

Effects of asthma on cell components in peripheral blood among smokers and non-smokers

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Summary

Background Eosinophils play a central role in asthma, but the interplay of the effects of smoking, eosinophils and asthma remains unclear.

Objective The primary objective of our study was to investigate the extent to which smoking modifies the effect of asthma on circulating eosinophils, CD4⁺ and CD8⁺ T cell counts.

Methods Data were collected semiannually between 1987 and 1994 from HIV-negative participants in the Multicenter AIDS Cohort Study. Asthma was defined by a questionnaire at baseline as a self-report of diagnosed asthma. A total of 1420 blood samples from 197 asthmatics and 15822 from 1997 non-asthmatics were collected.

Results Eosinophil levels were higher in asthmatics (28% of asthmatics had eosinophils $\geq 4\%$ and 16% of non-asthmatics) regardless of smoking history, but smoking modified the association between eosinophils and asthma. Namely, the odds ratios for eosinophils being $\geq 4\%$ in asthmatics to non-asthmatics decreased from 2.7 (95% CI: 2.0, 3.6) in never, to 2.1 (1.4, 3.1) in former, and to 1.5 (0.9, 2.3) in current smokers. Cross-sectional and longitudinal analyses coherently showed that smoking increased eosinophils in non-asthmatics, but the converse was true for asthmatics. In contrast, no differences in peripheral blood T cell counts between asthmatics and non-asthmatics were observed.

Conclusion Under the established link between increased eosinophils and asthma, these data indicate that smoking modified this relationship. This finding suggests that smoking plays a different immunological role in asthmatics and non-asthmatics.

Keywords CD4, CD8, effect modification, eosinophil, smoking

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Introduction

The effects of smoking on asthma are not fully understood. Some studies have found that smoking increases the risk of asthma attacks [1, 2], suggesting that inflammation of the mucosa and an increase in lung permeability secondary to smoking could augment antigen access to immunocompetent cells [3]. However, most studies have not demonstrated any association between smoking and asthma incidence [4–6]. More intriguing is the debate regarding the possible association between smoking cessation and asthma onset [7–9], which might indicate an immunological effect of smoking in the airways of asthmatics [10].

In the general population, smoking increases eosinophils in peripheral blood [11, 12]. Eosinophils play a central role in asthma [13], and both symptomatic and asymptomatic

asthmatics have increased numbers of eosinophils in peripheral blood [14]. Eosinophils are associated with decreased lung function in asthmatics [13, 15], severity of asthma [13], respiratory symptoms [16, 17] and bronchial hyper-responsiveness [18]. Eosinophilia is associated with higher mortality in the general population [19]. T lymphocytes initiate and program the immune response through the secretion of protein mediators. Thus, T cells are an important source of cytokines that may influence eosinophil growth and differentiation [20]. In bronchial lavage studies, smokers showed a dose-response reduction of the CD4⁺/CD8⁺ T cell ratio due to an increase of CD8, suggesting a disturbance of cellular immunoregulation in the lungs of smokers [21]. Studies on T cells in peripheral blood are controversial and have demonstrated both positive [22, 23] and negative [24, 25] associations between smoking and CD4⁺/CD8⁺ T cell ratio. However, these studies were cross-sectional and limited to small populations.

The Multicenter AIDS Cohort Study (MACS), an ongoing prospective study of the natural history of HIV infection,

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follows a large cohort of HIV-negative individuals. During semiannual follow-up with each individual, data on cell components of peripheral blood were collected, and smoking behaviour was ascertained. We have previously reported the effects of smoking on total white blood cells (WBC) within the non-HIV-infected individuals of the MACS [26], and we have also reported the interaction of HIV infection and smoking on the components of WBC [27]. Since a history of asthma was ascertained during the subject's initial visit, these data offered an opportunity to investigate the extent to which smoking modifies the effect of asthma on eosinophils, and on CD4⁺ and CD8⁺ T cell counts. The interrelations between eosinophils/T cells, smoking and asthma have not been evaluated previously.

