

Original article

Inflammation and functional outcome in diisocyanate-induced asthma after cessation of exposure

Background: The clinical outcome of diisocyanate-induced asthma has been found to be poor despite cessation of exposure. Our aim was to study the outcome of diisocyanate-induced asthma after initiation of inhaled steroid treatment at a mean period of 7 months (range 2–60 months) after cessation of exposure by following up lung function and bronchial inflammation.

Methods: Bronchoscopy was performed on 17 patients 2 days after a positive inhalation challenge test, after which budesonide 1600 µg a day was started. Bronchoscopy, spirometry, and histamine challenge tests were repeated at 6 months and on average 3 years. The results were also compared with those obtained from 15 healthy control subjects.

Results: Nonspecific bronchial hyperreactivity diminished significantly ($P = 0.006$); however, forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) values decreased, with a median yearly reduction of FEV1 of 79 ml. The count of mast cells in bronchial mucosa decreased ($P = 0.012$) and that of macrophages increased ($P = 0.001$). Interleukin-4 level in mucosa was during the first year significantly higher than in controls but its level decreased in the follow-up. Interleukin-6, interleukin-15, and tumour necrosis factor alpha messenger-RNA levels were significantly higher in hyperreactive patients than in nonhyperreactive patients at the end of the follow-up.

Conclusion: Our results indicate that inflammation may persist in diisocyanate-induced asthma despite inhaled steroid medication. However, TH2-type inflammation diminished. Persistent nonspecific bronchial hyperreactivity was associated with proinflammatory acting cytokines produced mainly by macrophages. Considering the poor prognosis of the disease the findings could be utilized to develop the follow-up and treatment of diisocyanate-induced asthma.

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The diisocyanates, toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI), and hexamethylene diisocyanate (HDI) are commonly used in the manufacture of plastics, paints and foam products. They are also the most common chemicals causing occupational asthma. Earlier study has shown that a considerable proportion of patients do not recover from occupational asthma (1).

In diisocyanate-induced asthma (DIA), bronchial inflammation, including lymphocytic infiltration and eosinophilia (2, 3), resembling that present in allergic asthma has been demonstrated. After cessation of exposure, a slight decrease in the count of mononuclear cells, eosinophils, and mast cells or a reduced thickness of subepithelial fibrosis has been found (4), suggesting a reversal of remodelling of the airway wall. However, respiratory symptoms and nonspecific bronchial hyper-

responsiveness (1, 5, 6) have been reported to persist for years in the majority of patients with DIA despite cessation of exposure. In the follow-up of lung function of DIA patients treated with inhaled steroids, a decrease in nonspecific bronchial hyperreactivity (NSBH) has typically been reported (6, 7).

In our previous study (7), the degree of lung function impairment and NSBH increased compared with the baseline results in patients with DIA 35% of whom used asthma medication and 7% were still exposed to diisocyanates at work. There was a need to increase or start asthma medication after a mean follow-up of 12 years.

Our aim was to follow up patients with DIA after cessation of exposure and to treat them with regular inhaled steroid medication to investigate the prognosis of DIA by spirometry and measurement of NSBH. In addition, the relation between lung function and markers

of inflammation was studied based on analysis of biopsies of bronchial mucosa and bronchoalveolar lavage (BAL) fluid specimens.

Methods

Study design

All new DIA patients at the Finnish Institute of Occupational Health (FIOH) during 1995–2001 were invited to enrol in this study; 17 patients chose to participate. Fiberoptic bronchoscopy and BAL were performed about 48 h after a positive inhalation challenge test to diisocyanates. Exposure to diisocyanates was ceased on average 7 months before the studies because of respiratory symptoms. Further exposure to diisocyanates was not permitted after diagnosis of DIA. After bronchoscopy, budesonide (AstraZeneca, Södertälje, Sweden) 1600 µg a day was started. The studies were repeated at 6 months and 2–3 years after the diagnosis. Information about compliance to use the medication was elicited from the patients by the doctor at the visits. The inflammatory findings of patients were compared with 15 healthy control subjects. Informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of FIOH.

Participants

Patients comprised 12 men and five women, who had been exposed to HDI ($n = 7$), MDI ($n = 7$), TDI ($n = 2$), or a combination of

HDI and MDI ($n = 1$). Their anthropometric data are presented in Table 1. Six patients had sometimes temperature in addition to asthma symptoms.

