Respiratory Medicine (2004) **98**, 858–864

respiratoryMEDICINE

Increased IgE-antibodies to *Staphylococcus aureus* enterotoxins in patients with COPD ☆

Gernot Rohde[a,*], Philippe Gevaert[b], Gabriele Holtappels[b], Irmgard Borg[a,c], Almut Wiethege[a], Umut Arinir[a], Gerhard Schultze-Werninghaus[a], Claus Bachert[b]

[a]*Division of Pneumology, Department of Internal Medicine, Allergology and Sleep Medicine, University Hospital Bergmannsheil, D-44789 Bochum, Germany*
[b]*Department of Otorhinolaryngology, Ghent University Hospital, Belgium*
[c]*Department of Experimental Pneumology, Ruhr-University Bochum, Germany*

Received 24 November 2003; accepted 10 February 2004

KEYWORDS
COPD;
Staphylococcus aureus;
Enterotoxins;
Superantigens;
IgE

Summary Recent evidence suggests that *Staphylococcus aureus* enterotoxins (SAEs) could modify airway disease by acting as superantigens, an immune response that can be monitored by detection of IgE antibodies to SAEs. We studied the expression of total IgE and specific IgE to SAEs using the Uni-CAP system in healthy controls, smokers without COPD and COPD patients. Only 1/10 controls (10%) and 1/16 smokers (6.3%) had IgE to SAEs compared to 7/18 patients with stable COPD (38.9%) and 21/54 patients with exacerbated COPD (38.9%). The IgE levels to SAEs of the patients with stable COPD (0.18 [0.05–26.2]kUA/l) and the patients with exacerbated COPD (0.09 [0.05–18.6]kUA/l) were significantly higher than those of smokers ($n = 16$; 0.05 [0.05–0.82]kUA/l) and controls ($n = 11$; 0.05 [0.05–0.9]kUA/l, $P < 0.05$). IgE to SAEs decreased significantly in the exacerbated patients during hospitalization (0.13 [0.05–18.3] vs. 0.05 [0.05–11]kUA/l, $P < 0.001$) going along with a significant increase in FEV_1 (38.1 [16.9–79.5] vs. 51.6 [15–80]%predicted, $P < 0.001$).Similarly to severe asthma, we found significantly elevated IgE to SAE in COPD patients. Our data for the first time suggest differences between healthy subjects, smokers and patients with established COPD regarding the role of bacterial products and point to a possible disease modifying role of SAEs.

Abbreviations: AEDS, atopic eczema/dermatitis syndrome; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; GOLD, global Initiative for chronic obstructive lung disease; IgE, immunoglobulin E; SAE, *Staphylococcus aureus* enterotoxins; SAE-IgE, IgE antibodies against SAE; SPA, staphylococcus-derived protein A; TCR, T-Cell Receptor; TSST, toxic shock syndrome toxin

☆-This work was supported by the Bundesministerium für Bildung und Forschung (BMBF) Grant #01GC 9802/8 (Gernot Rohde), by the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Grant A 12/5-KV-K17 (Claus Bachert), by grant FWO A12/5-KV-K17 (Claus Bachert), and by an unrestricted research grant from Aventis Pharma Germany.

*Corresponding author. Tel.: +49-234-3020; fax: +49-302-6420.
E-mail address: gernot.rohde@rub.de (G. Rohde).

