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Impact of Tobacco Smoke on Upper Airway Dendritic Cell Accumulation and Regulation by Sinonasal Epithelial Cells

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Abstract

Background—These studies examined the impact of environmental tobacco smoke (ETS) and active smoking on sinonasal dendritic cell (DCs) subsets in controls or patients with chronic rhinosinusitis with nasal polyps (CRSwNP). In subsequent *in vitro* investigations, we examined the influence of cigarette smoke extract (CSE) on human sinonasal epithelial cells' (HSNECs) ability to regulate DC functions.

Methods—Sinonasal tissue, blood and hair were collected from patients undergoing sinus surgery. Smoking status and ETS exposure were determined by hair nicotine. DC subsets were examined by flow cytometric analysis. Monocyte-derived dendritic cells (moDCs) were treated with conditioned medium from non-smoked exposed HSNEC (NS-HSNEC) or cigarette smoke extract-exposed HSNEC (CSE-HSNEC) to assess the impact of CSE exposure on HSNEC regulation of moDCs functions.

Results—Control subjects who were active smokers displayed increased sinonasal moDC and mDC1 and reduced mDC2 cells, while in those with CRSwNP only moDC and mDC2 were altered. ETS was found to increase only moDCs in patients with CRSwNP. *In vitro*, CSE stimulated HSNEC secretion of the moDC regulatory products CCL20, PGE2 and GM-CSF. CSE-exposure also promoted HSNECs to stimulate monocyte and moDC migration. MoDCs treated with CSE-HSNEC media stimulated an increase in antigen uptake and expression of CD80 and

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CD86. Lastly, CSE-HSNEC treated moDCs secreted increased levels of IL-10, IFN- γ and TSLP compared

Conclusions—Active smoking and to a lesser degree ETS, alters the sinonasal composition of DCs. A potential mechanism to account for this is that cigarette smoke stimulates HSNEC to induce moDC migration, maturation and activation.

Keywords

dendritic cell; environmental tobacco smoke; epithelial cell; sinusitis; tobacco smoke

Introduction

Chronic rhinosinusitis (CRS) affects up to 16% of the United States population and has few proven treatments (1). CRS with nasal polyps (CRSwNP) is the most difficult form of the disease to treat, and has a negative impact on quality of life that exceeds other chronic conditions, such as heart failure, angina and chronic obstructive pulmonary disease (2). One factor that has been identified as a potential cause and exacerbator of CRSwNP, as well as numerous chronic inflammatory diseases, is active smoking or exposure to environmental tobacco smoke (ETS). Studies have shown that perennial allergic rhinitis and tobacco use are associated with an increased risk of developing nasal polyposis (3). In addition cigarette smoke (CS) exposure decreases respiratory cell ciliogenesis which could contribute to decreased mucociliary clearance (4). ETS has also been shown to induce nasal irritation and congestion in children as well as increase nasal obstruction (5, 6). Although smoking rates have declined, 16.8% of the United States population continues to smoke, while another 25% are still exposed to ETS (7, 8). In patients with CRSwNP the ETS exposure rate is 10% while 8% are active smokers; a rate that has remained unchanged over the last seven years (9).

One of the first cell types to encounter tobacco smoke is airway epithelial cells. Airway epithelial cells can modulate the functions of a large number of cell types. One cell population particularly susceptible to regulation by airway epithelial cells are dendritic cells (DCs). DCs play a key role in a number of human airway diseases, including chronic obstructive pulmonary disease, asthma and CRS. Naïve, immature DCs are dispersed throughout the epithelial and sub-epithelial space and mature upon exposure to exogenous pathogens and by DC regulatory factors found in the local tissue microenvironment. In humans, there are three main subsets: myeloid DCs (mDC), plasmacytoid DCs (pDC) and monocyte-derived DCs (moDCs). mDCs have been shown in asthma models to induce airway hyper-responsiveness, eosinophilia, and Th2 cytokine production (10–12). pDC are of lymphoid lineage and produce high levels of interferon- α (IFN- α) and are most well known for their capacity to promote anti-viral immunity. When compared to mDC, pDC exhibit reduced immune stimulatory capacity and as such, they favor the generation of T-regulatory cells. Monocyte-derived DCs (moDCs) are found in low levels in steady state conditions, but are upregulated in the presence of inflammation. moDCs are highly susceptible to the influence of cytokine, chemokines and growth factors found in local tissue environment which strongly influences their initiation of T-cell responses (13, 14). In animal

models of asthma, and in humans with CRS, mDCs have been shown to play a critical role in driving Th2 inflammation (15–17).

