Is occupational asthma to diisocyanates a non–IgE-mediated disease?

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Background: Exposure to diisocyanates in the workplace is an important cause of occupational asthma. The majority of patients with diisocyanate-induced asthma have no detectable diisocyanate-specific IgE antibodies in serum. There has been much debate as to whether this is due to diisocyanate-induced asthma being mediated by non-IgE mechanisms or whether it is the result of using inappropriate conjugates.
Objective: We sought to determine whether RNA message for Cϵ, IL-4, and other associated inflammatory markers could be detected locally within the bronchial mucosa after diisocyanate challenge.
Methods: Fiberoptic bronchoscopic bronchial biopsy specimens were obtained at 24 hours after both a control and an active challenge in 5 patients with positive and 7 patients with negative inhalation test responses to diisocyanates. Using both immunohistochemistry and *in situ* hybridization, we determined mRNA for Cϵ, IL-4, IL-5, and other associated inflammatory markers.
Results: There was a striking absence of Cϵ and IL-4 mRNA-positive cells in bronchial biopsy specimens from patients challenged with diisocyanate (Cϵ median of 0 and interquartile range of 0-1.85; IL-4 median of 0 and interquartile range of 0-0.85). In contrast, there were increased numbers of IL-5–, CD25-, and CD4-positive cells and a trend toward an increase in eosinophils after active challenge with diisocyanate.
Conclusion: We found a striking absence of both bronchial Cϵ and IL-4 RNA message after inhalation challenge with diisocyanates, irrespective of whether the challenge test response was positive or negative. We propose that diisocyanate-induced asthma is a non–IgE-mediated disease, at least in patients in whom specific IgE antibodies to diisocyanates are undetectable. (J Allergy Clin Immunol 2006;117:663-9.)

Key words: *Occupational asthma, isocyanates, IgE*

Abbreviations used
HDI: Hexamethylene diisocyanate
IQR: Interquartile range
MDI: Diphenyl-methyl diisocyanate
TBS: Tris-buffered saline
TDI: Toluene diisocyanate

Diisocyanates are highly reactive, low-molecular-weight chemicals with widespread industrial applications. Toluene diisocyanate (TDI), diphenyl-methyl diisocyanate (MDI), and hexamethylene diisocyanate (HDI), in particular, are used to produce a variety of polyurethane-based products. Exposure to diisocyanates in the workplace is an important cause of occupational asthma, inducing disease in between 5% and 15% of exposed employees.[1-4] An estimated 20% of cases of occupational asthma in the United Kingdom[5] and 50% in Ontario (before the introduction of control measures)[6] are attributed to diisocyanate exposures.

For many years, it has been recognized that only a minority of cases of diisocyanate-induced asthma are accompanied by detectable levels of serum-specific IgE antibody.[7] In case series in which the diagnosis has been confirmed by means of specific inhalation testing, this proportion has been between 14% and 28%.[7-10] Diisocyanates are haptens and require conjugation to a carrier protein for the detection of specific IgE antibodies. Contemporary methods for generating diisocyanate-albumin conjugates have changed little since they were first described, and they remain poorly standardized. It is a matter of debate to what extent specific IgE antibody production is not detected because of inappropriate conjugates[11] and to what extent the disease is mediated by alternative immunologic or nonimmunologic mechanisms.

Significantly increased levels of Cϵ and IL-4 message are detected in the bronchial mucosa after active challenge in patients with asthma caused by high-molecular-weight allergens, such as grass pollen or house dust mite, and also in those with apparently intrinsic disease.[12] The production of IgE requires both IL-4 to target the ϵ gene for recombination and cross-linking of CD40 on the B cell with CD40 ligand on T lymphocytes or basophils to allow isotype switching to take place.[13] The somatic recombination of the immunoglobulin heavy chain cluster gives rise to mRNA for the ϵ heavy chain. In this study we set out to examine whether Cϵ, IL-4, and other associated inflammatory markers could be detected locally within the bronchial mucosa after diisocyanate challenge.

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TABLE I. Clinical summary of patients with diisocyanate-induced asthma

