Differential switching to IgG and IgA in active smoking COPD patients and healthy controls

Corry-Anke Brandsma*, Huib A.M. Kerstjens[#], Wouter H. van Geffen[#], Marie Geerlings*, Dirkje S. Postma[#], Machteld N. Hylkema* and Wim Timens*

ABSTRACT: Several studies have demonstrated the presence of B-cell follicles and autoantibodies in chronic obstructive pulmonary disease (COPD). It is unclear against which antigens this B-cell response is directed and whether it contributes to development or worsening of disease.

We assessed different B-cell subsets in blood and lung tissue from COPD patients and controls, and compared differences in B-cell responsiveness to stimulation with lung-specific antigens.

Active smoking induced an adaptive immune response with relatively high levels of (class-switched) memory B-cells in blood and immunoglobulin (Ig)G memory B-cells in the lung. COPD smokers showed more switching to IgG, whereas healthy smokers switched more to IgA. COPD patients had higher levels of memory B-cells in the lung and stimulation with lung-specific antigens induced higher numbers of anti-decorin antibody-producing cells in COPD patients compared with healthy controls.

Differential switching to IgG and IgA indicates that the adaptive immune response to smoke differs between COPD patients and healthy controls. A higher level of memory B-cells in the lungs of COPD patients may reflect an antigen-specific immune response, which could be directed against decorin, as suggested by the induction of anti-decorin antibody-producing cells in response to antigen-specific stimulation in COPD patients.

KEYWORDS: Cigarette smoke, decorin, immune response, lung tissue, memory B-cells

Chronic obstructive pulmonary disease (COPD) is a pulmonary disease characterised by a chronic inflammatory response in the lungs that is reflected by an influx of cells of the innate immune system, *i.e.* neutrophils and macrophages, as well as cells of the adaptive immune system, *i.e.* CD4- and CD8-positive T-cells, and B-cells [1, 2]. The exact mechanisms underlying this chronic inflammatory response in COPD have not yet been elucidated.

For a few years, there have been increasing indications of an antigen-specific and, possibly, auto-immune response in COPD. RICHMOND *et al.* [3] demonstrated that the presence of lymphoid follicles, described as bronchus-associated lymphoid tissue, was more prominent in smokers than in never-smokers. More recently, HOGG *et al.* [4] reported the presence of lymphoid follicles in the lungs of COPD patients and showed a relationship with disease severity. Furthermore,

we demonstrated the presence of oligoclonal B-cells with ongoing mutation in these follicles [5], indicating the presence of a specific B-cell response. Several reports have now confirmed the presence of B-cell follicles in COPD [6–10], some also in relation to disease severity. Notably, POLVERINO *et al.* [8] showed the presence of BAFF (B-cell-activating factor of the tumour necrosis factor family) in these follicles, which is a marker of B-cell survival and activation, and has been associated with autoimmune diseases [11, 12]. In addition to B-cells, the presence of oligoclonal CD4-positive T-cells has been demonstrated in the lung tissue of severe COPD patients [13] as well as an antigen-specific T-helper type 1 response to lung elastin [14]. Consistent with this, MOTZ *et al.* [15] recently demonstrated that chronic cigarette smoke exposure can generate T-cells in mice that are capable of inducing a COPD-like pathology upon transfer to naïve recipients lacking functional T- and B-cells. To our knowledge, the study by

AFFILIATIONS

*Depts of Pathology and Medical Biology, Groningen Research Institute for Asthma and COPD, and

[#]Pulmonary Diseases, Groningen Research Institute for Asthma and COPD, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

CORRESPONDENCE

C-A. Brandsma
Dept of Pathology and Medical Biology
University Medical Center Groningen and University of Groningen
P.O. box 30.001
9700 RB
Groningen
the Netherlands
E-mail: c.a.brandsma@umcg.nl

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MOTZ *et al.* [15] is the first functional study demonstrating smoke-induced autoreactivity with COPD-like pathology in mice.