Given that tobacco smoke is a significant exacerbator of CRSwNP (18), here we investigated the impact ETS exposure or active smoking on sinonasal DC populations both in CRSwNP and control sinonasal tissue. Given that airway epithelial cells have been shown to be potent regulators of DC functions, we also investigated the impact of cigarette smoke extract (CSE) on human sinonasal epithelial cell (HSNEC) regulation of monocyte-derived DC functions.

Methods

Patients & sample procurement

IRB approval was granted prior to initiation of the study (HR# 20077) and informed written consent was obtained from all participants. Blood, hair and sinus tissue were taken at the time of endoscopic sinus surgery. Patients were defined as having CRSwNP as described by the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (1). Patients with allergic fungal rhinosinusitis (AFRS) as defined by the Bent and Kuhn criteria (2) were included within the CRSwNP cohort. For controls, tissue was collected from subjects who were undergoing surgery for repair of cerebrospinal fluid leak or removal of non-hormone secreting pituitary tumor. Exclusion criteria include patients use of antibiotics, oral steroids or immunomodulatory agents within the preceding 30 days, aspirin exacerbated respiratory disease, other immunologic, renal, gastrointestinal, endocrine or skeletal disorders or pregnancy.. Per standard of care patients did utilize intranasal steroids prior to surgery. Self-reported smoking status was obtained at time of surgery and recorded in the pre-operative medical records. Hair samples were analyzed for nicotine previously described (19). Sinonasal tissue was collected, processed and stored as previously described (19–21). Blood from control patients was processed as previously described to obtain peripheral blood mononuclear cells (PBMCs) which were cryogenically preserved for later use (17).

Immunostaining and flow cytometric analysis for sinonasal DCs

Prior to staining, Single cell suspensions of sinonasal tissue were treated with FACS buffers containing 10% bovine serum albumin (BSA) plus 1% FcBlock (eBiosciences, San Diego, CA). MoDC were identified based on concomitant cell surface expression of HLA-DR with CD209 (DC-SIGN) and CD14 (17, 22, 23). pDCs were defined by positive staining for HLA-DR, CD123 and BDCA-4. Myeloid DCs were identified as mDC1 cells by expression of HLA-DR, CD11c and BDCA-1 or as mDC2 based on positive staining for HLA-DR, CD11c and BDCA-3 (24, 25). All flow cytometry antibodies were purchased from BD Biosciences (San Jose, CA) except anti-HLA-DR, which was purchased from R&D Systems (Minneapolis, MD). Matched isotype controls were used for each stain. Eight-parameter flow cytometric analysis was performed using a Guava 8HT flow cytometer. 7AAD positive cells (dead cells) were excluded from analysis.

Primary HSNEC culture and treatments

Control HSNEC cell cultures were established as previously described (19, 26, 27) from 16 individuals who reported to have never smoked and were confirmed by hair nicotine. Given

our prior studies that have identified that CRSwNP HSNEC are hyper responsive to inhaled stimuli (26), in these studies we focused solely on control subject-derived HSNEC. CSE was generated as previously described (19) and used at concentration of 5 or 10%. Subconfluent, epithelial cells were treated in 6-well dishes with vehicle control (BEGM) or CSE for 24 hours. After this time, cells were washed to remove excess CSE, fresh media added and cells were incubated for an additional 24 hours. This was done to collect HSNEC-conditioned media devoid of CSE so that we could examine the impact of only HSNEC-secreted products on DC functions. The two types of conditioned media used were defined as those from non-smoked HSNEC (NS-HNEC) or cigarette smoke extract-treated HSNEC (CSE-HSNEC). HSNEC conditioned media were collected and stored at -80°C until use.

DC procurement, differentiation and treatments

Monocytes from control patients were differentiated into moDCs as previously described (26). For controls, moDCs were treated with either their own culture medium (RPMI with no FBS) or HSNEC medium (BEGM). For experiments in which secretion of DC mediators was examined, moDCs were washed at the end of the three-day treatment period then cultured in the presence of fresh media for an additional 24 hours. This was done so that we could examine secretion of moDC mediators, induced by HSNEC treatment, but was devoid of any factor that would have been produced by HSNECs.

