

Decreased Sputum Mature Dendritic Cells in Healthy Smokers and Patients with Chronic Obstructive Pulmonary Disease

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Key Words

Chronic obstructive pulmonary disease • Dendritic cells • Immunophenotype • Smoking • Sputum

Abstract

Background: Asthmatics who smoke have decreased pulmonary mature dendritic cells (DCs). Chronic obstructive pulmonary disease (COPD) patients have an increased amount of pulmonary immature DCs. We hypothesized that healthy smokers and patients with COPD have decreased pulmonary mature DCs. **Methods:** We identified sputum DCs expressing the maturation markers CD83 and DC-lysosome associated membrane protein (DC-LAMP) and DC subpopulations (i.e. myeloid and plasmacytoid DCs) by flow cytometry in healthy smokers before they entered a smoking cessation trial (n = 30), in the same smokers after 6 months of smoking cessation (n = 11) and in COPD patients (n = 28, 14 current and 14 ex-smokers). 12 healthy never-smokers served as controls. DC numbers were expressed as percentage of total sputum CD45⁺ leukocytes. **Results:** CD83⁺ and DC-LAMP⁺ mature DCs were decreased in healthy smokers before they ceased smoking compared to after (p = 0.003 and p = 0.049, respectively) and in smokers before smoking

cessation compared to never-smokers (p = 0.027 and p = 0.028, respectively). COPD patients, both current and ex-smokers, showed decreased CD83⁺ mature DCs compared to never-smokers and smokers after cessation (p = 0.042 and p = 0.004, respectively). **Conclusions:** Cigarette smoking and COPD per se are associated with a decrease in pulmonary mature DCs. We speculate that this reduction is involved in the immunopathogenesis of smoking-related respiratory disorders, such as COPD.

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Introduction

Cigarette smoking is a major cause of morbidity and mortality worldwide and the main cause of chronic obstructive pulmonary disease (COPD). Despite extensive research, the pathogenesis of cigarette smoke-induced disorders remain largely undetermined. In the lungs of healthy smokers and COPD patients a characteristic inflammatory response is observed, which mainly consists of neutrophils, macrophages and T lymphocytes, specifically of the T cytotoxic subtype [1–3]. Lung T lymphocytes in patients with COPD secrete more interferon- γ ,

which is associated with the type 1 immune response [4–6]. However, the immunological mechanism(s) driving this characteristic amplified inflammatory response are not known.

Dendritic cells (DCs) have multiple functions in the immune responses of the lungs [7]. There are 2 major DC subtypes: myeloid DCs (MDCs), which are primarily involved in the initiation and control of adaptive immune responses and plasmacytoid DCs (PDCs), which are particularly involved in innate immunity, anti-viral responses and tolerance [8, 9]. A major event in the lifespan of DCs is their maturation. During this process DCs up-regulate lymph node homing receptors and lymphocyte stimulatory molecules and mature DCs subsequently migrate to draining lymph nodes to present the sequestered antigen(s) to T cells and induce adaptive immunity or tolerance [7]. Recently, we have hypothesized that pulmonary DCs may contribute to the pathogenesis of cigarette smoke-induced diseases, including COPD [10].

In support of this, cigarette smoke extract and nicotine suppress DC maturation in vitro [11, 12]. Data from animal studies are conflicting, with other studies showing increased and other studies showing decreased pulmonary MDC numbers and maturation after cigarette smoke exposure [13–16]. In humans little is known about the effects of smoking on DC maturation. One study showed that bronchoalveolar lavage fluid DCs from healthy smokers express low levels of lymph node homing receptors, but high levels of co-stimulatory molecules [17]. In asthmatics, smoking seems to decrease numbers of large airway CD83⁺ mature DCs [18], while in COPD patients active smoking is associated with decreased total DCs, as identified by morphology under electron microscopy [19].

Few things are known about the potential of pulmonary DCs to play a role in COPD pathogenesis. COPD patients show increased small airway langerin⁺ DCs and langerin is expressed predominately by immature DCs [20, 21]. Moreover, sputum supernatant from COPD patients suppresses DC maturation [22]. It is not known whether COPD patients have reduced numbers of pulmonary mature DCs.

