
Delayed asthmatic response: a new phenotype of bronchial response to allergen challenge and soluble adhesion molecules in the serum

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Background: Patients with bronchial asthma develop various types of asthmatic response to bronchial challenge with allergen, such as immediate asthmatic response, late asthmatic response, or delayed asthmatic response (DYAR), due to different immunologic mechanisms.

Objectives: To investigate the appearance and possible changes in the concentrations of soluble cell adhesion molecules during the DYAR, to explore the involvement of particular cell types in the mechanism(s) leading to DYAR, and to contribute to a fuller understanding of this clinical phenomenon.

Methods: The DYAR recorded in 28 patients ($P < .001$), appearing within 26 to 32 hours, reaching maximum within 32 to 48 hours, and resolving within 56 hours after the allergen challenge, was repeated 2 to 6 weeks later. The repeated DYAR ($P < .001$) was supplemented with blood cell counts and measurement of serum concentrations of soluble adhesion molecules by an enzyme-linked immunoassay.

Results: The prechallenge concentrations of soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble platelet endothelial cell adhesion molecule (sPECAM-1), soluble E-selectin, soluble L-selectin, soluble P-selectin, and soluble E-cadherin did not differ significantly from healthy controls. The DYAR was associated with the following changes in the serum: an increase of sICAM-1 at 6 and 12 hours and a decrease at 24 hours; an increase of sVCAM-1 at 24 and 36 hours; an increase of sPECAM-1 at 36 and 48 hours and a decrease at 56 and 72 hours; an increase of soluble E-selectin at 56 hours; an increase of soluble L-selectin at 56 and 72 hours; a decrease of soluble E-cadherin at 48 and 56 hours; and increased counts of blood leukocytes at 36, 48, and 56 hours, neutrophils at 24, 36, 48, and 56 hours, lymphocytes at 24, 36, and 48 hours, and monocytes at 6, 12, and 24 hours. The Th1/Th2 ratio in blood increased at 24, 36, 48, and 56 hours. The intracellular concentration of interferon γ , but not of interleukin 4, increased at 24, 36, 48, and 56 hours.

Conclusions: These results provide evidence of the involvement of neutrophils, Th1 lymphocytes, monocytes, platelets, and endothelial cells, upon participation of various adhesion molecules, in mechanism(s) underlying the clinical DYAR.

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INTRODUCTION

Allergic bronchial asthma is a typical multifaceted disorder involving various immunologic mechanisms.^{1–9} Patients with this disorder may develop different types of asthmatic response on allergen exposure, which can be simulated by bronchial provocation tests (BPTs).¹⁰ The immediate asthmatic response (IAR), the late asthmatic response (LAR), and the dual late asthmatic response (DLAR) types have already been investigated from various clinical, immunologic, and pharmacologic points of view.^{11–26}

An unusual asthmatic response appearing later than 24 hours after the BPTs was recorded in some of the patients who were examined at our department using routine diagnostic procedure.^{27,28} This response, beginning within 26 to 32 hours, reaching its maximum within 32 to 48 hours, and

resolving within 56 hours after the BPT with various inhalant allergens, was designated as a delayed asthmatic response (DYAR).^{27,28} The DYAR differs from the IAR and LAR in various aspects.^{4,11–13,23,24} The typical features of the DYAR, besides its appearance later than 24 hours after the allergen challenge, include the increased counts of leukocytes and neutrophils but not of eosinophils, the changes in the Th1/Th2 ratio in peripheral blood in favor of Th1 cells, increased concentrations of interferon γ (IFN- γ) but not of interleukin 4 (IL-4) in peripheral blood, and absence of serum concentration changes of the particular immunoglobulins.^{27,28}

Although these data would suggest an important, if not a key, role of the Th1 lymphocytes in the DYAR, the immunologic mechanisms underlying this response type are not yet sufficiently clarified. The purposes of this study, which is a continuation of our previous work,^{27,28} were (1) to investigate the appearance and possible changes in the concentrations of cell adhesion molecules during a DYAR; (2) to explore the involvement of particular cell types in the mechanism(s) leading to a DYAR; and (3) to contribute to a fuller understanding of this clinical phenomenon.