Assessment of moDC functions

Monocyte and moDCs were serum-starved (0.5% FBS) for 12 hours prior to the migration. Monocytes and moDC (5×10^4) were placed in 100 μL of media in the top half of a Transwell 96-well insert with 5.0 μm pore. The bottom chamber contained a 50:50 mix of phenol-free RPMI and BEGM or HSNEC-conditioned media. Migration was quantified by cell counting using a Guava 8HT flow cytometer set to record all events for 60 seconds. Non-specific migration to RPMI (containing no FBS) was subtracted from each patient's migrating fraction, then examined as the percent change compared to migration to BEGM.

Antigen uptake by moDC was assayed by uptake of FITC-conjugated dextran using previously described methods (26). Immunostaining and flow cytometric analysis was used to examine moDC co-stimulatory molecules CD80 and CD86 expression. Samples were assayed immediately following staining as described above and previously (26). IL-10, IL-12, IFN- γ and TSLP were conducted by ELISA.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 6.0 software (La Jolla, CA). A D'Agostino & Pearson omnibus test will be used to determine if data sets are normally distributed. An ordinary one-way ANOVA with post-hoc Tukey's multiple comparisons test was used to determine statistical significance in experiments with three or more experimental groups, except for CSE dose response analysis, in which post-hoc Dunnett's multiple comparison test was used as all values were compared to only control dosing. For migration experiments a paired t-test was used to determine statistical significance between migration of cells to NS-HSNEC and CSE-HSNEC conditioned media.

Results

ETS and active smoking alters sinonasal DC subsets

In these studies, we sought to address the impact of active smoking and exposure to ETS, on the sinonasal DC phenotypes. No difference in patient demographics was observed among the various groups and is shown in Table I. Initial comparisons between smoke naïve individuals demonstrated that CRSwNP patients has an increased percent of sinonasal moDCs ($p<0.0001$), mDC1s ($p<0.05$), mDC2s ($p<0.05$), as compared to control patients (Fig 1). Conversely, there were no differences in sinonasal pDCs between controls and patients with CRSwNP.

Next, we examined the impact of ETS or active smoking exposure on each of the sinonasal DC subsets. In controls, active smoking, but not ETS, was associated with an increased percentage of moDC, compared to smoke naïve control patients (Fig 2A). However, in patients with CRSwNP, both ETS exposure and active smoking was associated with a statistically significant increase in moDC as compared to smoke naïve patients with CRSwNP (Fig 2B). In controls, active smoking was associated with increase in the percent of sinonasal mDC1 cells (Fig 2C), while neither active smoking nor ETS exposure altered the percent of sinonasal mDC1 cells in patients with CRSwNP (Fig 2D). Active smoking was associated with decreased sinonasal mDC2 cells in both controls (Fig 2E) and patients with CRSwNP (Fig 2F). Lastly, sinonasal pDC cell percentages did not change significantly with ETS or active smoking in controls or patients with CRSwNP (Fig 2G & H, respectively). In comparisons of the impact of active smoking versus ETS exposure, in control subjects active smoking increased the percent of moDC and mDC1 present (Fig 2A and 2C, respectively). Taken together these results demonstrate that active smoking, and to a lesser degree ETS, alters the sinonasal balance of moDCs and both myeloid DC subsets, but does not alter pDCs.

Human sinonasal epithelial cells that are exposed to smoke (CSE) increase secretion of DC regulatory products

To determine optimal dosing of CSE, control HSNECs were treated for 24 hours with 5 or 10% CSE. After this time, HSENCs were washed; new media was added and allowed to incubate for an additional 24 hours. Secretion of three moDC regulatory products was examined and included Chemokine (C-C motif) ligand 20 (CCL20), Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Prostaglandin E2 (PGE2). CSE increased CCL20 secretion with 10% CSE treatment compared to control (Fig 3A). Likewise, GM-CSF was significantly elevated by treatment with 10% CSE, but not 5% CSE (Fig 3B). Lastly, HSNEC CSE treatment induced increases in PGE2 secretion at both doses (Fig 3C). These results demonstrate that CSE can induce HSNECs to produce a broad spectrum of moDC regulatory products. Furthermore, based on these data, 10% CSE treatment of HSNEC was used in our subsequent investigations.

Monocytes and moDCs are both recruited by HSNEC exposed to CSE

Next we examined the functional impact of CSE upon HSNEC recruitment of both monocytes and moDCs. Compared to media alone, NS-HSNEC induced an 8.5% increase in

monocyte migration (Fig 4A) and 8.6% increase in moDC migration (Fig 4B). Treatment of HSNEC with CSE significantly increased monocyte migration to 38% ($p<0.05$ vs NS-HSNEC). Likewise, moDC migration to CSE-HSNEC conditioned media was also increased to nearly 28% as compared to 8.6% migration to NS-HSNEC ($p<0.05$). Together these data demonstrate that exposure of HSNEC to CSE increases their ability to induce migration of both monocytes and moDCs.