In this paper we have hypothesized that: (1) active smoking in healthy smokers and (2) COPD per se are associated with reduced pulmonary mature DCs. For this reason we identified sputum DCs expressing 2 markers of maturation (CD83 and DC-lysosome associated membrane protein, DC-LAMP), in current smokers before and after smoking cessation and in COPD patients. Healthy never-smokers were used as controls. Furthermore, we have identified in our sputum samples DC sub-

Table 1. Subject characteristics

	Smokers	COPD patients	Never-smokers
n	30 (11)	28	12
Sex (M/F)	24/6 (7/4)	26/2	11/1
Age, years	51 ± 9 (49 ± 8)	60 ± 7 ^a	54 ± 11
Smoking history, pack-years	62 ± 31 (47 ± 15)	62 ± 26	–
FEV ₁ , % predicted	101 ± 12 (101 ± 10)	64 ± 12 ^b	103 ± 12
FEV ₁ /FVC, %	80 ± 5 (79 ± 4)	61 ± 6 ^b	80 ± 4
Inhaled steroids (yes/no)	0/30 (0/11)	17/11	0/12

Data are presented as mean ± SD or in absolute numbers. For smokers the figures not in parentheses represent results for all smokers before smoking cessation, those in parentheses represent results after smoking cessation (for those who stopped smoking).

^a p < 0.05 COPD patients vs. smokers; ^b p < 0.05 COPD patients vs. smokers and never-smokers.

populations that were previously identified in lung tissue, i.e. CD1c and blood dendritic cell antigen 3 (BDCA3) expressing myeloid DCs and BDCA2 expressing plasmacytoid DCs [23]. We did not identify immature DCs as these had already been quantified in healthy smokers and in COPD patients [20].

Materials and Methods

Subjects

Sputum was induced in a total of 30 healthy current smokers at the beginning of a smoking cessation trial and in 28 COPD patients (14 current and 14 ex-smokers). Recruitment of subjects was performed from those attending the smoking cessation clinic and the outpatient clinic of the University Hospital of Heraklion. Smokers who successfully quit smoking were longitudinally followed for 6 months. Sputum was again induced in those 11 subjects who succeeded 6 months of continuous smoking abstinence, as suggested by declaration, exhaled CO measurement and urine cotinine levels. Sputum was also induced in 12 healthy never-smokers, recruited by advertisement, who served as controls. We studied only non-allergic and non-atopic subjects (atopy assessed by skin prick tests). Subjects reporting a respiratory infection or an exacerbation during the previous 6 weeks were excluded. Subject characteristics are shown in table 1.

Written informed consent was obtained according to protocols approved by the hospital's medical ethics committee.

Sputum Induction and Flow Cytometry

Sputum was induced and processed, as previously described [24]. To standardize the time interval between sputum induction and last cigarette smoked in current smoking subjects, they were

Table 2. Monoclonal antibodies for flow cytometry

Specificity	Clone	Conjugate	Source
CD45	J.33	PE-Cy5	Beckman Coulter
CD14	RMO52	FITC	Beckman Coulter Immunotech, Marseille, France
CD19	J4.119	FITC	Beckman Coulter
CD1c	AD5-8E7	PE	Miltenyi Biotech, Bergish Gladbach, Germany
BDCA2	AC144	PE	Miltenyi
BDCA3	AD5-14412	PE	Miltenyi
CD83	HB15a	PE	Beckman Coulter
DC-LAMP	104.G4	PE	Beckman Coulter
Control	679.1Mc7	FITC	Beckman Coulter
Control	679.1Mc7	PE	Beckman Coulter
Control	679.1Mc7	PE-Cy5	Beckman Coulter

PE-Cy5 = Phycoerythrin-Cy5, FITC = fluorescein isothiocyanate, PE = phycoerythrin.

instructed to smoke the last cigarette 15 minutes before sputum induction. For identification of each DC subset by flow cytometry, approximately 10^6 sputum cells (at least 3×10^5 excluding squamous and dead cells) were stained with monoclonal antibodies. For labeling of CD14, CD19, CD45, CD83, CD1c, BDCA2 and BDCA3, extracellular stainings were performed. For DC-LAMP labeling, intracellular staining was performed. In 4 samples (1 obtained from a healthy smoker before smoking cessation, 2 from COPD patients and 1 from a never-smoker) staining for DC-LAMP⁺ mature DCs was not performed as there were an insufficient number of sputum cells. The antibodies used are listed in table 2. Extracellular stainings were performed by incubation of the cells with the appropriate antibodies for 30 min at 4–8°C. DC-LAMP intracellular staining was performed after 10 min pretreatment of the cells with 0.5% saponin and by incubation of the cells with anti-DC-LAMP in the presence of 0.5% saponin for 30 min at 4–8°C.

