

Short communication

The *in vivo* effect of isocyanate-induced asthma on basophil histamine release

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In vitro studies have suggested that basophils are involved in the asthmatic response to isocyanate. Classified by the type of airway response, three groups of patients exposed to isocyanate were studied: 1) nonreactive ($n=3$), 2) immediate responders ($n=4$), and 3) late responders ($n=4$). Basophil histamine release to concanavalin A (conA), FMLP, and anti-IgE was evaluated before and during the airway response. Histamine release significantly ($P=0.009$ for conA and 0.03 for anti-IgE) increased after exposure to isocyanate only in the group which had late bronchial response. Additionally, good correlation was found between baseline $FEV_1/FVC\%$ and degree of histamine release of basophils stimulated with conA. These findings support a role for basophils in the late asthmatic response to isocyanate.

**S. Kivity, V. Veintrov, E. Fireman,
O. Agami, Y. Lerman**

Allergy Unit, Institute of Pulmonary and Allergy Diseases, Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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S. Kivity, MD
Allergy Unit
Institute of Pulmonary and Allergy Diseases
Tel Aviv Sourasky Medical Center
6 Weizmann Street
Tel Aviv 64239
Israel

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Toluene diisocyanate (TDI) is a chemical widely used in the manufacture of polyurethane paints and plastics, substances to which a large number of workers are occupationally exposed (1), and which are well-recognized causes of occupational asthma.

The three basic patterns of asthmatic responses which are identifiable in sensitized workers – illustrated by the response to challenge with TDI in isocyanate-sensitized workers (1, 2) – are characterized by an immediate response, a delayed or late-onset reaction that usually begins 1–6 h after exposure, and a dual response with characteristics of both the immediate and the late-phase reactivity.

Various mechanisms for the development of the various reactions have been proposed (3); Butcher et al. demonstrated lymphocytic activation (4), while Bentley et al. (5) showed that the lymphocytes and eosinophils taken from the airways of patients with TDI-induced asthma were highly activated. Moreover, these lymphocytes were of the CD_8^+ type, secreting mRNA for interferon and interleukin-5.

The role of basophils in this reaction is less clear. An *in vitro* study of lymphocytes from this

type of asthmatic patient demonstrated a release of histamine-releasing factor (HRF) after incubation with isocyanate (6). HRF is known to augment histamine release from mast cells and basophils, which are essential in the asthmatic response.

In the following study, we evaluated the *in vivo* effect of isocyanate exposure on the basophil histamine release of patients with previously proven isocyanate sensitization.

Material and methods

Subjects

All subjects signed an informed consent document after approval by the local Helsinki committee. They were workers previously shown in our laboratory to have an asthmatic reaction to TDI. Based on the initial evaluation, four subjects had a late drop in FEV_1 (later than the first hour after exposure), four subjects had an immediate drop in FEV_1 (in the first 30 min after exposure), and three subjects had no reaction (less than 10% drop in FEV_1 after exposure). Most of the sensitized subjects (10/11=91%) stopped working with isocyanate due to their airway reaction.

Provocation challenge with TDI (BPT)

The subjects were exposed for 10–20 min to TDI at concentrations ranging from 0.007 ppm to 0.06 ppm in an exposure chamber (volume 9 m³), as proposed by Pepys & Hutchcroft (2). During exposure, peak TDI concentration was less than 0.02 ppm in most subjects, the exceptions being two for whom it reached 0.06 ppm in the last minute of exposure. We tested these levels in the other workers of the same factory and found equivalent results.

Spirometry (with a Fukuda spiro analyzer) was performed at baseline and every 15 min during the first hour after BPT, and then after every hour for the next 7 h. A venous blood sample was withdrawn at baseline and when a greater than 15% drop in FEV₁ occurred. In group II, the second blood sampling took place during the first hour after exposure; in group III, it was done from 1–6 h after exposure.

Blood sampling and histamine assay

Blood was sampled from a venflon cannula that was inserted into an antecubital fossa vein before challenge. First, 1 ml of blood was discarded and then 10 ml of blood was removed and transferred into polypropylene tubes containing 1 ml of 0.1 M EDTA. Direct reading of TDI was done calorimetrically with TLD-1 (MDA Scientific, Inc., USA).

Preparation of basophils and histamine assay were carried out as previously described elsewhere (7).

Statistical analysis

The percent histamine release (of the baseline and the postexposure values) of the three groups was compared by analysis of variance. The correlation between FEV₁/FVC% and percent histamine release was done by calculating the Pearson coefficient. A *P* value less than 0.05 was considered significant.

