Antinuclear autoantibodies are more prevalent in COPD in association with low body mass index but not with smoking history

H P J Bonarius,1,6 C A Brandsma,1 H A M Kerstjens,2 J A Koerts,1 M Kerkhof,3 E Nizankowska-Mogilnicka,4 C Roozendaal,5 D S Postma,2 W Timens1

ABSTRACT
Background Chronic obstructive pulmonary disease (COPD) is associated with a higher prevalence of antinuclear autoantibodies (ANAs). However, a significant subgroup of patients is ANA negative. It remains to be determined which patient groups carry autoantibodies.

Methods The association of smoking behaviour, disease status, gender, age and body mass index (BMI) with the presence of autoantibodies in the serum was determined in 124 patients with COPD and 108 non-COPD control subjects. In addition, the role of B cells in autoantibody generation in COPD was investigated by sequencing the antibody repertoire of B cells in the lungs of patients with COPD and of ex-smoking and never-smoking control subjects.

Results Patients with COPD had a significantly higher risk of being serum positive for ANAs (OR 3.12, 95% CI 1.68 to 5.76, p<0.001). ANAs were not significantly associated with age, smoking status, gender or pack-years of smoking. Within the COPD population, subjects with BMI <22 kg/m2 had a significantly higher risk of ANAs (OR 4.93, 95% CI 1.50 to 16.50, p=0.009) than those with normal or high BMI. The antibody repertoire of B cells in the lungs of patients with COPD had a high frequency of positively charged CD83 residues, a feature which is associated with self-reactive antibodies.

Conclusion The results show that COPD is a heterogeneous disease with respect to the prevalence of ANAs. ANAs are primarily associated with the presence of COPD and with low BMI, but not with smoking and forced expiratory volume in 1 s.

INTRODUCTION
Chronic obstructive pulmonary disease (COPD) is a widespread disease. In the USA alone, 15–25 million people (5–10% of the population) are affected.1 2 Despite its high and increasing prevalence and mortality, the pathogenesis of COPD remains unclear and there is no effective treatment available that halts the irreversible and progressive tissue destruction and small airway wall fibrosis which are characteristic of the disease.

The immune system is chronically activated in COPD. Markers of systemic inflammation such as C reactive protein and interleukin 6 are present in stable COPD and their levels relate to disease severity.3 Additionally, inflammatory cells are increased in different areas of the lung. The percentage of small airways containing neutrophils and B cells increases with disease severity, as defined by Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.4 Furthermore, there are more B cells in the large airways of patients with COPD with GOLD stage III compared with GOLD stage II.5 Lymphoid follicles consisting of B cells and follicular dendritic cells with adjacent T cells have been demonstrated in the parenchyma and in bronchial walls of patients with emphysema,6 and are present in the small airways in patients with severe and very severe COPD.4 We previously identified the presence of ongoing somatic mutations in the majority of these follicles, suggesting oligoclonal antigen-specific proliferation.6

Studies showing increased levels of antinuclear antibodies (ANAs)7 8 indicate an antigen-specific self-reactive response in patients with COPD. In addition, increased levels of anti-elastin autoantibodies and Th1 responses against elastin have been found in emphysema.6 This suggests the presence of antibody-mediated degradation of extracellular matrix which might contribute to sustained inflammation. Other papers reported no evidence for systemic autoantibodies directed against elastin peptides in chronic inflammatory lung disease.10 11 About 70% of patients with COPD were positive for ANAs,9 suggesting heterogeneity in the autoimmune response in COPD. In addition, serum samples of patients with COPD show a variety of ANA patterns (antinuclear, centromeric, speckled), possibly reflecting reactivity against different antigens.9

It is not yet clear which factors determine this heterogeneity. Age, gender and smoking have all been shown to have an effect on the prevalence of ANAs in the general population.12 However, these relations have not been investigated in COPD. We therefore investigated which patient characteristics (ie, smoking behaviour, gender, age, lung function and physical characteristics) determine the presence of (antinuclear) autoantibodies in COPD. In addition, as the increased numbers of B cells found in COPD lung tissue are likely to be involved in the generation of autoantibodies, we also analysed the antibody repertoire (immunoglobulin heavy chain) of lung tissue-derived B cells from patients with COPD and non-COPD controls.

