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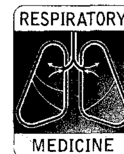

Peripheral blood lymphocyte cell subsets in subjects with chronic obstructive pulmonary disease: association with smoking, IgE and lung function

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In contrast to the numerous studies which show that lymphocytes play an important role in the pathogenesis of asthma, few studies have investigated the role of lymphocytes in the pathogenesis of chronic obstructive pulmonary disease (COPD). The aim of the present study was to investigate lymphocyte subsets in peripheral venous blood of smoking and non-smoking COPD patients and healthy controls. The interaction of smoking and IgE has also been assessed, and it was investigated whether a lower level of FEV_1 was associated with changes in lymphocyte subsets.

In the present study, peripheral venous blood lymphocyte subsets were investigated in 42 smoking and non-smoking, non-atopic subjects with a clear diagnosis of COPD (43–74 years) who all used bronchodilator therapy only, and in 24 normal, healthy control subjects (40–72 years).

No significant differences in lymphocyte subsets were found when either total groups or smoking subjects of both groups were compared. However, the percentage of $CD8^+$ lymphocytes (suppressor/ cytotoxic T-cells) was significantly higher in the non-smoking COPD subjects compared with the non-smoking, healthy control subjects (P<0·05). In addition, within the group of non-smoking COPD subjects, a higher CD4:CD8 ratio was associated with a higher FEV_1 as a percentage of predicted (% pred.) (r=0·55, P=0·01) and a lower total serum IgE (r= − 0·45, P=0·04). Within the group of smoking COPD subjects, a higher FEV_1 % pred. was associated with a higher percentage of $CD19^+$ lymphocytes (B-cells) (r=0·65, P<0·01).

The present study provides further evidence that the changes in the balance of T-cell subsets and IgE synthesis possibly plays a role in the pathogenesis of COPD.

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Introduction

Cigarette smoking is the most important risk factor for development and progression of chronic obstructive pulmonary disease (COPD) (1). However, only 15–20% of cigarette smokers develop clinically significant airways obstruction (2). Several mechanisms for an increased susceptibility in this subgroup of smokers have been suggested. Attention has been focused on the role of polymorphonuclear leukocytes (PMN) in the pathophysiology of COPD, as their numbers have been found to be increased in the bronchoalveolar lavage of smoking COPD patients (3–5). Cigarette smoke may directly recruit leukocytes to the lung, and indirectly via cytokine release from activated macrophages (6,7). Also, adhesion molecules, neuroendocrine cells and neuropeptides have been suggested as other possibly important factors in the pathogenesis of COPD (8,9).

Lymphocytes are known to modulate inflammatory processes in the airways of asthmatics (10–12). However, little has been published about their role in the pathogenesis of COPD (13–15). Moreover, smoking and non-smoking patients with different characteristics had been included in these studies (13–15), preventing firm conclusions. Studies in *healthy* subjects have

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demonstrated a considerable influence of cigarette smoking, age and gender on lymphocyte subsets (16–20). Since a distinct characterization of the patients in the latter studies has not been performed, it is not clear whether patients with COPD were included.

To investigate a role of lymphocytes and their activation in the pathogenesis of COPD, a study was designed to compare lymphocyte subsets in peripheral venous blood of smoking and non-smoking COPD patients with healthy controls. As smokers do have higher serum immunoglobulin E (IgE) levels, and high IgE levels have been associated with the development of COPD (21), the interaction of smoking and IgE have also been assessed. Finally, it was investigated whether a lower level of FEV_1 was associated with changes in lymphocyte subsets.

Methods

SUBJECTS

All COPD patients selected for this study met the following inclusion criteria: (1) aged 40 years or older, current, former or never-smokers without a history of asthmatic attacks, reporting either chronic cough with or without sputum production or dyspnoea when walking quietly on level ground, or both (22), and without other major diseases; (2) no atopy, defined by negative skin tests (Diephuis Laboratories, Groningen, The Netherlands), no detectable specific serum IgE to house dust mite (HDM), and total serum IgE levels <350 IU ml^{-1}; (3) forced expiratory volume in 1 s (FEV_1) more than 1 l and less than 70% of predicted (% pred.), without medication for the previous 8 h; FEV_1 after 40 mg ipratropium bromide less than 80% pred.; (4) the concentration of methacholine causing a 20% decrease of FEV_1 from baseline (PC_{20}) less than 8 mg ml^{-1}; (5) no upper respiratory tract infection or exacerbation within 6 weeks before the start of the study.