CSE-exposed HSNECs stimulate increased moDC antigen uptake and co-stimulatory molecule expression

Given the importance of antigen uptake in driving DC maturation, we next examined the impact of CSE on HSNEC regulation of moDCs uptake of dextran-FITC. Compared to DCs cultured in media alone, those treated with conditioned media from NS-HSNEC displayed no increase in dextran-FITC uptake (Fig 5A). However, treatment of moDCs with CSE-HSNEC more than doubled antigen uptake and was significantly higher than media alone ($p<0.01$) or NS-HSNEC treated moDCs ($p<0.01$).

Next, we assessed expression of the DC co-stimulatory molecules CD80 and CD86. Expression of CD80 did not change with NS-HSNEC treatment as compared to media control (Fig 5B). Treatment of moDCs with CSE-HSNEC results in a doubling of the expression of CD80 compared to control ($p<0.01$) or NS-HSNEC ($p<0.01$). In addition, CD86 expression was unaffected by NS-HSNEC treatment (Fig 5C), expression was elevated significantly by CSE-HSNEC treatment ($p<0.01$ vs control or NS-HSNEC treatment).

moDCs have altered cytokine secretion following CSE-HSNEC treatment

Lastly, we examined the impact of CSE-HSNEC on the secretion of DC-derived immune modulatory products. Compared to control treated DCs, those treated with NS-HSNEC did not display any significant changes in the secretion of IL-10, IL-12 (p70) or IFN- γ (Fig 6A, B, D). However, NS-HSNEC did induce a significant increase in moDC secretion of TSLP compared to media alone (Fig. 6D). The most robust secretion of cytokines by moDCs followed treatment, with CSE-HSNEC. As shown in Figs. 6A & B, both IL-10 and IFN- γ level, were nearly doubled with CSE-HSNEC treatment and were significantly elevated compared to control ($p<0.05$) or NS-HSNEC treatments ($p<0.05$). moDC thymic stromal lymphopoietin (TSLP) secretion was also modestly increased above control levels with NS-HSNEC ($p<0.05$) with the highest expression seen in CSE-HSNEC treated ($p<0.05$, vs control or NS-HSNEC treatment respectively). The only cytokine examined in which NS-HSNEC or CSE-HSNEC treatment has no impact was IL-12 (p70) (Fig 6D). Collectively, these data demonstrate that HSNEC smoke exposure promotes moDC maturation, migration and cytokine production in a contact-independent manner.

Discussion

The immune response in CRSwNP is a complex interplay between environmental exposures and an aberrant host response of which DCs are hypothesized to play a critical role. What remains unclear is how environmental exposures, including ETS and active smoking, alter

the balance in sinonasal DCs phenotypes and their functions. Similar to our previous studies of moDCs in circulation, here we noted an increase in the moDCs in the sinonasal mucosa of patients with CRSwNP (17). We also demonstrate that compared to control subjects, patients with CRSwNP have increased populations of sinonasal myeloid DC subsets. Our results were comparable to those of Poposki et al and Pezato et al, both of which observed an increase in the percent myeloid and mDC1 and mDC2 cells in the mucosa of CRS patients as compared to normal controls (28, 29). However, these results differ from Lee et al., which observed an decrease presence myeloid DCs in patients with CRSwNP (30). We similarly did not observe any significant differences in the percent of sinonasal pDCs, though there was a trend towards fewer pDCs in the sinonasal tissue of patients with CRSwNP. This trend is comparable to that described by Pezato et al, but counters the increase in pDCs presented by Poposki and colleagues. Differences between our study and those of Lee and colleagues may be due in part to their inclusion of a significant number of patients taking oral steroids. In our study, patients were excluded if they had taken oral steroids as steroids have been shown to block monocyte to DC differentiation as well as DC maturation and migration (31). Differences in the methods by which the tissue were processed as well as the markers used to define pDCs, may also account for the varying outcomes between studies.

