

Immunologic Aspects of Isocyanate Asthma: IL-1 β , IL-3, IL-4, sIL2R, and sICAM-1

Leslie C. Grammer, Martha A. Shaughnessy, Cynthia Steichen Kabalin, Paul R. Yarnold,* Jean-Luc Malo,† and André Cartier‡

ABSTRACT

This pilot study investigated serum levels of cytokines and soluble receptors during five positive and five negative isocyanate inhalational challenges. Serum was obtained from 10 individuals with symptoms compatible with isocyanate asthma before isocyanate challenge and the day following their maximal change in pulmonary function after isocyanate challenge. Serum levels of interleukin 1 β , interleukin 3, interleukin 4, soluble interleukin 2 receptor, and soluble intercellular adhesion molecule 1 were measured and compared. Interleukin 1 β , interleukin 3, and interleukin 4 were not detected. The mean soluble interleukin 2 receptor and soluble intercellular adhesion molecule 1 levels were not statistically different before and after challenge or between groups. In summary, the cytokines studied are not detectable in peripheral blood during isocyanate inhalation challenge; soluble interleukin

2 receptor and soluble intercellular adhesion molecule 1 are both detectable but do not change significantly after a positive isocyanate challenge. (Allergy and Asthma Proc 19:301–305, 1998)

Study of the pathogenic mechanisms of occupational asthma may generate useful information relative to the pathogenesis of nonoccupational asthma.¹ More than 140 low molecular weight chemicals (<1 kd) have been reported to cause occupational asthma.² In structure activity studies, the universal property that sensitizing chemicals share is the ability to chemically interact with human macro-molecules.³ Low molecular weight occupational agents such as the acid anhydrides can give rise to asthma through haptenization of respiratory tract proteins and subsequent induction of specific IgE antibodies. Diisocyanates may also act as haptens and induce specific IgE; however, in many cases of asthma due to diisocyanates, the immunologic mechanism has not been elucidated.⁴ There is a higher prevalence of positive antibody, either specific IgE or IgG in diisocyanate workers that are inhalation challenge positive than in those that are not. However, specific antibody cannot be found in all challenge positive individuals. There is some evidence that a cellular mechanism resulting in histamine releasing factor (HRF) production may be important.^{6,7} Studies suggesting relationships between HLA haplotypes and development of diisocyanate asthma also implicate an immunologic mechanism of some type.⁸

Rather than focusing on antigen specific immune mechanisms in asthma, some investigators have evaluated the role of inflammatory cells and their cytokines in asthma. Several investigators have reported elevated levels of some cytokines but not others in symptomatic asthma airways.^{9,10} Other groups have reported elevated cytokines and soluble cytokine receptors in peripheral blood. Granulocyte macrophage colony stimulating factor (GM-CSF),¹¹ interleukin-

*From the Division of Allergy-Immunology, Department of Medicine, and the Ernest S. Bazley Asthma and Allergic Diseases Center of Northwestern Memorial Hospital and Northwestern University Medical Center, the *Division of General Internal Medicine, Department of Medicine, Northwestern University Medical School, Chicago, Illinois USA, and ‡Hôpital du Sacré-Coeur de Montreal, Service du Pneumologie, Montreal, Quebec, Canada Supported by the Ernest S. Bazley Grant to Northwestern Memorial Hospital and Northwestern University Medical School, and †Centre québécois d'excellence en santé respiratoire*

Martha A. Shaughnessy, an important contributor to the Allergy-Immunology Division of Northwestern University for 16 years, died September 9, 1997

Address correspondence and reprint requests to Dr. Leslie C. Grammer, Northwestern University Medical School, Division of Allergy-Immunology MC-S207, 303 East Chicago Avenue, Chicago, IL 60611

4(IL-4),¹²⁻¹⁴ soluble Fcε receptor II (sFcεRII),¹² and soluble interleukin 2 receptor (sIL2R)^{12,14} have been reported to be elevated in peripheral blood of patients with asthma. Interleukin 1β (IL-1β), tumor necrosis factor (TNF), and interferonγ (IFNγ) have been reported to be undetectable.¹¹

Another aspect of inflammation that has been evaluated in asthma is the role of adhesion molecules, the rationale being that intercellular adhesion molecule-1(ICAM-1) is known to be important in T cell recognition and eosinophil and T cell adhesion to endothelium. In a primate asthma model, a monoclonal antibody against ICAM-1 attenuated airway eosinophilia and hyperresponsiveness.¹⁵ Soluble ICAM has been reported to be elevated in asthma.¹⁶ There is also a reported of sICAM being higher during asthma attacks as compared to stable conditions.¹⁷

Isocyanate asthma has many characteristics of an immunologic disease. There is a latency period, only a small percentage of exposed individuals is affected, and a very low exposure results in disease. While many immunologic associations have been reported with isocyanate asthma, the responsible immunopathologic process has not yet been conclusively identified. A synopsis of the immunologic findings in isocyanate asthma can be found in Table I.