Control subjects were normal, smoking or non-smoking, healthy volunteers of either sex, aged 40 years or older taking no medication, without a history of atopy or bronchial obstruction and therefore not satisfying the ATS criteria for asthma or COPD (22), with no detectable specific IgE to HDM, and total serum IgE within the normal range. The FEV_1 had to be greater than 80% pred.

Chronic obstructive pulmonary disease patients visited the hospital on two separate days. On the first day, lung function and PC_{20} methacholine were assessed. Theophylline was discontinued for 48 h, and other bronchodilators were discontinued for 8 h before each visit to the hospital. The use of inhaled corticosteroids was stopped 4 weeks before the start of the study; oral corticosteroids, cromolyn sodium, nedocromil sodium or anti-histamines were not used. Within 2 weeks, on the second day, peripheral venous blood was drawn to investigate the lymphocyte subsets. The control subjects visited the hospital on 1 day. After an extensive history had been taken to exclude any pulmonary symptoms, lung function was carried out, and blood was withdrawn for determination of specific and total serum IgE and lymphocyte subsets. Subjects, either COPD patients or healthy controls, who smoked cigarettes at the time of the study were considered to be 'current' smokers; all others were considered to be non-smokers (smoking cessation >1 yr or never-smokers). The study was approved by the Hospital Medical Ethics Committee and all subjects gave their written informed consent to participate.

BRONCHIAL PROVOCATION

Spirometry was performed using a calibrated water-sealed spirometer (Lode BV, Groningen, The Netherlands) according to standardized guidelines. Baseline FEV_1 and forced vital capacity (FVC) were measured until three reproducible recordings were obtained. Highest values were used for analysis. Reference values are those of the European Community for Coal and Steel (23). FEV_1 was expressed as a percentage of predicted FEV_1 (FEV_1 % pred.).

A solution of methacholine bromide was administered as an aerosol generated from a starting volume of 3 ml in a DeVilbiss 646 nebulizer (De Vilbiss Co., Somerset, PA, U.S.A.), connected to the central chamber of an inspiratory–expiratory valve box. Solution output was 0·13 ml min^{-1}. After inhalation of 0·9% sodium chloride, subjects inhaled doubling concentrations of methacholine bromide for 2 min, ranging from 0·0367 to 256 mg ml^{-1}, at 5-min

intervals. FEV_1 was measured 30 and 90 s after each inhalation until it was less than 80% of the pre-challenge value. PC_{20} values were determined by linear interpolation between the last two data points on the logarithmic concentration–response curve.

SAMPLING OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral venous blood samples (30 ml) were anticoagulated with EDTA. Blood mononuclear cells were separated using 'Ficoll-Paque' density gradient centrifugation (Pharmacia Fine Chemicals). Mononuclear cells were resuspended in ammonium chloride for 5 min at 4°C, and centrifugated at 970 ***g*** for 5 min at the same temperature. Subsequently, the mononuclear cells were washed twice in phosphate-buffered saline (PBS) and 0·1% glucose, and centrifugated at 970 ***g*** for 5 min, and subsequently at 170 ***g*** for 10 min, both at 4°C. The mononuclear cell concentration was determined by using a Coulter Counter, and viability, as tested by Trypan blue exclusion, was always higher than 95%. Suspensions of 100 ml cells ($0{\cdot}4 \times 10^6$ cells ml^{-1} in PBS) were taken for further lymphocyte-subset determination by flow cytometry.