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METHODS

Patients

Twenty-eight of the 55 asthma patients examined at the Department of Allergology and Immunology, Institute of Medical Sciences "De Klokkenberg", Breda, The Netherlands, between 1999 and 2000 and developing a DYAR to BPTs (Fig 1)²⁸ were randomly selected and volunteered to participate in this study (Tables 1, 2, 3, and 4). Some of the data concerning the characteristics of these 28 patients were already presented in the previously published article as a part of the summarized data related to all 55 DYAR patients.²⁸

These patients, who were 20 to 45 years of age, had reversible bronchial constriction alternating with symptom-free periods, without any restrictive changes in their pulmonary function (Global Initiative for Asthma classification: step 2, mild persistent asthma).²⁹ They had no airway infections and did not use any oral corticosteroids or immunotherapy. They were examined by means of a routine diagnostic procedure, serving also as inclusion-exclusion criteria (Tables 1 and 4). This procedure consisted of various diagnostic parameters, including bronchial histamine threshold according to the European and international standard procedures^{10,30,31} modified by us, single determination of the cellular adhesion molecules in peripheral blood (Table 5), and routine BPTs with inhalant allergens ($n = 67$) (Tables 1, 2, and 3) and phosphate-buffered saline (PBS) control challenges ($n = 28$).^{10,28} The allergens for the BPTs were chosen with respect to the positive skin test results (positive skin response of any

type) and positive or suspect disease history for involvement of a certain allergen in the patients' bronchial asthma (Tables 2 and 3). The initial BPTs have always been performed with allergenic extracts diluted 1:10 (Table 3), and when results were negative, the BPTs with undiluted extracts (our standard concentrations) were followed 4 to 6 days later (Table 2 and 3).

All BPTs were performed during a period when the patients had no manifest bronchial symptoms, outside the allergen-relevant season and during hospitalization. Inhaled corticosteroids and long-acting β_2 -sympathomimetics were withdrawn at 4 weeks; cromolyn, nedocromil sodium, leukotriene antagonists, and xanthine derivatives 2 weeks; and other drugs 48 hours before the BPTs. If the forced expiratory volume in 1 second values decreased after allergen challenge with 50% or more with respect to the predicted values, the patients ($n = 5$) were treated with a single dose of 200 to 400 μg of salbutamol aerosol.²⁸

In these 28 patients, BPTs with the same allergens and PBS control challenges were repeated 2 to 6 weeks later.²⁸ The repeated DYARs and PBS controls (Fig 1) were supplemented with counting of particular cell types (Table 6 and Fig 2A) and recording of the cell adhesion molecules in the peripheral blood (Table 7 and Fig 2B). A 5-day interval was always inserted between the end of the preceding test and the begin of the following test to prevent the carryover effects and to allow patient recovery. The local ethical committee approved this study, and an oral informed consent was obtained from all participants.