Methods

Participants

A detailed description of the MACS population and study design has been provided elsewhere [28]. Briefly, a cohort of homosexual men from four US centres was followed semiannually. Seropositivity to HIV was determined, at each visit, by ELISA and confirmed with Western blot. At entry, 2195 participants were seropositive; and of the 3427 seronegative subjects, 573 had seroconverted as of December 2000. In this study, we focused on the 2854 participants who remained seronegative up to December 2000.

Although the first of the semi-annual MACS visits took place in 1984, and blood was collected at each visit, for the purposes of this study we restricted attention to data collected between visits 8 and 22, when a uniform questionnaire on smoking was used. Hence, the time of follow-up spanned 7 years, from visit 8 in 1987–1988 to visit 22 in 1994–1995. Of the 2854 participants who remained seronegative up to December 2000, a total of 2519 (88%) attended at least one visit during this time. During each visit, blood was collected for haematological studies, including complete blood counts and automated differential, and to determine the percentage and number of T lymphocytes by flow cytometry, with a comprehensive quality control programme among the four study sites [29]. Of the 2519 individuals seen between visits 8 and 22, 2194 (87%) provided haematological information regarding eosinophils and T cells, and answered the smoking questionnaire. This group comprised the study population for the analysis reported here. Data were available for 17 242 person-visits over the 8-year period of follow-up. Smoking behaviour was assessed at each visit in three categories (i.e., never/former/current). Individuals were classified as current smokers if they reported at least one cigarette daily during the previous month (92% of cases classified as current smokers) or if they reported occasional smoking (8%).

Asthma was defined at baseline based on the answer to the question 'Has a doctor or other medical practitioner ever told you that you had asthma?'

Statistical analysis

The first step in data analysis was to describe the study variables in asthmatics and non-asthmatics, both at baseline

and at all person-visits. To accomplish the primary aim of examining the interplay of smoking and asthma on cell components in peripheral blood, we first analysed all person-visits in the strata of six categories defined by asthma/no asthma and never/former/current smoking, with asthma as a fixed variable assessed at baseline and smoking as a time-dependent variable incorporating behavioural changes over time. To determine how the putative differences in cell components in asthmatics and non-asthmatics might be affected by smoking, we tested the statistical significance of differences for continuous variables and of odds ratios for binary outcomes (e.g., eosinophils $\geq 4\%$). To combine data for all visits formally (i.e., conceiving the visits as a series of cross-sectional studies) and to incorporate the intrinsic correlation of cell components measured in the same individual over time (of no prior interest to this study), we used robust methods for the calculation of confidence intervals [30]. The association of asthma to cell components was measured using a cut-off point of 4% for eosinophils, which corresponds to approximately the 15th percentile and the clinical criteria of eosinophilia [11], and for the CD4⁺/CD8⁺ T cell ratio we used 1.8 (the median) as cut-off for the analysis. To determine how cell components changed according to changes in smoking behaviour, we formed all the possible pairs of visits that were 6 months apart and used standard methods for comparisons of means and proportions.

Results

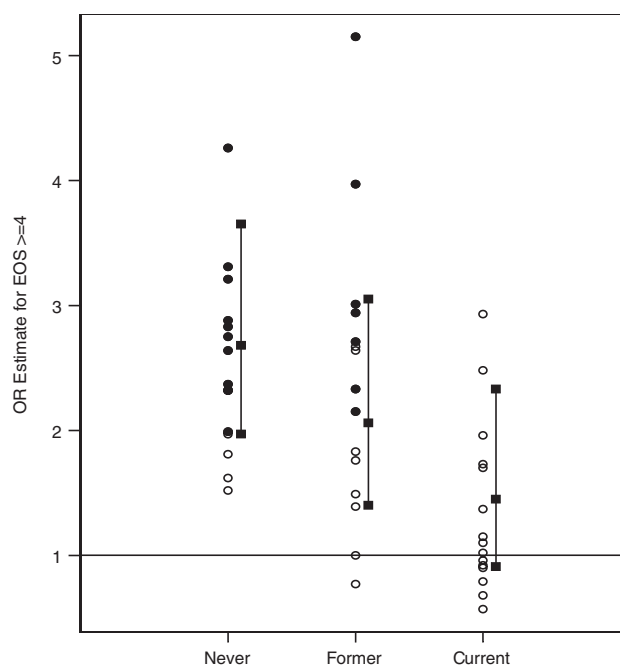
Of the 2194 individuals in our study population at their initial MACS visit, 197 (9%) reported having been identified with a history of asthma during their initial MACS visit. Table 1 shows descriptive statistics for asthmatics and non-asthmatics. The two groups were similar in age, but participants who lived in Los Angeles and non-white subjects had slightly higher rates of asthma. In the follow-up period from 1987 to 1994–1995, the 197 asthmatics and the 1997 non-asthmatics contributed 1420 and 15822 person-visits, respectively. Using person-visits as the units of analysis, the asthmatic group had a lower proportion of current smokers. Regarding cells in peripheral blood, the groups did not differ in white blood counts or T cell subsets (i.e., CD4⁺ and CD8⁺), but asthmatics did show significantly higher levels of eosinophils (190 counts/mL (2.8%) in asthmatics and 148 counts/mL (2.2%) in non-asthmatics, $P < 0.05$), both in the mean values and in the percent of person-visits with eosinophils $\geq 4\%$ (27.6% in asthmatics and 16.1% in non-asthmatics).