0954-6111/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2004.02.012

Introduction

COPD is characterized by progressive airflow limitation caused by chronic inflammation in the peripheral airways and lung parenchyma.[1] Infectious agents are an important factor in the pathophysiology of COPD. The most common pathogens recovered are viruses[2] and bacteria.[3] The most important bacteria found during acute exacerbations of COPD are pneumococci, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus*.[4–6] Moreover, chronic bacterial colonization in the lower respiratory tract of patients with chronic bronchitis has been shown.[7] Recent evidence suggests that *Staphylococcus aureus* enterotoxins (SAE) could modify airway disease by acting as superantigens.[8] SAE are a group of high-molecular weight pyrogenic proteins that have in common an extremely potent stimulatory activity for T-lymphocytes, including $CD4^+$, $CD8^+$, and gamma $delta^+$ T-cells, of several species.[9,10] Superantigens exert this effect on the T-cells by cross-linking the variable part on the beta chain of the T-cell receptor (TCR) with MHC class II molecules on accessory or target T-cells, outside the peptide-binding groove area,[11] although binding to MHC class I molecule has also been observed in a MHC class II-negative epidermal cell line.[12] This leads to stimulation of up to 20–25% of the naive T-cell population in a non-specific way, compared with stimulation of only about 0.1% of the T-cell population via the conventional allergen-specific MHC-restricted route utilizing both TCR-Vα and β chains.[13] Staphylococcal protein A (SPA) as well as the SAE TSST-1 have also been demonstrated to possess B-cell superantigen moieties, inducing polyclonal IgE synthesis.[14,15] In COPD, $CD8^+$ T-cells are found in elevated numbers in the peripheral airways[16] and their number correlates with airflow limitation.[17] It is therefore possible that there may be an interaction of these $CD8^+$ T-cells with staphylococcal enterotoxins. Moreover, IgE antibodies to SAE were found significantly more frequently in severe difficult to treat asthma, which shares some features with COPD, compared to controls. These antibodies were linked to the severity of inflammation, concentrations of IgE antibodies, and corticosteroid dependence.[8] Therefore we sought to determine a possible role of bacterial superantigens in COPD.

Patients and methods

Four different groups of patients were investigated. Healthy controls were in generally good health and had no respiratory symptoms. Spirometry was normal, they were non-smoking and the skin prick test was negative to common allergens (*D. pteronyssinus*, *Alternaria tenuis*, grass and birch pollen, cat). They were recruited from the personnel of the hospital. Smokers had smoked for more than 10 pack years and were still active, and had chronic symptoms like cough and phlegm but did not report about dyspnea. The spirometry was normal ($FEV_1 > 80\%$, $FEV_1/FVC > 70\%$). This group was recruited by the help of a newspaper advertisement from the population of Bochum. There was no history of COPD, allergic disease (asthma, rhinitis or dermatitis), upper respiratory tract infection in the previous month, use of any systemic or topical medication in these two groups. The third group were patients with stable COPD hospitalized for other reasons than COPD. The diagnosis of COPD was based on the GOLD criteria.[18] They were at least GOLD stage 2 and did not have an exacerbation within the last 30 days prior to hospital admission, and had no changes in therapy within the last 14 days (including inhaled and oral medication). The last group were exacerbated COPD patients, characterized by worsening in dyspnoea, cough and expectoration,[19] which were hospitalized for acute exacerbation. There were no limitations regarding the necessary medication before admission. During hospitalization, patients were treated with systemic corticosteroids and most of the patients received a course of antibiotic therapy. Exclusion criteria for both COPD groups were bronchial asthma and dyspnea of other origin (cardiovascular, broncho-pulmonary [e.g. pneumonia, interstitial lung disorders], pleural, or others [e.g. upper airways obstruction, neuromuscular, anemia]).

The study was approved by the Ethical Committee of the Ruhr-University of Bochum, Germany. Written informed consent was obtained from all subjects before inclusion into the study.

Data collection

Smoking habits and current medication were recorded from all subjects.

Diagnostic Methods

Spirometry

Spirometric tests were performed using a Jaeger Flowscreen device (E Jaeger, Würzburg, Germany). The best out of three trials was selected and data were compared with reference values.[20] Forced

expiratory volume in 1 s (FEV_1), before and after inhalation of two puffs of salbutamol (200 μg) delivered by a metered dosed inhaler were assessed.

Phadiatop, total and specific IgE

Blood was allowed to clot at room temperature for 20–30 min, centrifuged at 1500 × *g* for 10 min at 4°C, separated and stored in aliquots at −80°C until analysis. All supernatants and sera were assayed for an IgE screening for inhalant allergens (Phadiatop), total and specific IgE by the Uni-CAP system (Pharmacia & Upjohn, Upsala, Sweden). Specific IgE was determined for a mix of *S. aureus* enterotoxins (SEA, SEC, and TSST-1) as described before.[8] No non-specific IgE reactivity to the SAE mix ImmunoCAP was found for non-antibody active IgE (E myeloma) at concentrations up to 1000 kU/l. Samples positive for IgE antibody to the SAE mix tested negative to a control ImmunoCAP without allergen.