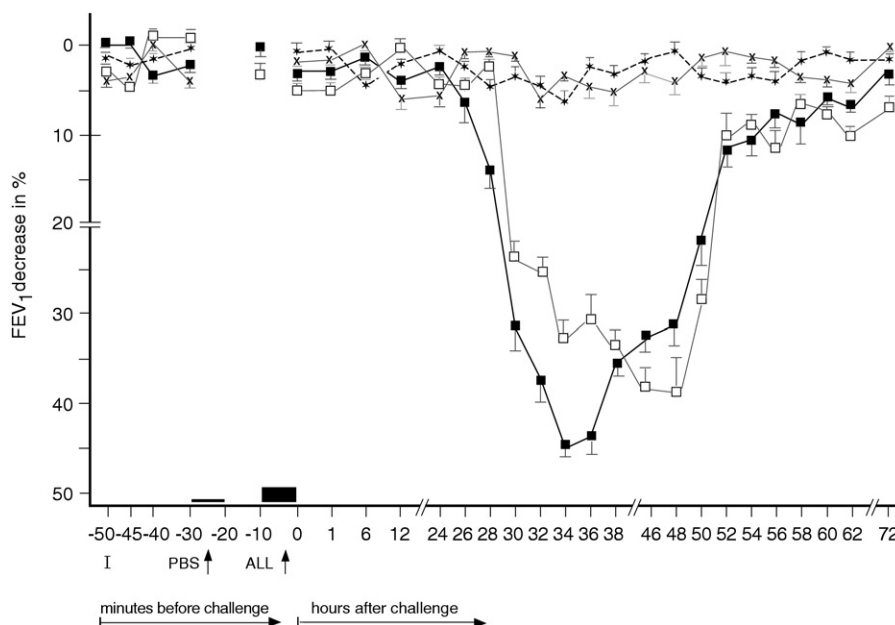


Figure 1. Mean (SEM) delayed asthmatic response (DYAR) to allergen challenge and phosphate-buffered saline (PBS) control challenge. The mean percentage changes in the forced expiratory volume in 1 second (FEV_1) values calculated from 28 DYAR allergen challenges and 28 PBS control challenges. ■, Initial DYAR; □, repeated DYAR; ☆, initial PBS; +, repeated PBS; I, initial (baseline) values; ALL, allergen challenge.

Table 1. Characteristics of the Patients and Control Subjects

	Patients with DYAR (n = 28)	Control subjects			
		IAR (n = 23)	LAR (n = 25)	DLAR (n = 16)	Healthy subjects (n = 24)
Age, mean (SD), y	30 (5)	27 (4)	31 (8)	29 (6)	28 (7)
Sex, M/F	15/13	12/11	11/14	9/7	10/14
Disease history, mean (SD), y	4.0 (1.7)	4.3 (1.9)	5.5 (1.5)	6.0 (2.4)	0
No. of asthmatic attacks per month, mean (SD)	4 (1)	3 (1)	4 (2)	2 (1)	0
FEV ₁ , mean (SD), % predicted	95.1 (6.8)	93.5 (5.2)	96.5 (4.8)	94.1 (3.9)	98.3 (6.1)
FVC, mean (SD), % predicted	99.2 (5.1)	102.4 (3.7)	98.5 (7.9)	106.2 (7.1)	103.4 (4.3)
Blood leukocyte count, mean (SD), $\times 10^9/L^a$	8.3 (0.8)	7.1 (0.5)	7.4 (0.3)	7.7 (0.6)	6.9 (1.4)
Blood eosinophil count, mean (SD), $\times 10^6/L^b$	307 (34)	528 (71) ^h	493 (86) ^h	515 (69) ^h	223 (55)
Blood neutrophil count, mean (SD), $\times 10^9/L^c$	6.9 (0.5)	5.8 (1.0)	6.2 (1.1)	7.0 (0.9)	6.5 (1.2)
Blood thrombocyte count, mean (SD), $\times 10^9/L^d$	317 (42)	331 (35)	364 (51)	335 (49)	308 (18)
Blood differential count, mean (SD), $\times 10^9/L^e$					
Basophils	0.11	0.14	0.15	0.13	0.12
Eosinophils	0.25	0.7	0.4	0.6	0.3
Neutrophils	6.6	4.3	4.7	5.1	4.5
Monocytes	0.5	0.4	0.25	0.3	0.18
Lymphocytes	2.9	1.0	1.3	1.5	1.1
Erythrocyte sedimentation rate ^f	12	8	12	11	10
Bronchial histamine threshold, mg/mL ^g					
≤ 4.0	4	2	3	1	0
8.0	6	9	5	2	0
16.0	7	5	7	8	1
32.0	6	7	6	3	1
> 32.0	5	0	4	2	22
BPT (total/positive)	2–3/1	2/1–2	2–3/1	2/1–2	0
Th1/Th2, mean (SD), %	8.4 (3.1)	7.0 (2.5)	7.6 (3.8)	8.1 (3.9)	8.5 (3.4)
IFN- γ , mean (SD), pg/mL	178 (90)	165 (102)	184 (111)	191 (86)	199 (95)
IL-4, mean (SD), pg/mL	22.4 (4.1)	23.8 (5.5)	22.9 (5.1)	19.7 (6.5)	23.4 (4.7)