After bronchoscopy, for the first year, standard medication was used, but after 1 year, adjustments in medication were allowed. For two patients, the inhaled steroid was changed to fluticasone dipropionate (Glaxo, Evreux, France) 1000 µg a day. The dose of budesonide at the last control varied from 400 to 2400 µg/day (mean 1257 µg/day). For five patients, also formoterol (AstraZeneca) 24 µg a day and for one patient salmeterol (Glaxo) 100 µg a day had been started. Changes in medication were made based on patients' individual symptoms and PEF monitoring.

Most (76%) of the patients were nonsmokers or ex-smokers. Two of the seven ex-smokers had quit smoking more than 20 years earlier (Table 1). During the present follow-up, smokers did not change their smoking habits.

Bronchoscopy was performed once on eight nonsmoking, two ex-smoking, and five smoking control subjects (Table 1). Subjects with lung diseases or any other diseases, and those with allergic symptoms were excluded. An acute respiratory infection was an exclusion criterion. All had normal blood haemoglobin levels, leucocyte counts and spirometry results, and histamine challenge test showed no bronchial hyperreactivity.

Lung function methods

Flow-volume spirometry was performed with a rolling-seal spirometer (Mijnhardt BV, Bunnik, the Netherlands) connected to a microcomputer (Medikro MR-3; Medikro, Kuopio, Finland), using

Table 1. Age, gender, atopy and smoking data of patients and controls. Exposure and challenge data are also provided for the patients

Parameter	Patients ($n = 17$)	Controls ($n = 15$)
Gender	Male (12) Female (5)	Male (5) Female (10)
Age (years) (mean $\pm$ SD)	42.8 $\pm$ 12.7	35.5 $\pm$ 10.9
Duration of exposure to isocyanates (years)	12.2 $\pm$ 9.6 (range 0.5–33)	–
Duration of asthma symptoms (years)	6.5 $\pm$ 6.2 (range 0.33–20)	–
Last exposure to diisocyanates before examinations (months)	7.4 $\pm$ 14.1 (range 0.2–60)	–
Atopy in prick testing or in phadiatop®	5 (29%)	2 (13%)
Smoking	Nonsmokers (6) Ex-smokers (7) Smokers (4)	Nonsmokers (8) Ex-smokers (2) Smokers (5)
Pack years (quitting, years), mean $\pm$ SD	Ex-smokers 20.4 $\pm$ 16.5 (9.1 $\pm$ 11.1*) Smokers 16.8 $\pm$ 13.1	Ex smokers 5.9 $\pm$ 4.1 (5 $\pm$ 1.4) Smokers 25.1 $\pm$ 11.8
Sensitizing agent	HDI ($n = 7$) MDI ($n = 7$) TDI ($n = 2$) HDI and MDI ($n = 1$)	No diisocyanate exposure
Diisocyanate-specific IgE-positive	3 (17.6%)	
Challenge test reaction to diisocyanates	Immediate 5 (29%) Late 12 (71%)	Not studied
PEF reduction in specific challenge test (l/min) (mean $\pm$ SD)	23.2 $\pm$ 9.0	
FEV ₁ (l) $\pm$ SD; (% of predicted)	3.79 $\pm$ 0.86 (97.4 $\pm$ 9.7)	3.69 $\pm$ 0.79 (93.5 $\pm$ 14.6)
FVC (l) $\pm$ SD; (% of predicted)	4.5 $\pm$ 1.0 (97.9 $\pm$ 8.8)	4.9 $\pm$ 0.98 (102.1 $\pm$ 13.0)
FEV ₁ reduction in specific challenge test (l) (mean $\pm$ SD)	22.7 $\pm$ 10.0	
Challenge test concentration (mg/m ³ NCO) (mean $\pm$ SD)		
HDI-monomer	0.0096 $\pm$ 0.0001	
HDI-prepolymer	2.25 $\pm$ 1.37	
MDI-monomer	0.0013 $\pm$ 0.00 001	
TDI-monomer	0.069	

*Range 1–28 years.

HDI, hexamethylene diisocyanate; MDI, diphenyl methane diisocyanate; TDI, toluene diisocyanate.

the reference values of Viljanen (8). The flow-volume curve was formed with the envelope method with curves obtained from at least three successive forced expiratory breathing manoeuvres, using the standards of the European Respiratory Society (ERS) (9). The histamine challenge test was performed according to the Sovijärvi et al.' method (10), and forced expiratory volume in 1 s (FEV1) values were monitored with a Vitalograph S bellows spirometer (Vitalograph, Buckingham, UK). Provocative dose causing a 15% reduction in FEV1 was measured. Bronchial hyperreactivity was graded as follows: strong PD15 < 0.1 mg, moderate PD15 0.1–0.4 mg, slight PD15 0.4–1.6 mg, and no hyperreactivity PD15 > 1.6 mg. For statistical calculations a PD15 value was graphically extrapolated for those with PD15 > 1.6 mg. Diffusing capacity and total lung capacity were measured with single breath method using Viljanen reference values (8).