The purpose of the current pilot study was to determine whether cytokines IL-1β, interleukin 3 (IL-3), or IL-4, a soluble cytokine receptor, sIL2R, or a soluble adhesion molecule, sICAM-1 are elevated in peripheral blood after a positive isocyanate inhalation challenge as compared to a negative challenge. If any were demonstrated to be elevated, those findings would have obvious pathogenetic and diagnostic implications.

METHODS

Subjects

Ten subjects who had specific inhalation challenge (SIC) with diisocyanates were studied. Demographics and inhalation challenge data of the study subjects are listed in Table II. Details of the specific inhalation challenge methodology are outlined elsewhere.²⁹ One control challenge and one graded isocyanate challenge were performed. Subjects were not taking medication for asthma. The diagnosis was based on a ≥20% drop in FEV₁ on the isocyanate day with minimal, ≤10% variability on the observation day. The study was approved by the institutional committee on human research and appropriate informed consent was obtained from the subjects.

Serum Samples

Blood was drawn from study subjects before SIC and the day after maximal change in pulmonary function. Serum was separated from cells, frozen, shipped on dry ice, and stored at -20°C until immunologic assays were performed.

TABLE I

Immunology of Diisocyanate Asthma	
Immunologic Observation	Reference
Low prevalence specific IgE	19
Positive antibody, especially IgG more likely in challenge positive individuals	20
No change in plasma histamine or complement in challenge positive individuals	21
Peripheral blood mononuclear cells from those with isocyanate asthma have increased production of antigen specific HRF and MCP-1 in response to antigenic stimulation	22
HLA MHC Class II alleles associated with development of and protection from isocyanate asthma: aspartic acid at position 57 may be key in HLA-DQB1	23
ECP increased in peripheral blood after positive TDI challenge	24
Preferential Vβeta IL and Vβeta 5 expression in isocyanate asthma	25
Bronchial biopsies from isocyanate asthma CD8+ cells that produce IL-5 and IFN-gamma	26
Bronchial biopsies from isocyanate asthma have increased levels of expression of CD25 and VLA-4	27
Bronchial biopsies from isocyanate asthma have thickened basement membrane, increased eosinophil, and increased CD3+CD45+ cells	28

Cytokine and Soluble Factor Assays

To measure serum levels of IL-1β, IL-3, and IL-4, an enzyme-linked immunosorbent assay (ELISA) was used. The kits were obtained from R & D Systems, Minneapolis, MN. To measure serum levels of sIL2R and sICAM-1, an ELISA was used. The sIL2R kit was obtained from AMAC, Inc. (Westbrook, ME), and the sICAM-1 kit was obtained from Bender MedSystems (Vienna, Austria). Each sample was assayed in duplicate on 2 different days. A positive control standard curve was also performed. The OD of each well was read on an Artek automated ELISA reader (Artek Systems Corp., Farmindale, NY).

Statistical Analysis

Mean levels of sICAM-1 and sIL2R were each compared between- and within-groups using a 2 (positive versus negative challenge) by 2 (pre- versus post-test) factorial mixed-model analysis of variance.³⁰ Pearson correla-

TABLE II

Demographic and Challenge Data of Study Subjects

Number	Sex	Age	Duration of Exposure (years)	Duration of Symptoms (years)	Smoking Status*	Atopy**	Diagnosis***	Temporal Reaction to Challenge	FEV ₁ L (%pred)	PC ₂₀ (mg/ml)
1	M	48	36	8	NS	N	OA	Dual (-32%)	2.72 (74.42)	0.25
2	M	59	30	10	S	N	OA	Late (-22%)	1.95 (66.45)	0.3
3	M	53	30	5	ES	P	OA	Immediate (-23%)	2.59 (79.87)	0.5
4	M	52	39	1	NS	N	OA	Immediate (-39%)	3.38 (120.44)	2.25
5	M	28	0.58	0.33	S	N	OA	Late (-27%)	4.23 (110.17)	>128
6	F	51	13	2	ES	N	no OA		1.76 (57.82)	109
7	M	41	17	5	ES	N	no OA		2 (65.34)	3.2
8	F	27	0.83	0.75	NS	N	no OA		3.63 (126.91)	>128
9	M	35	15	8	S	N	no OA		3.76 (84.81)	>128
10	M	26	2.6	1.6	NS	N	no OA		4.8 (82.99)	2.5

*NS = non smoker, ES = ex smoker, S = smoker.