MONONUCLEAR CELL STAINING AND FLOW CYTOMETRY

Isolated mononuclear cells were prepared for two-colour immunofluorescence analysis with monoclonal antibodies anti-CD3 (Leu-4), anti-CD4 (Leu-3a), anti-CD8 (Leu-2a), anti-CD19 (Leu-12), CD45RO (UCHL1), anti-CD56 (Leu-19) and anti-CD16 (Leu-11c) (Becton-Dickinson, Mountain View, CA, U.S.A.) conjugated to phycoerythrin (PE) or fluorescein isothiocyanate (FITC) to identify T-cells, T-helper/inducer cells ($CD4^+$), T-cytotoxic/suppressor cells ($CD8^+$), B-cells ($CD19^+$), activated or memory T-cells ($UCHL1^+$) and natural killer (NK) cells, respectively. The final cell concentration in each sample was adjusted to 1×10^6 cells ml^{-1} in PBS and 0·5% bovine serum albumin (BSA). The cells were centrifugated at 530 ***g*** for 5 min at 10°C and subsequently incubated for 30 min at 4°C with appropriate dilutions of monoclonal antibodies. Unspecific binding was checked using FITC and PE-labelled control antibodies. After the cells had been washed twice with PBS and 0·5% BSA, they were centrifugated at 530 ***g*** for 5 min at 4°C. After the addition of PBS with 0·5% BSA, the incubated cells were stored in the dark at 4°C until analysis.

Flow cytometry was performed on a Becton-Dickinson FACS Analyser, equipped with an argon laser and interfaced with a Hewlett-Packard Consort 30 computer. For each (combination of) monoclonal antibody (antibodies), 10 000 mononuclear cells were counted and gated with forward and sideward scatter to detect lymphocytes from other leukocytes. Subsequently, the lymphocyte gate was set on the image of the combined monocyte marker CD14 (Leu-m3) and the total leukocyte marker CD45 (Hle-1) scatter in order to exclude contamination from erythrocytes and debris, and to exclude monocytes and granulocytes.

MEASUREMENT OF TOTAL SERUM IgE

Total serum IgE (expressed in IU ml^{-1}) was measured using an enzymatic *in vitro* test system (Phadezym RAST®) based on the Radio Allergo Sorbent Test principle for determination of circulating IgE antibodies in human serum in a sample of blood (normal values <120 IU ml^{-1}, but smokers may have higher values).

STATISTICAL ANALYSIS

Skewness of distributions was assessed with Kolmogorov-Smirnov tests. To get a normal distribution, IgE was logarithmically transformed, which was also necessary for the proportion of CD19 positive lymphocytes by using a logistic transformation for proportions (*p*): $y=\ln[p/(1-p)]$. Lymphocyte cell subsets are expressed as a percentage of lymphocytes, whereas $UCHL1^+$ CD3 or $UCHL1^+$ CD4 cells are expressed in percentages of the proportion of $CD3^+$ or $CD4^+$ T-cells which is UCHL1 positive. PC_{20} values were analysed after base 2 logarithmic transformation, one log unit being one dose step in concentration.

Differences between (sub)groups of patients with COPD and healthy control subjects were

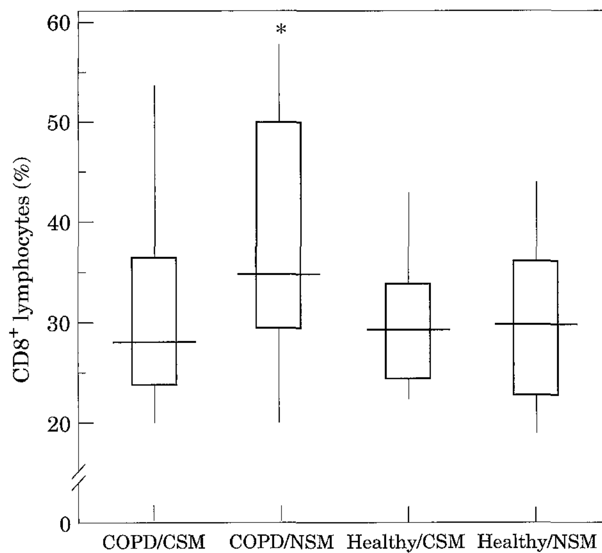

FIG. 1. *Non-smoking chronic obstructive pulmonary disease (COPD) patients with a significantly higher percentage of $CD8^+$ lymphocytes than non-smoking healthy controls ($P<0.05$). Horizontal lines represent median values, bars represent the interquartile ranges and vertical lines represent the full range of values. CSM, current smokers; NSM, non-smokers.

determined by analysis of co-variance taking *age, gender* and *pack-years* as co-variables. Multiple linear regression analysis with stepwise method was used to identify variables or groups of variables that were most strongly associated with the dependent variable FEV_1 % pred. Two-tailed tests have been used throughout at the 95% level of significance. All analysis were performed with the SPSS/PC+ package (24). All the data are expressed in mean ± SD, except for the data presentation in Fig. 1 for which medians and interquartile ranges were also used.