Specific challenge test methods

The diagnosis was based on specific inhalation challenge testing, as described thoroughly elsewhere (7). Specific challenge test with MDI monomer (Merck 820797), TDI monomer (Merck 808264), or HDI-containing substance (monomer and prepolymer; Autocryl, Glasurit, Deltron, Dupont 250S, Teknodur, Permacron) was performed. FEV1 and peak expiratory flow (PEF) values were monitored until the following morning using a spirometer. The control challenge test was conducted for 13 patients with polyol (hydrogenated decene oligomers, Nexbase 200 6FG; Neste PAO NV, Beringen, Belgium), for one with a solution containing 0.5% NaCl, 0.275% Na-bicarbonate, 0.4% phenol (Yliopistoapteekki, Helsinki, Finland), and for three with paint without the diisocyanate hardener. The criterion for a positive challenge test was at least a 20% reduction in FEV1 or PEF, provided that the control challenge test was negative. Immediate reactions with a 15–20% reduction were considered positive if symptoms, such as dyspnoea, were simultaneously present. Eleven patients had been using inhaled steroids, and the medication was discontinued a mean of 17.5 days (range 6–60 days) before the examinations.

Bronchoscopic methods

Bronchoscopy was performed after local anaesthesia with 2% xylocain with a bronchofiberscope (Olympus BF-XT30; Olympus, Aizu, Japan), and BAL was performed and analysed according to Taskinen et al. (11). The biopsies from bronchial mucosa were taken from the right lower or upper lobe or from the left upper lobe subsegmental carinas. For BAL, the bronchoscope was wedged into the right middle lobe, and a 20-ml aliquot of physiologic saline was instilled to the bronchus and aspirated immediately in the same syringe; this was repeated 10 times. BAL fluid was centrifuged, and the cytological picture was studied from the cell fraction by routine methods of the pathologic laboratory of the FIOH (12).

Immunohistochemistry

One bronchial mucosal biopsy specimen was immediately fixed in 10% neutral buffered formaline and another was snap-frozen in liquid nitrogen. Liquid nitrogen-frozen specimens were embedded in Tissue Tek, stored at –70°C, and sectioned in 6 µm slices with a cryostat. Specimens stained with haematoxylin–eosin were used to evaluate tissue morphology. Monoclonal antibodies (DAKO, Glostrup, Denmark) against neutrophil elastase (clone NP45), CD163 (clone Ber-Mac3), CD3 (clone T3-4B5), mast cell tryptase (clone AA1), CD4, CD8 and interleukin 4 (IL-4) were used to

identify neutrophils, macrophages, T lymphocytes, mast cells, CD4⁺ and CD8⁺ T cells and IL-4 secreting cells. To detect eosinophils, mouse monoclonal antibody (mAb) EG2 (Pharmacia, Uppsala, Sweden) was used. Staining, visualization, photographing and cell calculations were performed as described earlier (13).

Real-time quantitative RT-PCR assay

Total RNA from BAL cells was extracted using Trizol Reagent (Gibco BRL, Paisley, UK) according to the manufacturer's protocol. DNAaseI treatment, RNA quantification, cDNA synthesis and real-time quantitative PCR were performed as described earlier (14). PCR primers and probes were obtained as predeveloped separate assay reagents (CCL11, CCL5, IL-18, IL-13, IL-6, TNF-β, CCL20, CCL22, CCL4, IL-16, CCL3) or the Cytokine Gene Expression Plate I with preattached primers and probes (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN-γ and TNF) was used.

The results are expressed as relative units (fold differences). Calculations were performed according to manufacturer's instructions exactly as described earlier (14).

Statistical methods

Several parameters were nonnormally distributed in our data, and therefore, nonparametric tests were applied. Wilcoxon one-sample rank test was used when comparing results of patients' lung function, cell count, cytokine, etc., between examination phases. Results of patients' examinations were compared with those of controls using the Wilcoxon test for two independent samples. We made several simultaneous comparisons and used Bonferroni correction of the p-values to indicate a statistically significant result.

The rates of PEF variation between the different stages of follow-up were compared using chi-square tests.