METHODS
Lung tissue
Human lung tissue was obtained from surgical resection material of eight patients with COPD and eight without the disease.15 In case of tumours,
only lung tissue distant from the tumour was included. Tissue from patients with severe emphysema was obtained from lung resections in case of single or double lung transplantation or lung volume reduction surgery. COPD was confirmed by the combination of forced expiratory flow in 1 s (FEV1) and FEV1/forced vital capacity (FVC) ratio following GOLD criteria,14 and the presence of emphysema was based on histological examination of lung tissue performed by an experienced pulmonary pathologist (WT).

**Serum analysis**

A total of 252 serum samples were analysed for ANAs. Samples were derived from patients with COPD without (n=50) and with (n=94) a history of smoking and control subjects with normal lung function without (n=57) and with (n=71) a history of smoking. Age and gender were matched whenever possible in these four subject groups. The majority of the samples were obtained from two population-based studies—the European Community Respiratory Health Study (ELON)15 (84 samples) and the Burden Of Lung Disease (BOLD)16 (68 samples). Fifty-seven samples were obtained from a previous study which included age-matched controls17 and 25 samples were obtained from patients with COPD undergoing lung transplantation. Patients with asthma were excluded. None of the subjects had signs or symptoms of any autoimmune disease. All subjects were characterised by lung function, smoking status and smoking history (pack-years). Smoking status was indicated as never-smoker or ever-smoker, the latter including current and former smokers. Age, gender, height and weight were known for all subjects, except for the BOLD study where weight was not recorded.

**Autoantibody analysis**

Serum levels of three types of autoantibodies were measured: ANAs, antineutrophil cytoplasm antibody (ANCA) and antibodies against other tissue antigens (anti-mitochondrial, anti-smooth muscle, anti-parietal cell and anti-liver-kidney microsomal).

Indirect immunofluorescence (IIF) was performed to screen for ANAs according to standard routine diagnostic procedures as used at the Laboratory for Clinical Immunology, University Medical Center, Groningen, The Netherlands. A commercial kit (HEP2000 IFA kit, ImmunoConcepts, Sacramento, California, USA) was used according to the guidelines of the manufacturer’s specifications. For screening purposes, the ANA measurements were performed in two different dilutions (1:40 and 1:80). Samples that were positive in this screening assay were retested in twofold dilutions ranging from 1:40 to 1:640. This means that all positive samples were tested in duplicate. Serum samples with a titre of ≥1:80 were considered positive. Fluorescence patterns were reported as homogeneous, coarse speckled, fine speckled, nucleolar or the anti-SSA pattern that is typical of Hep2000 cells.

ANCAs directed against myeloperoxidase (anti-MPO antibodies) were determined by ELISA as described previously. IIF was performed to screen for antibodies against other tissue antigens according to standard routine diagnostic procedures as used for detection of anti-mitochondrial, anti-smooth muscle, anti-parietal cell and anti-liver-kidney microsomal autoantibodies at the Laboratory for Infectious Diseases, Groningen, The Netherlands.

**Analyses of IgH repertoire of B cells isolated from human lung tissue**

CD20-positive B cell follicles were harvested from 20 μm thick frozen sections by laser microdissection (PALM GmbH, Bernried, Germany). The IgH repertoire was then determined by PCR, cloning of the PCR pools in E coli and sequencing as described previously. Annotation of IgH CDR3 (length, translation, DH and JH families) was performed with V-Quest open access software.

**Statistical analysis**

A two-sided Fisher exact test was used to test the statistical significance of contingency tables. The two-tailed Mann–Whitney test was used for testing statistical significance of differences between the frequency of positively charged CDR3 amino acid residues.