Results

PATIENTS

Forty-two COPD patients and 24 normal, healthy, control Caucasian subjects satisfied the inclusion criteria. Healthy control subjects differed significantly in that they were younger, had a smaller number of pack-years and better lung function (selection criterion). This was also the case when the smoking, healthy control subjects were compared with the smoking COPD patients, whereas the non-smoking, healthy controls only had a significantly better lung function (Table 1).

Within the COPD group, current smokers had a significantly higher total serum IgE than non-smokers. Within the group of non-smoking COPD patients, total serum IgE turned out to be negatively associated with FEV_1 % pred. ($r=-0.44$, $P=0.04$). This association was not found within the other three subgroups. Within the group of healthy controls, the current smokers were significantly younger and had higher total serum IgE than the non-smokers.

LYMPHOCYTE MARKERS

Differences Between COPD and Healthy Control (Sub)Groups (Fig. 1 and Table 2)

Mean percentages of lymphocyte cell subsets of the COPD and healthy control groups were not significantly different. No significant differences were found between smoking COPD patients and smoking healthy controls. Non-smoking COPD patients had a significantly higher percentage of $CD8^+$ lymphocytes than non-smoking healthy controls (38.2 ± 11.7 and 30.2 ± 7.8, respectively, $P<0.05$). No significant differences in activation of $CD3^+$ or $CD4^+$ lymphocytes between the COPD patients and healthy controls, or between the four subgroups was found.

Relationship with Smoking (Table 2)

Within the healthy control group, smokers had a significantly lower percentage of $CD16^+/CD56^+$ lymphocytes than non-smokers (12.7 ± 4.1 and 16.7 ± 4.2, respectively, $P<0.05$). The percentage of $CD19^+$ lymphocytes was not significantly different (12.1 ± 4.4 and 9.2 ± 4.2, respectively). Otherwise, lymphocyte cell subsets were comparable. Within the COPD group, smokers had a significantly higher percentage of $CD19^+$ lymphocytes than non-smokers (12.3 ± 5.6 and 7.5 ± 3.3, respectively, $P<0.01$). The percentage of $CD16^+/CD56^+$ lymphocytes was not significantly different (13.4 ± 7.5 and 17.8 ± 7.6, respectively).

Relationship with FEV_1 % pred. (Figs 2 and 3)

When pooling patients with COPD and healthy controls in a multiple linear regression model,

TABLE 1. Subject characteristics

Subjects with COPD	Current smokers (n=21)	Non-smokers (n=21)
Age (years)	58·8 ± 8·0	62·1 ± 6·9
Gender (F/M)	1/20	2/19
FEV_1 (% pred.)	49·1 ± 12·5	49·4 ± 11·6
FEV_1/FVC (%)	45·1 ± 10 5	46·5 ± 11·6
Reversibility (ΔFEV_1 % pred.)	8·4 ± 5·0	5·4 ± 3·3
PC_{20} methacholine (mg ml^{-1})*	0·3	0·5
Total IgE (IU ml^{-1})*	60·9†	35·2
Atopy	Negative	Negative
Current/former/never-smoking	21/0/0	0/16/5
Cigarettes (day^{-1})	14·1 ± 9·9	0
Pack-years	24·2 ± 8·8	18·8 ± 15·3
Healthy control subjects	**Current smokers (n=9)**	**Non-smokers (n=15)**
Age (years)	46·1 ± 5·3‡‖	57·5 ± 8·8
Gender (F/M)	1/8	2/13
FEV_1 (% pred.)	103·9 ± 10·4‖	106·6 ± 10·6§
FEV_1/FVC (%)	76·5 ± 5·3‖	76·0 ± 6·6§
Total IgE (IU ml^{-1})*	53·5	32·1
Atopy	Negative	Negative
Current/former/never-smoking	9/0/0	0/14/1
Cigarettes (day^{-1})	11·8 ± 7·1	0
Pack-years	13·4 ± 9·7‖	13·0 ± 11·0