Cytokine levels between hyperreactive and nonhyperreactive patients were compared with Kruskal–Wallis variance analysis.

All the analyses were carried out with spss for windows Release 12.01 (SPSS Inc., Chicago, IL, USA).

Results

Lung function studies

The mean duration (± SD) of follow-up was 33.5 ± 13.1 months. Between the first and the third examinations, a significant reduction in forced vital capacity (FVC) values was found, an almost significant in FEV1 values (Table 2). The median (range) yearly decline of FEV1 was 78.7 (+161; –429) ml in all patients, in smokers 38 (+161; –133) ml, in ex-smokers 79 (+48; –109) ml and in nonsmokers 160 (+15; –429) ml. One nonsmoking patient with a 429-ml yearly FEV1 reduction had a normal spirometry and was nonhyperreactive and almost symptomless, with a diurnal PEF variation less than 10%, the reason why his doctor had reduced his budesonide dosage to 400 µg/day. Two other nonsmoking patients showing at least a 150-ml yearly FEV1 reduction used budesonide 800 µg/day in the third examination. They had only occasional asthma symptoms, but one of them was hyperreactive and showed

Table 2. Durations of the follow-ups and the results of lung function tests of patients (mean $\pm$ SD) during follow-up

	First examination (baseline) (<i>n</i> = 17)	Second examination (<i>n</i> = 15)	Third examination (<i>n</i> = 12)	Change between examinations		
				1/2	2/3	1/3
Follow-up duration (months)						
Mean ($\pm$ SD)		7.9 $\pm$ 3.0	33.5 $\pm$ 13.1			
Range		5–13	18–56			
PEF variation (%)						
<10%	5 (29%)	5 (33%)	4 (33%)	ns	ns	ns
10–19%	10 (59%)	7 (47%)	6 (50%)			
$\geq$ 20%	2 (12%)	2 (13%)	2 (17%)			
Positive bronchodilation test (number)	2	1	0			
FVC (l)	4.9 $\pm$ 0.98	4.81 $\pm$ 0.92	4.6 $\pm$ 0.78	ns	0.013	0.015
FVC (% of predicted)	102.1 $\pm$ 13.0	100.1 $\pm$ 9.7	97.5 $\pm$ 7.7	ns	ns	ns
FEV1 (l)	3.69 $\pm$ 0.79	3.54 $\pm$ 0.74	3.4 $\pm$ 0.6	ns	ns	0.023
FEV1 (% of predicted)	93.5 $\pm$ 14.6	90.3 $\pm$ 12.1	87.1 $\pm$ 11.2	ns	ns	ns
MMEF (l/s)	2.9 $\pm$ 1.17	2.69 $\pm$ 0.98	2.4 $\pm$ 0.84	ns	ns	ns
MMEF (% of predicted)	64.6 $\pm$ 21.7	61.3 $\pm$ 22.3	55.1 $\pm$ 17.0	ns	ns	ns
MEF50 (l/s)	3.69 $\pm$ 1.16	3.30 $\pm$ 1.13	2.93 $\pm$ 0.94	ns	ns	ns
MEF50 (% of predicted)	67.5 $\pm$ 18.1	63.7 $\pm$ 20.4	57.1 $\pm$ 17.3	ns	ns	ns
FEV1/FVC ratio (%)	74.8 $\pm$ 7.0	73.8 $\pm$ 7.9	75.3 $\pm$ 9.5	ns	ns	ns
DL (% of predicted)	90.2 $\pm$ 16.8	nd	97.8 $\pm$ 12.5			ns
DL/VA (% of predicted)	95.1 $\pm$ 16.3	nd	92.6 $\pm$ 10.2			ns
TLC (% of predicted)	89.4 $\pm$ 11.0	nd	101.6 $\pm$ 6.8			ns
Serum total IgE (kU/l)	88.8 $\pm$ 57.2	nd	88.8 $\pm$ 57.1			ns
Bronchial hyperreactivity						
No	4 (24%)	8 (53%)	6 (50%)			
Slight	8 (47%)	5 (33%)	5 (42%)			
Moderate	3 (18%)	1 (7%)	–			
Not studied	1 (6%)	2 (13%)	1 (8%)			
PD15	0.89 $\pm$ 1.78	2.0 $\pm$ 2.32	2.76 $\pm$ 2.76	0.006	ns	0.005

FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; MMEF, maximal mid expiratory flow; MEF50, maximal expiratory flow at the level where 50% of FVC remains exhaled; DL, diffusing capacity; DL/VA, specific diffusing capacity; TLC, total lung capacity; nd, not done; ns, not significant.

