CD11a/CD18) and its ligand CD54 (ICAM-1) in human DCs (Figure 3, A and B). In addition, nicotine also increased the expression of the MHC class II molecule HLA-DR and induced the expression of the DC maturation marker CD83 in human DCs (Figure 3B). Nicotine had similar effects on murine DCs. In murine DCs, nicotine increased the expression of CD54, the MHC class II molecule I-A^d, and the costimulatory molecule CD40 (Figure 3C).

Nicotine induced a marked increase in the secretion of the proinflammatory cytokine IL-12 p40 by DCs as detected by ELISA (Figure 4A). These effects of nicotine were blocked by mecamylamine and the selective α_7-nACh receptor antagonist α-bungarotoxin. The proinflammatory effect of nicotine caused a compensatory upregulation of the antiinflammatory cytokine IL-10 (Figure 4B).

Nicotine Augments the Ability of DCs to Stimulate T Cells

We next determined whether nicotine could enhance the ability of DCs to stimulate T cells by use of allogeneic MLRs

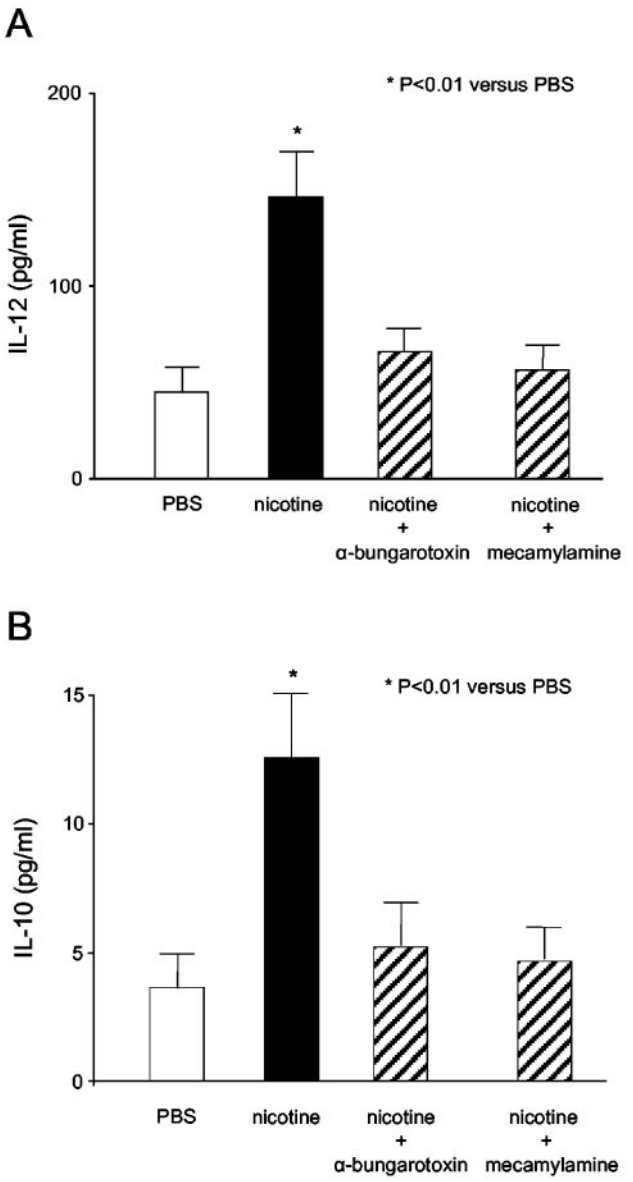

Figure 4. Nicotine-induced IL-12 p40 (A) and IL-10 (B) production in human DCs is delivered through nACh receptors. DCs were preincubated 30 minutes before nicotine stimulation (10^{-7} mol/L) with specific α_7-nACh receptor antagonist α-bungarotoxin and broad-spectrum nACh receptor antagonist mecamylamine. Both inhibitors were used at 10^{-7} mol/L. IL-12 p40 secretion in response to nicotine stimulation could be strongly inhibited in presence of α-bungarotoxin and mecamylamine. Data are mean±SEM (n=3).