An Elite Coulter cytometer was used for 3-color flow cytometry. Data were acquired in list mode and analyzed using Epics Elite. CD1c expressing MDCs were identified as CD45^{pos}CD14^{low/neg}CD19^{neg}CD1c^{pos}, PDCs were identified as CD45^{pos}CD14^{low/neg}BDCA2^{pos}, BDCA3 expressing MDCs were identified as CD45^{pos}CD14^{neg}BDCA3^{high}, mature DCs were identified as CD45^{pos}CD14^{neg}CD83^{pos} and as CD45^{pos}CD14^{neg}DC-LAMP^{pos} (fig. 1). Gating was performed on CD45-positive cells with relatively low side scatter (SS), which reflects the relatively low granularity of pulmonary DCs [23]. CD14^{high} high auto-fluorescent macrophages and monocytes were excluded. CD14^{low} cells were included in the analysis of CD1c expressing MDCs and PDCs because low CD14 expression has been observed in pulmonary DCs [23]. B cells are known to express CD1c. For this reason, CD19^{pos} B cells were excluded from the analysis of CD1c expressing MDCs. Due to the reported expression of BDCA3 by monocytes, as well as by CD1c⁺ MDCs and PDCs, CD14^{low} cells were excluded and only BDCA3^{high} cells were included in the analysis of BDCA3 ex-

pressing MDCs [25, 26]. CD14^{low} cells were also excluded from the analysis of mature DCs, to overcome the problem of possible induction of CD83 and DC-LAMP expression in monocytes/macrophages after our sputum processing manipulations. Staining with isotype controls was designed so that the same strategy used to detect the DC-specific marker was applied to the negative control sample. DC numbers were expressed as percent of CD45⁺ sputum leukocytes.

Statistical Analysis

Normality was tested by the Shapiro-Wilk test. Variables were non-normally distributed. Differences between the same smokers before and after smoking cessation ($n = 11$) were tested using the Wilcoxon signed ranks test. Differences between COPD patients ($n = 28$), the whole group of smokers before smoking cessation ($n = 30$), smokers after smoking cessation ($n = 11$) and never-smokers ($n = 12$) were tested using the Kruskal-Wallis analysis of variance followed by the Mann-Whitney U test where appropriate. The software SPSS 16.0 for windows (SPSS Inc., Chicago, Ill., USA) was used. Probability values <0.05 were considered as statistically significant.

Results

Smokers before versus after Smoking Cessation

CD83⁺ and DC-LAMP⁺ DCs were decreased in smokers before smoking cessation compared to after ($n = 11$; $p = 0.003$ and $p = 0.049$, respectively; Wilcoxon signed ranks; fig. 2, table 3). The ratios of CD83⁺ and DC-LAMP⁺ DCs to total DCs were also decreased.

CD1c⁺ MDCs, BDCA3^{high} MDCs and BDCA2⁺ PDCs, as well as total DCs (i.e. CD1c⁺ MDCs + BDCA3^{high} MDCs + PDCs), were not significantly altered in smokers after smoking cessation (table 3).

Comparison between COPD Patients, Smokers before and after Smoking Cessation, and Never-Smokers

CD83⁺ mature DCs and DC-LAMP⁺ mature DCs were different between groups ($p = 0.003$ and $p = 0.02$, Kruskal-Wallis followed by Mann Whitney U). CD83⁺ and DC-LAMP⁺ mature DCs were decreased in healthy smokers before smoking cessation ($n = 30$) compared to never-smokers ($n = 12$; $p = 0.027$ and $p = 0.028$, respectively, fig. 2, table 3). The ratio of CD83⁺ mature to total DCs was also decreased in healthy smokers before smoking cessation compared to never-smokers. CD83⁺ DCs were decreased in COPD patients ($n = 28$) compared to smokers after smoking cessation ($n = 11$) and never-smokers (table 3). This could not be due to steroid treatment or current smoking because the subgroups of steroid naïve COPD patients and ex-smoker COPD patients also showed decreased CD83⁺ mature DCs compared to