With respect to the autoantigens that may drive autoimmunity, the presence of autoantibodies against HEp-2 epithelial cells [16, 17], bronchial epithelial cells [16], endothelial cells [16, 18], elastin [14], cytokeratin 18 [19] and several immunogenic peptides [20] has been demonstrated in patients with COPD. It should be noted, however, that we [21] and others [22–24] could not demonstrate this anti-elastin autoantibody response in COPD. Recent data demonstrating that long-term cigarette smoke exposure can induce antibodies in mice that can induce COPD-like pathology upon transfer into naïve recipients supports a possible pathogenetic role for autoantibodies in COPD [25].

Interestingly, cigarette smoking has been demonstrated as a risk factor for the development of several autoimmune disorders [26], including rheumatoid arthritis (RA) [27], systemic lupus erythematosus (SLE) [28], primary biliary cirrhosis (PBC) [29] and multiple sclerosis [30]. Moreover, a recent study showed that patients with several autoimmune disorders, such as RA, SLE, Graves' disease, PBC, polymyalgia rheumatica and psoriasis, had an increased risk of developing COPD [31]. Together, these findings imply a role of smoking in autoimmune pathogenesis.

Although there is convincing evidence for the presence of B-cell follicles and autoantibodies in COPD, it is still unclear to which antigens these B-cells respond, and whether this response is pathological in humans and contributes to the development or worsening of COPD.

We investigated whether B-cells from COPD patients and healthy individuals respond differently to stimulation with lung-specific antigens with respect to plasma cell differentiation and antibody production. Given the previous results of LEE *et al.* [14], our own recent data regarding autoantibodies against elastin, collagen and decorin [21, 32], and our previous findings regarding a decreased presence of decorin in COPD [33, 34], we chose elastin, collagen and decorin as lung-specific antigens in this study.

Since we previously demonstrated that current smoking can have a pronounced effect on the adaptive immune response [35], equal numbers of current smokers, ex-smokers and never-smokers were included in this study. As a follow-up to our previous findings [35], we analysed different B-cell subsets in lung tissue from COPD patients and non-COPD controls as well as analysing immunoglobulin (Ig)G expression on memory B-cells.

MATERIALS AND METHODS

For detailed description of the material and methods, see the online supplementary material.

Subjects

For the isolation of leukocytes from blood, 23 COPD patients with post-bronchodilator forced expiratory volume in 1 s (FEV₁)/forced vital capacity (FVC) <70% and FEV₁ <80% predicted were included, as were 36 healthy individuals without pulmonary symptoms and with FEV₁/FVC >70% and FEV₁ >90% pred. All were male, >40 yrs of age, and had

no major comorbidities and a negative skin-prick test or Phadiatop test (Phadia, Uppsala, Sweden). Exacerbations or the use of corticosteroids in the previous 6 weeks were not allowed. Smokers and ex-smokers (no smoking in the previous year) had to have ≥10 pack-yrs of exposure. Approval was obtained from the local medical ethics committee (University of Groningen, Groningen, the Netherlands) and participants gave written informed consent.

For the isolation of leukocytes from lung tissue, 14 COPD patients and nine non-COPD controls were included. Lung tissue was derived from patients undergoing surgery for lung transplantation or pulmonary carcinoma or from donor lungs. The study protocol was consistent with national ethical and professional guidelines [36].

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated and used for flow cytometry analysis, immunocytochemical staining on cytopins and stimulation experiments (fig. S1). Lung tissue single-cell leukocyte suspensions were freshly isolated using a slightly adapted protocol as described previously in a mouse model (online supplementary material) [32, 37].