Results

The study group comprised 11 men aged 21–67 years, and the years of exposure ranged from 3 to 30 years. The baseline FEV₁/FVC% ranged from 59% to 96% (74 ± 11%, mean ± SD).

The spirometric changes following the provocation with isocyanate are shown in Table 1, in which the results are expressed as means ± SD. There was no significant change in spirometry up to 7 h after exposure in the patients in group I. Group II included four patients with an immediate drop in their FEV₁; patient no. 7, who had a 66% drop in

FEV₁, received two puffs of salbutamol by metered-dose inhaler, and this was followed by a quick return to baseline value. No further change in the spirometry of each patient in this group was seen. Group III included four patients who showed no immediate change but only a late change in FEV₁, which peaked at 2–4 h after exposure. Only patient no. 8 (45% drop in FEV₁) required salbutamol by inhalation (postexposure – baseline values/baseline % values).

Table 2 illustrates the mean percent difference of histamine release from basophils after TDI exposure. No significant difference was seen between the baseline histamine release following the triggers in the three groups. There were only mild changes in histamine release following all triggers among patients without response to TDI, as well as in those who had immediate responses. However, in the group with late responses, there was an increase in histamine release from basophils taken during the late response. While a relative increase was observed after all triggers, it was statistically significant only after 20 µg concanavalin A (conA) and anti-IgE.

Table 3 lists the correlation between pulmonary function (FEV₁/FVC%) and the matched percent histamine release from basophils from all the patients, including baseline as well as postexposure values. There was a consistent negative Pearson correlation with all the triggers used, meaning that there was an inverse correlation between FEV₁/FVC% and histamine release. This became statistically significant only with 20 µg/ml conA and 10⁻⁷ and 10⁻⁶ M FMLP.

Discussion

This study demonstrated that isocyanate-induced asthmatic reaction increases histamine release from basophils of patients only when a late reaction occurs, and not during the immediate response or in exposed but asymptomatic subjects. This study confirmed the finding of Herd & Bernstein (6), who reported that diisocyanate-HSA antigens stimulated the *in vitro* synthesis of HRF in workers with isocyanate asthmatic response. No mention was made in this study of the difference between those subjects who had an immediate or a late asthmatic reaction. The changes in basophil releasability seen in our study could be explained by the release of HRF shown in Herd & Bernstein's study (6). *In vitro* production of HRF has been described after specific stimulation with protein allergens, including dust mite, grass, and ragweed and in response to streptokinase/streptodornase antigens (8, 9).

The mechanism by which isocyanate induces sensitization is unknown, but clinical and functional

Table 1. Spirometric values before and after exposure to TDI

	No.	Baseline			First hour			1-6 h		
		FEV ₁ (l)	FVC (l)	FEV ₁ /FVC	FEV ₁ (l)	FVC (l)	% Δ FEV ₁	FEV ₁ (l)	FVC (l)	% Δ FEV ₁
GI	1	2.48	3.65	68%	2.41	3.39	-2	2.78	3.82	+12
	2	3.49	3.64	96%	3.28	3.49	-6	3.30	3.43	-5
	3	2.75	3.83	72%	2.73	3.80	-1	2.70	3.75	-2
GII	4	2.43	3.10	79%	1.55	2.45	-36	2.22	3.10	-3
	5	2.32	3.95	59%	1.47	2.33	-36	2.29	3.71	0
	6	2.05	2.81	73%	1.70	2.69	-17	2.16	2.67	+5
	7*	3.69	4.33	85%	1.24	1.89	-66	3.47	4.15	-6
GIII	8*	1.15	2.6	58%	1.54	2.7	+33	0.83	1.99	-28
	9	2.44	3.26	75%	2.42	3.22	0	2.00	3.37	-18
	10	3.24	4.45	73%	3.20	4.55	-1	2.23	3.13	-31
	11	3.56	4.42	81%	3.65	4.55	+1	2.00	3.86	-48

GI: group I, Nonresponders; GII: group II, immediate responders; GIII: group III, late responders.

*Patients no. 7 and 8 received salbutamol by inhalation after drop in FEV₁.