(Figure 5A). Nicotine-stimulated DCs markedly increased the T-cell secretion of IL-2, consistent with T-cell activation. The effect of nicotine (10^{-7} mol/L) was similar to that of the potent activator LPS (100 ng/mL). Nicotine-stimulated monocytes also induced IL-2 production in allogeneic T lymphocytes but less efficiently than DCs (Figure 5B). To determine whether nicotine stimulation is also effective in promoting antigen-specific responses, we studied its effects on the ability of OVA-exposed murine DCs to stimulate proliferation in DO11.10 OVA-transgenic T cells (Figure 5C). Nicotine significantly enhanced the T cell–stimulatory capacity of syngeneic murine DCs presenting OVA peptide

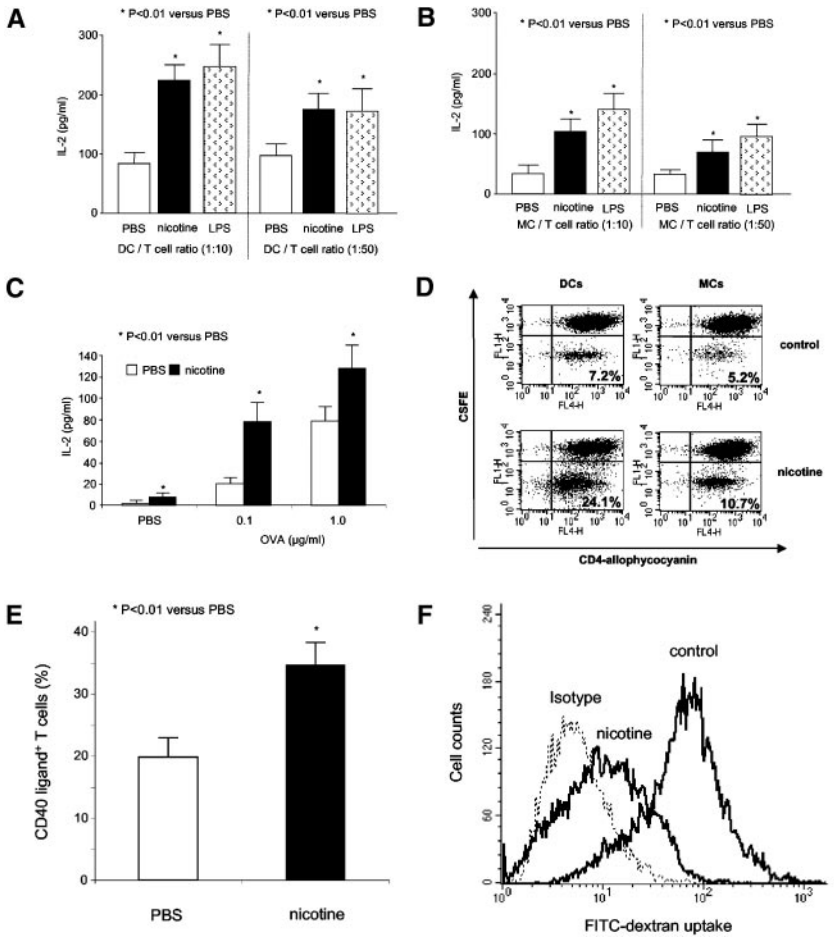

Figure 5. Functional improvement of antigen-presenting cells after stimulation with nicotine (10^{-7} mol/L). In MLRs of human DCs mixed with allogeneic T lymphocytes, nicotine-prestimulated DCs (black bars) induced T-cell activation, which was measured as secretion of T cell–specific cytokine IL-2. Similar activation was obtained by standard doses (100 ng/mL) of LPS (stippled bars) (A). Similar T cell–stimulatory effects of nicotine could be found for monocytes (MCs) along with allogeneic T cells but to a lesser extent than DCs (B). To also prove stimulating effects of nicotine for antigen-specific assays, we used OVA-peptide–loaded murine DCs in combination with OVA-transgenic T cells (C). To assess T-cell proliferation in response to allogeneic nicotine-prestimulated human DCs or monocytes, we measured loss of incorporated CSFE labeling by FACS analysis, resulting in cells with lower levels of fluorescence of CSFE. To detect T helper cells, we performed a dual staining together with allopyocyanine-labeled CD4 antibodies (D). Nicotine-stimulated DCs significantly enhanced CD40L (CD154) expression on T cells in DC–T-cell cocultures as assessed by CD3-FITC/CD40L-PE double stainings (E). Enhanced T-cell–stimulatory capacity of nicotine-stimulated human DCs is paralleled by a decrease in internalization of soluble antigens such as FITC-dextran (F). Data are given as mean±SEM (n=3) and representative FACS analyses out of 3 independent experiments are chosen.