Statistical analysis

The primary objective of this study was to compare the levels of IgE to SAE in healthy controls, smokers and patients with and without acute exacerbation of COPD. Secondary objectives were to correlate the findings to clinical parameters. Continuous data were checked for normal distribution using the Kolmogorov–Smirnov test. The data were of non-parametrical distribution and results were expressed as median and range. Differences between groups were assessed by Kruskal–Wallis test. To further analyse significant differences between two individual groups a pair wise comparison by two-sided Mann–Whitney *U*-test was performed. The two-sided Wilcoxon test was used for paired variables. For discrete variables, frequencies and percentages were reported and compared by χ^2 test or Fisher's exact test where appropriate. The Yates correction procedure was applied to all comparisons. For correlation analysis the Spearman–Rho coefficient was calculated. All significance level were set to 5%. Data were analysed and processed on SPSS Version 10.0 on a Windows 98 operating system.

Results

Patients

Eleven healthy controls, 16 smokers, 18 patients with stable COPD and 54 patients with acute exacerbation of COPD were included (Table 1). None of the healthy controls, all of the smokers, 4/18 (22%) of the stable COPD patients and 22/54 (41%) of the exacerbated COPD patients were active smokers. The number of pack years ranged from 35 to 43 in the latter three groups without significant differences between the groups. Neither healthy controls nor smokers received any medication. There were no significant differences between stable and exacerbated COPD patients concerning medication with inhaled or systemic steroids. The FEV_1 was normal in healthy controls and smokers, whereas there was a significant reduction in patients with stable COPD (52.9 [26.8–71.3]%predicted, $P<0.001$). FEV_1 in exacerbated patients was significantly further decreased compared to stable patients (38.1 [16.8–79.5]%predicted, $P<0.001$). None of the patients with COPD had a reversibility in FEV_1 of more than 15% predicted.

Phadiatop

IgE screening for inhalant allergens (Phadiatop) was positive in 20% of healthy controls, in 28% of

Table 1 Characteristics of the patients.

	Healthy controls ($n = 11$)	Smokers ($n = 16$)	Stable COPD ($n = 18$)	AE-COPD ($n = 54$)
Age *Median [range]*	28 [23–38]	48.5 [46–56]	67 [53–86]	69 [43–83]
Smoking *n (%)*	0	16 yes (100%)	4 no (22%) 10 ex (56%) 4 yes (22%)	7 no (13%) 25 ex (46%) 22 yes (41%)
Pack-Years *Median [Range]*	0	38 [15–92]	43 [1–80]	35 [1–88]
Inhaled and/or systemic corticosteroids *n(%)*	0	0	13 (72%)	44 (82%)
FEV_1%predicted *Median [Range]*	99.0 [90.1–125.9]	98.4 [75.6–124]	52.9 [26.8–71.3]	38.1 [16.8–79.5]
Reversibility (%) *Median [Range]*	n.d.	n.d.	3.6 [-2.8–14.4]	0.0 [-22.9–13.9]

patients with stable COPD and in 25% of patients with exacerbated COPD without any significant differences between these groups.

Total IgE

Total IgE levels of the COPD group as a whole were not significantly higher than in healthy controls (56.1 [5.3–2440] vs. 37.8 [8.4–236]kUA/l, $P = 0.361$). When healthy controls, smokers, patients with stable and exacerbated COPD were analysed by Kruskal–Wallis test no significant differences between the groups were detected ($P = 0.163$, Table 2 and Fig. 1).