Using all person-visits as the units of analysis, Table 2 describes the distribution of cells in peripheral blood for asthmatics and non-asthmatics in the strata for never, former and current smokers. Eosinophil levels were higher in asthmatics ($P < 0.05$) regardless of smoking history. Among non-asthmatics, eosinophil levels increased with smoking, from 2.09% among the never group, to 2.23% among former smokers and to 2.35% among current smokers (2.4% in smokers of more than 1 pack/day). However, among asthmatics, the trend was in the opposite direction; namely, levels of eosinophils decreased from 2.91% in never, to 2.88%

Table 1. Description of study variables by asthma in seronegative participants in the MACS (1984–1994)

	Asthma in 1984 (<i>n</i> = 197)	No asthma in 1984 (<i>n</i> = 1997)
Mean age (years)	34.5 ± 9.5	34.1 ± 8.1
Center, <i>n</i> (%)		
Baltimore	43 (21.8%)	544 (27.2%)
Chicago	41 (20.8%)	390 (19.5%)
Los Angeles	73 (37.1%)	514 (25.7%)
Pittsburgh	40 (20.3%)	549 (27.5%)
Race, <i>n</i> (%)		
White	180 (91.4%)	1,865 (93.4%)
Non-white	17 (8.6%)	132 (6.6%)
No. of person-visits in the follow-up (1987–1994) (<i>n</i>)	1420	15 822
Smoking, <i>n</i> (%)		
Never	713 (50.2)	7361 (46.5)
Former	406 (28.6)	4239 (26.8)
Current	301 (21.2)	4222 (26.7)
< 1 pack/day	114 (37.9)	1911 (45.3)
≥ 1 pack/day	187 (62.1)	2308 (54.7)
Cells, mean ± SD		
White blood cell (WBC) counts/mL	6701 ± 1745	6714 ± 1807
% Eosinophil among WBC	2.83 ± 2.36	2.20 ± 1.76
≥ 4%	27.6%	16.1%
% CD4 ⁺ among lymphocytes	46.90 ± 7.78	46.25 ± 7.93
% CD8 ⁺ among lymphocytes	27.18 ± 6.98	27.27 ± 7.37
Ratio CD4 ⁺ /CD8 ⁺ T cell	1.88 ± 0.83	1.90 ± 0.79

in former and 2.58% in current smokers (2.4% in smokers of more than 1 pack/day). The opposite directionality of the effect of smoking on eosinophils among asthmatics and non-asthmatics led to an attenuation of the increase of eosinophils among asthmatics. Specifically, the overall difference of 0.63 reported in Table 1 was modified by smoking as follows: 0.82 for never, 0.65 for former and only 0.23 for current smokers (*P* for effect modification: <0.05). This effect modification was even more pronounced using the percentage of person-visits with eosinophils ≥4%. Specifically, the odds ratios for eosinophils ≥4% were 2.5, 2.1 and 1.2 among never, former

**Fig. 1.** Association (odds ratio, OR) between eosinophils (EOS ≥ 4%) and asthma by smoking status per visit. The vertical lines portray the 95% CI for the OR at ALL visits using robust methods for the evaluation of standard errors. Each dot depicts the OR at each visit. Full dots where *P* < 0.05.

and current smokers, respectively (being 0.85 in smokers of more than 1 pack/day).