323 to 339 to OVA-transgenic T lymphocytes. Compared with vehicle-treated cells, nicotine-treated human DCs increased the proliferation of allogeneic $CD4^+$ T cells by more than 3-fold; a similar effect was observed in nicotine-treated monocytes (2.2-fold induction) (Figure 5D). Similarly, nicotine stimulation led to a 2.3-fold increase in proliferation of syngeneic OVA-specific DO11.10 transgenic T cells. Similarly, nicotine stimulation led to a 2.3-fold increase in proliferation of syngeneic OVA-specific DO11.10 transgenic T cells. In addition, nicotine-stimulated DCs significantly increased expression of CD40L (CD154) on T cells in DC–T-cell cocultures (Figure 5E). Acquisition of T-cell–stimulatory capacity in maturing DCs is inversely correlated to their capacity for endocytosis of antigens such as FITC-dextran. Consistent with its effect to activate DCs, nicotine reduced their capacity for endocytosis (Figure 5F).

Nicotine Stimulation Activates MAPK and Akt Pathways in Human DCs

To gain insight into nicotine-induced signaling, we determined whether MAPKs and Akt might be involved in nicotine-induced signaling pathways in human DCs. Nicotine rapidly increased the phosphorylation of p38 MAPK, an effect that was visible within 5 minutes (Figure 6A). Maximal activation of p38 MAPK was achieved by 15 minutes (547±86% increase), declining after 30 minutes. Phosphorylation of the MAPK ERK reached a maximal induction (332±34% increase) after 30 minutes of nicotine stimulation (Figure 6B). Activation of the Akt pathway, however, peaked relatively late, at 60 minutes, with a 418±78% increase (Figure 6C).

Role for MAPK and Akt in the Nicotine-Induced Upregulation of the Costimulatory Molecule CD86 in Human DCs

Having defined signaling pathways that are activated in nicotine-stimulated human DCs, we examined the role of

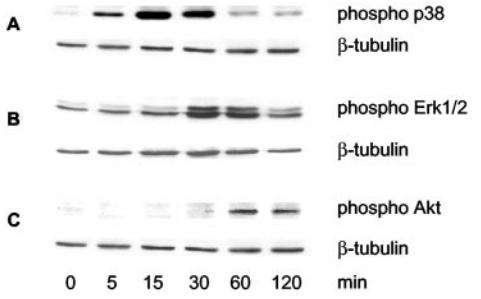

Figure 6. Nicotine (10^{-7} mol/L) activates MAPK and Akt pathways in human DCs. Phosphorylation of p38 MAPK (A), ERK (B), and Akt (C) is shown in representative Western blots (n=5). Tubulin is used as loading control.