ORs and 95% CIs for the association between the presence of ANAs and several independent variables were calculated using logistic regression analysis. The associations were studied univariately and after adjustment for age, gender, smoking, BMI, COPD status or country of residence if the variable was a confounder. A confounder was defined as a variable whose removal from the model caused a change in the estimated coefficient of at least 10% in multiple logistic regression analysis.

BMI was entered into the logistic regression model as a dichotomous variable defining ‘low BMI’ as <22 kg/m². This provided the best goodness of fit using Akaike information criteria compared with models with BMI as a continuous variable or as a categorical variable (<22, 22–24, 24–26, 26–28 and ≥28 kg/m²).

Statistical testing was performed at a significance level of p=0.05. SPSS software Version 14.0 was used for statistics.

**RESULTS**

**Participant characteristics**

Table 1 shows the characteristics of never-smokers and ever-smokers among subjects with and without airway obstruction. The never-smoking COPD patient group contained significantly more women and were older than the other groups and generally had less severe COPD compared with the COPD ever-smoker group.

**Effect of smoking, COPD (severity), gender, age and lung function on the presence of ANAs**

Figure 1A shows that significantly more patients with COPD than controls were serum positive for ANAs (44% vs 22%, p=0.001). This difference between COPD and non-COPD controls remained significant in subgroups of never-smokers (4/30 ANA-positive in COPD group vs 7/37 in non-COPD group; p=0.019) and of ever-smokers (40/94 ANA-positive in COPD group vs 17/71 in non-COPD group; p=0.013). Within the COPD group there was no significant difference in the percentage positive for ANAs between never-smokers and ever-smokers (p=0.855).

The median titre in both the ANA-positive COPD group and the ANA-positive control group was 1:80. The most frequently observed fluorescence pattern was coarse speckled (81% of patients with COPD and 80% of controls), although other samples showed a nucleolar pattern.

Table 2 shows the univariate and adjusted ORs for ANAs for the independent variables analysed. COPD significantly increased the probability of being ANA-positive (OR 5.12, 95% CI 1.68 to 7.56). Gender, age and smoking status had no significant effect on the presence of ANAs. FEV1% predicted, FEV1/FVC ratio and pack-years of smoking had no relationship with the presence of ANAs (table 2).
Effect of BMI and disease on autoantibodies

Figure 2 shows the distribution of subjects and the frequency of subjects serum positive for ANAs categorised according to BMI. In the COPD group, those in the lowest BMI category (<22 kg/m²) had a high chance of being serum positive for ANAs (figure 2D), but this was not the case for non-COPD controls (figure 2C).

Figure 1B shows that patients with COPD with low BMI had significantly higher probability (0.11–1.56) of being ANA-positive than patients with normal BMI (p=0.017); the OR for low BMI was 4.93 (95% CI 1.50 to 16.15) (table 2).

Subjects with both COPD and a low BMI (n=16) had a much higher risk of being ANA-positive than subjects with normal BMI (OR 8.61, 95% CI 2.21 to 33.59). For subjects with COPD and normal BMI (n=68), no significant association was found with the presence of ANAs (OR 1.81, 95% CI 0.66 to 4.94). However, this tendency towards an interaction between COPD and BMI was not significant in a logistic regression model (p=0.273). In healthy subjects there was no difference between ANA serum levels for low BMI compared with normal BMI (OR 1.11).

ANCAs and other autoantibodies

ANCAs were measured in 46 patients with COPD and 8 non-COPD controls and all samples were negative. Anti-mitochondrial, anti-smooth muscle, anti-parietal cell and anti-liver-kidney microsomal were measured in 12 patients with COPD and all samples were negative.