Peripheral blood levels of CD4⁺ T cells were higher, and CD8⁺ T cells were lower in asthmatics and non-asthmatics who reported a history of smoking (Table 2). The increase in CD4⁺ T cells produced by smoking was more pronounced in asthmatics than in non-asthmatics, resulting in significantly (*P* < 0.05) higher levels of CD4⁺ T cells in asthmatics than in non-asthmatics among former and current smokers. However, the increase in CD4⁺ T cells was slight and did not result in significant differences in the CD4⁺/CD8⁺ T cells ratio between asthmatics and non-asthmatics at any strata of smoking.

To determine possible trends over time, we further stratified by visit (i.e., we carried out a separate analysis for each of the

Table 2. Cross-sectional association: cells (counts and percent) by smoking (never, former, current) and asthma at ALL visits by 2194 seronegative participants in the MACS (1987–1994)

	Never		Former		Current	
	Asthma	No asthma	Asthma	No asthma	Asthma	No asthma
Person-visits	713	7361	406	4239	301	4222
WBC mean counts/ml	6370	6339	6658	6532	7543	7551
Eosinophils % in WBC						
Mean	2.91*	2.09	2.88*	2.23	2.58*	2.35
≥ 4%	29.3%*	14.4%	28.6%*	16.3%	22.3%	18.9%
T cells, mean						
%CD4 ⁺ †	45.49	45.05	47.65*	46.33	49.24*	48.26
%CD8 ⁺ †	27.78	27.64	26.66	27.12	26.46	26.77
CD4 ⁺ /CD8 ⁺	1.81	1.80	1.97	1.89	2.03	2.00

WBC: white blood cells.

**P* < 0.05 comparing asthma/no-asthma within the smoking strata.

†Percent in total lymphocytes.

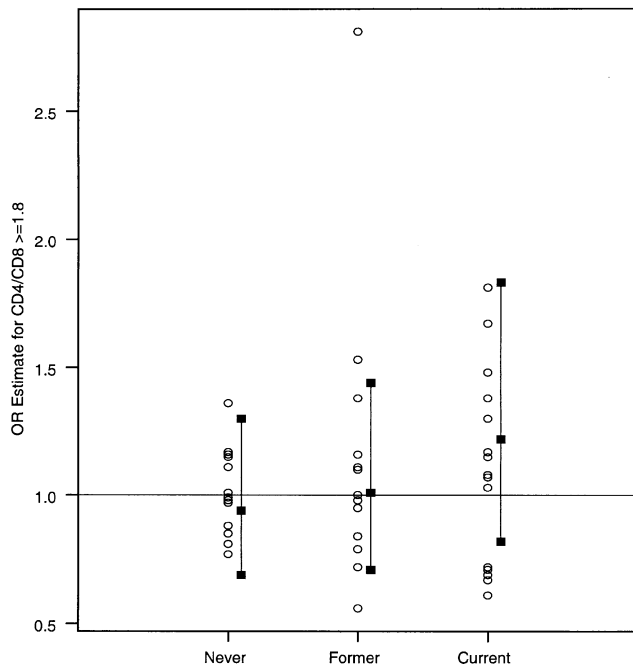


Fig. 2. Association (odds ratio, OR) between $CD4^+/CD8^+$ T cell ratio (measured per $CD4^+/CD8^+$ T cell ≥ 1.8) by smoking status per visit. The vertical lines portray the 95% CI for the OR at ALL visits using robust methods for the evaluation of standard errors.