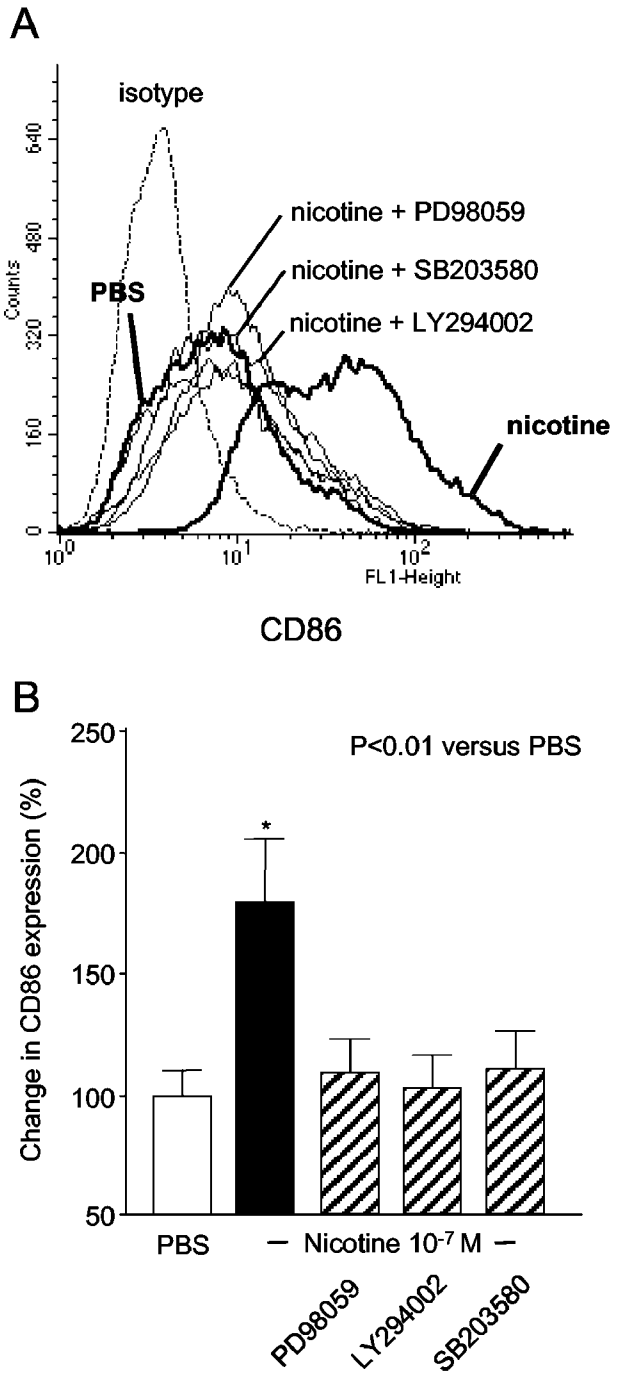

Figure 7. Inhibitors of MAPK and PI3K abrogate effect of nicotine on CD86 expression. A, Human DCs were pretreated with vehicle or p38 MAPK inhibitor SB203580 (10 μmol/L), MEK inhibitor PD98059 (50 μmol/L), and PI3K inhibitor LY294002 (10 μmol/L) 30 minutes before stimulation with nicotine (10^{-7} mol/L). Representative FACS analyses out of 3 are shown, and data are presented as mean±SEM. B, Histographic presentation of FACS data.

these signaling pathways in nicotine-induced expression of the costimulatory molecule CD86. The MAP kinase (MEK) inhibitor PD98059 and the inhibitor of p38 MAPK SB203580 each abolished the nicotine-induced increase in CD86 expression (Figure 7). Likewise, nicotine-induced CD86 upregulation was completely dependent on activation of PI3K/Akt pathways, because it was abrogated by the PI3K inhibitor LY294002 (Figure 7).

DC Homing to Atherosclerotic Lesions

To investigate whether nicotine affects DCs in vivo, DCs were labeled with CSFE and injected into apoE-deficient mice. Atherosclerotic plaques above the aortic valve at the level of the sinus of Valsalva are the most advanced lesions in apoE-deficient mice. Sections of the aortic root were analyzed with fluorescent microscopy for CSFE-labeled DCs that were adherent to or within the atherosclerotic plaques. The majority of the labeled DCs were detected in regions inside the plaque, suggesting transmigration of the labeled DCs across the endothelial cell layer (Figure 8, A and B). Forty-eight hours after intravenous injection, DC homing to atherosclerotic lesions was significantly enhanced by nicotine. Adjusting for differences in plaque area, we found significantly more DCs in the nicotine group (34.2±10.9 DCs/lesion) than the control group (11.0±5.1; $P<0.001$). In contrast, atherosclerotic lesions in apoE-deficient mice without DC injection showed only background fluorescence (data not shown).