Level of significance ($P < 0.02$).

slight obstruction in spirometry. The other had normal spirometry and was nonhyperreactive. The fourth non-smoker was hyperreactive, although he received budesonide 1600 μ g/day. He had a median yearly increase of FEV1 by 15 ml.

The rate of PEF variation did not change significantly between the examination phases. The PD15 value increased significantly between the first and second and between the first and third examinations (Table 2). Hyperreactivity level decreased in three patients from moderate to slight, in two patients remained slight from the first to the third examination, and three patients became nonhyperreactive. Two patients were nonhyperreactive throughout the follow-up. At the end of the study, one patient using budesonide 1600 μ g/day, three patients using budesonide 800 μ g/day, and one using fluticasone 1000 μ g/day were hyperreactive.

At the third examination phase, all patients had occasional asthma symptoms and used short-acting betasymphathomimetic medication on demand.

Increased FEV1 decline or persistence of hyperreactivity did not associate with the causative isocyanate or smoking.

Histological specimen from bronchial mucosa

The number of neutrophils or activated eosinophils in patients did not significantly change in follow-up.

The number of mast cells in the first examination in the patients was at the same level as in controls. An almost significant reduction in mast cells occurred between the first and second examinations ($P = 0.012$) (Data not shown).

The macrophage count was in patients in the second and third examinations significantly higher than in controls (Fig. 1).

The CD3 lymphocyte count was similar; the level of CD8 cells slightly higher than in controls. The level of CD4 cells in patients was almost significantly lower than in controls during the follow-up ($P = 0.05$ – 0.01). The ratio CD4:CD8 rose nonsignificantly during the follow-up; mean (SD), 0.87 (0.43), 1.16 (0.75) and 1.78 (1.78) corresponding to the examination phases, controls 13.47 (26.9) (data not shown).

Compared with the control subjects the patients had significantly higher numbers of IL-4 secreting cells in the first and second examination (Fig. 2). The number of

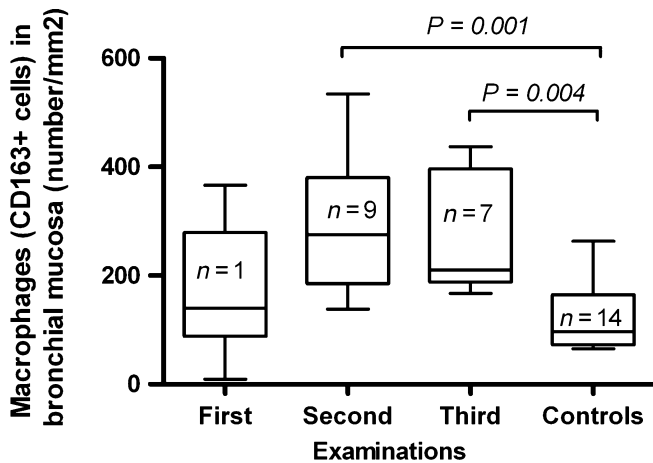


Figure 1. Histological findings in bronchial mucosa during three examination phases for patients and in healthy controls. Level of significance, $P < 0.01$.

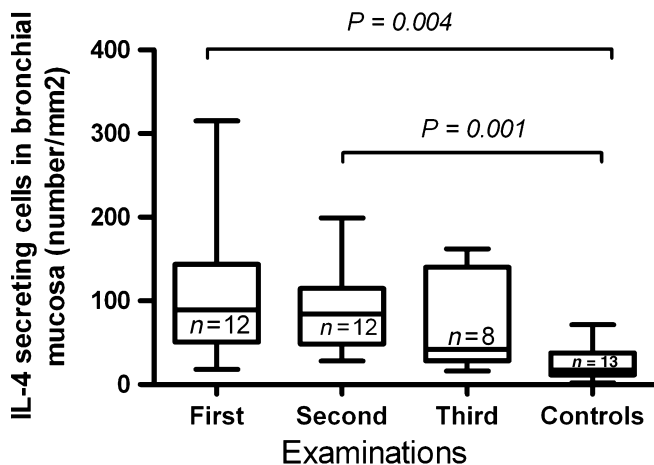


Figure 2. Immunohistochemical findings in bronchial mucosa during the three examination phases for patients and in healthy control subjects. Level of significance, $P < 0.01$.

IL-4 secreting cells was reduced in the follow-up, but the change was not significant.

