Bronchial responsiveness, eosinophilia, and short term exposure to air pollution

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Abstract

The number of capillary blood eosinophils and the prevalence of bronchial hyper-responsiveness (BHR) were compared between schoolchildren living in a polluted area (Årdal) and unpolluted area (Lærdal).

In Årdal there is an aluminium smelter emitting sulphur dioxide and fluoride to the environment. Daily measurements of these pollutants in ambient air were available. The mean number of eosinophils in Årdal was $220 \times 10^6/l$ compared with $106 \times 10^6/l$ in Lærdal. The prevalence of BHR was 15.9% in Årdal and 11.8% in Lærdal. The odds ratio of having BHR in relation to these pollutants during the last 24 hours were: 1.12 (95% confidence interval (CI) 1.01 to 1.24) by increasing sulphur dioxide with $10 \mu g/m^3$, and 1.31 (95% CI 1.07 to 1.60) when fluoride exposure increased with $1 \mu g/m^3$. Similarly, these exposures were associated with a decrease in eosinophils of $-21 \times 10^6/l$ (95% CI -36 to -6) and $-52 \times 10^6/l$ (95% CI -98 to -8), respectively, in atopics.

It is hypothesised that recent exposure to irritants induces changes in the airways leading to BHR in addition to recruitment of eosinophils to the airways in atopic subjects.

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western Norway (Årdal and Lærdal). In Årdal (index area) there is an aluminium plant emitting air pollutants to the environment, whereas Lærdal (reference area) is an agricultural municipality with a low level of air pollution. In a previous report of the same cohort of schoolchildren we found that the prevalence of bronchial hyperresponsiveness (BHR) was positively associated with exposure to air pollutants during the first three years of life.⁸

The objective of the present study was to investigate the effect of recent (during the last 24 hours to 30 days) exposure to sulphur dioxide and fluoride on blood eosinophils and bronchial responsiveness.

Subjects and methods

STUDY AREA

Both Årdal and Lærdal are valleys with mountains with an altitude of 1200-1500 m on each side. In Årdal, there are two towns separated by a 12 km long lake: Årdalstangen and Øvre Årdal. The size of the population in these two towns is about 2500 and 4000 respectively. The Lærdal valley is about 40 km long and people live at points throughout the length of the valley. About 25% of the population are farmers and the total population is some 2300 people. In Lærdal, no industry emits air pollutants and the traffic is sparse. The climate is very similar in the two valleys: the annual rainfall ranges from 490 to 690 mm and the mean temperature is about $-3^\circ C$ in January and $14^\circ C$ in July.

STUDY POPULATION

All the pupils in the first, third, and fifth grades (that is, aged 7-13 years) in Årdal and Lærdal were invited to participate in a cross sectional study during the 1989-92 winter seasons. One of the parents attended the examination with the child. All the children and their parents were informed about the aims of the study and the test procedures. The protocol was approved by the regional ethics committee. In all, 620 from 645 eligible pupils attended the clinical examination, that is, the overall response rate was 96.1%. The mean (SD) age was 9.3 (1.7) years, 8.2% had ever had asthma, and a positive skin prick test was found in 17.8%.

QUESTIONNAIRES

Before the clinical examination, the parents received a respiratory questionnaire,⁹ including questions about the familial history of asthma or hay fever, respiratory symptoms,

The number of blood eosinophils is increased in subjects who are atopic compared with those who are non-atopic.¹ An accumulation of eosinophils is reported in the bronchoalveolar lavage fluid during allergen induced late phase reaction.² This influx of eosinophils to the airways is associated with a temporal increase in bronchial responsiveness accompanied by a decrease in peripheral eosinophils.³ The number of eosinophils in bronchoalveolar fluid is also positively correlated with bronchial responsiveness in asthmatic adults and children.^{3,4}

Results from experimental studies in guinea pigs have indicated that eosinophils accumulate in the lower airways after exposure to ozone⁵ and nitrogen dioxide.⁶ It is thus likely that non-immunological agents can stimulate eosinophils. Studies in humans of the effect of different environmental exposures on the number of blood eosinophils are recommended.⁷

We counted capillary blood eosinophils and measured bronchial responsiveness to methacholine in schoolchildren in two valleys in

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bronchitis before 2 years of age, and the parents' smoking habits at the time of the examination.