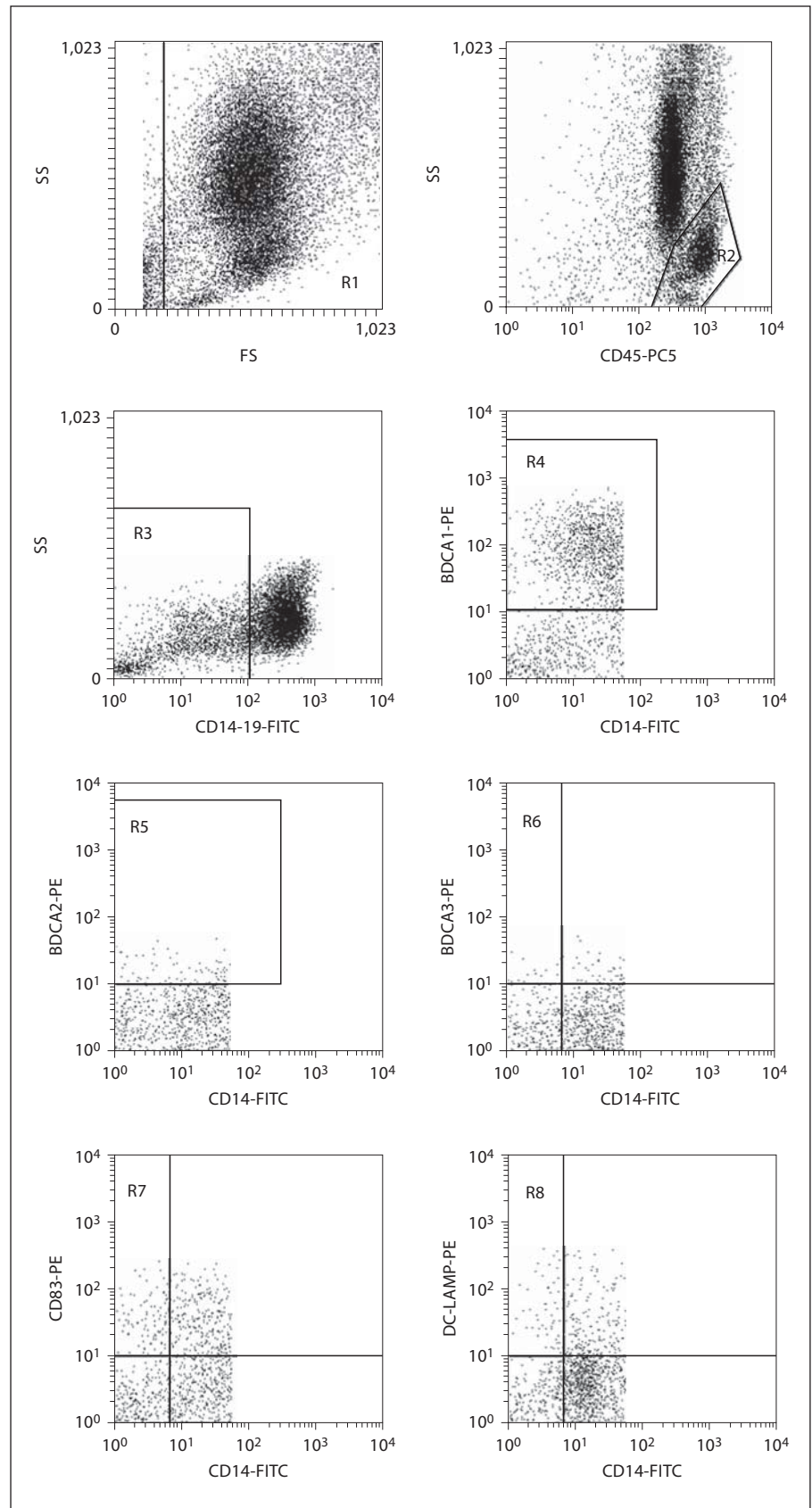


Fig. 1. R1: gating in forward scatter (FS) and side scatter (SS) modes. R2: gating of CD45^{pos} leukocytes with relatively low SS. R3: gating to exclude CD14^{high} monocytes/macrophages and CD19^{high} B cells. R4: gating of CD14^{low/neg}BDCA1(CD1c)^{pos} MDCs. R5: gating of CD14^{low/neg}BDCA2^{pos} plasmacytoid DCs. R6: gating of CD14^{neg}BDCA3^{high} MDCs. R7: gating of CD14^{neg}CD83^{pos} mature DCs. R8: gating of CD14^{neg}DC-LAMP^{pos} mature DCs.

