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# Brief exposures to $NO_2$ augment the allergic inflammation in asthmatics

C. Barck,[a,*] J. Lundahl,[b] G. Halldén,[b] and G. Bylin[a]

[a] *Division of Respiratory Medicine and Allergology, Department of Medicine, Karolinska Institute at Huddinge University Hospital, S-141 86 Stockholm, Sweden*

[b] *Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden*

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**Abstract**

Exposure to high ambient levels of nitrogen dioxide ($NO_2$) enhances the airway reaction in humans to allergen, measured as decreased pulmonary function. We tested whether this $NO_2$ effect is associated with an increased inflammatory response to allergen in the airways. To mimic real-life conditions, in which exposure to high ambient levels of $NO_2$ occurs only during short periods of time but often several times a day, we used a repeated-exposure model. On day 1, 18 subjects with allergic asthma were exposed, in randomized order, to purified air or to 500 $\mu g/m^3$ $NO_2$ for 15 min, and on day 2 for $2 \times 15$ min. Allergen was inhaled 3–4 h after the $NO_2$ exposures on both days. Symptoms, pulmonary function, and inflammatory response in sputum and blood were measured daily. Eosinophil cationic protein in both sputum and blood increased more from day 1 to day 3 after $NO_2$ + allergen than after air + allergen, whereas eosinophil counts did not differ. The change in myeloperoxidase was significantly greater after $NO_2$ + allergen than after air + allergen in blood but not in sputum. This finding was not accompanied by raised levels of neutrophils in sputum and blood. Symptoms and pulmonary function were equally affected by $NO_2$ + allergen and air + allergen. We conclude that two to three brief exposures to ambient levels of $NO_2$ can prime circulating eosinophils and enhance the eosinophilic activity in sputum in response to inhaled allergen. This might be an important mechanism by which air pollutants amplify the inflammatory reactions in the airways.


*Keywords:* Nitrogen dioxide; Allergen; Induced sputum; Eosinophils; Eosinophil cationic protein

## 1. Introduction

Pollen is the most common allergen in allergic rhinitis patients in many countries (Scadding and Church, 2002). Many of these patients also have symptoms of asthma during the pollen season. The decrease in pulmonary function caused by inhalation of pollens and other allergens is amplified in asthmatics by pre-exposure to outdoor air pollutants such as nitrogen oxides and ozone (Molfino et al., 1991; Jörres et al., 1996; Tunnicliffe et al., 1994).

In controlled human studies, nitrogen dioxide ($NO_2$) has been shown to enhance the asthmatic response to inhaled allergen, after both single and repeated exposures (Tunnicliffe et al., 1994; Jörres et al., 1995; Strand et al., 1997, 1998). However, the reduction in forced expiratory volume in 1 s ($FEV_1$) caused by $NO_2$ exposure before allergen has been only moderate. It can therefore be questioned whether this limited $NO_2$ effect on $FEV_1$ can cause asthma exacerbations of clinical significance. On the other hand, if $NO_2$ also causes an enhanced allergic inflammation in the bronchi, it is more likely that $NO_2$ exposure can be linked to serious deteriorations in asthma.

The doses of $NO_2$ and allergen in an environmental chamber study ought to mimic outdoor exposure. The individual exposure pattern to environmental $NO_2$ is often characterized by relatively long periods of exposure to low or moderate levels of $NO_2$ alternating with short periods of higher exposure, for example, in road tunnels (Svartengren et al., 2000), in streets with heavy traffic (Bascom et al., 1996), or in kitchens with gas stoves (Jarvis et al., 1996).

*Corresponding author. Fax: +46-8-7117306.
*E-mail addresses:* charlotte.barck@medhs.ki.se (C. Barck), joachim.lundahl@ks.se (J. Lundahl), gunilla.hallden@ks.se (G. Halldén), gunnar.bylin@medhs.ki.se (G. Bylin).

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We proposed that brief, repeated exposures to high ambient levels of $NO_2$ and low doses of allergen, similar to what people may encounter in daily life, might augment an allergic inflammatory reaction in the airways. We therefore measured the allergic inflammatory response in the lower airways in patients with allergic asthma subjected to repeated short exposures to $NO_2$ followed by a low dose of allergen.