BLOOD EOSINOPHIL CELL COUNT AND SKIN PRICK TEST

Capillary blood smears were collected and eosinophils were counted using a Fuchs-Rosenthal chamber.¹⁰ The result was given as the mean of two parallel counts, measured as $\times 10^6$ cells/l. Of the 620 subjects who participated in the study, a capillary blood sample was obtained from 434 (70.0%) subjects.

Skin prick tests were performed on 556 (89.7%) of the 620 subjects who attended the examination using lancets coated with eight common aeroallergens (Phazet, Pharmacia, Uppsala, Sweden).¹¹ Five doctors were trained to perform the skin prick test; one of them (VS) tested subjects in both areas. The following allergens were used: birch, cat, cladosporium, dog, horse, house mite, mugwort and timothy, in addition to histamine and saline references. The weal size was recorded after 15 minutes and given as the mean of the long axis and its perpendicular and was regarded as positive if it was ≥ 3 mm. Those who had a positive reaction to at least one of the skin prick tests were considered to have atopy.

LUNG FUNCTION AND BRONCHIAL RESPONSIVENESS

Spirometry was performed using a pneumotachograph (Vitalograph, Birmingham) which was calibrated daily to 5 litres using a 1 litre syringe. The test was accepted if the difference between the best and the second best test varied by less than 5% or 100 ml, whichever was larger. The result was given as forced expiratory volume in one second (FEV₁) as a percentage of predicted using the predicted values published by Cotes.¹² Methacholine challenge was performed using a shortened version of the protocol suggested by Cockcroft *et al.*^{13,14} All the subjects whose baseline FEV₁ was $\geq 70\%$ of predicted¹² were invited to perform the challenge test. The response to the methacholine challenge was expressed as PC₂₀: that is, the concentration that caused a fall in FEV₁ of 20% from baseline calculated by linear interpolation on the log linear dose response curve. If FEV₁ was less than 70% of predicted,¹² the subject was asked to perform a reversibility test. After two inhalations of 0.1 mg of salbutamol a second spirometry measurement was made five minutes later. A subject was regarded as having BHR if PC₂₀ ≤ 8 mg/ml or FEV₁ increased by more than 10% at the reversibility test. Bronchial responsiveness was also expressed as the log transformed dose response slope as suggested by O'Connor *et al.*¹⁵

ASSESSMENT OF ENVIRONMENTAL EXPOSURE

In Øvre Årdal there is an aluminium smelter producing 180 000 tonnes of primary aluminium annually. The main emissions from the plant

are sulphur dioxide, fluoride, and particulates. The concentration of sulphur dioxide and fluoride in ambient outdoor air has been measured every day at one station in both towns since 1978, using equipment for continuous sampling. Two types of sampler have been used: one equipped with an absorption solution for sulphur dioxide and one with an alkaline filter for the absorption of fluoride. Both kinds of samplers were constructed exclusively to absorb gas. The sampler shifted from one bottle (sulphur dioxide) and filter (fluoride) to the next every 24 hours at 10 am, that is, the daily mean was measured. The subjects attended the examination between 8 am and 3 pm. Hence, the measurements of sulphur dioxide and fluoride on the previous day could be regarded as estimates of the mean exposure to these agents during the last 24 hours. The majority of the population in Årdal live less than 2 km from the location of the samplers. Data on temperature and humidity were also available.

The exposure of the subjects was classified as follows. In each subject living in the index area two indices of exposure to sulphur dioxide and fluoride were used: (i) current exposure: exposure during the last 24 hours prior to the examination and (ii) last month exposure: exposure during 1–30 days before the survey. The median and 10th–90th centiles of the exposure to these agents are listed in table 1.