15 semi-annual visits) and quantified the relationships between asthma and eosinophil levels and $CD4^+/CD8^+$ T cell ratio by the odds ratios of eosinophils $\geq 4\%$ and $CD4^+/CD8^+$ T cells ≥ 1.8 in asthmatics to non-asthmatics. Figs 1 and 2 depict the odds ratios among never, former and current smokers. To combine all the visits into a summary measure, we used robust methods for the calculation of confidence intervals due to the intrinsic correlation of eosinophils and $CD4^+/CD8^+$ T cell ratios measured in the same individual over time. The summary odds ratios (95% C.I.) for eosinophil being $\geq 4\%$ in asthmatics to non-asthmatics decreased from 2.7 (2.0–3.6) in never, to 2.1 (1.4–3.1) in former and to a non-significant odds ratio of 1.5 (0.9–2.3) in current smokers (Fig. 1). In contrast, the pattern for the $CD4^+/CD8^+$ T cells ratio showed a slight increase in smokers, but none of the odds ratios were statistically significant as the three confidence intervals contained the null value of 1 (Fig. 2).

To determine how the percentage of eosinophils among WBC and $CD4^+/CD8^+$ T cell ratios varied according to

changes in smoking status, we formed all the possible pairs of visits six months apart. The outcomes of primary interest were differences in eosinophil and $CD4^+/CD8^+$ T cell levels (Table 3). Most of the subjects (91%) did not change smoking behaviour between visits. Individuals who remained in the same smoking category between visits maintained the same eosinophil levels. Consistent with the data in Table 2 and Fig. 1, asthmatics who started or resumed smoking demonstrated a decrease in the percentage of eosinophils among WBC (mean change = -0.207), but non-asthmatics demonstrated an increase (mean change = 0.039). Similarly, those asthmatics who quit smoking showed an increase in eosinophils (mean change = 0.174), while non-asthmatics showed a decrease (mean change = -0.041). Owing to the relatively small number of individuals who changed smoking behaviour (9%), the test for effect modification was not statistically significant ($P = 0.29$). Changes in the $CD4^+/CD8^+$ T cell ratio as a function of changed behaviour between visits were even less significant.

To determine the percentage of subjects exhibiting eosinophils $\geq 4\%$ according to changes in smoking behaviour, we carried out a parallel analysis using the proportion of discordant pairs between two consecutive visits. The relative risk of an increase in eosinophils $> 4\%$ from one visit to the next was 0.90 (0.32–2.46) for asthmatics and 1.39 (0.95–2.07) for non-asthmatics who resumed smoking, not quantifiable (i.e., unquantifiably high) for asthmatics and 0.92 (0.51–1.65) for non-asthmatics who quit smoking.

Discussion

Higher peripheral blood eosinophil and $CD4^+$ T cells in asthmatics are not new. What this study adds is that persons with asthma had circulating eosinophil levels two times greater than non-asthmatics if they never smoked, but similar levels if they smoked, demonstrating that smoking attenuates increased eosinophilia in asthma. Similarly, changes in smoking behaviour within any 6-month period led to reversal of eosinophil levels in asthmatics compared to non-asthmatics. Thus, as expected, starting to smoke increased the proportion of eosinophils in individuals without asthma, but decreased the proportion of eosinophils in individuals with asthma. Also, as expected, smoking cessation decreased the proportion of eosinophils in non-asthmatics, but increased it in asthmatics. In contrast, this study did not observe a difference in peripheral blood T cell percentages between

Table 3. Mean change $\pm$ SD for differences in percent eosinophils and $CD4^+/CD8^+$ T cell ratio according to the change in smoking during the study follow-up, 1987–1994

Smoking	No. of pairs		Percent eosinophils		Ratio $CD4^+/CD8^+$	
	Asthma	No asthma	Asthma	No asthma	Asthma	No asthma
Never–never	502	5300	0.040 ± 2.24	-0.002 ± 1.69	0.006 ± 0.54	0.010 ± 0.51
Former–former	236	2573	0.051 ± 2.05	0.017 ± 1.66	-0.002 ± 0.51	0.020 ± 0.54
Current–current	190	2884	0.011 ± 1.77	-0.016 ± 1.71	-0.018 ± 0.51	0.017 ± 0.63
Never/former–current	58	777	-0.207 ± 2.47	0.039 ± 1.62	-0.001 ± 0.57	-0.025 ± 0.55
Current–Former	23	267	0.174 ± 1.15	-0.041 ± 1.82	-0.083 ± 0.80	0.014 ± 0.56

asthmatics and non-asthmatics, although the $CD4^+/CD8^+$ T cell ratio increased moderately with smoking. A moderate increase in this ratio, related to quantity of smoking, had previously been observed [10, 26].