Discussion

The present study suggests an important role for nicotine in DC activation. This was evidenced by the upregulation of nACh receptors, costimulatory molecules such as CD86 and CD40, MHC class molecules such as HLA-DR, and adhesion molecules such as CD54. Moreover, nicotine enhances the ability of DCs and monocytes to stimulate T-cell proliferation and cytokine production in allogeneic MLRs and antigen-specific assays. This indicates that nicotine enhances adaptive immunity and pathophysiological processes that involve adaptive immunity, such as atherosclerosis. Indeed, we demonstrate that nicotine significantly enhanced DC homing to atherosclerotic lesions.

DCs act as sentinels, which are highly specialized in sampling antigens from sites of inflammation.[12,21] In atherosclerosis, possible antigens relevant for the initiation and progression of atherosclerotic lesions might be oxidized LDL, β_2-glycoprotein I, and heat-shock proteins.[22] These antigens are actually self-antigens, modified by oxidation in the case of LDL. Prolonged presentation of self-antigens by DCs is crucial for the development of destructive autoimmune responses.[23] In autoimmune disease, antigen-specific activation of autoimmune T cells is based on the interactions of T-cell receptors with self-antigens presented by MHC molecules on DCs. The capacity of DCs to stimulate T cells depends on their stage of maturation: after DCs picked up antigens at their immature phagocytic stage, DCs move to the regional lymph nodes while undergoing maturation.[12] Maturation boosts their capacity to efficiently present antigens to T cells and is mediated by upregulation of costimulatory molecules such as CD86 (B7.2) and CD40. Costimulation by the ligands CD80/86 and its receptor CD28 on T cells is required for efficient T-cell stimulation.[24] Lack of costimulation leads to T-cell anergy and apoptosis. Moreover, MHC class II molecules are upregulated and the possibility of DC–T-cell encounters is favored by enhanced expression of adhesion

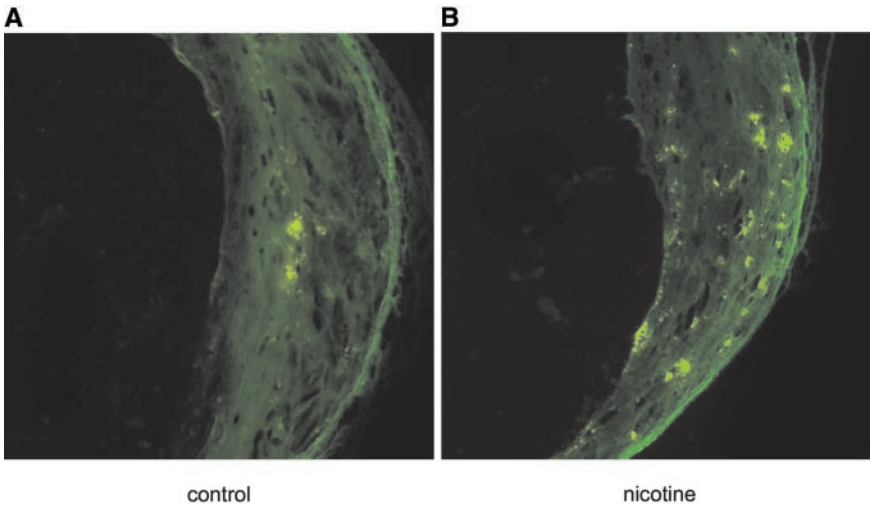

Figure 8. Murine model of atherosclerosis: cross sections of aortic root of mice treated with vehicle (A) or nicotine (B). Homing of CSFE-labeled DCs was significantly enhanced by nicotine treatment (subcutaneous injection of 1 μg nicotine per 10 g body weight twice daily). Representative pictures are shown (n=3).

molecules. In parallel, activated DCs secrete cytokines and chemokines to recruit more immune cells to the site of inflammation. Thus, T cells present in atherosclerotic lesions may be nonspecifically activated T cells or those that have been expanded within lymph nodes in recognition of the presented antigen.