Table 2. Histamine release from basophils after TDI exposure

Releaser	Group	Mean difference (in %) of histamine release	SD%	P value
SR	I	9.00	5.29	0.1597
	II	-6.25	7.45	
	III	3.75	13.0	
ConA 10 µg/ml	I	-0.33	1.52	0.9987
	II	-0.66	2.08	
	III	-0.25	16.64	
ConA 20 µg/ml	I	2.00	2.82	0.009*
	II	-11.33	10.01	
	III	12.25	3.77	
FMLP 10 ⁻⁷ M	I	15.66	11.50	0.100
	II	-20.33	17.15	
	III	43.00	46.87	
FMLP 10 ⁻⁶ M	I	17.00	24.43	0.1821
	II	-18.00	23.38	
	III	28.00	44.38	
FMLP 10 ⁻⁵ M	I	22.00	34.22	0.1547
	II	-15.00	17.35	
	III	30.00	38.43	
Anti-IgE	I	-6.33	5.50	0.0292*
	II	-23.75	14.86	
	III	14.25	20.93	

Mean percent difference was calculated from difference in release after and before exposure divided by baseline value. Group I: no response; group II: immediate response; group III: late response.

SR: spontaneous release.

*Statistically significant.

abnormalities in this form of occupational asthma are similar to those found in allergen-induced asthma (10).

In our study, a statistically significant enhanced histamine release from basophils was found only after conA and anti-IgE, although all other triggers used show the same tendency. Another interesting aspect of the data is the inverse correlation found between FEV₁/FVC%, a reliable parameter of

Table 3. Correlation between matched FEV₁/FVC% and basophil histamine release

Trigger	No. of measurements*	Pearson coefficient α**	Significance P value α
		β	β
SR	25	-0.1558	0.45
		-0.2252	0.27
ConA 10 µg/ml	25	-0.1156	0.58
		0.1481	0.58
ConA 20 µg/ml	25	-0.2909	0.15
		-0.3913	0.05
FMLP 10 ⁻⁷ M	20	-0.4538	0.04***
		-0.3846	0.127
FMLP 10 ⁻⁶ M	25	-0.2654	0.20
		-0.2452	0.28
FMLP 10 ⁻⁵ M	25	-0.3810	0.053***
		-0.3506	-0.101
Anti-IgE	25	-0.1588	0.44
		0.2653	0.20

*All measurements of FEV₁/FVC% and its matched histamine release were included.

**Correlation with measured FEV₁/FVC% was examined.

***Significant correlation.

β Logarithm was of measured FEV₁/FVC% and histamine release.

airway flow limitation, and histamine release from basophils. The trend was seen with all the triggers, but was significant only with FMLP and conA. The smallness of the group studied may be the reason for the lack of significant correlation with the other triggers. This interesting finding has been shown in

previous studies on asthmatic patients, as reported by Alam et al. (11), who showed good correlation between HRF taken from asthmatics and the degree of bronchial hyperactivity (11).

We concluded that late asthmatic reaction after TDI inhalation is associated with increased basophil histamine release, an effect which could be of major importance in inducing this airway response.

References

1. Chan Yung M, Lam S. Occupational asthma. *Am Rev Respir Dis* 1986;133:686-703.
2. Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975;112:829-59.
3. Sietta M, Distefano A, Maestraeli P, et al. Airway mucosal inflammation in occupational asthma induced by toluene di-isocyanate. *Am Rev Respir Dis* 1992;145:160-8.
4. Butcher BT, O'Neil CE, Reed MA, Salvaggio JE. Radioallergosorbent testing of toluene di-isocyanate-reactive individualities using *p*-tolylisocyanate antigen. *J Allergy Clin Immunol* 1980;66:213-16.
5. Bentley AM, Maestrelli P, Sietta M, Fabbri LM, Durham SR, Kay AB. Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate induced asthma. *J Allergy Clin Immunol* 1992;89:821-8.
6. Herd ZL, Bernstein DI. Antigen-specific stimulation of histamine releasing factors in di-isocyanate-induced occupational asthma. *Am J Respir Crit Care Med* 1994;150:988-94.
7. Beaven NA, Jacobsen S, Horakova Z. Modification of the enzymatic isotope assay of histamine and its application to measurement of histamine in tissues, serum and urine. *Clin Chim Acta* 1972;37:91-4.
8. Goetzel EJ, Foster DW, Payan DG. A basophil activating factor from human T-lymphocytes. *Immunology* 1984;93:227-34.
9. Alam R, Lett-Brown MA, Forsythe OA, et al. Monocyte chemotactic and activating factor is a potent histamine releasing factor for basophils. *J Clin Invest* 1992;89:723-8.
10. Fabbri LM, Maestrelli MP, Sietta M, Mapp CE. Airway inflammation during late asthmatic reactions induced by toluene di-isocyanate. *Am Rev Respir Dis* 1992;145:375-86.
11. Alam R, Kuna P, Rozmieccki J, Kuzminska B. The magnitude of the spontaneous production of histamine releasing factor (HRP) by lymphocytes *in vitro* correlates with the state of bronchial hyperactivity in patients with asthma. *J Allergy Clin Immunol* 1987;79:105-8.

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