There was a rather high correlation between NSBH and IL-4 in the second examination ($\rho = -0.561$, $P = 0.058$; $n = 12$). In the third examination NSBH correlated with CD3 ($\rho = -0.880$, $P = 0.021$) and CD4 cells ($\rho = -0.941$, $P = 0.005$) ($n = 6$, both).

BAL fluid examinations

The results of the cytological examinations of BAL fluid are given in Table 3. Total cell count was significantly reduced between the first and second examinations.

The relative lymphocyte count became in patients in the second and third examinations significantly lower than in the first examination. There were, however, no

significant differences between patients and controls. The relative eosinophil count in the first examination was almost significantly higher in patients than in controls, and decreased in the second and third examinations.

The neutrophil count or the relative macrophage count did not change during follow-up. The basophil count was almost zero in all patients, as well as in healthy subjects.

Cytokine and chemokine mRNA studies

Cytokines on chemokines in BAL cells (please see list in the Methods section) did not significantly change or differ from controls during the follow-up.

No systematic significant associations were observed between spirometric variables or NSBH and cytokines or chemokines during the follow-up in correlation analyses. However, when we compared cytokine and chemokine levels between hyperreactive patients and nonhyperreactive patients in the third examination, IL-6, IL-15 and TNF- α were significantly higher in the former group (Fig. 3). The chemokines CXCL8 (IL-8), CCL3 (MIP1 α) and CCL5 (RANTES) were also higher in hyperreactive patients than in nonhyperreactive patients but the difference did not reach statistical significance (data not shown).

Discussion

In follow-up of DIA after cessation of exposure and during inhaled steroid treatment, NSBH diminished, but FEV1 and FVC showed a progressive decrease. After diagnosis of DIA, the number of eosinophils in BAL fluid of asthma patients was suggestively elevated compared with controls and in the follow-up the counts of eosinophils in BAL fluid and mast cells and IL-4 secreting cells in bronchial mucosa decreased, which is in agreement with earlier study (15).

Although the degree of NSBH decreased significantly during the follow-up, at the end of the period a slight NSBH remained in almost half of the patients. We found that proinflammatory cytokines IL-6 and TNF- α as well as Th1 cytokine IL-15 were significantly higher in the hyperreactive patients, which is a new finding.

Maghni et al. (16) described IL-8 to be higher in induced sputum of patients with occupational asthma with no improvement (i.e. those with persisting NSBH) compared with those without hyperreactivity. However, the causes of occupational asthma were not given, although cases caused by both high and low molecular weight agents were included. While this study (16) was carefully performed, a limited number of inflammatory markers were examined. Also here, IL-8 showed a tendency to be higher in hyperreactive patients. However, when several cytokines or chemokines were investigated,

Table 3. Results of bronchoalveolar lavage fluid cell analysis of patients and controls; median (range).

Parameter	First examination (baseline) (n = 17)	Second examination (n = 15)	Third examination (n = 12)	Controls (n = 15)	1/2	2/3	1/3	1/C	2/C	3/C
Cell count (million/l)	203.0 (59–528)	128.0 (74–424)	163.0 (34–482)	168.0 (82–464)	0.005	ns	ns	ns	ns	ns
Lymphocytes (%)	22.0 (9–46)	11.0 (2–21)	11.5 (2–36)	18.0 (1–29)	0.007	ns	0.004	ns	ns	ns
Eosinophils (%)	1.0 (0–29)	1.0 (0–11)	1.0 (0–3)	0.5 (0–1)	ns	ns	ns	0.012	ns	ns
Neutrophils (%)	1.0 (0.5–10)	2.0 (0–7)	2.0 (1–5)	2.0 (0–8)	ns	ns	ns	ns	ns	ns
Macrophages (%)	77.0 (42–88)	87.0 (72–97)	87.0 (57–98)	80.0 (69–97)	ns	ns	ns	ns	ns	ns

Level of significance ($P < 0.01$).