IgE- antibodies to SAE

Only 1/10 controls (10%) and 1/16 smokers (6.3%) had IgE to SAE compared to 7/18 patients with stable COPD (38.9%) and 21/54 patients with exacerbated COPD (38.9%). Smokers had significantly less frequently IgE to SAE compared to patients with stable COPD ($P = 0.043$) and compared to patients with exacerbated COPD ($P = 0.031$). When the absolute levels were analysed by Kruskal–Wallis, there were significantly higher levels in both, the stable and the exacerbated COPD patients compared to either healthy controls or smokers (Fig. 2).

Correlation of total IgE and SAE-IgE

There was a significant correlation between total and specific IgE levels ($r = 0,62$; $P < 0.001$) in the COPD patients. However, six subjects demonstrated increased total IgE of more than 200 kUA/l, but did not show IgE antibodies to SAE, indicating that the test was independent from total IgE levels, and that other than the tested staphylococcal superantigens might be involved in some cases. This correlation was not found in healthy controls or smokers.

Effect of hospitalization on IgE levels to SAE in AE-COPD patients

IgE levels to SAE were determined at admission to and closely before discharge from the hospital in 44 AE-COPD patients (mean duration 15.5 [3–45] days). SAE-IgE levels decreased significantly in these patients during hospitalization (0.13 [0.05–18.3]kUA/l vs. 0.05 [0.05–11]kUA/l, $P < 0.001$). Along with this decrease there was a significant increase in FEV_1 in these patients (38.1 [16.9–79.5] vs. 51.6 [15–80]%predicted, $P < 0.001$) and we found an inverse correlation between these changes ($r = -0.216$, $P = 0.033$).

Discussion

Similar to findings in unstable asthma[8] we here describe significantly higher serum levels of specific IgE antibodies against staphylococcal enterotoxins in COPD patients compared to healthy controls and

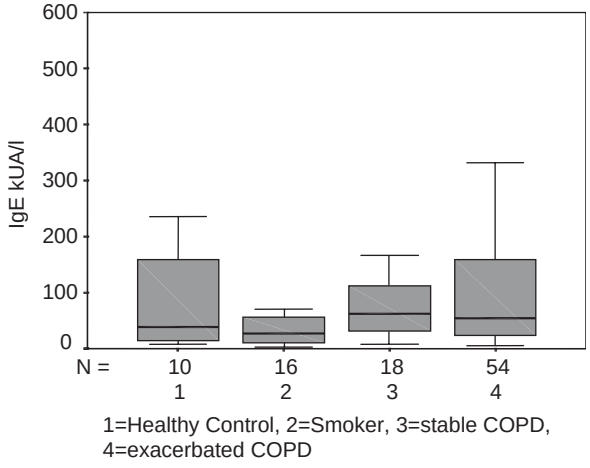

Figure 1 Serum levels of total IgE. Box-and-whisker plot: the central box represents the values from the lower to upper quartile (25–75 percentile), the middle line the median and the whiskers the highest and lowest value respectively exclusive outliers.

Table 2 Total IgE, IgE to SAE and Phadiatop.

	Healthy controls ($n = 11$)	Smokers ($n = 16$)	Stable COPD ($n = 18$)	AE-COPD ($n = 54$)
Total IgE (kUA/l) Median [Range]	37.8 [8.4–236]	26.5 [2.9–388]	62.2 [7.4–1476]	54.1 [5.3–2440]
IgE to SAE *n* pos./*n* total [%]	1/10 [10%]	4/16 [25%]	10/18 [56%]	27/54 [50%]
Phadiatop *n* pos./*n* total [%]	2/10 [20%]	n.d.	5/18 [28%]	5/20 [25%]