Flow cytometry analysis

1×10^6 PBMCs or lung leukocytes were stained with Alexa Fluor® 700-conjugated anti-CD20, allophycocyanin (APC)- and Cy7-conjugated anti-CD27, phycoerythrin (PE)- and Cy7-conjugated anti-CD38, APC-conjugated anti-CD138, biotinylated anti-IgM followed by PE-conjugated streptavidin (BD Biosciences, San Jose, CA, USA), PE- and Cy5-conjugated anti-IgG (eBioscience, Vienna, Austria), fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (BD Bioscience), FITC-conjugated anti-CD14 (IQ Products, Groningen, the Netherlands) and FITC-conjugated anti-CD16 (IQ Products). Fluorescent staining of the cells was measured on a LSR-II flow cytometer (BD Biosciences) and data were analysed using FlowJo software (Tree Star Inc., Ashland, OR, USA). First, lymphocytes were gated based on cell size (forward scatter) and density (side scatter). This population was used as input population for the analyses. Subsequently, cells expressing CD3, CD14 and CD16 were excluded from the analysis to remove T-cells, monocytes, macrophages and granulocytes. Next, the different B-cell subsets were distinguished based on the expression of CD20, CD27, IgM and IgG. Total B-cells were analysed based on CD20 expression, and total memory B-cells were analysed based on co-expression of CD20 and CD27 (fig. S2a). Within the total CD20 population, class-switched memory B-cells were classified as CD27 positive and IgM negative (fig. S2b). Within the memory B-cell population, IgG-positive memory B-cells, IgM-positive memory B-cells and memory B-cells negative for IgG and IgM could be distinguished (fig. S2c).

PBMC stimulation

2×10^6 PBMCs from COPD patients and healthy controls were stimulated for 6 days with lung elastin, lung collagen (both $1 \mu\text{g}\cdot\text{mL}^{-1}$; Elastin Products, Owensville, MI, USA) and recombinant human decorin ($1 \mu\text{g}\cdot\text{mL}^{-1}$; R&D Systems, Minneapolis, MN, USA) in combination with a mixture of interleukin (IL)-10 ($50 \text{ ng}\cdot\text{mL}^{-1}$), IL-4 ($10 \text{ ng}\cdot\text{mL}^{-1}$) (both Peprotech, Rocky Hill,

NJ, USA), IL-2 ($1.25 \text{ pg}\cdot\text{mL}^{-1}$; R&D Systems) and anti-CD40 ($5 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$; eBioscience) (cytokine mix) to stimulate plasma cell differentiation and Ig production (referred to hereafter as extracellular matrix (ECM) plus cytokine mix stimulation). A double dose of this cytokine mix served as positive control (referred to hereafter as cytokine mix stimulation) and no stimulants as negative control.

Immunocytochemistry

IgA-, IgG- and IgE-expressing B-cells and plasma cells were identified in cytopins from PBMCs or stimulated cells using immunocytochemistry. 1,000 cells were counted per cytospin and counts were expressed as the percentage of positive cells.

IgG, IgA and IgM ELISA

IgA levels were determined using an ELISA kit (Bethyl Laboratories, Montgomery, TX, USA). For detection of IgM and IgG, plates were coated with anti-human Ig (Southern Biotech, Birmingham, AL, USA) followed by incubation with the culture supernatants and alkaline phosphatase-conjugated anti-human IgG or IgM. Staining was visualised using p-nitrophenylphosphate as a substrate.

Antigen-specific ELISPOT analysis

96-well filter plates were coated with lung elastin, lung collagen or recombinant human decorin, and incubated overnight with 0.5×10^6 stimulated cells per well. The following day, plates were incubated with biotinylated rabbit anti-human IgG followed by peroxidase-conjugated streptavidin. Spots were visualised with 3-amino-9-ethylcarbazole substrate and counted using an automated reader.

Statistical analyses

Multiple linear regressions were used to determine whether COPD, current smoking or both in combination affected the different parameters from the PBMC experiments. When significant effects of COPD or current smoking were found or a significant interaction was present between COPD and current smoking, additional Mann-Whitney U-tests (MWUs) were used for specific *post hoc* analyses. For the lung tissue experiments, MWUs were used to compare COPD patients

with non-COPD controls and current smokers with ex- and never-smokers. A value of $p < 0.05$ was considered significant.