Antibody repertoire of B cells in the lung

We examined the antibody repertoire sequences of B cells in the lung in order to test for characteristics of self-reactivity: Self-reactive antibodies against DNA and nuclear targets (ANAs) have a high frequency of cationic residues in the hypervariable part of the heavy chain, the complementarity-determining region 3 (IgH-CDR3). 21–23

First, IgH sequences of antibodies against nuclear antigens from published data were compared with non-self-reactive antibodies to quantify this characteristic for ANA reactivity. Antibodies from patients and control subjects were cloned, sequenced and the ANA reactivity for each monoclonal was tested by the HEp-2 assay. 24 25 HEp-2-reactive antibodies

Table 1 Characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Healthy never smokers</th>
<th>Healthy ever smokers</th>
<th>COPD never smokers</th>
<th>COPD ever smokers</th>
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<tbody>
<tr>
<td><strong>(A) Grouped by disease status and smoking history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Subjects (n)</td>
<td>37</td>
<td>71</td>
<td>30</td>
<td>94</td>
</tr>
<tr>
<td>Women (%)</td>
<td>54.1</td>
<td>45.1</td>
<td>80.0</td>
<td>36.2</td>
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<tr>
<td>Age (years)</td>
<td>55.9 ± 8.5</td>
<td>55.7 ± 9.3</td>
<td>68.1 ± 10.3</td>
<td>60.4 ± 7.3</td>
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<td>FEV₁/FVC (%)</td>
<td>112.6 ± 20.3</td>
<td>101.2 ± 16.1</td>
<td>73.0 ± 20.7</td>
<td>58.8 ± 23.2</td>
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<td>Current smokers (%)</td>
<td>—</td>
<td>46.5</td>
<td>—</td>
<td>46.8</td>
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<tr>
<td>Pack-years smoking</td>
<td>—</td>
<td>29.6 ± 39.6</td>
<td>0</td>
<td>36.9 ± 18.7</td>
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<td><strong>(B) Grouped by disease status and body mass index (BMI).</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects (n)</td>
<td>55</td>
<td>6</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 3.3</td>
<td>20.9 ± 0.6</td>
<td>26.8 ± 3.4</td>
<td>21.1 ± 1.3</td>
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<tr>
<td>Women (%)</td>
<td>40.0</td>
<td>66.7</td>
<td>33.8</td>
<td>50.0</td>
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<tr>
<td>Age (years)</td>
<td>56.4 ± 7.2</td>
<td>53.3 ± 4.1</td>
<td>60.0 ± 6.9</td>
<td>59.9 ± 7.8</td>
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<tr>
<td>FEV₁/FVC ratio × 100</td>
<td>110.8 ± 17.5</td>
<td>108.2 ± 5.9</td>
<td>56.4 ± 21.5</td>
<td>52.3 ± 22.6</td>
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<tr>
<td>Pack-years smoking</td>
<td>14.7 ± 14.9</td>
<td>16.8 ± 10.5</td>
<td>36.3 ± 20.5</td>
<td>34.6 ± 18.0</td>
</tr>
</tbody>
</table>

Values are expressed as percentage or mean ±SD.

BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV₁%, forced expiratory volume in 1 s (% predicted); FVC, forced vital capacity.

Figure 1 Antinuclear antibodies (ANAs) in patients with chronic obstructive pulmonary disease (COPD) and healthy controls grouped by (A) disease status and smoking history and (B) disease status and BMI. For each group the percentage of patients positive for ANAs is given.
Table 2 Univariate and adjusted ORs for the presence of antinuclear antibodies

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.05 (1.02 to 1.08)</td>
<td>1.02 (0.98 to 1.06)</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>1.04 (0.61 to 1.79)</td>
<td>0.888 (0.60 to 1.42)</td>
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<tr>
<td>COPD status (COPD/no COPD)</td>
<td>2.69 (1.52 to 4.74)</td>
<td>3.12 (1.68 to 5.76)</td>
</tr>
<tr>
<td>Smoking status</td>
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<td></td>
</tr>
<tr>
<td>Never</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Ex-smoking</td>
<td>1.02 (0.52 to 2.03)</td>
<td>1.62 (0.44 to 7.47)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.32 (0.66 to 2.64)</td>
<td>2.06 (0.48 to 8.82)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>1.00 (0.99 to 1.01)</td>
<td>1.01 (0.98 to 1.03)</td>
</tr>
<tr>
<td>BMI (&lt;22/≥22)</td>
<td>3.56 (1.39 to 9.09)</td>
<td>3.63 (1.34 to 9.86)</td>
</tr>
</tbody>
</table>