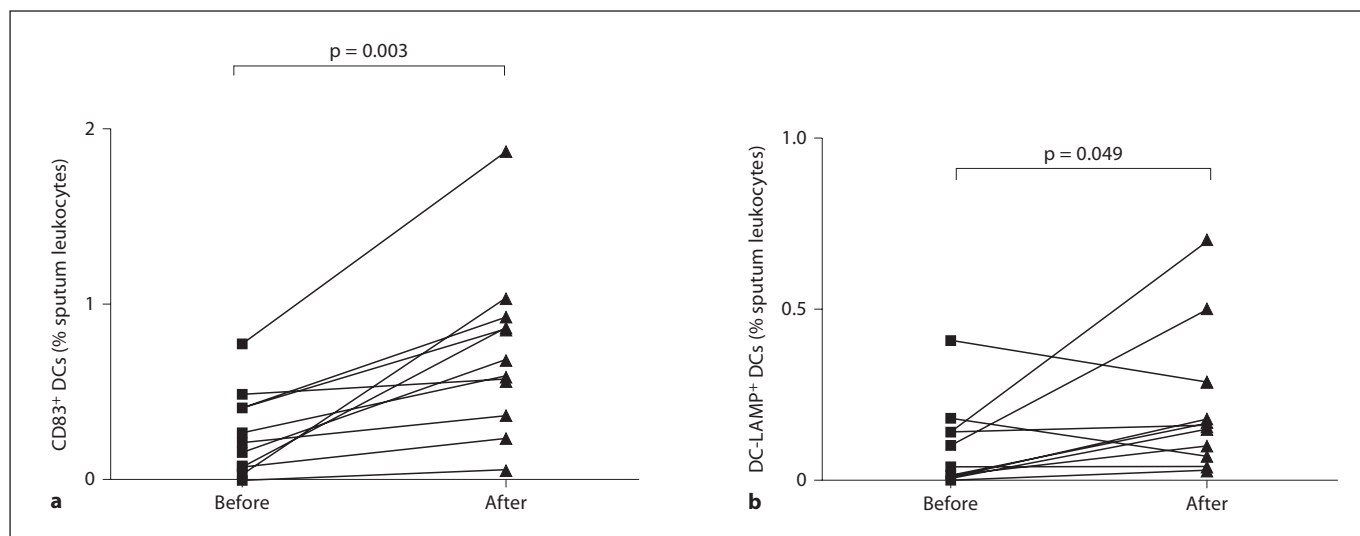


Fig. 2. CD83⁺ mature DCs (a) and DC-LAMP⁺ mature DCs (b), expressed as percent sputum CD45⁺ leukocytes in smokers before and after smoking cessation (n = 11).

Table 3. Results of flow cytometric analysis of sputum DCs

	Smokers		COPD patients (n = 28)	Never-smokers (n = 12)
	total before SC (n = 30)	before SC/after SC (n = 11)		
Total DCs	1.15 (0.04–4.49)	1.45 (0.04–4.48)/1.11 (0.37–3.07)	0.94 (0.08–4.08)	0.79 (0.24–2.9)
CD1c ⁺ MDCs	0.95 (0.03–3.95)	0.97 (0.03–2.3)/0.48 (0.12–1.50)	0.71 (0.02–3.67)	0.60 (0.20–1.35)
BDCA3 ^{high} MDCs	0.03 (0–2.01)	0.03 (0–2.01)/0.02 (0–1.80)	0.06 (0–0.33)	0.04 (0.00–0.32)
PDCs	0.14 (0.01–1.11)	0.09 (0.01–1.11)/0.23 (0.07–0.84)	0.14 (0.01–0.39)	0.08 (0.03–1.89)
CD83 ⁺ DCs	0.25 (0–1.38) ^a	0.21 (0–0.78)/0.69 (0.06–1.87) ^b	0.29 (0.04–0.89) ^{c, d}	0.48 (0.11–1.39)
CD83 ⁺ DCs/total DCs	0.22 (0–1.14) ^a	0.21 (0–1.14)/0.60 (0.16–1.78) ^b	0.33 (0.05–0.81) ^{c, d}	0.54 (0.14–4.19)
DC-LAMP ⁺ DCs	0.07 (0–0.56) ^a	0.04 (0–0.41)/0.16 (0.03–0.70) ^b	0.12 (0.01–0.9)	0.23 (0.01–1.48)
DC-LAMP ⁺ DCs/total DCs	0.05 (0–1.14) ^a	0.05 (0–1.14)/0.20 (0.03–0.49)	0.11 (0.01–1.73)	0.18 (0.04–2.03)

Data are presented as median (range). All DC numbers are expressed as percent sputum CD45⁺ leukocytes. SC = Smoking cessation. Total DCs = CD1c⁺ myeloid DCs (MDCs) + BDCA3^{high} MDCs + PDCs.