Abbreviations: BPT, bronchial provocation test with allergens (per patient indicates total/positive); DLAR, dual late asthmatic response; DYAR, delayed asthmatic response; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IFN- γ , interferon γ ; IAR, immediate asthmatic response; IL-4, interleukin 4; LAR, late asthmatic response.

^a Reference range, 4.0 to 10.0 $\times 10^9/L$.

^b Reference range, less than 300 $\times 10^6/L$.

^c Reference range, 2.0 to 7.2 $\times 10^9/L$.

^d Reference range, 150 to 400 $\times 10^9/L$.

^e Reference range, basophils, less than 0.2 $\times 10^9/L$; eosinophils, less than 0.5 $\times 10^9/L$; neutrophils, 2.0 to 7.2 $\times 10^9/L$; monocytes, 0.2 to 1.0 $\times 10^9/L$; lymphocytes, 1.0 to 4.0 $\times 10^9/L$.

^f Reference range, less than 20 mm in 1 hour.

^g Reference range, greater than 32.0 mg/mL, as determined according to the European standard procedure.

^h $P < .05$ compared with healthy subjects.

Control Subjects

Twenty-three asthmatic patients demonstrating an IAR, 25 patients developing an LAR, 16 showing a DLAR, and 24 healthy subjects (Tables 1, 2, 4, and 5) volunteered to participate as control subjects.

Allergens

Dialyzed and lyophilized allergen extracts (Allergopharma, Reinbek, Germany) diluted in PBS were used in concentrations of 100 to 500 BU/mL for skin tests and 1,000 to 3,000 BU/mL for BPTs (Tables 2 and 3). The concentrations recommended by the manufacturer were 500 BU/mL for skin tests and 5,000 BU/mL for the BPTs.^{27,28}

Skin Tests

Scratch tests with allergenic extracts in concentrations of 500 BU/mL were performed and the results evaluated after 20 minutes. If the results were negative, then intradermal tests in concentrations of 100 BU/mL and 500 BU/mL were performed and evaluated 20 minutes and 6, 12, 24, 36, 48, 72, and 96 hours after the intradermal injection. A skin wheal (> 7.0 mm in diameter) occurring after 20 minutes was qualified as a positive immediate skin response, the skin infiltration appearing between 6 and 12 hours as a late skin response, and the skin induration observed later than 48 hours as a delayed skin response.^{11,12,27,28}

Table 2. Allergens Causing Particular Types of Asthmatic Responses

	Concentration, BU/mL	Responses due to the diluted (1:10) or undiluted allergen extracts [total No. of responses to allergen]				
		Patients investigated (n = 28)		Control subjects (n = 64)		
		DYAR (n = 28)	NAR (n = 39)	IAR (n = 23)	LAR (n = 25)	DLAR (n = 16)
<i>Dermatophagoides pteronyssinus</i>	100/1,000	2/7 [9]	12/12 [12]	3/5 [8]	2/8 [10]	1/6 [7]
Animal danders						
Dog	300/3,000	0/1 [1]	1/1 [1]	1/1 [2]	0/2 [2]	0/1 [1]
Cat	100/1,000	2/1 [3]	1/1 [1]	1/2 [3]	0/1 [1]	1/0 [1]
Guinea pig	200/2,000	0/1 [1]	00/1 [1]	0/1 [1]	0/1 [1]	
Hamster	200/2,000	0	0	0/1 [1]	0	0
Horse	200/2,000	0	2/2 [2]	0	0	0
Feathers						
Pigeon	300/3,000	0/1 [1]	1/1 [1]	0	0/2 [2]	0
Parakeet	300/3,000	0/2 [2]	1/1 [1]	1/0 [1]	0	0
Canary	300/3,000	0	1/1 [1]	0	1/0 [1]	0
Parrot	300/3,000	0	0	0	0	0/1 [1]
<i>Aspergillus fumigatus</i>	100/1,000	0/1 [1]	2/2 [2]	0	0/1 [1]	0
<i>Aspergillus niger</i>	100/1,000	0	1/1 [1]	0	0	0
Pollen ^a						
Grass mix 1	100/1,000	3/5 [8]	7/7 [7]	1/3 [4]	0/3 [3]	1/4 [5]
Grass mix 2	100/1,000	1/0 [1]	3/3 [3]	0/1 [1]	1/1 [2]	0/1 [1]
Tree mix	300/3,000	0/1 [1]	1/1 [1]	0/1 [1]	0	0
Weed mix	100/1,000	0	1/1 [1]	0	0/1 [1]	0
Birch	100/1,000	0	2/2 [2]	1/0 [1]	0/1 [1]	0
Poplar	200/2,000	0	3/3 [3]	0	0	0