COPD, chronic obstructive pulmonary disease.
*Geometric mean.
†P significantly different in smoking compared with non-smoking COPD subjects (P<0·05).
‡P significantly different in smoking compared with non-smoking, healthy control subjects (P<0·01).
§P significantly different in non-smoking, healthy controls compared with non-smoking COPD subjects (P<0·01).
‖P significantly different in smoking, healthy controls compared with smoking COPD subjects (P<0·01).

FEV_1 % pred. was negatively associated with smoking (B= − 15·2, P=0·03), age (B= − 1·2, P=0·004), pack-years (B= − 0·5, P=0·06) and $CD8^+$ lymphocytes (B= − 0·7, P=0·04) (r^2=0·32), suggesting that non-smoking patients with low FEV_1 (COPD) have a higher percentage of $CD8^+$ lymphocytes. Within the group of non-smoking COPD patients, FEV_1 % pred. was significantly associated with the percentage of $CD4^+$ lymphocytes (r=0·58, P=0·007) and the CD4:CD8 ratio (r=0·55, P=0·01) (Fig. 2), also without the outlier (CD4:CD8 ratio 3.1). These associations were not significant in the group of smoking COPD patients, and the groups of non-smoking and smoking, healthy control subjects.

Within the group of smoking COPD patients, FEV_1 % pred. was significantly associated with the percentage of $CD19^+$ lymphocytes (r=0·65, P<0·01) (Fig. 3). No significant association

TABLE 2. Lymphocyte subsets in subjects with chronic obstructive pulmonary disease (COPD) and healthy controls, subdivided into smokers and non-smokers

Cell surface antigen	COPD smokers	COPD non-smokers	Healthy control smokers	Healthy control non-smokers
CD3	69·0 ± 10·9	70·3 ± 10·3	71·4 ± 8·1	70·0 ± 8·1
CD4	41·1 ± 10·4	36·9 ± 10·4	44·0 ± 5·2	43·1 ± 8·1
CD8	31·2 ± 9·6	38·2 ± 11·7§	29·9 ± 6·4	30·2 ± 7·8
CD4:CD8 ratio	1·5 ± 0·6	1·1 ± 0·6	1·5 ± 0·4	1·6 ± 0·6
CD19	12·3 ± 5·6	7·5 ± 3·3‡	12·1 ± 4·4	9·2 ± 4·2
CD16/CD56	13·4 ± 7·5	17·8 ± 7·6	12·7 ± 4·1	16·7 ± 4·2†
UCHL1 CD3*	62·2 ± 13·8	56·3 ± 12·6	59·4 ± 13·1	58·7 ± 12·0
UCHL1 CD4*	65·2 ± 17·3	59·3 ± 15·7	61·9 ± 15·2	58·3 ± 16·3

Values are expressed as a percentage of lymphocytes.
*Percentage of the proportion $CD3^+$ or $CD4^+$ T-cells which is UCHL1 positive.
†$P<0{\cdot}05$ comparing healthy control non-smokers with healthy control smokers.
‡$P<0{\cdot}01$ comparing COPD non-smokers with COPD smokers.
§$P<0{\cdot}05$ comparing COPD non-smokers with healthy control non-smokers.