During the winter of 1991, the concentration of sulphur dioxide in Lærdal was measured using the same type of equipment as in Årdal and analysed at the same laboratory. The median was $2.5 \mu\text{g}/\text{m}^3$ (10th–90th centiles 0.1–11.1) in 44 observations (that is, about 1/10 of the level in Årdal). At the same time, the concentration of fluorides was not detectable.

STATISTICAL ANALYSES

Univariate analyses (*t* tests and χ^2 statistics) were used to investigate the association between each of the outcomes and the exposure variables in the index group. The association between the outcome and each of the following covariates were examined: age, gender, atopy, familial history of asthma or hay fever, FEV₁ (as % of the predicted value), bronchitis before 2 years of age, temperature, humidity, parental smoking, and presence of animals in the household.

Multivariate analyses were used in order to control for extraneous effects. A covariate was included if the univariate test had a *p* value ≤ 0.25 .¹⁶ The full model was reduced by backward elimination using the partial F test as the selection criterion¹⁷ in the multiple regression model and the log likelihood test in the logistic

Table 1 Median exposure ($\mu\text{g}/\text{m}^3$) to sulphur dioxide and fluoride in the index group (10th and 90th centiles in parentheses)

Time of exposure	Sulphur dioxide	Fluoride
Last 24 hours	22.2 (1.9–85.3)	1.0 (0.4–3.3)
Last 1–30 days	32.9 (20.9–43.8)	1.6 (0.8–2.8)

Table 2 Mean eosinophils, the prevalence of BHR, and some covariates in different exposure categories during the last 24 hours in the index area (Årdal) and the reference area (Lærdal)

	Exposure to sulphur dioxide in the index area ($\mu\text{g}/\text{m}^3$)				Reference area
	<20	20-29	30-39	≥ 40	
No of subjects*	189	85	67	100	141
Eosinophils ($\times 10^6/\text{l}$)					
Atopics	378	290	182	215	181
Non-atopics	198	197	190	188	95
BHR (%)	12.4	13.9	16.7	19.0	11.8
FEV ₁ (% of predicted)	100.4	104.5	100.1	99.6	98.1
Age (years)	8.9	9.3	9.2	9.6	9.5
Atopy (%)	20.4	25.0	16.4	19.0	14.5
Bronchitis before 2 years of age (%)	14.4	23.5	11.9	17.0	20.6

*Exposure to 38 subjects in the index group were missing.

regression model.¹⁶ The covariates were removed from the model unless the reduced model deviated from the initial model at a significance level of 5% or the coefficients of the exposure variables changed by more than 20% compared with the full model. In these analyses, sulphur dioxide and fluoride exposure data were used as continuous variables. The analysis of eosinophils was performed using the SYSTAT statistical package,¹⁸ whereas the LOGIT module of SYSTAT¹⁹ was used for the logistic regression analysis of BHR. The fit of the model was assessed using the Hosmer-Lemeshow test.²⁰

Results

The univariate analyses indicated that the number of blood eosinophils was higher in subjects living in Årdal ($220 \times 10^6/\text{l}$) compared with those living in Lærdal ($106 \times 10^6/\text{l}$) ($p < 0.001$), at the time of the examination. The prevalence of BHR in the index area was 15.9% compared with 11.8% in the reference area ($p = 0.27$).

The relation between eosinophils and exposure to sulphur dioxide during the last 24 hours before the examination are shown in table 2. A negative dose response relation between eosinophils and exposure to sulphur dioxide (table 2) and fluoride during the last 24 hours was indicated. A dose response relation between the prevalence of BHR and exposure to these pollutants during the last 24 hours was indicated (table 2).

The remaining analyses of the association between the outcome variables and exposure variables were restricted to the subjects in the index area using multivariate methods. These analyses were performed using indices of

Table 3 Mean (SE) change in the number of eosinophils (as $\times 10^6/\text{l}$) in relation to sulphur dioxide and fluoride exposure, adjusted for atopy, obtained by multiple regression

Time lag between examination and exposure	Exposure variable	
	Sulphur dioxide†	Fluoride
Last 1-30 days	16 (7)*	26 (11)
Current	-4 (3)	-13 (7)*
Atopy	129 (29)***	121 (29)***
Atopy $\times$ current	-17 (7)*	-28 (17)

†The change in eosinophils by increasing sulphur dioxide exposure by $10 \mu\text{g}/\text{m}^3$. Both covariates were entered as continuous variables.