|                          | COPD group |          |
| Age (years)              | 1.03 (0.98 to 1.07) | 0.97 (0.90 to 1.04) |
| Gender (F/M)             | 0.97 (0.47 to 1.97) | 0.49 (0.15 to 1.58) |
| FEV1%pred                | 1.01 (1.00 to 1.03) | 1.00 (0.97 to 1.03) |
| FEV1/FVC ratio (%)       | 0.99 (0.96 to 1.02) | 0.98 (0.93 to 1.04) |

| Smoking status           |           |          |
| Never                    | Reference | Reference |
| Ex-smoking               | 0.64 (0.26 to 1.61) | 1.71 (0.16 to 18.32) |
| Current smoking          | 1.14 (0.45 to 2.90) | 2.63 (0.24 to 28.93) |
| Pack-years               | 1.00 (0.98 to 1.01) | 0.99 (0.96 to 1.03) |
| BMI (<22/≥22)            | 4.30 (1.37 to 13.47) | 4.93 (1.50 to 16.15) |

*Adjusted for age, gender, COPD status, smoking status, low BMI or country of residence if the variable was a confounder of the studied association.

BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV1%, forced expiratory volume in 1 s (% predicted); FVC, forced vital capacity.

Figure 2  Body mass index (BMI) distribution and antinuclear antibodies (ANAs) in patients with chronic obstructive pulmonary disease (COPD) and controls with normal lung function. Distribution of subjects stratified by BMI in (A) the control group (n=61) and (B) the COPD group (n=84). Values are given as percentage of total subjects in each group. (C,D) Percentage of ANA-positive subjects within each category for (C) controls and (D) patients with COPD.

Table 3 shows the patient characteristics. The VDJ regions of the rearranged IgH alleles were amplified by PCR, cloned and sequenced (figure 3A,B). In total, we found 138 unique CDR3 (protein) sequences, 79 of which were derived from patients with COPD and 59 from non-COPD controls. Lung B cell clones

(n=166) contained, on average, 7.8% cationic residues in IgH-CDR3 while antibodies not directed against nuclear antigens (n=265) only had 5.7% cationic residues (p<0.021, figure 3D). B cell aggregates were isolated by laser microdissection from lung sections of patients with COPD and non-COPD patients.

Figure 2  Body mass index (BMI) distribution and antinuclear antibodies (ANAs) in patients with chronic obstructive pulmonary disease (COPD) and controls with normal lung function. Distribution of subjects stratified by BMI in (A) the control group (n=61) and (B) the COPD group (n=84). Values are given as percentage of total subjects in each group. (C,D) Percentage of ANA-positive subjects within each category for (C) controls and (D) patients with COPD.
from patients had, on average, 10.0% cationic CDR3 residues which was significantly more than in non-COPD patients (6.7%, \( p < 0.013 \); figure 3C).

**DISCUSSION**

It has been reported previously that a significant number of patients with COPD have antibodies against self-antigens but some apparently do not develop an autoimmune response. We have quantified the fraction of patients that are positive for ANAs and other autoantibodies, and investigated which characteristic of the patient population is associated with higher ANAs. We found that 44% of the patients with COPD were ANA-positive, significantly more than the prevalence of 22% in controls. The presence of ANAs was not associated with smoking behaviour. Among the patients with COPD, higher ANAs were more often found in patients with low BMI. Finally, we observed that the antibody repertoire of B cells in the lungs of patients with COPD has a high frequency of positively charged CDR3 residues, a feature which is associated with self-reactive antibodies.