**P = positive, N = negative.

***OA = occupational asthma.

TABLE III

Soluble Factors Before and After Specific Inhalation Challenge Separately by Group (Positive versus Negative Challenge)

Group	Subject	sICAM (pg/mL)		sIL2R (pM)	
		Pre	Post	Pre	Post
Positive	1	313	281	40	28
	2	331	369	33	34
	3	406	394	57	56
	4	300	350	24	25
	5	438	406	46	48
	Mean	358	360	40	38
	SD	61	49	13	13
Negative	6	481	481	49	47
	7	369	406	17	14
	8	M	M	25	24
	9	381	369	61	60
	10	200	263	22	25
	Mean	358	380	35	34
	SD	117	91	19	19

Positive = positive challenge; Negative = negative challenge; SD = standard deviation; M = Missing.

tions between pre- and post-test values of sICAM-1 and sIL2R were used to estimate the temporal reliability of these indices, separately by group (positive versus negative challenge).³¹ Finally, single-subject statistical analysis was used to determine whether sICAM-1 or sIL2R levels were significantly different at pre- versus post-test, separately for each patient.³² Because of the small sample size and corresponding low statistical power, a generalized (per-comparison) Type I error rate of $p < 0.05$ was used to establish statistical significance.

RESULTS

The levels of IL-1 β , IL-3, and IL-4 were below detectable limits before and after maximal pulmonary function change from SIC. The levels of sIL2R and sICAM-1 before and after maximal pulmonary function change from SIC are listed in Table III.

Concerning mean sICAM-1 levels, analysis of variance indicated that there were no significant differences either between groups or across testings, and also that there was no significant group by testing interaction: all $F(1, 7) < 0.97$, $p < 0.36$ (Table III).

Analysis of variance indicated that there were no significant differences either between groups or across testings for mean sIL2R levels, and also that there was no significant group by testing interaction: all $F(1, 8) < 0.88$, $p < 0.38$ (Table III).

For patients with a positive challenge, pre-post sICAM-1 levels were marginally positively correlated ($r = 0.77$, $p < 0.065$), and sIL2R levels were significantly positively cor-

related ($r = 0.90$, $p < 0.04$). For patients with a negative challenge, both sICAM-1 levels ($r = 0.98$, $p < 0.02$) and sIL2R levels ($r = 0.99$, $p < 0.0004$) were significantly positively correlated.

DISCUSSION

Diisocyanates are among the most common causes of occupational asthma worldwide.⁷ It is probably the most mechanistically perplexing cause of occupational asthma since its immunologic mechanism remains elusive despite 2 decades of study. Evidence exists for an important role for cellular immunity from both in vitro cell and in vivo biopsy studies.³³ To be specific, T cells may have some role in diisocyanate asthma.

Two types of murine TH cell types, TH₁ and TH₂, have been defined by cytokine profiles. These same general cell types also have been described in humans.³⁴ In general, TH₁ cells produce IL-1, IL-2, IL-3, and IFN γ whereas TH₂ cells produce IL-1, IL-3, IL-4, and IL-5. We therefore elected to measure IL-1 β , IL-3, and IL-4 to attempt to find evidence of cytokines from either TH cell type. Another group had reported inability to detect IL-1 β in severe asthma, which we also did not detect.¹¹ IL-3 has not been studied in asthma previously. IL-4, however, has been reported in one study to be elevated in asthmatics, both atopic and nonatopic.¹² We are unsure of the exact reason that IL-4 was not elevated in these individuals. Perhaps TH cells, in particular TH₂ cells are not important in diisocyanate asthma. Another possibility is that the day after a maximal response may not be the ideal timing, and serial blood samples around the time of the maximal response may be necessary for cytokine detection. Cytokines might be more easily detected in BAL fluid than in serum; however, BAL was not collected in this study.

Some molecules like IL-2R and MHCII increase in number on the surface of T cells after antigen activation. The receptor for IL-2 appears on activated T cells 18–24 hours after activation. In the absence of continued antigenic stimulation, it is shed from the cell surface and its soluble fragment, sIL2R can be measured in serum. It has been reported to correlate reliably with disease activity in a number of clinical entities including autoimmune inflammatory disorders, transplant rejection, and a few infectious disorders.³⁵ In this population, levels did not change in a statistically significant manner. This could be indicative of immune activation being unimportant in diisocyanate asthma. Alternatively, perhaps the timing was not optimal and additional blood samples at 36 and 48 hours might reveal elevated levels of sIL2R.

ICAM-1 is inducible on endothelial cells within a few hours of exposure to a number of cytokines including IL-1, TNF, and IFN γ .³⁶ In the absence of cytokine stimulation, the receptor is shed and sICAM-1 can be measured in serum. ICAM-1 on endothelial cells is correlated with in-

filtration of lymphocytes into inflammatory lesions and is upregulated in a variety of conditions including transplant rejection and allergic inflammation.³⁷ There was no statistically significant change in sICAM-1 in this population. Perhaps additional serum samples at 36 or 48 hours would also be useful. In addition, a larger sample size might be helpful to increase statistical power.

In summary, in this pilot study the cytokines that we studied were not detectable in peripheral blood before or after a positive SIC with diisocyanates. Both sIL2R and sICAM-1 are detectable and correlate with each other, but did not change significantly after positive SIC.

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