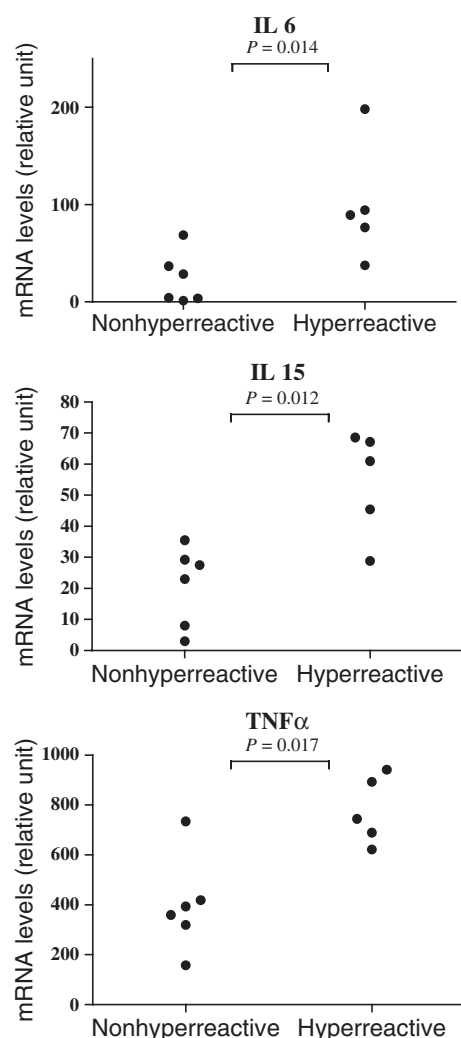


Figure 3. Levels of proinflammatory cytokines IL6 and TNF- α , and Th1 cytokine IL15 in bronchoalveolar lavage (BAL) cells in the third examination phase in hyperreactive ($n = 5$) and nonhyperreactive ($n = 6$) patients. Level of significance, $P < 0.05$.

IL-6, TNF- α and IL-15 differentiated between hyperreactive and nonhyperreactive patients. Our study indicates that inflammation is important in persistent hyperreactivity in DIA. Further, additional markers for follow-up of asthmatic inflammation in DIA are given.

TNF- α is an important regulator of asthmatic inflammation, and its expression has been related to the severity of asthma (17). Earlier there have been suggestions on the role of TNF- α and some other proinflammatory cytokines in chronic TDI-induced asthma (18). Our results indicate that TNF- α may have an important role in NSBH in DIA. Thus, our results conform in humans the findings of animal studies where TNF- α was found to have a central role both in inflammatory processes and in inducing bronchial hyperreactivity (19) in DIA. It has also been shown that treatment with anti-TNF antibodies is helpful in refractory asthma (20), the reason why our findings suggest that TNF- α -antagonizing medication might be useful in the treatment of DIA.

Cytokine IL-15, activates mast cells, eosinophils, and CD8 T cells (21), as well as decreases the apoptosis of CD4 memory T cells (22) and eosinophils (23). Blocking IL-15 has been demonstrated to prevent bronchial hyperreactivity (21), which is consistent with our findings.

IL-6 is a proinflammatory cytokine that promotes T-cell activation and proliferation. The role of IL-6 in remodelling and its association with NSBH are complicated. IL-6 has been reported to be elevated in symptomatic hyperreactive nonallergic asthmatics (24), and a local synthesis or release of IL-6 has been suggested in TDI-induced asthma (24, 25); our findings support this theory.

All these cytokines associated with bronchial hyperreactivity are known to be produced by macrophages, although also some other cells can release them. The increased count of macrophages in bronchial mucosa might be crucial for persistence of NSBH in DIA. Macrophages have been found to act as antigen-presenting cells, suppressing Th2-dependent humoral response and increasing Th1-oriented cellular immunity (22). However, there is also some evidence on the role of macrophages in the pathophysiology of asthma. Macrophage activity has been found to be higher in asthmatics than in healthy subjects, and even an association of macrophage activity with the severity of asthma has been found (26). There are also earlier reports on the association of macrophages on bronchial hyperreactivity (27). None of the patients with NSBH was a current smoker, the reason why tobacco smoking seems an unlikely factor for persistent NSBH.

In the second examination, simultaneously with the increase in the number of macrophages, the count of IL-4 secreting cells was elevated in bronchial mucosa. We cannot exclude that increased IL-4 would affect macrophage function in some aspects. Earlier, IL-4 has been found to activate macrophages (28) and to alter the balance within macrophage subpopulations (29).

In the third examination phase, the count of IL-4 secreting cells was diminished and then there was an indication that CD3 and CD4 lymphocytes would be more important in NSBH than IL-4. Count of CD4 cells remained at a lower level than in controls during the whole follow-up. However, there was a gradual increase in the CD4/CD8 ratio which is explained here by a slight increase in CD4 helper lymphocytes. Also CD3 cells slightly, nonsignificantly, increased at the third examination. Because the correlation between CD4 or CD3 cells and NSBH was found in a small number of cases, the results shall be dealt with cautiously. However, these findings are in accordance with earlier study where lymphocytic activation has been found to be important in chronic DIA (19, 30).