Nicotine at physiologically relevant concentrations promotes progression of advanced atherosclerotic lesions, an effect that was inhibited in part by the specific COX-2 inhibitor rofecoxib.[8] Rofecoxib has powerful antiinflammatory properties that, together with its antiangiogenic effect, may have contributed to the inhibition of lesion progression. In our experiments, nicotine had a maximal immunostimulatory effect at concentrations between 10^{-8} and 10^{-7} mol/L, which can be found in the plasma of smokers.[25] Because atherogenesis is known to rely on both innate and adaptive immune responses, it is tempting to speculate that nicotine-enhanced activation of DCs might contribute to T-cell recruitment and acceleration of inflammation in the atherosclerotic lesions. Indeed, soluble factors such as IL-12 released by activated DCs are well established to contribute to the formation of atherosclerotic lesions and plaque destabilization.[26] In addition, nicotine stimulated DC expression of CD40. Ligation of CD40 can induce IL-12 production in DCs.[14] Moreover, genetic disruption of the CD40/CD40 ligand system has been described to prevent the initiation and progression of atherosclerosis.[27]

Antagonists of the α_7-nicotinic receptors blocked the effects of nicotine on DC maturation. The nACh receptors are ligand-gated ionotropic channels consisting of α-subunits or a combination of α- and β-subunits, each of which has a unique pharmacological profile.[28] Intriguingly, we observed a marked expression of the homopentamer α_7-nACh receptor in DCs. The α_7-nACh receptor appears to play a crucial role in the observed effects of nicotine on DC maturation. The specific α_7-nACh receptor antagonist α-bungarotoxin abrogated the stimulatory effects of nicotine, an effect similar to what we observed for the nonselective nACh receptor antagonist mecamylamine.

The downstream effector pathways by which nicotine activated DCs appear to rely on the activation of several kinases. Here, we demonstrated activation of ERK1/2, p38 MAPK, and Akt in DCs by nicotine. Activation of ERK 1/2 and Akt is consistent with the effect of nicotine in other cell types, which is mediated via the α_7-nACh receptor.[29–31] However, p38 MAPK has not yet been involved in activation by nicotine.[32] Notably, the p38 MAPK pathway is involved in the LPS-induced upregulation of costimulatory molecules via stimulation of transcription factors such as nuclear factor-κB.[33]

Finally, nicotine profoundly enhanced homing of DCs to atherosclerotic lesions in vivo, which supports the hypothesis that the proatherogenic effect of nicotine may be mediated in part by recruiting DCs to sites of chronic inflammation such as atherosclerotic plaques. DCs clustering with T cells have been identified in atherosclerotic lesions of apoE-deficient mice and form clusters with T cells.[34]

Because tobacco smoke consists of more than 4000 chemical constituents, it is impossible to predict the effect of nicotine within this complex mixture of components. Although we show that nicotine has a potent immunostimulatory effect, there are several molecules in cigarette smoke that may be toxic to DCs (eg, cadmium, reactive oxygen species; see Powell[35] for review). The net effect of cigarette smoke on endothelial function may be quite different from that of nicotine alone. In addition, our results are in contrast to several studies showing a decrease in immune responses in the presence of nicotine.[7] However, these studies used much higher doses of nicotine that, in our experiments, showed cytotoxic effects, which cannot be reversed by nACh receptor antagonists (Figure 2).

In conclusion, the present study indicates that nicotine enhances adaptive immunity, an effect that is mediated by nicotinic ACh receptors on DCs and monocytes. Nicotine activates these antigen-presenting cells, inducing the expression of surface molecules involved in inflammation and augmenting T-cell proliferation and cytokine secretion. These effects of nicotine together with the enhanced recruitment of DCs to atherosclerotic lesions may contribute to pathophysiological processes involved in the growth and destabilization of atherosclerotic plaques.

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