Abbreviations: DLAR, dual late asthmatic response; DYAR, delayed asthmatic response; IAR, immediate asthmatic response; LAR, late asthmatic response; NAR, negative asthmatic response.

^a Grass pollen mix 1 was made up of *Dactylis glomerata*, *Lolium perenne*, *Phleum pratensis*, and *Poa pratensis*; grass pollen mix 2, *Festuca pratensis*, *Holcus lanatus*, *Agrostis alba*, and *Anthoxanthum odoratum*; tree pollen mix, *Betula pendula*, *Corylus avellana*, *Juniperus communis*, and *Salix alba*; weed pollen mix, *Artemisia vulgaris*, *Plantago lanceolata*, *Rumex acetosa*, and *Taraxacum officinale*.

Bronchial Provocation Tests

The BPTs were performed using spirometry (Spirograph D-75; Lode Instruments, Groningen, The Netherlands) recording the forced vital capacity and forced expiratory volume in 1 second values. The aerosolized allergen extracts and PBS were inhaled by means of the Wiesbadener Doppel Inhalator (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, Germany) at an airflow of 10 L/min. The nebulizer output was 0.12 to 0.14 mL/min, and the aerosol particles had a median mass diameter of 2.8 to 3.6 μ m.

The BPTs were performed according to the European standard^{10,32} by a modified schedule^{27,28}: (1) initial (baseline) values recorded at 0, 5, and 10 minutes; (2) PBS control values recorded at 0, 5, and 10 minutes after a 10-minute PBS inhalation; and (3) inhalation of allergen aerosol for 2 $\times$ 5 minutes, with inserted spirometry measurement, followed by recording of FEV₁ and FVC values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and the every hour up to the 12th hour and every second hour during the 22nd- and 38th-hour and the 46th- and 56th-hour interval.²⁸ The control PBS challenge was performed applying the same schedule as that of the BPT with allergens. The BPT and PBS challenges were performed according to a double-blind schedule. A 5-day interval has

always been inserted between the end of the previous and the beginning of the following test.^{27,28}

In 28 DYAR patients, the changes in the cell adhesion molecule blood concentrations were measured during the repeated DYAR and PBS challenges before and at 1, 6, 12, 24, 36, 48, 56, and 72 hours after the challenge (Table 7).

Supplementary Parameters

Th1/Th2 ratio values in peripheral blood were determined by means of flow cytometry, and the intracellular IFN- γ and IL-4 in the peripheral blood mononuclear cells were estimated by means of enzyme-linked immunosorbent assay (ELISA) kits, as described previously.²⁸

Determination of the Soluble Adhesion Molecules in the Serum

The samples of venous blood (5 mL) were kept at room temperature for 1 hour to allow them to coagulate and then centrifuged at 3,000 $\times$ g at 4°C for 15 minutes. Serum aliquots were stored at -70°C until analysis. The soluble adhesion molecules were estimated in the serum by commercial enzyme-linked immunoassay kits, according to the manufacturers' recommendations, by a double-blind schedule. The soluble intercellular adhesion molecule 1 (sICAM-1), soluble