RESULTS

Participant characteristics

The characteristics of the subjects included in the PBMC and lung tissue analyses are shown in table 1. COPD patients included in the PBMC analyses were older than the healthy individuals and had more pack-years of smoking exposure when compared with healthy current and ex-smokers.

B-cell subsets in peripheral blood

As we demonstrated previously [35], the percentages of total and memory B-cells were lower in COPD patients compared with healthy controls and the percentages of memory and class-switched memory B-cells were higher in current smokers compared with the ex- and never-smokers (fig. S3).

In our previous study [35], class-switched memory B-cells were identified as being CD27 positive and IgM negative, which gives no information on actual isotype switching. Therefore, we now also analysed IgG expression on memory B-cells. The percentage of IgG-positive memory B-cells was higher in COPD smokers compared with COPD ex-smokers (MWU $p=0.036$) (fig. 1). This effect of current smoking was not present in the healthy controls. Linear regression analyses found a significant positive interaction between current smoking and COPD ($p=0.008$), which means that the effect of current smoking is different in COPD patients compared with healthy controls. In contrast to IgG, there was a lower percentage of IgM memory B-cells in COPD smokers compared with COPD ex-smokers (MWU $p=0.003$) (fig. 1) and again this effect of current smoking was not present in healthy controls. Linear regression analysis for IgM memory B-cells showed a negative interaction between current smoking and COPD ($p=0.038$). There was a higher percentage of IgG- and IgM-negative (and, thus, IgA- or IgE-positive) memory B-cells in current smokers compared with ex- and never-smokers, irrespective of COPD (smoke effect $p=0.007$) (fig. 1).

TABLE 1 Participant characteristics

	Smoking status	Subjects n	Age yrs	Smoking exposure pack-yrs	FEV1 % pred
PBMC study					
COPD	CS	12	67 (45–76)*	39 (28–75)*	65 (49–79)
	ES	11	73 (44–81)*	45 (15–75)*	70 (38–76)
Healthy	CS	11	52 (45–64)	22 (12–50)	109 (99–150)
	ES	13	59 (51–67)	23 (12–45)	107 (96–128)
	NS	12	58 (52–74)	0	110 (94–129)
Lung tissue study					
COPD	2 CS/11 ES/1 UNK	14	61 (48–69)	38 (11–90) [#]	25 (16–77)
Non-COPD control	5 CS/3 ES/1 NS	9	61 (53–77)	30 (0–75) [#]	103 (90–116) [†]

Data are presented as median (range), unless otherwise stated. FEV1: forced expiratory volume in 1 s; % pred: % predicted; PBMC: peripheral blood mononuclear cell; COPD: chronic obstructive pulmonary disease; CS: current smoker; ES: ex-smoker; NS: never-smoker; UNK: unknown smoking status. [#]: smoking exposure was unknown for three COPD patients and four non-COPD controls; [†]: FEV1 was unknown for the two donor lungs. *: $p < 0.05$ for COPD patients versus healthy controls.

FIGURE 1. Memory B-cells in peripheral blood. Percentages of a) immunoglobulin (Ig)G-positive, b) IgM-positive, and c) IgG- and IgM-negative memory B-cells in the peripheral blood of chronic obstructive pulmonary disease (COPD) patients and healthy individuals are shown. p-values represent Mann-Whitney U-tests. *: $p < 0.05$; #: $p = 0.05$; †: $p = 0.08$.