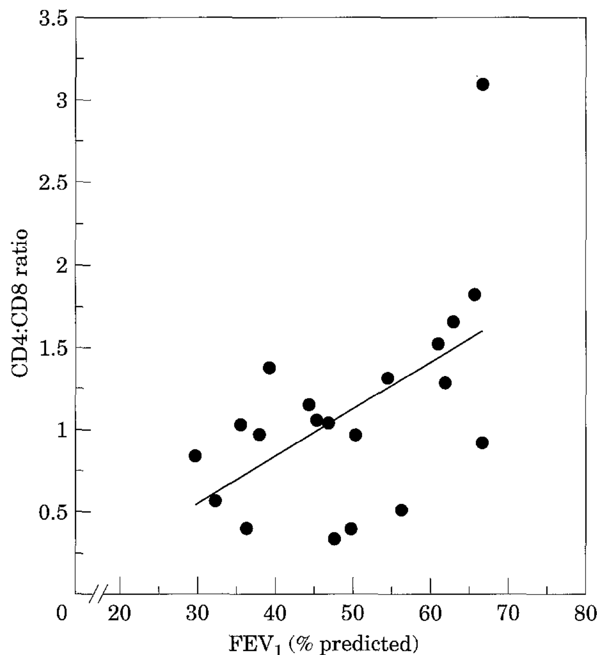

FIG. 2. Relationship between FEV_1 % predicted and the CD4:CD8 ratio within the group of non-smoking chronic obstructive pulmonary disease patients (r=0·55, P=0·01).

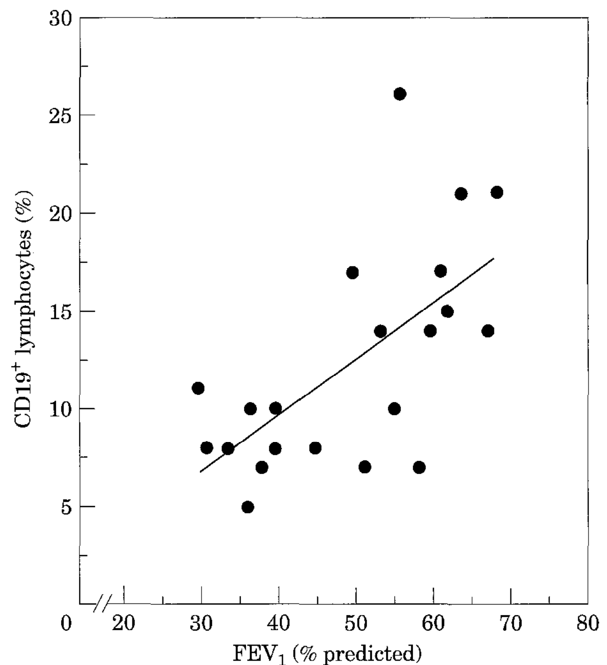

FIG. 3. Relationship between FEV_1 % predicted and $CD19^+$ lymphocytes within the group of smoking chronic obstructive pulmonary disease patients (r=0·65, P<0·01).

between FEV_1 % pred. and $CD19^+$ lymphocytes was found within other (sub)groups.

Relationship with Serum IgE (Fig. 4)

Within the group of non-smoking COPD subjects, total serum IgE was negatively associated with the percentage of $CD4^+$ lymphocytes (r= − 0·48, P=0·03) and with the CD4:CD8 ratio (r= − 0·45, P=0·004) (Fig. 4).The association between the percentage of $CD8^+$ lymphocytes and total serum IgE was more pronounced within the group of smoking COPD

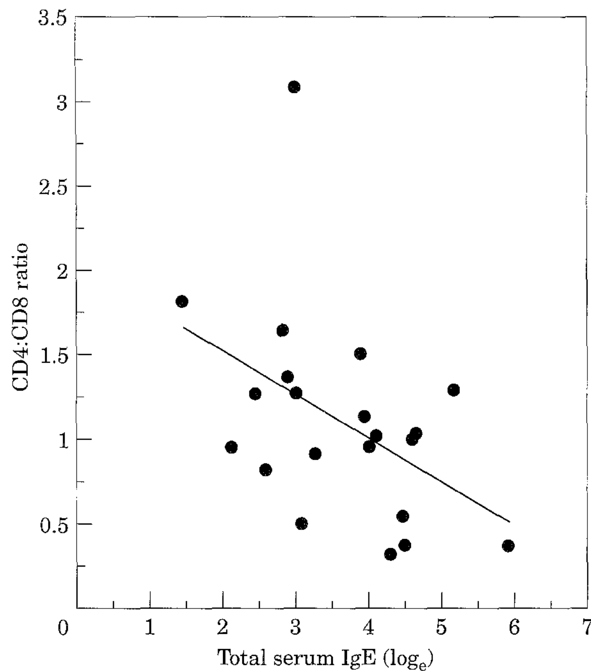

FIG. 4. Relationship between total serum immunoglobulin E and CD4:CD8 ratio within the group of non-smoking chronic obstructive pulmonary disease patients ($r = -0{\cdot}45$, $P=0{\cdot}04$).

subjects ($r=0{\cdot}50$, $P=0{\cdot}02$). No other significant association between total serum IgE and lymphocyte subsets was found within other (sub)- groups.