Table 3. Survey of the DYAR and NAR and Positive Skin Test Results in 28 Study Patients

Allergen	Concentration, BU/mL diluted (1:10)/undiluted	DYAR (n = 28)				NAR (n = 39)			
		Positive responses diluted (1:10)/undiluted	Positive skin responses (n = 28)			Negative responses diluted (1:10)/undiluted	Positive skin responses (n = 39)		
			ISR	LSR	DYSR		ISR	LSR	DYSR
<i>Dermatophagoides pteronyssinus</i>	100/1,000	2/7	5	1	3	12/12	9	2	1
Animal danders									
Dog	300/3,000	0/1	0	0	1	1/1	1	0	0
Cat	100/1,000	2/1	1	0	2	1/1	1	0	0
Guinea pig	200/2,000	0/1	1	0	0				
Hamster	200/2,000								
Horse	200/2,000					2/2	1	1	0
Feathers									
Pigeon	300/3,000	0/1	0	0	1	1/1	0	1	0
Parakeet	300/3,000	0/2	1	0	1	1/1	1	0	0
Canary	300/3,000					1/1	0	1	0
Parrot	300/3,000								
<i>Aspergillus fumigatus</i>	100/1,000	0/1	0	1	0	2/2	1	1	0
<i>Aspergillus niger</i>	100/1,000					1/1	0	1	0
Pollen									
Grass mix 1	100/1,000	3/5	6	0	2	7/7	4	1	2
Grass mix 2	100/1,000	1/0	1	0	0	3/3	1	1	1
Tree mix	300/3,000	0/1	0	0	1	1/1	1	0	0
Weed mix	100/1,000					1/1	1	0	0
Birch	100/1,000					2/2	1	1	0
Poplar	200/2,000					3/3	2	1	0

Abbreviations: DYAR, delayed asthmatic response; DYSR, delayed skin response; ISR, immediate skin response; LSR, late skin response; 0 = no response; NAR, negative asthmatic response.

vascular cell adhesion molecule 1 (sVCAM-1), soluble E-selectin, soluble L-selectin, soluble P-selectin, and soluble E-cadherin were measured by means of R&D Systems kits (Minneapolis, Minnesota), whereas soluble platelet endothelial cell adhesion molecule (sPECAM-1) was measured by means of a Bender MedSystems kit (Wien, Austria). All measurements were performed in duplicate. The detection limits of the enzyme-linked immunoassay kits were as follows: sICAM-1, 0.25 ng/mL; sVCAM-1, 1.26 ng/mL; E-selectin, 0.03 ng/mL; L-selectin, 0.3 ng/mL; P-selectin, 0.5 ng/mL; E-cadherin, 0.09 ng/mL; and PECAM-1, 0.06 ng/mL. The interassay coefficients of variations for these kits were less than 7%, and the intra-assay coefficients of variations were less than 8%.

Statistical Analysis

The initial and repeated DYAR and PBS controls were statistically analyzed by means of polynomials fitted to the mean curves over time: 8 time points within 120 minutes and 22 time points up to 72 hours after the challenge. The hypotheses were tested by the generalized multivariate analysis of variance model.³³ The postchallenge cellular adhesion molecule values recorded at each of the time points during the DYAR and PBS challenges in individual patients were compared with the prechallenge cellular adhesion molecule values and evaluated by Wilcoxon matched-pair signed rank test. The

mean postchallenge cellular adhesion molecule values measured at each of the time points during the DYAR were compared with the corresponding PBS values and statistically evaluated by the Mann-Whitney *U* test. $P < .05$ was considered statistically significant.

RESULTS

The 28 patients, in whom 67 BPTs with various inhalant allergens and 28 PBS control challenges were performed, developed 28 positive DYARs and 39 negative asthmatic responses (Tables 1, 2, and 3). None of the patients developing a positive DYAR demonstrated other types of asthmatic response to BPTs with other allergens. The concentrations of allergenic extracts producing the positive DYARs and the negative responses are presented in Table 3.