The number of asthmatics who changed their smoking habits between two consecutive visits was small, and we could not exclude differences in cells due to chance. However, the consistency of the cross-sectional analysis, demonstrating a statistically significant negative interaction, and the longitudinal analysis, demonstrating a qualitative negative interaction, suggests that the interaction found in the longitudinal analysis was not likely due to random variation. A second limitation of the study relates to the potential misclassification of asthma. Asthma diagnosis was based on a single question and we did not have information on asthma treatment. However, the longitudinal analysis of subjects who changed smoking status reduces the influence of the potential misclassification of asthma diagnosis, since comparisons were made within the same subjects. A third limitation refers to the lack of information on asthma onset/remission over the follow-up period. However, incidence rates in adulthood are expected to be rather low. Finally, the study did not have enough power to assess the effect of intensity of smoking in a fine manner. However, when smokers of more than 1 pack/day were grouped aside from smokers of less than 1 pack/day, the effect modification of smoking was even larger.

Halonen et al. [11] first described the relationship between smoking and an increase in circulating eosinophils in 1982. The fact that smoking also increases IgE [12, 31] suggests that smoking has a profound effect on the immunological system. Allergies to tobacco, an increase of alveolar epithelial permeability to allergens, reduction of immune receptor activity of alveolar macrophages, alteration of T cell function, and changes in the $CD4^+/CD8^+$ T cell ratio (10) are just a few of these effects. The fact that smoking produces only a small change in the $CD4^+/CD8^+$ T cell ratio at the peripheral level does not rule out organ level effects, as studies with bronchoalveolar lavage have observed [21]. Recent findings in a general population study showed that the association between smoking and specific IgE is different for different allergens (e.g., negative association of smoking with IgE antibodies to grass and cat, but positive with antibodies to mites), suggesting that the immunological impact of smoking is complex [32].

What is happening in the peripheral blood may be different at the airway level. However, studies including sputum induction observed that non-smoking asthmatics had a higher proportion of sputum eosinophils than smoking asthmatics [38, 39], which is in agreement with our results in peripheral blood.

How, then, can we explain the paradoxical effect of starting/quitting smoking on the level of circulating eosinophils in asthmatics compared to non-asthmatics? Can inflammation in non-smoking asthmatics be different than in smoking asthmatics, which in turn may have a different inflammation than smoking non-asthmatics? This apparently counterintuitive finding agrees with some other epidemiological data. In a general population survey, smoking was found to increase bronchial hyper-responsiveness only in non-atopic individuals and the authors reported a negative

interaction between smoking and atopy [33]. Furthermore, in a recent longitudinal study, a strong association was found between smoking and onset of asthma among non-atopic subjects [34]. It is not known whether smoking directly affects bone marrow, or if its effect on the equilibrium of immunologic receptors and inflammatory mediators remains at the humoral level. Thus, one explanation for these findings is that smoking has different immunological effects in subjects with and without atopy.

An increase in eosinophils in non-asthmatic smokers supports the hypothesis that smoking may enhance the risk of adult onset asthma. Not all epidemiological studies, however, have observed an association between smoking and asthma incidence [7, 35–37]. On the other hand, cessation of smoking in non-asthmatics has been shown to reduce eosinophils, which agrees with recent findings from a Lung Health Study demonstrating that non-asthmatics who quit smoking had a lower prevalence of pulmonary symptoms [8]. A small study of asthmatics showed a worsening of symptoms following smoking cessation, leading the authors to suggest the occurrence of an immunological disequilibrium in the airways of asthmatics when exposure to tobacco smoke is altered [40]. This finding was attributed to misdiagnosis of the symptoms that prompted smoking cessation rather than the appearance of symptoms after cessation. In light of the present results showing an increase of eosinophils in asthmatics who quit smoking, and the relationship between eosinophils and symptoms [16], the findings could indeed be causal, and their mechanistic basis merits further biological research.