Discussion

This study has investigated peripheral venous blood lymphocyte subsets of non-atopic individuals with COPD and healthy controls in order to find evidence that lymphocytes may be implicated in the disease entity, COPD. Mean percentages of lymphocyte cell subsets of the COPD and healthy control groups as a whole were not significantly different. No significant differences in lymphocyte subsets were observed between smoking COPD patients and smoking healthy controls. This may be due to the fact that current smoking itself affects the proportion of lymphocyte cell subsets (16–20), and that non-smoking patients with COPD may be more severely affected by their disease as has been suggested by Fletcher *et al.* (2). By comparing non-smoking groups, a *clearer* interpretation of differences in lymphocyte cell subsets in smoking-affected and non-affected individuals is possible. The present results show that the percentage of $CD8^+$ lymphocytes was significantly higher in the non-smoking COPD subjects (Fig. 1).

In contrast to the present findings in COPD, Walker *et al.* (11) found peripheral blood $CD8^+$ lymphocytes of non-smoking, non-atopic asthmatics to be decreased in blood, and increased in bronchoalveolar lavage fluid (BAL). In asthmatic patients who all developed early-phase responses after inhalation of allergen, Gonzalez *et al.* (12) found a discrepancy between lymphocyte subsets measured in peripheral blood and bronchoalveolar lavage, suggesting that mobilization of suppressor T-cells into the lung occurred after inhalation of allergen. Therefore, in the present study, it is not clear whether it may be assumed that peripheral blood lymphocyte cell subsets reflect ongoing processes in the lung. It is also conceivable that the higher percentages of peripheral venous blood lymphocyte subsets of non-smokers in the present study are in fact reflecting lower percentages of lymphocyte subsets in the lung. This would be in contrast to the findings of Fournier *et al.* (13) who found a significant increase in $CD8^+$ lymphocytes in the airway wall in *smoking* patients with chronic bronchitis when compared with healthy control subjects. However, the patients' characteristics of the study of Fournier *et al.* (13) are different from the non-smoking COPD patients of the present study in that they were heavy smokers with better lung function.

An explanation for the role of $CD8^+$ lymphocyte subsets in patients with COPD may be their involvement in immunity to infectious agents (25), and COPD patients are known to be vulnerable to infections. An increase of blood $CD8^+$ lymphocytes might thus reflect an overshooted reaction in an attempt of the immunological system to restitute normal immune functions. Blood $CD8^+$ lymphocytes have also been found to be increased in healthy heavy smokers (18,20). The increased $CD8^+$ lymphocytes returned to lower values when subjects stopped smoking. In the present study, it is conceivable that the $CD8^+$ lymphocytes of the non-smoking COPD subjects did not return to lower values after the moment they stopped smoking because they developed COPD.

Multiple regression analysis showed that non-smoking COPD patients with low FEV_1 have a significant higher percentage of $CD8^+$ lymphocytes. Moreover, within the group of non-smoking COPD patients, a low FEV_1 % pred. was significantly associated with both a lower percentage of $CD4^+$ cells and a lower CD4:CD8 ratio (Fig. 2). This may suggest that alterations in the balance of T-cell subsets are associated with the degree of airflow obstruction, especially in non-smoking patients with COPD.

In the group of non-smoking COPD subjects, significant associations of total serum IgE were found with the percentage of $CD4^+$ lymphocytes and the CD4:CD8 ratio (Fig. 4). This may suggest that alterations in the balance of T-cell subsets are also implicated in IgE synthesis. This may be true within the group of smoking COPD patients, considering the significant association between total serum IgE and the percentage of $CD8^+$ lymphocytes.