* $p < 0.05$, *** $p < 0.001$.

sulphur dioxide and fluoride in separate models (tables 3 and 4). Regarding the analysis of eosinophils as the dependent variable atopy remained in the final model in addition to the exposure variables. Using these exposure variables as continuous covariates, a significant positive association between eosinophils and sulphur dioxide and fluoride exposure during the last 30 days before the examination was found (table 3). After inclusion of atopy and exposure during the last 30 days, the product term between atopy and exposure during the last 24 hours to sulphur dioxide was significant (table 3); that is, the association between eosinophils and exposure was restricted to the atopics. The association between eosinophils and the exposures during the last 24 hours were therefore performed in atopic and non-atopic subjects separately. In atopic subjects, increasing sulphur dioxide with $10 \mu\text{g}/\text{m}^3$ and fluoride with $1 \mu\text{g}/\text{m}^3$ was associated with a decrease in eosinophils of $-21 \times 10^6/\text{l}$ (95% CI -36 to -6) and $-52 \times 10^6/\text{l}$ (95% CI -98 to -8), respectively.

In accordance with the criteria of model reduction, atopy, bronchitis before 2 years of age, age, and lung function were included as covariates in the final logistic model of BHR, that is, exposure to environmental tobacco smoke was deleted during the backward elimination of the model. In the first step the relation between the prevalence of BHR and sulphur dioxide and fluoride was investigated separately (table 4). A positive relation was found between the prevalence of BHR and exposure to sulphur dioxide and fluoride during the last 24 hours, whereas no association with previous exposure was found. The product term between exposure during the last 24 hours and atopic status was not significant; that is, there was no difference between atopic and non-atopic subjects. The Hosmer-Lemeshow test of the models showed no significant deviation between the observed and expected numbers ($p = 0.67-0.99$).

Multiple regression of the log transformed dose response slope revealed a significant association with fluoride exposure ($p = 0.003$), but not with sulphur dioxide exposure ($p = 0.17$). The R^2 for the former model was 9.2% and 5.3% for the latter model.

There was a significant positive association between the prevalence of BHR and atopy (odds ratio (OR) 2.7, 95% CI 1.4 to 5.5) and bronchitis before 2 years of age (OR 2.3, 95% CI 1.1 to 4.7). The prevalence of BHR was negatively correlated to FEV₁ as % of predicted (OR 0.7 by increasing FEV₁ with 5%, 95% CI 0.6 to 0.8) and age (OR 0.6 by increasing age with 2 years, 95% CI 0.4 to 0.9).

Table 4 OR (95% CI) of BHR by increasing sulphur dioxide by $10 \mu\text{g}/\text{m}^3$ and fluoride by $1 \mu\text{g}/\text{m}^3$, adjusted for age, atopy, bronchitis before 2 years of age and lung function, obtained by logistic regression

Time lag between examination and exposure	Exposure variable	
	Sulphur dioxide	Fluoride
Last 24 hours	1.12 (1.01 to 1.24)	1.31 (1.07 to 1.60)
Last 1-30 days	0.94 (0.73 to 1.21)	0.97 (0.86 to 1.08)

Discussion

We have found that exposure to sulphur dioxide and fluoride during the last 24 hours is associated with a decrease in the blood eosinophils in atopic subjects and an increase in the prevalence of BHR, the latter relation being independent of the atopic status.

The results from the Hosmer-Lemeshow test showed that the logistic regression seemed to fit the data quite well.¹⁶ On the other hand, the multiple linear regression could only explain 5–9% of the variance, indicating that the use of the logistic models were more appropriate than the linear regression models. The logistic regression revealed a significant association between BHR and both indices of exposure.