In control subjects, active smoking was associated with an increased percentage of moDCs and mDC1s while the percent of mDC2s was decreased. ETS had no impact on control DC subsets. Patients with CRSwNP appeared to be more sensitive to the effects of smoke exposure, as ETS increased moDC sinonasal populations. Human studies examining the impact of acute smoke exposure on DC populations in bronchoalveolar lavage fluid also found an increase in myeloid DCs after exposure to tobacco smoke (32). Murine studies though have suggested that both acute and chronic tobacco smoke exposure are associated with an increase in myeloid DCs (33, 34). Studies that examined total DCs instead of specific DC subpopulations (as reported in the current manuscript), have presented opposing data. For example, in an analysis of total DCs using CD83 expression to identify DCs, smoking was associated with a decrease in DCs in current smokers. Moreover, following smoking cessation total DC levels were comparable to non-smokers (35). Likewise, Liao et al., demonstrated that in the small airway there was a modest decrease in the presence of CD83⁺ DCs in active smokers as compared to non-smokers (36). Given our work demonstrating that smoke had opposing effects on each of the myeloid DC subsets, total myeloid DC counts may not give an accurate representation of tissue populations.

Following the observation of elevated moDCs in the sinonasal mucosa of CRSwNP patients with ETS exposure and active smokers, we next shifted our focus to the role of HSNEC in regulating moDC functions. As a number of previous reports have looked at the direct effects of tobacco smoke or smoke components on DCs [reviewed in (37)], we designed our studies to assess the effects of CSE on HSNEC regulation of DC functions. While DCs can interact directly with smoke and antigens through pseudopodia extended between epithelial cells, most moDCs have been shown to reside in the subepithelium (38) and as such would have limited direct encounters with inhaled stimuli. However, because we did not utilize air liquid interface cultures, we cannot assess how ciliated epithelium would regulate moDC functions, which is thus a limitation of these studies. In addition to direct antigenic stimulation, DC activation can occur indirectly (bystander activation) by pro-inflammatory

mediators such as IL-4, IFN- α , IFN- β , TNF- α or by epithelial derived mediators such as PGE2 and GM-CSF (39–42). As we observed in these studies, HSNEC produce, in a dose dependent manner, a variety of DC regulatory products in responses to smoke exposure including; CCL20, GM-CSF, and PGE2.

The presence of DCs in airway tissues arises primarily through two distinct mechanisms. One is via direct recruitment to the tissues and the other is by the recruitment of monocytes that are differentiated in situ to become DCs. In light of this, we examined the ability of HSNECs to induce the recruitment of monocytes and moDCs, and determined that impact of epithelial CSE exposure on these processes. We observed that both immature moDCs and to an even greater degree monocytes, increased their migration in response to CSE-HSNEC as compared to NS-HSNEC conditioned medium. These observations are consistent with murine acute smoking studies, which have shown that monocytes migrating to the lung in response to tobacco smoke play a critical role in tissue remodeling [46].

Murine smoking models have demonstrated conflicting reports the cigarette smoke can reduce MHC II, CD80 and CD86 expression (43) while others suggest cigarette smoke can elevate expression of these molecules (44). Previous *in vitro* studies have shown that when DCs were directly treated with CSE, they increase their CD80 and CD86 expression (45). Furthermore, we observed that CSE induced HSNEC to secrete products that increase moDC CD80 and CD86 co-stimulatory molecule expression. One possible explanation for these differences may be related to the mode of stimulation. Here we demonstrate indirect stimulation, via HSNEC, promotes upregulation of co-stimulatory molecule expression whereas direct had no effect.

In addition to co-stimulatory molecule expression, DC secretion of T-cell regulatory proteins was examined. Literature with regard to the impact of tobacco smoke on the production of IL-10 is highly variable and cell specific. For example, in DCs cultured from peripheral blood mononuclear cells, ETS exposed infants demonstrated a decrease in IL-10 compared to infants with no ETS exposure (46). Here we observed that compared to NS-HSNEC treatment, moDC IL-10 was increased with CSE-HSNEC conditioned media treatment. TSLP has been reported to be released by DCs in response to pathogens and allergen stimulation (47) and found to be elevated in murine smoking models (48). Our data presented here suggests that DCs may serve as a source of the elevated TSLP observed smokers. In agreement with prior reports that tobacco smoke can induce mixed Th1/Th2 immune responses, we also detected that CSE-HSNEC treatment increased DC secretion of IFN- γ . While classically thought of as a cytokine produced by T-cells, multiple reports have shown that DCs can produce IFN- γ (49, 50).