The DYAR began within 26 to 32 hours, reached its maximum within 32 to 48 hours, and resolved within 56 hours (Fig 1).²⁸ The initial and repeated DYARs were statistically significant in comparing both the postchallenge with the prechallenge forced expiratory volume in 1 second values ($P < .001$ and $P < .001$, respectively) and with the PBS control values ($P < .001$ and $P < .001$, respectively). No significant differences were found between the initial and the repeated DYARs ($P > .10$). The other 39 BPTs resulted in 39

Table 4. Single Diagnostic Parameters

Parameter	Patients with DYAR (n = 28)	Control patients			Healthy subjects (n = 24)
		IAR (n = 23)	LAR (n = 25)	DLAR (n = 16)	
Positive skin response ^a					
Immediate	15	19	12	12	1
Late	2	4	13	4	0
Delayed	11	0	0	0	0
Increased total IgE in the serum ^b	0	4	1	3	0
Positive specific IgE in the serum ^c	0	17	5	10	0
Increased total IgG in the serum ^d	0	1	11	6	0
Increased IgG subclasses in the serum ^e					
IgG1	0	0	2	1	0
IgG2	0	1	0	0	0
IgG3	0	0	4	3	1
IgG4	0	0	5	2	0
Increased total IgM in the serum ^f	0	0	0	0	0
Increased total IgA in the serum ^g	0	0	0	0	0
Thorax radiograph ^h	1	1	2	0	0

Abbreviations: DLAR, dual late asthmatic response; DYAR, delayed asthmatic response; IAR, immediate asthmatic response; LAR, late asthmatic response.

^a Produced by intracutaneous (intradermal) skin test.

^b Reference range, less than 500 IU/mL.

^c Reference range, greater than 0.70 U/mL (more than class 1).

^d Reference range, less than 15.0 g/L.

^e Reference ranges: IgG1, less than 5.0 g/L; IgG2, less than 2.6 g/L; IgG3, less than 0.4 g/L; and IgG4, less than 0.5 g/L.

^f Reference range, less than 3.8 g/L.

^g Reference range, less than 3.2 g/L.

^h Thorax radiograph is the number of patients demonstrating pronounced bronchovascular markings on the radiographs.

negative asthmatic responses ($P > .20$) despite positive skin test results (Tables 2 and 3).

The 28 DYAR patients demonstrated delayed dyspnea and wheezing in 100%, positive immediate skin responses in 56% and delayed skin responses in 39%, decreased bronchial histamine threshold in 78%, and no changes in the immunoglobulin serum concentrations in 100% (Tables 1 and 4).²⁸

The DYAR was accompanied by significant changes ($P < .05$) in peripheral blood: (1) increased counts of total leuko-

cytes at 36, 48, and 56 hours and neutrophils at 12 and 24 hours and (2) increased numbers of neutrophils at 24, 36, 48, and 56 hours, lymphocytes at 24, 36, and 48 hours, and monocytes at 6, 12, and 24 hours in blood differential counts (Table 6 and Fig 2A).²⁸ No significant changes in the eosinophil and basophil counts were measured in the peripheral blood during the DYAR (Table 6).

The DYAR was also associated with significant changes in the ratio of Th1/Th2 cells in peripheral blood in favor of Th1

Table 5. Single Determination of the Prechallenge Adhesion Molecule Concentrations in the Serum^a