In conclusion, we feel that this study underscores the complexity of the relationships between smoking, asthma and eosinophils in peripheral blood, and suggests that smoking plays a different immunological role in asthmatics and non-asthmatics.

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References

- 1 Sunyer J, Antó JM, Sabriá J et al. Risk factors of soybean epidemic asthma. The role of smoking and atopy. *Am Rev Respir Dis* 1992; 145:1098–102.

- 2 O'Connor GT, Sparrow D, Segal MR, Weiss ST. Smoking, atopy, and methacholine airway responsiveness among middle-aged and elderly men. The Normative Aging Study. *Am Rev Respir Dis* 1989; 140:1520–6.
- 3 Holt PG. Immune and inflammatory function in cigarette smokers. *Thorax* 1987; 42:241–9.
- 4 Siroux V, Pin I, Orszynszyn MP, Le Moual N, Kauffmann F. Relationships of active smoking to asthma and asthma severity in EGEA study. *Eur Respir J* 2000; 15:470–7.
- 5 Troisi RJ, Speizer FE, Rosner B, Throchopoulos D, Willet WC. Cigarette smoking and incidence of chronic bronchitis and asthma in women. *Chest* 1995; 108:1557–61.
- 6 Vesterinen E, Kaprio J, Kostenvuo M. Prospective study of asthma in relation to smoking habit in 1472 adults. *Thorax* 1998; 43:534–9.
- 7 Flodin U, Jönsson P, Ziegler J, Axelson O. An epidemiologic study of bronchial asthma and smoking. *Epidemiology* 1995; 6:503–5.
- 8 Kanner RE, Connett JE, William DE, Buist AS. Effect of randomized assignment to a smoking cessation intervention and changes in smoking habits on respiratory symptoms in smokers with early chronic obstructive pulmonary disease: the Lung Health Study. *Am J Med* 1999; 106:410–6.
- 9 Godtfredsen NS, Lange P, Prescott E, Osler M, Vestbo J. Changes in smoking habit and risk of asthma: a longitudinal population based study. *Eur Respir J* 2001; 18:549–54.
- 10 Johnson D, Houchens DP, Kluwe WM, Craig DK, Fisher GL. Effects of mainstream tobacco smoke on the immune system in animals and humans: a review. *CRC Crit Rev Toxicol* 1990; 20:369–95.
- 11 Halonen M, Barbee R, Lebowitz MD, Rurrows B. An epidemiological study of the interrelationships of total serum immunoglobulin E, allergy skin test reactivity and eosinophilia. *J Allergy Clin Immunol* 1982; 69:221–8.
- 12 Taylor RG, Gross E, Holland F, Pride NB. Smoking, allergy and the differential white blood cell count. *Thorax* 1985; 40:17–22.
- 13 Bousquet J, Chanez P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Eng J Med* 1990; 323:1033–9.
- 14 Schwartz J, Weiss ST. Prediction of respiratory symptoms by peripheral blood neutrophils and eosinophils in NHANES I. *Chest* 1993; 104:1210–15.
- 15 Horn BR, Robin ED, Theodore J, Van Kessel A. Total eosinophil counts in the management of bronchial asthma. *N Eng J Med* 1975; 229:1152–5.
- 16 Jansen DF, Schouten JP, Vonk JM et al. Smoking and airway hyperresponsiveness especially in the presence of blood eosinophilia increase the risk to develop respiratory symptoms. *Am J Respir Crit Care Med* 1999; 160:259–64.
- 17 Lewis S, Ian P, Stringer J, Knox AJ, Weiss ST, Britton J. The relation between peripheral blood leukocyte counts and respiratory symptoms, atopy, lung function, and airways responsiveness in adults. *Chest* 2001; 119:105–14.
- 18 Taylor LA, Luksza AR. Peripheral blood eosinophil counts and bronchial hyperresponsiveness. *Thorax* 1987; 42:452–6.
- 19 Hoppers JJ, Schouten JP, Weiss ST, Postma DS, Rijcken B. Eosinophilia is associated with increased all-cause mortality after a follow-up of 30 years in a general population sample. *Epidemiology* 2000; 11:261–8.