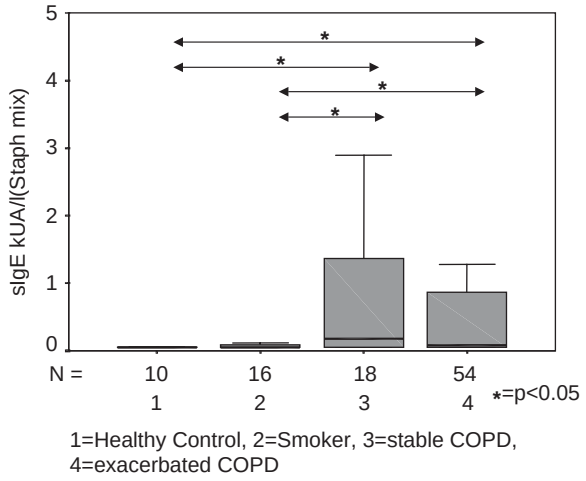

Figure 2 Serum levels of IgE to SAE. Box-and-whisker plot. The central box represents the values from the lower to upper quartile (25–75 percentile), the middle line the median and the whiskers the highest and lowest value respectively exclusive outliers. Arrows are drawn between groups with significant differences.

smokers. Regarding the clinical presentation of the patients and the spirometric data showing no reversibility of airflow obstruction after bronchodilator use, we are sure that none of our COPD patients could have suffered from asthma. It is important to notice that there were no significant differences in total IgE antibody levels or in IgE to common inhalant allergens (Phadiatop) between healthy controls and patients with COPD. Moreover, none of these subjects suffered from allergic symptoms. This underlines the fact, that production of IgE to SAE is of non-atopic origin, but rather reflects the superantigen activity on B- and T-cells as described before.[11–15] Looking at the median age of the four different groups one might argue that aging may influence levels of IgE to SAE. However, it is not probable that these differences in age play a role in the differences in IgE to SAE-levels reported. The main changes in the adaptive immune system occur in the T-cells compartment.[21,22] Nevertheless changes both in the B-cell germline-encoded repertoire and the age-associated decrease in somatic hypermutation of the B-cell antigen receptors are now known to be critically affected by helper T-cell aging. But these effects would rather cause a diminished antibody production whereas we found increased antibodies to SAE. Therefore this would cause a conservative mistake rather underestimating the differences in IgE to SAE-levels between the groups. Moreover the B-cell number is strictly regulated and despite the decreased output of B-cells by the bone marrow does not decline during aging. Self-renewal of peripheral B-cells is sufficient to assure the stability of peripheral B-cell number.[23] Therefore the differences in age between the groups are not responsible for the increase in IgE to SAE in patients with COPD.

The levels of IgE to SAE were significantly elevated not only in the exacerbated group but also in patients with stable COPD. This may be an expression of the bacterial colonization observed in the lower airways in COPD including *S. aureus*.[24] However, not the mere presence, but rather the release of enterotoxins from staphylococcal strains might be important. In other chronic diseases like atopic eczema/dermatitis syndrome (AEDS) there is much greater *S. aureus* colonization in the skin of patients (80–100%) than in normal healthy subjects (5–30%). *S. aureus* isolated from the skin of at least 65% of the AEDS patients secrete superantigens SEA, SEB, SEC, SED and TSST-1, which penetrate the epidermis and dermis after breakdown of the epithelial barrier, where they interact with different cells of the immune system, leading primarily to a T-cell-dependent inflammation.[25] Although *S. aureus* is less frequently found in COPD, a similar way of activation may be given in COPD. However, the data presented here are only preliminary and do not allow to conclude on a mechanism. Future studies will have to address this issue. Interestingly, in mice, staphylococcal enterotoxins have been shown to induce lymphocyte-dependent airway inflammation.[26] $CD8^+$ lymphocytes are found in COPD in elevated numbers in the peripheral airways[16] and their number correlates with airflow limitation.[17] It is therefore possible that there may be an activation of these $CD8^+$ T-cells by staphylococcal enterotoxins. In the patients with exacerbated COPD there was an inverse correlation between levels of IgE to SAE and FEV_1 during hospitalization supporting this hypothesis.