A significant group of patients with COPD has been reported to carry serum autoantibodies against elastin or antinuclear targets by three independent studies, whereas other reports have not shown an antibody response against elastin in patients with COPD. Feghali-Bostwick and coworkers reported that 68% of 47 patients with COPD were serum positive for ANAs whereas Hodson and Turner-Warwick found 28% of 50 patients with severe chronic bronchitis to be serum positive for ANAs. Our study in a larger population of patients with COPD (n=124) shows that 44% were ANA positive. Thus, although the sample sizes are small, all these studies show that a significant group of

**Table 3** Characteristics of patients and controls contributing surgical resection material

<table>
<thead>
<tr>
<th></th>
<th>COPD patients</th>
<th>Non-COPD controls</th>
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</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Women (%)</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.4±5.7</td>
<td>59.4±7.8</td>
</tr>
<tr>
<td>FEV1/FVC ratio (%)</td>
<td>35.0±24.8</td>
<td>85.5±16.3</td>
</tr>
<tr>
<td>Ex/no smokers</td>
<td>8/0</td>
<td>4/4</td>
</tr>
<tr>
<td>Pack-years</td>
<td>35.0±24.8</td>
<td>12.2±14.0</td>
</tr>
</tbody>
</table>

Number, average values and SDs are shown.

FEV1%, forced expiratory volume in 1 s (% predicted); FVC, forced vital capacity.

Figure 3 IgH repertoire analysis of lung-infiltrated B cells. (A) B cell follicles in 20 \( \mu \)m thick frozen lung sections were stained (CD20) and harvested by laser microdissection. (B) DNA was isolated and IgH CDR3 regions were amplified in semi-nested PCR. Oligonucleotides are shown by short arrows. The oligoclonal pool of CDR3 amplicons was then cloned individually in \( E. \text{coli} \), sequenced and the amino acid composition of the CDR3 was determined. (C) Frequency of cationic amino acids (shown in %) in the CDR3 region of antibodies sequenced from lung-infiltrated B cells. Each dot represents the average values of 10–30 antibodies from one patient. (D) Frequency of cationic amino acids (shown in %) in the CDR3 region of antibodies from Barbas et al\(^{19}\) and Wardemann et al\(^{20}\). These were characterised for reactivity against antinuclear targets. Each represents the average value of 10–50 antibodies from one subject.
patients with COPD develops an autoimmune response against
antinuclear targets. The clinical relevance should now be tested in
larger cohorts.

It remains unclear how immune tolerance is broken in patients
with COPD and autoimmunity against nuclear antigens arises.
Our data on lung tissue suggest that lung B cells may play a role.
We show for the first time that lung B cells of patients with
COPD produce antibodies that carry a high frequency of posi-
tively charged amino acids in the IgH-CDR3, a feature which is
also observed with ANAs. We therefore hypothesise that the
development of COPD is associated with infiltration of B cells in
the lung that are directed at infectious material or residues of
destroyed lung tissue. B cell follicular structures then arise in
which germinal centres can develop, enabling B cell isotype
switching and affinity maturation by somatic hypermutations.
With ongoing smoking, long-term inflammation and increasing
lung damage in COPD, inflammatory cells are exposed to nuclear
antigens derived from damaged epithelial cells and probably
many other antigenic protein residues of damaged cells and
structures.25 26 This exposure may last for years, even after
smoking cessation,26 and can cause the unintended recognition
of (nuclear) antigens by somatically hypermutated antibodies
that were originally targeted to non-self antigens.

Our data thus suggest that B cells in the lungs of patients
with COPD generate antibodies (or antibody receptors) which
share a feature that is characteristic for ANAs—positively
charged residues in the IgH CDR3 region, one of the regions in
antibodies that is critical for antigen binding. Of interest, B cells
present in the lungs of non-COPD patients have this charac-
teristic significantly less frequently.

As smoking was found to be related to the prevalence of ANA
in a large cohort study of the general (non-COPD) population13
and as COPD is recognised mostly as a smoking-related disease,
one might expect that the autoantibody levels are related to
smoking history in COPD as well. In our cohort of patients with
COPD and controls we did not find such a relationship.
This could be due to sample size limitations because we analysed only
232 subjects compared with 2875 subjects in the above-
mentioned cohort study. Furthermore, differences in study
design and in genetic background (Caucasian vs Japanese) may
have played a role.