## 2. Materials and methods

### 2.1. Subjects

Participating in the study were 18 subjects with mild asthma and allergy to birch or timothy pollen. Half of the subjects were recruited from the allergy outpatient clinic at Huddinge University Hospital, and half from advertising in a newspaper. There were 10 men and 8 women with a mean age of 32 years (range 23–48; Table 1). Extrinsic asthma was based on a typical history of attacks of dyspnea during the pollen season and airway hyper-responsiveness to histamine. The diagnosis of seasonal allergy to either birch or timothy pollen was confirmed at an inclusion test by a positive skin prick reaction ($>3$ mm) and a positive bronchial challenge with the relevant allergen. These inclusion tests were done at least 4 weeks before the study began.

All 18 subjects had an immediate asthmatic reaction, and 5 of them had a late asthmatic reaction, defined as $\geqslant 15\%$ decline in $FEV_1$ 3–10 h after the allergen challenge. All subjects used inhaled $\beta_2$-agonist as needed, and 11 used inhaled steroids during the pollen season. The interval between steroid use and the study start was at least 2 months, and none had any steroid or other anti-inflammatory medication during the study period, which was out of pollen season. All subjects had an acute-phase reaction (CRP) $<10$ mg/L.

### 2.2. Study design

The study was designed to mimic a conceivable exposure to ambient concentrations of $NO_2$ and pollen allergen during a couple of days (Fig. 1). On the first day, the subjects were briefly exposed to $NO_2$ once before allergen inhalation. On the second day, the subjects were briefly exposed twice to $NO_2$ before allergen.

The study had a single-blinded, crossover design, with each subject acting as his or her own control. The subjects were exposed to 500 $\mu g/m^3$ (260 ppb) $NO_2$ or to filtered air for 15 min on day 1 and for $2 \times 15$ min on day 2 (the same type of exposure on days 1 and 2). On day 1 the exposure to $NO_2$ or air was followed 4 h later by a bronchial challenge with an individually titrated dose of allergen (see later). On day 2, the time between the second exposure to $NO_2$ or air and the allergen challenge was 3 h. The time interval of $\sim 4$ h between $NO_2$ or air and allergen was based on previous studies by our group (Strand et al., 1997; Barck et al., 2002).

Table 1
Characteristics of subjects and inhaled allergen dose

| Subject | Age (years) | Smoking[a] | Allergen inhaled[b] | $FEV_1$ at inclusion (% pred) | Histamine[c] $PD_{SRaw\ 100\%}$ (μg) | Allergen[d] $PD_{SRaw\ 100\%}$ (SQ units) | Allergen dose[e] (SQ units) |
|---|---|---|---|---|---|---|---|
| 1 | 32 | N | B | 90 | 402 | 231 | 96 |
| 2 | 38 | E | B | 117 | 223 | 128 | 55 |
| 3 | 29 | N | B | 102 | 737 | 790 | 330 |
| 4 | 25 | N | T | 90 | 172 | 446 | 220 |
| 5 | 31 | N | B | 89 | 549 | 178 | 82 |
| 6 | 26 | N | T | 87 | 803 | 423 | 220 |
| 7 | 48 | N | T | 102 | 803 | 939 | 386 |
| 8 | 31 | N | B | 105 | 461 | 111 | 55 |
| 9 | 41 | E | B | 88 | 129 | 676 | 276 |
| 10 | 26 | E | B | 104 | 37 | 169 | 68 |
| 11 | 44 | E | T | 104 | 43 | 41 | 18 |
| 12 | 23 | N | B | 115 | 338 | 461 | 220 |
| 13 | 25 | N | T | 104 | 23 | 18 | 11 |
| 14 | 35 | N | B | 95 | 128 | 137 | 68 |
| 15 | 32 | N | B | 110 | 178 | 99 | 42 |
| 16 | 29 | N | B | 107 | 102 | 26 | 11 |
| 17 | 25 | N | B | 106 | 84 | 495 | 220 |
| 18 | 28 | N | B | 87 | 588 | 609 | 274 |
| Range | 23–48 | | | 87–117 | 23–803 | 18–939 | 11–386 |

[a] N, never smoker; E, ex-smoker.
[b] B, birch; T, timothy.
[c] Provocative dose of histamine causing a 100% increase in specific airway resistance.
[d] Provocative dose of allergen causing a 100% increase in specific airway resistance. SQ, standard quality units.
[e] Total allergen dose given after air/$NO_2$ during days 1 and 2.