A possible drawback of this study may be seen in the lack of microbiologic data showing *S. aureus* colonization and production of enterotoxins within the lower airways, but *S. aureus* is often found as part of the normal microflora of the human skin, the upper respiratory tract, especially the vestibulum nasi, and the intestinal tract. In patients with COPD there is bacterial colonization also in the lower airways including *S. aureus*.[24] Moreover, during exacerbations of COPD, *S. aureus* has been found as a pathogen.[5,6] Nevertheless there are no prospective studies investigating staphylococcal carriage in patients with COPD. However, there are studies looking at nasal staphylococcal carriage. The nasal vestibule is the ecologic reservoir for S. aureus, with about 25% of the population

being permanent carriers.[27] A large proportion of the population (approximately 60%) harbours *S. aureus* intermittently, and the strains change with varying frequency. Notably, only a minority of people (approximately 20%) almost never carries *S. aureus*.[27] The differences could be due to host factors and/or to competition between members of the nasal flora (e.g. *Corynebacterium* spp.).[28] Indeed, a higher incidence of *S. aureus* colonization of skin or mucous membranes may be due to injuries (e.g.smoking), abnormal leukocyte function and inflammation (e.g. atopic dermatitis), viral infections (e.g. influenza), metabolic abnormalities (e.g. diabetes mellitus and uraemia) and miscellaneous other conditions (e.g. malnutrition, old age, malignancies, etc.).[27] In elderly hospitalized patients 20%[29] to 33%[30] are concerned. Added to the information given above it is very likely that numbers are even higher in patients with COPD. We think that IgE to SAEs found in serum actually are induced by *S. aureus* localized in the lower airways of these patients. Alternatively, the source of SAEs may also be the nose and sinuses, and droplets from the nose containing SAEs could be inhaled. However, it has been shown for other bacteria like *Streptococcus pneumoniae* and *Haemophilus influenzae* that elevated levels of specific IgE can be found.[31,32]

In conclusion, this is the first study showing elevated IgE antibodies directed against *S. aureus* enterotoxins in the serum of patients with COPD, serving as a marker for previous or current contact of the immune system to SAEs. Our data indicate an immunological reaction to superantigens as a possible trigger of chronic inflammation in COPD, which needs further study.

Acknowledgements

The authors are indebted to B. Schärling, S. Werner and L. Dretaki-Schnackenberg (all University Hospital Bergmannsheil, Department of Internal Medicine, Division of Pneumology, Allergology and Sleep Medicine, Germany) for assistance in collecting and processing the samples, and to S.G.O. Johansson (Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden) for the donation of the SAE mix ImmunoCAP.

References

1. Barnes PJ. Chronic obstructive pulmonary disease. *N Engl J Med* 2000;**343**(4):269–80.
2. Rohde G, Wiethege A, Borg I, Kauth M, Bauer TT, Gillissen A, et al. Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalization—a case-control study. *Thorax* 2003;**58**(1):37–42.
3. Sethi S. Bacterial infection and the pathogenesis of COPD. *Chest* 2000;**117**(5 Suppl 2):286S–91S.
4. Sethi S, Evans N, Grant BJB, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. *N Engl J Med* 2002;**347**(7):465–71.
5. Vogel F. Parenteral cephalosporins for the treatment of lower respiratory tract infections. *Infection* 1993;**21** (Suppl 1):S28–34.
6. Medici TC, von Graevenitz A, Shang H, Bohni E, Wall M. Gram stain and culture of morning and 24 h sputum in the diagnosis of bacterial exacerbation of chronic bronchitis: a dogma disputed. *Eur Respir J* 1988;**1**(10):923–8.
7. Monsó E, Rosell A, Bonet G, Manterola J, Cardona PJ, Ruiz J, et al. Risk factors for lower airway bacterial colonization in chronic bronchitis. *Eur Respir J* 1999;**13**:338–42.
8. Bachert C, Gevaert P, Howarth P, Holtappels G, van Cauwenberge P, Johansson SG. IgE to *Staphylococcus aureus* enterotoxins in serum is related to severity to asthma. *J Allergy Clin Immunol* 2003;**111**(5):1131–2.
9. Bachert C, Gevaert P, van Cauwenberge P. *Staphylococcus aureus* enterotoxins: a key in airway disease? *Allergy* 2002;**57**:480–7.
10. Fleischer B. Superantigens. *APMIS* 1994;**102**(1):3–12.
11. Mollick JA, McMasters RL, Grossman D, Rich RR. Localization of a site on bacterial superantigens that determines T-cell receptor beta chain specificity. *J Exp Med* 1993;**177**(2): 283–93.
12. Haffner AC, Zepter K, Elmets CA. Major histocompatibility complex class I molecule serves as a ligand for presentation of the superantigen staphylococcal enterotoxin B to T-cells. *PNAS* 1996;**93**(7):3037–42.
13. Hong SC, Waterbury G, Janeway Jr CA. Different superantigens interact with distinct sites in the V beta domain of a single T-cell receptor. *J Exp Med* 1996;**183**(4): 1437–46.
14. Hofer MF, Harbeck RJ, Schlievert PM, Leung DY. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 1999;**112**: 171–6.
15. Kristiansen SV, Pascual V, Lipsky PE. Staphylococcal protein A induces biased production of Ig by VH3-expressing B lymphocytes. *J Immunol* 1994;**153**:2974–82.
16. Saetta M, Di Stefano A, Turato G, Facchini F, Corbino L, Mapp CE, et al. $CD8^+$ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;**157**(3):822–6.
17. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of $CD8^+$ T lymphocytes with FEV_1. *Am J Respir Crit Care Med* 1997;**155**(3):852–7.
18. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary. *Am J Respir Crit Care Med* 2001;**163**(5):1256–76.
19. Anthonisen NR, Manfreda J, Warren CPW, Hershfield ES, Harding GKM, Nelson NA. Antibiotic therapy in acute exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med* 1987;**106**(2):196–204.
20. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J. Lung volumes and forced ventilatory flows.