Larramendy *et al.* (19) demonstrated that blood peripheral $CD8^+$ and $CD19^+$ lymphocytes (B-cells) of smoking healthy subjects contained an increased frequency of micronuclei compared with non-smoking healthy subjects. This may be regarded as $CD19^+$ and $CD8^+$ lymphocytes being more sensitive for changes to smoking, which may be important in the development of COPD. As shown in other studies (17,20,26–28), healthy and COPD smokers had a higher percentage of $CD19^+$ lymphocytes and total serum IgE than healthy and COPD non-smokers. The effects of smoking may trigger the $CD19^+$ lymphocytes to produce IgE. One may hypothesize that IgE and/or $CD19^+$ lymphocytes play a role in COPD. Several studies have shown that increased IgE levels in current smokers are negatively associated with FEV_1 % pred. (17,26–28). Although this IgE–FEV_1 relationship is thought to be a general phenomenon in current smokers, the present study indicates that IgE may also be of importance in non-smoking COPD patients. In a recent paper, Villar *et al.* (21) have also suggested that IgE may be of significance in the pathogenesis of smoking-related airways obstruction. Bosken *et al.* (14) demonstrated that patients with airways obstruction had significantly more $CD19^+$ lymphocytes in the airway wall than patients without airway obstruction. In the present study, the percentage of $CD19^+$ lymphocytes was not different in the COPD (sub)groups compared with the healthy control (sub)groups. However, within the smoking COPD group, a lower FEV_1 % pred. was found in subjects with a lower percentage of $CD19^+$ lymphocytes (Fig. 3). In this respect, the present findings may support the hypothesis that $CD19^+$ lymphocytes are mobilized into the lung, especially in COPD patients with a low FEV_1 % pred., resulting in a lower percentage of $CD19^+$ lymphocytes in peripheral blood.

In both smoking COPD and smoking, healthy control subjects, percentages of $CD16^+/CD56^+$ lymphocytes (NK cells) were decreased when compared with the non-smoking COPD and non-smoking, healthy control subjects. Tollerud *et al.* (29) also demonstrated that cigarette smoking is associated with a decrease in the proportion of circulating NK cells. As NK cells are considered to play an important role in anti-tumour immunity, a decrease of these cells may contribute to an increased risk of malignancies.

Some general remarks on the present study have to be mentioned. Several studies, investigating the role of airway inflammation in COPD, have studied patients with and without airway obstruction, and even allergic patients may have participated. In the present study, non-atopic patients with COPD were selected. All COPD patients had chronic airway obstruction, most of them with only little airway reversibility (ΔFEV_1 $<10\%$ pred.). A small number of COPD patients (5) and one control patient had never smoked. The subject characteristics of the never-smoking patients with COPD were not significantly different from the former smokers. Occupational diseases may have played a role in the development of COPD. However, only two male patients frequently came into contact with dust. It has also been suggested that neuropeptides may cause COPD, even in patients who have never smoked cigarettes (9). In a recent study, Lusuardi *et al.* (30) demonstrated the presence of airway inflammation in subjects with chronic bronchitis and airway obstruction (mean FEV_1 64% pred.) who had never smoked. In the present study, the healthy control subjects were not extensively matched with the COPD patients. This has been accounted for by means of co-variance analysis, adjusting for gender, age

and the number of pack-years. Some findings in the present study, especially the influence of current smoking on IgE and lymphocyte subsets in patients with COPD and healthy controls, do correspond to the findings in epidemiological studies (16,27–29). This suggests the allowance of investigating lymphocyte subsets in relatively small groups of smoking and non-smoking patients.

In summary, the present study described some peripheral blood immunological alterations which may play a role in the pathogenesis of COPD. It is evident that a complex interaction of smoking, changes in balances of T-cell subsets and IgE synthesis may be responsible for the development of COPD. However, the exact mechanisms for development of COPD have yet to be elucidated. Further studies especially investigating airway tissues of smokers and non-smokers with COPD and healthy controls, by following smokers who stop smoking, are necessary to evaluate the results of the present study.

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