Our findings support the hypothesis that the presence of
autoantibodies is associated with COPD and not (or not only)
with smoking per se. First, the presence of ANAs in patients
with COPD was not associated with smoking history, smoking
status or pack-years of smoking. Second, our group of non-
smoking patients with COPD had a significantly higher preva-
lence of ANAs than the non-smoking controls. It therefore
seems likely that the increased presence of autoantibodies in
COPD is also associated with the disease itself and is not only
caused by smoking. As indicated above, this should be further
validated by testing in a larger cohort of patients with COPD
and controls.

Within the COPD study group we found no association of
ANAs with lung function or with COPD stage as defined by
GOLD criteria. This is in agreement with data reported previ-
ously50 and suggests that an autoimmune response is associated
with having developed COPD as such and not with the severity
of the disease.

Interestingly, our study showed that the presence of ANAs
was significantly associated with a low BMI. Patients with
COPD with a BMI <22 kg/m² had a 4.9 times higher proba-
bility of being ANA-positive whereas this was not the case in
non-COPD controls. Thus, an association between COPD and
autoimmunity occurs particularly in people with a low BMI.
Although we only observed a significant positive association
between a low BMI and the presence of ANAs in subjects with
COPD (OR 4.3, 95% CI 1.4 to 13.5) and not in subjects
without COPD (OR 1.2, 95% CI 0.1 to 11.4), we found no
statistical proof of effect modification when an interaction
term of COPD and low BMI was included in the logistic
regression analysis. This is probably due to the low number of
available subjects with low BMI, especially in the group
without COPD (n=6).

An autoantibody response has recently been associated with
low BMI in patients with COPD.6 Using immunoprecipitation
of antibodies purified from COPD patients with human cells,
Feghali-Bostwick et al identified a 130 kD autoantigen which
was associated with low BMI in patients with COPD.6 It is
possible that this 130 kd autoantigen is a nuclear antigen which
is also picked up in the ANA assay. This could partly explain
why patients with COPD with low BMI are ANA-positive. A
possible way to investigate this further is to isolate or clone
antibodies from patients with COPD and test their reactivity
against both antinuclear (eg, Hep-2 or antigen-specific nuclear
targets) and 130 kD and other proteins in immunoprecipitation
assays. Second, our findings are interesting in view of a previous
observation50 that patients with COPD with a predominantly
emphysematous phenotype (ie, low attenuation areas on the CT
scan) had a significantly lower BMI range (20.2±2.8 kg/m²) than
patients with predominantly airway thickening (23.0±2.6 kg/
²). It remains to be determined whether patients with COPD
with a high percentage of low attenuation areas are also ANA-
positive. Together these data suggest an association between
three characteristics shared by one specific group of patients
with COPD: an emphysema-dominant phenotype, low BMI and
self-reactivity against nuclear antigens.

Our findings may help to achieve a better understanding of
the multifactorial and complex disorder of COPD and to divide
this heterogeneous population into relevant phenotypes.
Previous data suggested the existence of a patient subpopulation
characterised by low BMI, an emphysema-dominant phenotype
and an unidentified autoimmune response. Our data extend
these observations and suggest that this subpopulation is asso-
ciared with an autoimmune response against antinuclear targets
and, additionally, that smoking behaviour and gender are not
linked to this subpopulation. It will be interesting to determine
whether the response to other autoantigens shares the same
characteristics. Interestingly, even non-smoking individuals with
lung function values compatible with COPD have increased
levels of ANAs. None of these subjects had other complaints or
clinical findings indicative of autoimmune disease. This suggests
that either environmental tobacco smoke or other environ-
mental factors or endogenous factors contribute to COPD
development and that this is associated with elevated ANAs.

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Antinuclear autoantibodies are more prevalent in COPD in association with low body mass index but not with smoking history


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