^a p < 0.05, smokers before SC vs. never-smokers; ^b p < 0.05, smokers before vs. after SC; ^c p < 0.05, COPD patients vs. never-smokers; ^d p < 0.05 COPD patients vs. smokers after SC.

smokers after smoking cessation and never-smokers (steroid naïve patients vs. healthy smokers after smoking cessation p = 0.019, steroid naïve patients vs. never-smokers p = 0.036, ex-smoker patients vs. healthy smokers after smoking cessation p = 0.016, ex-smoker patients vs. never-smokers p = 0.049, data not shown). Moreover, CD83⁺ DCs were not different between current and ex-smoker COPD patients and they were not different between ste-

roid-treated versus steroid-naïve COPD patients (p = 0.427 and p = 0.817, respectively; data not shown).

CD1c⁺ MDCs, BDCA3^{high} MDCs, BDCA2⁺ PDCs and total DCs, were not different between groups (p = 0.141–0.603, Kruskal-Wallis; table 3). They were also not different between the subgroups of steroid-treated versus steroid-naïve COPD patients (p = 0.643–0.926; data not shown). However, when the subgroups of current and ex-

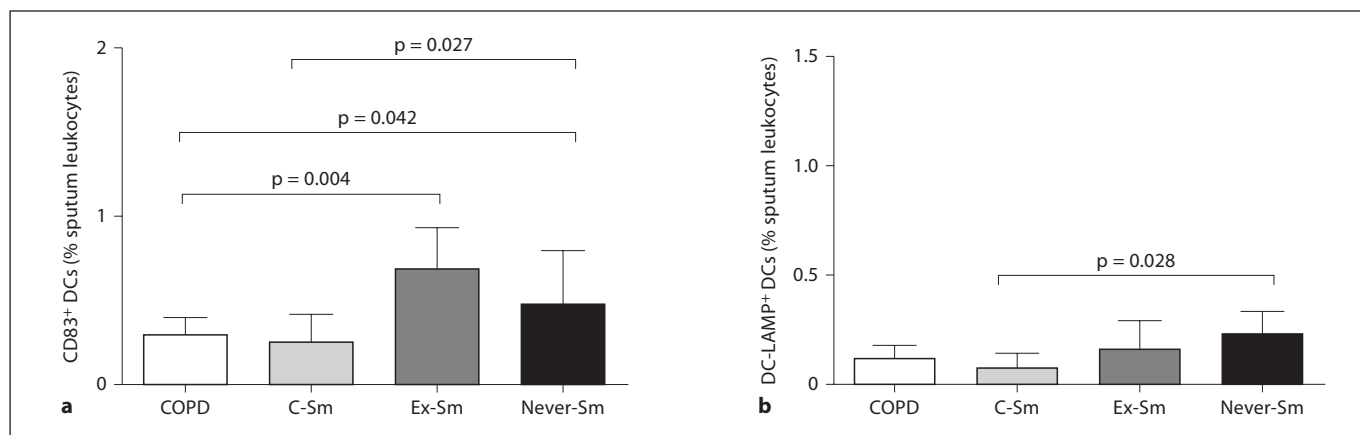


Fig. 3. CD83⁺ mature DCs (a) and DC-LAMP⁺ mature DCs (b), expressed as percent sputum CD45⁺ leukocytes in COPD patients (n = 28), all smokers before smoking cessation (C-Sm; n = 30), smokers after smoking cessation (Ex-Sm; n = 11) and never-smokers (Never-Sm; n = 12). Columns correspond to median values and interquartile range.