Because the effect of current smoking on IgG-positive memory B-cells was different between COPD patients and healthy controls, while the percentage of class-switched memory B-cells was higher in both COPD smokers and healthy smokers, we anticipated that there could be a difference in the effect of current smoking in COPD patients

FIGURE 2. Immunoglobulin (Ig)A positive B-cells in peripheral blood. a) Percentages of IgA-positive cells in peripheral blood of chronic obstructive pulmonary disease (COPD) patients and healthy individuals. *: $p < 0.05$ by Mann-Whitney U-test. b) Representative image of cells stained positively for IgA (red staining).

and healthy controls for the percentage of IgA- or IgE-positive memory B-cells. Therefore, we assessed percentages of IgA- and IgE-positive cells using immunocytochemistry on the same cells as used in the flow cytometry analyses. We found a higher percentage of IgA-positive B-cells in current smokers compared with ex- and never-smokers (smoke effect $p = 0.003$) (fig. 2). This effect of current smoking was mainly driven by the significant difference between healthy smokers and healthy never smokers (MWU $p = 0.039$), because there was no difference between COPD smokers and COPD ex-smokers (MWU $p = 0.1$). Additionally, COPD patients had a lower percentage of IgA-positive B-cells than healthy controls (COPD effect $p = 0.022$).

COPD patients also had a lower percentage of IgE-positive B-cells than healthy controls (COPD effect $p = 0.001$) (fig. S4), but there was no effect of current smoking on IgE-positive B-cells.

FIGURE 3. Immunoglobulin (IgA) antibody levels in supernatant. Total IgA antibody levels in supernatants from peripheral blood mononuclear cells derived from chronic obstructive pulmonary disease (COPD) patients and healthy individuals that were cultured for 6 days a) without stimulation, b) with extracellular matrix (ECM) plus cytokine mix stimulation or c) with cytokine mix stimulation are shown. p-values represent Mann–Whitney U-test. *: $p < 0.05$; #: $p = 0.09$; †: $p = 0.06$.

Antibody production by PBMCs

To study the response of B-cells to stimulation with lung-specific antigens, PBMCs were cultured for 6 days with a mixture of ECM proteins and cytokine mix. Without stimulation, PBMCs from current smokers produced higher IgA levels

than PBMCs from ex- and never-smokers (smoke effect $p = 0.02$). This effect of current smoking was mainly caused by higher IgA levels in healthy smokers compared with healthy never-smokers (MWU $p = 0.01$) (fig. 3), because there was no difference in IgA levels between COPD smokers and

FIGURE 4. Anti-decorin immunoglobulin (IgG) antibody-producing cells. The numbers of anti-decorin IgG antibody producing cells in peripheral blood mononuclear cells derived from chronic obstructive pulmonary disease (COPD) patients and healthy individuals that were cultured for 6 days with extracellular matrix (ECM) plus cytokine mix stimulation are shown for a) all the subgroups and b) COPD compared with healthy controls. *: $p < 0.05$ by Mann–Whitney U-test.

COPD ex-smokers (MWU $p=0.3$). After stimulation with ECM plus cytokine mix or cytokine mix alone, the effect of current smoking on IgA production was more pronounced than without stimulation (smoke effect: $p=0.001$ (ECM); $p=0.002$ (cytokine mix)) (fig. 3). PBMCs from healthy smokers produced higher levels of IgA compared with healthy ex-smokers (MWU: $p=0.007$ (ECM); $p=0.01$ (cytokine mix)) and healthy never-smokers (MWU: $p=0.01$ (ECM); $p=0.01$ (cytokine mix)). For COPD smokers, there was a trend for higher IgA levels compared with COPD ex-smokers (MWU: $p=0.06$ (ECM); $p=0.09$ (cytokine mix)).

Without stimulation, PBMCs from current smokers produced lower levels of IgG than ex- and never-smokers (smoke effect $p=0.027$) (fig. S5a). This effect was mainly caused by the lower levels of IgG in healthy current smokers compared with healthy ex- and never-smokers. After stimulation with ECM plus cytokine mix or cytokine mix alone, there was no effect of current smoking or COPD on IgG production (fig. S5a). There were trends towards lower IgM production by PMBCs from COPD patients compared with healthy controls for all culture conditions (COPD effect: $p=0.065$ (no stimulation); $p=0.06$ (ECM); $p=0.05$ (cytokine mix)) (fig. S5b).