Agent	Age (y)	Occupation	Total IgE (kU/L)	Specific IgE* (kU/L)	Atopic	Duration away from exposure (mo)	Maximum % change in FEV_1	PC_{20} Control	PC_{20} Active	Challenge result
HDI	38	Paint sprayer	392.2	<0.35 <0.35 <0.35	Y	48	−28%	0.18	0.15	+
HDI	35	Paint sprayer	16.2	<0.35 <0.35 <0.35	Y	1	−32%	>16	5.8	+
HDI	21	Paint sprayer	116	<0.35 <0.35 <0.35	Y	1	−60%	4.7	4.2	+
HDI	38	Injection molder	22.2	<0.35 <0.35 <0.35	Y	<1	−40%	0.3	0.16	+
HDI	28	Paint sprayer	110.5	<0.35 <0.35 0.5	N	3	−20%	>16	>16	+
HDI	27	Injection molder	32.2	<0.35 <0.35 3.5	Y	5	0	>16	13	−
HDI	39	Health and safety	79.9	<0.35 <0.35 <0.35	Y	2	0	3.6	2.7	−
MDI	31	Molding technician	274.8	<0.35 <0.35 <0.35	Y	<1	0	>16	>16	−
HDI	35	Paint sprayer	118.9	<0.35 <0.35 <0.35	Y	<1	0	>16	>16	−
HDI	38	Paint sprayer	27.2	<0.35 <0.35 <0.35	N	<1	0	6.2	>16	−
HDI	35	Paint sprayer	4.73	<0.35 <0.35 <0.35	N	6	0	>16	>16	−
HDI	41	Paint sprayer	329.4	<0.35 <0.35 <0.35	N	11	0	2.8	3.0	−
Control (n = 6)	31.5		46.0	ND	N		0	>32		ND

ND, Not done.
*HDI, MDI, and TDI, respectively.

METHODS

Bronchoscopy study design

Patients were recruited from a specialist clinic after referral by their general practitioner or occupational health physician. All patients with suspected diisocyanate-induced asthma in whom there was a clinical indication for specific inhalation testing were invited to take part. In this way we recruited 12 patients (all male) between 1997 and 2003; their clinical details are summarized in Table I, and duration of exposure before onset of symptoms and duration of symptoms before leaving work are summarized in Table II.

The study was approved by the Ethics Committee of Royal Brompton and Harefield Hospital, and each patient provided informed consent.

Occupational inhalational testing

Single-blind, controlled inhalation tests were undertaken in an exposure chamber under medical supervision designed to reproduce, as far as practicable, appropriate workplace exposures. Carefully regulated active or control (inactive) exposures were arranged on consecutive days until a diagnosis was reached. Nine patients worked as spray painters and were exposed to an HDI hardening agent, which was omitted from the paint mixture during control challenges. Three patients worked in injection molding, 2 exposed to HDI and 1 to MDI; workplace conditions for the former were reproduced by squirting air through a mixture of the active and inert components, with the active component being omitted during control challenges. MDI exposure was reproduced by heating crystals of the diisocyanate in a sand bath for 15 minutes. Both active and control challenges were monitored in every case by using continuous measurement of airborne diisocyanate concentrations, these being maintained within the appropriate short-term exposure limit values. Responses to each day's challenge were assessed by FEV_1 measurements at 0, 5, 10, 15, and 30 minutes after the exposure and hourly thereafter for up to 12 hours. In addition, nonspecific bronchial responsiveness to histamine was measured at admission and 24 hours after each exposure, apart from the days when bronchoscopy was undertaken.[14] A reproducible and sustained dual or late-phase decrease in FEV_1 of greater than 15% from baseline value, an increase in nonspecific bronchial reactivity after active challenge alone, or both was considered a positive response.

Fiberoptic bronchoscopy

All but 2 patients underwent fiberoptic bronchoscopy 24 hours after a control exposure and again 24 hours after an active exposure. Two patients had one bronchoscopy only, one after a control challenge and the other after an active challenge. On bronchoscopy days, histamine response testing was omitted from the usual schedule. Subjects were premedicated with nebulized salbutamol (2.5 mg), intravenous atropine (0.6 mg), and midazolam (5-10 mg) 10 minutes before the procedure. Oxygen was delivered throughout through nasal cannulae, with oxygen saturation monitored by means of pulse oximetry. The nose and throat were anesthetized with topical lignocaine spray and 4% lignocaine applied through the bronchoscope channel to the vocal cords, with 2% lignocaine used to anesthetize the upper airways. We used an Olympus model BF P20 bronchoscope (Olympus Company, Tokyo, Japan) to obtain 4 to 8 biopsy specimens from segmental divisions of the major bronchi, with bronchoalveolar lavage carried out just before the taking of biopsy specimens. All bronchoscopies and procedures were carried out by the same experienced operator.

Skin prick testing

Skin prick tests were undertaken with physiologic saline solution, histamine, grass pollen, cat, and *Dermatophagoides pteronyssinus* (Allergopharma, Reinbeck, Germany). Wheal sizes were recorded after 10 minutes by measuring the mean of their longest and midpoint orthogonal internal diameters; a positive response was one with a mean wheal diameter of at least 3 mm greater than that obtained in response to saline solution. Atopy was defined as a positive response to tests with one or more extracts of cat, grass pollen, or *D pteronyssinus.*

Specific IgE antibodies

Specific IgE antibodies to diisocyanates were measured with Unicap (Sweden Diagnostics, Milton Keynes, United Kingdom). In only 2 patients were measurements greater than 0.35 kU/L (Table I).

Biopsy specimens

Biopsy specimens were divided into 2 halves. One half was immediately mounted in OCT compound (VWR; Lutterworth, Leics, United Kingdom), snap-frozen by means of immersion in