- 20 Corrigan CJ. T cells in asthma. In: Holgate ST, Busse WW, eds. *Inflammatory mechanism in asthma*. New York: Marcel Dekker, 1998:343–59.
- 21 Costabel U, Bross KJ, Reuter C, Ruhle K, Matthys H. Alterations in immunoregulation T-cell subsets in cigarette smokers. *Chest* 1986; 90:39–44.
- 22 Silverman NA, Potvin C, Alexander JC, Chretien PB. *In vitro* lymphocyte reactivity and T-cell level in chronic cigarette smokers. *Clin Exp Immunol* 1975; 22:285–92.
- 23 Tollerud DJ, Clark JW, Brown LM et al. The effects of cigarette smoking on T-cell subsets: a population-based survey of healthy Caucasians. *Am Rev Respir Dis* 1989; 139:1446–51.
- 24 Petersen BH, Steimel LF, Callaghan JT. Suppression of mitogen induced lymphocyte transformation in cigarette smokers. *Clin Immunol Immunopathol* 1983; 27:135–40.
- 25 Tollerud DJ, Brown LM, Blattner WA, Mann DL, Pankiw-Tros L, Hoover RN. T cell subsets in healthy black smokers and nonsmokers. *Am Rev Respir Dis* 1991; 144:612–6.
- 26 Sunyer J, Munoz A, Peng Y t al. Longitudinal relation between smoking and white blood cells. *Am J Epidemiol* 1996; 144:734–41.
- 27 Park LP, Margolick JB, Giorgi JV et al. Influence of HIV-1 infection and cigarette smoking on leukocyte profiles in homosexual men. *J Acquir Immune Defic Syndr* 1992; 5:1124–30.
- 28 Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. *Am J Epidemiol* 1987; 126:310–8.
- 29 Giorgi JV, Cheng HL, Margolick JB et al. Quality control in the flow cytometric measurement of T- lymphocyte subsets: the Multicenter AIDS Cohort Study experience. *Clin Immunol Immunopathol* 1990; 55:173–86.
- 30 Diggle P, Liang K, Zeger SL. Analysis of longitudinal data. New York: Oxford Science Publications, 1994:143–5.
- 31 Burrows B, Halonen M, Lebowitz MD, Knudson RA, Barbee RA. The relationship of serum immunoglobulin E, allergy skin tests, and smoking to respiratory disorders. *J Allergy Clin Immunol* 1982; 70:199–204.
- 32 Jarvis D, Chinn S, Luczynska C, Burney P. The association of smoking with sensitization to common environmental allergens: results from European Community Health Survey. *J Allergy Clin Immunol* 1999; 104:934–40.
- 33 Sunyer J, Anto JM, Kogevinas M, Soriano JB, Tobas A, Munoz A, and the Spanish Group of the European Study of Asthma Smoking and bronchial responsiveness in non-atopic and atopic young adults. *Thorax* 1997; 52:235–8.
- 34 Plaschke PP, Janson C, Norrman E, Bjornsson E, Ellbjar S, Jarvholm B. Onset and emission of allergic rhinitis and asthma and the relationship with atopic sensitization and smoking. *Am J Resp Crit Care Med* 2000; 162:920–4.
- 35 Larson L. Incidence of asthma in Swedish teenagers: relation to sex and smoking. *Thorax* 1995; 50:260–4.
- 36 Ronmark E, Lundback B, Jonsson E, Jonson AC, Lindstom M, Sandstrom T. Incidence of asthma in adults – report from the Obstructive Pulmonary Disease in Northern Sweden Study. *Allergy* 1997; 52:1071–8.
- 37 Lebowitz MD. Smoking habits and changes in smoking habits as they relate to chronic conditions and respiratory symptoms. *Am J Epidemiol* 1977; 105:534–43.
- 38 Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mold asthma. *Chest* 2001; 120:1917–22.
- 39 Chalmers GW, MacLeod KJ, Little SA, Thomson L, McSharry C, Thomson NC. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. *Thorax* 2002; 57:226–30.
- 40 Hillerdal G, Rylander R. Asthma and cessation of smoking. *Clin Allergy* 1984; 14:45–7.