In studies monitoring lung function in DIA with a corresponding duration of follow-up (3, 5, 6), the persistence of NSBH has varied between 58% and 80% without treatment with inhaled steroids. In our study, the presence of NSBH at the end of the follow-up was 45%. Inhaled steroids are known to reduce NSBH (9). Maestrelli et al. (6) found that NSBH in DIA decreased with inhaled steroid treatment compared with placebo medication. In their study, two patients continuing therapy with inhaled steroids further increased the PD20 values. In this study, a reduction in NSBH was found already in the second examination and most clearly after 3 years of treatment with inhaled steroids, which is consistent with above research (6). This indicates that intensive, long-term treatment with inhaled steroids is needed in DIA.

Despite diminishing NSBH, a significant impairment of lung function was measured. Compared with our earlier paper on DIA (7), where only one-third of patients used regular inhaled steroid medication, two-thirds of patients were smokers, and some were still exposed to diisocyanates, the yearly FEV1 reduction is here higher, and the result is the opposite to what we had expected. However, in the previous paper, the follow-up duration was about 10 years. Although the main recovery of lung function in occupational asthma is anticipated to occur during the first 2 years after cessation of exposure (31), a gradual improvement later on could be one explanation for the question why the yearly FEV1 decline was smaller after a longer follow-up.

The median decrease in FEV1 exceeded the normal yearly reduction in smoking subjects reported earlier (32). In occupational asthma caused by several allergens, Anees et al. (33) found a 27-ml yearly reduction of FEV1 1 year after cessation of exposure, irrelevant of the

causative agent resembling the yearly decline of healthy adults. Unfortunately, they did not provide the individual results of their 35 patients with DIA.

As in an earlier study (33), smoking did not explain the rapid lung function reduction. Surprisingly, the most rapid decline of lung function was seen in nonsmokers. The number of smokers, nonsmokers, and ex-smokers that could be followed up over the entire study period was only four in each group, which reduces the power of this comparison.

One possible cause of increased functional impairment might be the rather long period of exposure to diisocyanates and long duration of asthma symptoms before the diagnosis in several patients. Earlier study has found the beneficial effect of inhaled steroids to be greater with early diagnosis and onset of treatment (34).

Because NSBH did not disappear in all patients insufficient medication could be suspected. A similar high-dose budesonide treatment was used for all patients for 1 year, after which for some patients the dose and for two patients also the steroid was changed. It was not possible to have a standard medication for 3 years for all patients; in some cases, there was even a need to increase the medication. NSBH was also present in some patients receiving a high dose of inhaled steroids, and one patient receiving budesonide 400 µ/day showed no NSBH. In addition, increased FEV1 reduction which was seen most clearly in nonsmokers could not be foreseen based on spirometry or histamine challenge results, which often were normal. These results indicate that monitoring of lung function may be insufficient for following up DIA patients. Possibly also inflammatory condition should be monitored.

In some patients, treatment with long-acting betasymptomimetics (LAB) had been initiated, which may have influenced airway remodelling; however, the exact effects of these medicines on the remodelling process or on NSBH have not yet been established (35). Of our patients using LABs, three showed increased FEV1 decline, but most had normal FEV1 decline or even an improvement of FEV1 during follow-up.

Because almost half of the patients had slight lymphocytosis in the baseline BAL examinations and some also temperature in addition to asthma symptoms, slight alveolar reaction could be suspected at the diagnosis phase. Two patients had a slight reduction in diffusing capacity in the baseline studies, but there were no radiological indications on alveolitis. This slight reduction in diffusing capacity might also have been caused by obstruction. In the third examination phase, all patients had normal diffusing capacity values. As is presented in Table 2, total lung capacity increased, and maximal expiratory flow at the level where 50% of FVC remains exhaled (MEF50) and maximal mid expiratory flow (MMEF) decreased in the follow-up giving an impression of peripheral obstruction. FEV1/FVC ratio simultaneously slightly increased, which probably represents a

slight functional restriction. The development of a real restrictive process is unlikely.

In conclusion, after cessation of diisocyanate exposure and during inhaled steroid medication, the degree of bronchial hyperreactivity in patients with DIA decreased, but FEV1 reduction progressed. A reduction of inflammatory cells of the Th2 pathway was found, but the count of macrophages remained elevated. Patients with persistent bronchial hyperreactivity continued to have bronchial inflammation, especially associated with pro-inflammatory acting cytokines IL-6, IL-15 and TNF- α

which are known to be produced by macrophages. These findings may indicate new possibilities in therapy and follow-up of DIA.

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