Plasma cell differentiation of PBMCs

Stimulation with cytokine mix resulted in more IgA plasma cells in current smokers than in ex- and never-smokers (smoke

effect $p=0.034$) (fig. S6). This effect was not present after stimulation with ECM plus cytokine mix.

After stimulation with ECM plus cytokine mix or cytokine mix alone, fewer IgG-positive plasma cells were present in COPD patients compared with healthy controls (COPD effect: $p=0.038$ (ECM); $p=0.017$ (cytokine mix)) (fig. S6).

Induction of antigen-specific antibody-producing cells

Stimulation with ECM plus cytokine mix resulted in more anti-decorin IgG antibody-producing cells in COPD patients compared with healthy controls (MWU $p=0.03$; COPD effect $p=0.08$) (fig. 4). This difference was not present after stimulation with cytokine mix alone and there were no differences in anti-elastin or anti-collagen antibody-producing cells (data not shown).

B-cell subsets in lung tissue

To investigate whether our findings regarding circulating B-cells in blood are a good reflection of the situation in the lung, we investigated the presence of the same B-cell subsets in lung tissue from COPD patients and non-COPD controls.

COPD patients had higher percentages of memory B-cells in lung tissue than non-COPD controls (MWU $p=0.035$) (fig. 5). There were no differences in the percentages of total B-cells, class-switched memory B-cells and IgG-positive memory B-cells

FIGURE 5. B-cell subsets in lung tissue: chronic obstructive pulmonary disease (COPD) patients *versus* non-COPD controls. Percentages of a) total B-cells, b) memory B-cells, c) class-switched memory B-cells and d) immunoglobulin (Ig)G-positive memory B-cells in lung tissue are shown for COPD patients and non-COPD controls.

*: $p<0.05$ by Mann-Whitney U-test.

FIGURE 6. B-cell subsets in lung tissue: current smokers versus ex- and never-smokers. Percentages of a) total B-cells, b) memory B-cells, c) immunoglobulin (Ig)G-positive memory B-cells and d) IgM-positive memory B-cells in lung tissue are shown for current smokers and ex- and never-smokers. *: $p < 0.05$ by Mann-Whitney U-test.

between COPD patients and non-COPD controls (fig. 5). When investigating the effects of current smoking on B-cell subsets in lung tissue, we found a higher percentage of IgG-positive memory B-cells in current smokers than in ex- and never-smokers (MWU $p = 0.001$) (fig. 6). There was no effect of current smoking on the percentages of total B-cells, (class-switched) memory B-cells and IgM-positive memory B-cells (fig. 6).

DISCUSSION

This study showed that active smoking induces an ongoing adaptive immune response that is reflected by relatively high levels of (class-switched) memory B-cells in peripheral blood and higher percentages of IgG-positive memory B-cells in the lung. In addition, COPD smokers showed more switching to IgG in peripheral blood, while healthy smokers switched more to IgA. Furthermore, COPD patients had higher levels of memory B-cells in lung tissue than non-COPD controls and, after stimulation with lung-specific ECM proteins, higher numbers of anti-decorin IgG antibody-producing cells were present in PBMCs derived from COPD patients when compared with healthy controls.

The increase in IgA-positive B-cells in the peripheral blood of current smokers was consistent with higher levels of IgA production by PBMCs from current smokers and higher numbers of IgA plasma cells in response to cytokine mix stimulation in current smokers. The effect of current smoking