The difference in eosinophils and bronchial responsiveness between the subjects living in the index area and the reference area could be due to differences between populations. This explanation is, however, unlikely. Firstly, an association between eosinophils and bronchial responsiveness and environmental exposure was found in subjects living in the index area. Moreover, the prevalence of BHR in subjects with the lowest exposure during the last 24 hours was very close to the prevalence of BHR in the reference group. The possibility that systematic differences between the subjects within the index group could explain these findings appears unlikely. In fact, the inclusion of several potential confounders did not dilute the associations.

Furthermore, the relation between bronchial responsiveness and exposure to air pollutants appears to be influenced by the time lag between the exposure and the effect. We found that exposure during the first three years of life in these children increased the prevalence of BHR at school age.⁸ On the other hand, the exposure during the last 24 hours, but not during the last month, appeared to increase bronchial responsiveness. It therefore seems likely that exposure to air pollutants in infancy has a long term effect on bronchial responsiveness, whereas exposure during the last 24 hours in school age exert a short term effect which is transient.

It has been suggested that epithelial damage leads to inflammatory changes that are accompanied with BHR.^{21–22} The bronchial responsiveness appears to be related to the numbers of eosinophils and mast cells in the bronchoalveolar fluid of asthmatics.²³ Furthermore, it is hypothesised that air pollution induces epithelial damage resulting in mediator release and BHR.²⁴ The finding that exposure to sulphur dioxide and fluoride during the last 24 hours was associated with increased prevalence of BHR, indicate that these irritants may induced mucosal changes leading to BHR. It appears that this response to the exposure has the same magnitude in atopic as non-atopic subjects. Our results indicate, however, that the eosinophils in atopic subjects react differently from non-atopics to irritant exposure. One may speculate if changes in the respiratory tree is associated with BHR trigger eosinophils in atopic subjects to migrate to the airways.

A positive relationship between eosinophils and 30 day exposure was found. Similar associations have, actually, been found using experimental designs.^{3–25} In these studies the number of blood eosinophils increased on the next day after allergen exposure compared with the day of the exposure. The authors suggest that the exposure stimulate the recruitment of eosinophils from the bone marrow to the blood. It is possible that exposure to air pollution for several day may have a similar effect. The analyses indicated that the strength of association to sulphur dioxide and fluoride was different in terms of bronchial responsiveness and eosinophils. Firstly, these exposure variables were highly correlated; that is, the analyses do not differentiate adequately between these two covariates. Secondly, both sulphur dioxide and fluoride might merely be indices of some other agent which is correlated to them.

The relation between peripheral eosinophils and environmental exposure to non-allergens has been investigated in only a few studies in humans. An increase in the number of blood eosinophils was found in adult smokers compared with non-smoking French policemen²⁶ and in the male offspring of smoking parents.²⁷ The positive association between peripheral eosinophils and environmental exposure in this study is in agreement with the first of these studies.

The relation between outdoor exposure to pollutants and respiratory symptoms, BHR, and atopy has been studied in polluted areas in Eastern Europe with less polluted areas in the West.^{28–30} In these studies, the prevalence of atopy^{28–30} and BHR²⁸ was higher in the West than in the East although the former area was most polluted. In one of these studies,²⁹ domestic crowding appeared to protect against atopic sensitisation. After adjustment for atopy, these differences decreased,^{28–29} indicating that the Western lifestyle is an independent risk factor for sensitisation that explain an increased prevalence of asthma and BHR. In our study, however, the comparisons were made between different exposure groups living in the index area. Furthermore, adjustment for atopy was included in the analyses. It thus seems likely that air pollution may increase bronchial responsiveness when other extraneous factors are taken into account.

In summary, high exposure to irritants like sulphur dioxide and fluoride during the last 24 hours is associated with an increase in bronchial responsiveness. In atopic subjects this increase in bronchial responsiveness is associated with a decrease in the peripheral eosinophils cell count. Exposure during the last month is associated with a raised number of blood eosinophils.

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