Report working party, Standardization of lung function tests, European Community for steel and coal, Official statement of the European Respiratory Society. *Eur Resp J* 1993;**6**: 5–40.
21. Pawelec G, Barnett Y, Forsey R, Frasca D, Globerson A, McLeod J, et al. T-cells and aging, January 2002 update. *Front Biosci* 2002;**7**:d1056–183.
22. Effros RB. Ageing and the immune system. *Novartis Found Symp* 2001;**235**:130–9.
23. Weksler ME, Szabo P. The effect of age on the B-cell repertoire. *J Clin Immunol* 2000;**20**(4):240–9.
24. Soler N, Ewig S, Torres A, Filella X, Gonzalez J, Xaubet A. Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease. *Eur Respir J* 1999;**14**(5):1015–22.
25. Breuer K, Kapp A, Werfel T. Bacterial infections and atopic dermatitis. *Allergy* 2003;**56**:1034–41.
26. Herz U, Ruckert R, Wollenhaupt K, Tschernig T, Neuhaus-Steinmetz U, Pabst R, et al. Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness—a model for non-allergic asthma. *Eur J Immunol* 1999;**29**:1021–31.
27. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia, Study Group. *N Engl J Med* 2001;**344**(1):11–6.
28. Lina G, Boutite F, Tristan A, Bes M, Etienne J, Vandenesch F. Bacterial competition for human nasal cavity colonization: role of staphylococcal agr alleles. *Appl Environ Microbiol* 2003;**69**(1):18–23.
29. Corbella X, Dominguez MA, Pujol M, Ayats J, Sendra M, Pallares R, et al. *Staphylococcus aureus* nasal carriage as a marker for subsequent staphylococcal infections in intensive care unit patients. *Eur J Clin Microbiol Infect Dis* 1997;**16**(5):351–7.
30. Parnaby RM, O'Dwyer G, Monsey HA, Shafi MS. Carriage of *Staphylococcus aureus* in the elderly. *J Hosp Infect* 1996;**33**(3):201–6.
31. Kjaergard LL, Larsen FO, Norn S, Clementsen P, Skov PS, Permin H. Basophil-bound IgE and serum IgE directed against *Haemophilus influenzae* and *Streptococcus pneumoniae* in patients with chronic bronchitis during acute exacerbations. *APMIS* 1996;**104**(1):61–7.
32. Bloom JW, Halonen M, Dunn AM, Pinnas JL, Burrows B. Pneumococcus-specific immunoglobulin E in cigarette smokers. *Clin Allergy* 1986;**16**(1):25–32.