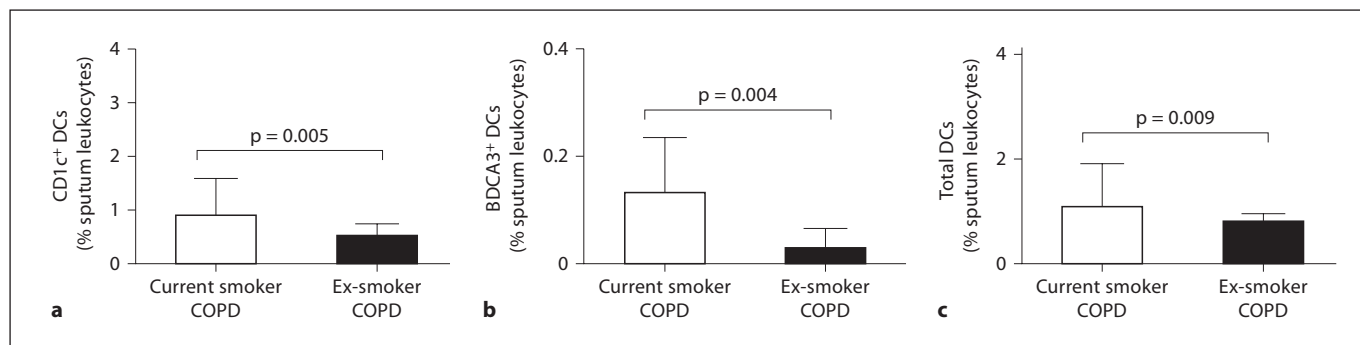


Fig. 4. CD1c⁺ MDCs (a), BDCA3^{high} MDCs (b) and total DCs (CD1c⁺ MDCs + BDCA3^{high} MDCs + PDCs; c), expressed as percent sputum CD45⁺ leukocytes in current smoker COPD patients (n = 14), and ex-smoker COPD patients (n = 14). Columns correspond to median values and interquartile range.

smokers with COPD were compared, CD1c⁺ MDCs, BDCA3^{high} MDCs and total DCs were increased in current smokers with COPD versus ex-smokers with COPD (p = 0.005, p = 0.004 and p = 0.009, respectively; fig. 4).

Discussion

The present study showed that active cigarette smoking in healthy subjects and COPD *per se* are associated with a decrease in airway mature DCs. Importantly, airway mature DC numbers in healthy smokers return at the never-smokers' levels after 6 months of smoking cessation.

In a previous study we showed that bronchial CD83⁺ mature DCs are decreased in asthmatic smokers compared to asthmatics who do not smoke or to healthy never-smokers [18]. Further to active smoking in asthmatics, smoking in healthy subjects and COPD *per se* seem to be associated with reduced pulmonary CD83⁺ mature DCs. Our results come in agreement with those recently reported by Demedts et al. [20] about increased airway immature DCs in COPD. However, there have been opposing findings from other authors. Specifically, Bratke et al. [17] showed that bronchoalveolar lavage fluid myeloid DCs from smokers compared to those from never-smokers show increased expression of the maturation markers CD80 and CD86, similar expression of CD83 and lower

expression of the lymph node homing receptor CCR7. CCR7 expression is up-regulated on mature DCs. It is possible that smoking differentially affects the expression of different molecules, specifically since some molecules, like CD83 and CCR7, are expressed later during DC maturation. Importantly, CCR7 expression on DCs negatively correlated with severity of airflow limitation in smokers [17], which fits well with our finding of decreased DCs expressing the maturation marker CD83 in patients with obstructive airway disease.

Although the present study is largely descriptive and the mechanism(s) of the reported reduction in DC maturation were not investigated, based on existing literature we may speculate that smoking increases levels of neutrophil elastase (NE), which suppresses DC maturation, while it inhibits secretory leukocyte protein inhibitor, which could reverse the NE-induced suppression on DC maturation [22, 27, 28]. Smoking may also inhibit epithelial cell excretion of cytokines critical to DC maturation [29, 30]. Finally, smoking may directly suppress pulmonary DC maturation through its components [11, 12].

It could be argued that the reported reduction in CD83⁺ mature DCs in COPD patients is also due to the effect of active smoking and not to the disease process itself. However, we found no difference in CD83⁺ mature DCs between current and ex-smokers with COPD, while ex-smokers with COPD had also significantly fewer CD83⁺ DCs compared to healthy smokers after smoking cessation and never-smokers. A plausible explanation is that high levels of NE or TGF- β in the lungs of COPD patients, even in those who have ceased smoking, suppress expression of CD83 on DCs [22, 31, 32]. Or CD83 expression on DCs could be down-regulated by interaction of DCs with epithelial cells and alveolar macrophages [29, 33].

Reduced numbers of pulmonary mature DCs in healthy smokers and COPD patients may impair their immune responses towards respiratory tract infections [34]. Specifically, reduced CD83 expression on DCs may suppress their ability to induce T cell responses [35]. Likewise, after smoking cessation in healthy smokers the observed return of mature DC numbers to the never-smoker levels may enhance the ability of pulmonary DCs to respond efficiently to infections and may attenuate airway inflammation [36].