Conclusions

The results of these studies suggest that tobacco smoke can alter the DC subsets found in the sinonasal mucosal tissues. Furthermore, we demonstrate that after CSE exposure, HSNEC can induce DC maturation, migration, co-stimulatory molecule expression and alterations in cytokine production that likely result in the inflammatory milieu found in CRS which is exacerbated by tobacco smoke exposure. Lastly, these studies also demonstrate that DCs

alternatively activated by HSNEC are capable of secreting inflammatory cytokines. The results of these studies provide mechanism insights as to how tobacco smoke exacerbates upper airway inflammation. Future studies may examine how these activated DCs alter T-cell cytokine production and regulatory functions.

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Figure 1. Analysis of sinonasal DCs by subset and diagnosis

DC subsets were defined by staining for the following markers: moDCs = HLA-DR⁺CD209⁺CD14⁺, mDC1 = HLA-DR⁺CD11c⁺CD1c⁺, mDC2 = HLA-DR⁺CD11c⁺CD141⁺, pDC = HLA-DR⁺CD123⁺CD304⁺. Cell viability was determined by 7AAD. Statistics shown are unpaired t-test between indicated groups. Line indicates arithmetic mean of each group. *p<0.05 between indicated groups.

Figure 2. Active smoking alters moDC & myeloid DC infiltrate; ETS alters only moDC infiltrate in CRSwNP

Mean percent of DCs in sinonasal tissue by diagnosis, ETS exposure and active smoking. Statics shown indicating significance compared to control using post-hoc Tukey's multiple comparison test * $p < 0.05$ between indicated groups. Line indicates arithmetic mean of each group.

Figure 3. HSNEC production of moDC regulatory products is increased in response to CSE exposure

Control HSNECs were treated with varying doses of cigarette smoke extract (CSE). Cells were treated for 24 hours, then CSE was removed and supernatant was collected after an additional 24-hour incubation. Mean $\pm$ SD with statistics shown indicating significance compared to control using post-hoc Dunnett's multiple comparison test. * $p < 0.05$ vs control. $n = 4$ patients/factor shown.

Figure 4. CSE increases monocyte and moDC migration to HSNEC conditioned media
(A) Monocytes and (B) moDC migration to conditioned media from non-smoked HSNEC conditioned media (NS-HSNEC) or conditioned media from cells treated with 10% CSE (CSE-HSNEC). Data shown are mean $\pm$ SD with percent change compared to cells migrating to non-conditioned media (BEGM). * $p < 0.05$ vs NS-HSNEC. Migration shown is the HSNEC conditioned media from 4 different subjects.

Figure 5. CSE-exposed, HSNEC-conditioned media stimulates moDC to increase antigen uptake and costimulatory molecule expression

(A) CSE treatment of control HSNEC induces them to secrete products that causes moDC antigen uptake. * $p < 0.01$ vs media alone (control). Quantification of mean fluorescent intensity (MFI) for (B) CD80 and (C) CD86 expression. Data shown are mean $\pm$ SD with moDCs from $n=4$ different donors and are representative of three separate experiments. * $p < 0.01$ vs media alone (control) ** $p < 0.01$ vs non-smoked HSNEC conditioned media (NS-HSNEC).

Figure 6. CSE-exposed HSNEC conditioned media alters moDC secretion of select cytokines

Following treatment for 3 days, moDC were washed and allowed to incubate in new medium to examine their secretion of immune modulatory products by ELISA. * $p < 0.05$ vs media alone (control) ** $p < 0.05$ vs non-smoked HSNEC conditioned media (NS-HSNEC). Data shown are mean $\pm$ SD with moDCs from $n=3$ different donors and are representative of three separate experiments.

Patient Demographics

Table 1

	Control Naïve	Control ETS	Control AS	CRSwNP Naïve	CRSwNP ETS	CRSwNP AS	p value
n	12	5	8	8	9	7	
Gender (%)							
Men	41.7	40.0	25.0	75.0	22.2	71.4	0.4962
Women	58.3	60.0	75.0	25.0	77.8	28.6	
Age, mean $\pm$ SD	58.7 $\pm$ 17.4	55.6 $\pm$ 10.0	53.3 $\pm$ 7.6	43.9 $\pm$ 19.8	41.9 $\pm$ 21.4	46.3 $\pm$ 12.4	0.2369
Race (%)							
White	50.0	80.0	62.5	50.0	33.3	57.1	0.7209
African-American	50.0	20.0	37.5	50.0	66.6	42.9	

CRSwNP = chronic rhinosinusitis with nasal polyps. ETS = Environmental tobacco smoke. AS = active smoker.