on IgG-positive memory B-cells in the peripheral blood of COPD patients was not consistent with our findings regarding IgG production and differentiation to IgG plasma cells *in vitro*. However, it was very similar to what we found in lung tissue, suggesting that in this respect, the peripheral blood compartment is a good reflection of the lung compartment. Although we had relatively low numbers of currently smoking COPD patients and non-COPD controls in the lung tissue analyses, we demonstrated a clear increase in IgG memory B-cells in current smokers. Due to the low numbers of current smokers, it was not possible to perform separate analyses for COPD patients and non-COPD controls. Therefore, we could not test whether a similar difference in IgG response to smoking existed in COPD and control lung tissue, as found in peripheral blood. Nevertheless, given the higher percentage of total memory B-cells in lung tissue of COPD patients, it is likely that the total number of IgG memory B-cells will be highest in lung tissue from COPD smokers. The higher levels of memory B-cells in lung tissue from COPD patients compared with non-COPD controls correspond with previous immunohistochemical studies demonstrating higher numbers of B-cells and B-cell follicles in COPD [4, 38], and provide further support for an antigen-specific immune response in the lungs of COPD patients.

Our findings regarding the higher numbers of anti-decorin IgG antibody-producing cells in stimulated PBMCs from COPD patients are consistent with these findings and may point to

decorin as potential antigen. Although the differences in anti-decorin antibody-producing cells were not very large, we suppose that the effect is specific because the difference was only present after stimulation with lung-specific ECM and was only present for decorin. Decorin is an important matrix protein in the lung that is known to interact with fibrillar collagens, contributing to collagen fibre network formation [39, 40], and can bind transforming growth factor- β , neutralising its pro-fibrotic actions [41, 42]. Loss of decorin in the lung may contribute to loosening of the collagen fibre network and higher levels of active transforming growth factor- β . As we have shown that decorin is consistently reduced in its presence and production in COPD [33, 34, 43], it will be of interest to evaluate whether a humoral autoimmune response may be involved. The next step is to also perform ELISPOT analysis on lung tissue to find out whether these anti-decorin IgG antibody-producing cells are also prominent in the lungs of COPD patients.

The observation that COPD smokers express more IgG and healthy smokers more IgA is novel and intriguing, and suggests that the smoke-induced adaptive immune response is different in COPD patients and healthy controls. Regarding our findings in healthy smokers, switching to IgA is typical for mucosal immune responses [44] and it can be envisaged that the high levels of circulating IgA-positive B-cells and the higher IgA production, as found in response to *ex vivo* stimulation in current smokers, is a direct reflection of the ongoing immune triggering of the airways by smoking. The observation that this effect was only present in the current smokers and not in ex-smokers supports this.

Regarding our findings in COPD patients, isotype switching to IgG can have several causes. It can be a reflection of immune responses against bacterial or viral infections, but more exciting is the possibility of a specific (auto)immune response against neoantigens. These neoantigens may be continuously induced by (long-term) smoking in the damaged COPD lung and, to a lesser extent, in a healthy smoker's lungs [5, 45]. The recent findings of KIRKHAM *et al.* [46] demonstrating that carbonyl modification of proteins by exposure to chronic oxidative stress, such as cigarette smoking, can trigger an antibody mediated immune response support this theory. Interestingly, as smoking was already implicated in autoimmune pathogenesis [26, 31], our current results suggest that smoking may also be a risk factor of an autoimmune component in the development of COPD.

In conclusion, we showed that active smoking induces relatively high levels of (class-switched) memory B-cells in peripheral blood and increased percentages of IgG-memory B-cells in the lung. The smoke-induced adaptive immune response in blood is different in COPD patients and healthy controls, *i.e.* healthy smokers shift towards an IgA response, whereas when COPD is also present, this response is shifted more towards IgG. The smoke-induced IgA response is probably the result of a mucosal immune response evoked by the constant triggering of the airways in smokers. The IgG response in COPD smokers may reflect a specific (auto)immune response against smoke-induced neoantigens or a specific immune response against microbial pathogens. Additionally, we found a higher level of memory B-cells in the lungs of COPD patients, which may reflect an antigen-specific immune response that could be directed against decorin.

These findings are novel and may be of clinical relevance given the profound effects of active smoking on the adaptive immune system, particularly given the different effect of smoking in COPD patients. Moreover, we provide further support for the presence of an antigen-specific immune response in COPD.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

A statement of interest for D.S. Postma can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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