Total DC numbers and their subpopulations (i.e., MDCs and PDCs), were not significantly altered after smoking cessation and were not different between COPD patients and healthy subjects. However, total DCs were increased in current smokers with COPD compared to

ex-smokers with COPD, due to an increase in CD1c and BDCA3 expressing MDCs. Moreover, there was a non-significant trend towards increased CD1c⁺ MDCs in healthy smokers before versus after smoking cessation ($p = 0.131$; table 3). These findings may suggest that smoking increases numbers of pulmonary MDCs. Other studies have also shown that smoking up-regulates synthesis and release of a variety of DC-attracting chemokines [15, 16, 29, 30]. The increased MDCs could be involved in the pathogenesis of cigarette smoke-induced diseases through the release of metalloproteinases [37]. They may also perpetuate inflammatory processes by recruiting T cells, by stimulating already primed T cells or/and by preserving antigens – foreign or self – for presentation to T cells for several months [7, 10].

Limitations

Methodology. We consider our results to be robust because mature DCs were identified in sputum using 2 different markers (CD83 and DC-LAMP), and results for both of these markers agreed that mature DCs are decreased in smokers. Although one may argue that some of the CD14^{low} cells included in the analysis of CD1c and BDCA2 expressing DCs were monocytes, pulmonary DCs do express CD14 at low levels, while the DC-specific markers CD1c and BDCA2 are not expressed *in vivo* by monocytes [23]. We did not exclude T cells and natural killer cells from our analysis, because none of these cells has been shown *in vivo* to express CD1c, CD83 or BDCA2/3 on their surface, or DC-LAMP intracellularly. Moreover, our preliminary experiments confirmed that sputum T cells and natural killer cells do not express any of these markers. Furthermore, although there are reports about possible expression of BDCA3 by CD1c⁺ MDCs and PDCs [10, 26], Demedts et al., as well as our own group have not detected BDCA3/CD1c or BDCA2/CD1c co-expression by pulmonary DCs [9, 23, 38]. Whatsoever, we have only included BDCA3^{high} cells in the present analysis of BDCA3 expressing MDCs. Finally, we expressed sputum DCs in percentages and not in absolute numbers. Admittedly, an increase or decrease in cell percentages does not necessarily mean an increase or decrease in absolute numbers and had we shown differences in absolute DC numbers this would strengthen our results. However, absolute DC numbers partly reflect differences in absolute total cell numbers, which may greatly vary among different subject samples. For this reason we believe that differences in DC percentages supply essential information about true differences in DC numbers.

Interpretation. We also acknowledge that there are potential limitations to the interpretation of our results. First, a proportion of COPD patients were on inhaled steroid treatment, which could affect DC numbers and maturation [39]. However, we found no differences between steroid-treated and steroid-naïve COPD patients. Moreover, the subgroup of steroid-naïve COPD patients also showed decreased mature DCs. Second, although there was no difference in smoking history (pack-years) between COPD patients and healthy smokers, COPD patients were significantly older. However, the literature on potential alterations of DCs with aging is scarce [40]. To the best of our knowledge there are no published data about reduced CD83 and DC-LAMP expression on DCs with aging. Importantly, the age difference between COPD patients and never-smokers was not statistically significant. Third, the number of control subjects included in the study is relatively small compared to the patients with COPD. This is mainly due to significant difficulties in the recruitment of healthy control subjects. Unfortunately, we could not do power analysis prior to the initiation of the study, because there were no previous data about numbers of DCs in sputum samples from patients with COPD. However, we were still able to detect differences between the control group and the patient group. Fourth, sputum samples mainly the large airways, and therefore our findings cannot be extrapolated to the small airways and alveoli. However, induced sputum is an important tool in the study of pulmonary DCs, as it may provide an adequate cellular specimen for the study of DCs, while being less invasive compared to bronchoalveolar lavage. Fifth, including TGF- β and NE sputum levels would strengthen the results of the study. However,

a correlation does not always imply a cause and effect relationship. Moreover, we still consider our observational data to be important because they are novel and may support future functional studies which will clarify whether TGF- β and NE are implicated in the observed alterations of DC maturation in COPD patients and healthy smokers. Finally, it may be argued that the observed decreases in mature DC numbers are merely due to increases in immature DC numbers and not to true alterations in the DC maturation process. However, this could not be our case because we expressed mature DC numbers as a proportion of total sputum cells and not as a proportion of DCs.

Conclusion

We have shown that COPD and active smoking in healthy subjects are associated with decreased airway mature DCs. We may speculate that this reduction is involved in the immunopathological processes that take place in the lungs of COPD patients and healthy subjects who smoke. Importantly, our results suggest that parameters of airway immunity which are altered by smoking can be partly restored in healthy smokers after 6 months of smoking cessation.

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