Parameter	Patients with DYAR (n = 28)	Control patients			Healthy subjects (n = 24)
		IAR (n = 23)	LAR (n = 25)	DLAR (n = 16)	
sICAM-1	115.7 (13.8)	173.5 (38.3) ^b	190.6 (24.5) ^b	180.1 (27.0) ^b	112.7 (15.3)
sVCAM-1	521.0 (53.7)	566.0 (42.7)	753.8 (54.4) ^c	605.5 (32.7)	545.5 (44.1)
sPECAM-1	17.5 (3.0)	15.3 (4.1)	18.4 (2.8)	17.7 (3.2)	16.3 (2.4)
sE-selectin	32.1 (5.5)	57.6 (5.2) ^b	29.9 (3.6)	34.0 (2.6)	35.9 (6.5)
sL-selectin	619.0 (41.4)	628.0 (39.3)	667.5 (45.0)	640.0 (27.5)	681.6 (53.0)
sP-selectin	45.1 (7.7)	48.2 (4.7)	40.3 (3.2)	41.4 (3.0)	43.8 (6.9)
sE-cadherin	6.1 (3.2)	5.5 (1.9)	6.4 (2.5)	5.3 (2.1)	5.7 (2.11)

Abbreviations: DLAR, dual late asthmatic response; DYAR, delayed asthmatic response; IAR, intermediate asthmatic response; LAR, late asthmatic response; s, soluble; sICAM-1, soluble intercellular adhesion molecule 1; sPECAM-1, soluble platelet endothelial cell adhesion molecule; sVCAM-1, soluble vascular cell adhesion molecule 1.

^a Data are presented as mean (SD) in nanograms per milliliter.

^b $P \leq .05$ compared with healthy controls.

^c $P < .05$ compared with healthy subjects.

Table 6. Mean (SEM) Differential Cell Counts in Peripheral Blood During the DYAR and PBS Responses

	Before the challenge	After the challenge, h							
		1	6	12	24	36	48	56	72
Leukocytes ^a									
DYAR	8.6 (0.8)	10.5 (0.5)	10.9 (0.4)	10.4 (0.9)	11.5 (0.7) ^g	12.9 (0.5) ^h	13.2 (0.6) ^h	12.1 (0.7) ^h	11.0 (0.5)
PBS	7.9 (0.5)	6.6 (0.7)	7.8 (1.0)	9.3 (0.8)	7.2 (0.5)	8.3 (1.1)	9.0 (1.1)	7.1 (1)	08.4 (0.9)
Eosinophils ^b									
DYAR	299 (30)	331 (27) ^g	309 (16)	320 (42)	270 (33)	255 (28)	269 (35)	215 (22)	284 (34)
PBS	261 (28)	293 (36)	277 (25)	302 (35)	288 (41)	283 (17)	295 (23)	278 (19)	268 (25)
Neutrophils ^c									
DYAR	6.7 (0.5)	6.5 (0.3)	7.0 (0.9)	8.9 (0.7) ^h	8.6 (0.7) ^h	8.1 (1.0) ^g	6.3 (0.4)	6.6 (0.5)	6.5 (0.6)
PBS	5.8 (1.0)	6.0 (0.8)	6.5 (0.4)	6.9 (0.8)	6.4 (0.5)	6.2 (0.3)	6.8 (0.7)	6.4 (0.3)	6.0 (0.2)
Thrombocytes ^d									
DYAR	335 (41)	342 (39)	328 (43)	383 (46)	345 (45)	478 (49) ^g	471 (31) ^g	306 (20)	322 (31)
PBS	280 (30)	314 (27)	305 (19)	297 (25)	351 (34)	315 (30)	303 (22)	269 (38)	291 (24)
Differential cell count ^e									
Basophils									
DYAR	0.1	0.1	0.12	0.09	0.1	0.08	0.1	0.09	0.1
PBS	0.07	0.09	0.06	0.05	0.08	0.05	0.03	0.02	0.06
Eosinophils									
DYAR	0.35	0.4	0.35	0.4	0.36	0.4	0.2	0.35	0.3
PBS	0.12	0.4	0.29	0.23	0.21	0.28	0.15	0.17	0.13
Neutrophils									
DYAR	6.2	5.8	6.9	7.2	8.8 ^h	9.6 ^h	8.9 ^h	8.8 ^h	7.0
PBS	5.1	5.0	5.5	5.9	6.4	6.0	5.3	7.1	5.8
Monocytes									
DYAR	0.2	0.3	1.0 ^h	1.9 ^h	2.7 ^h	0.4	0.7	0.5	0.3
PBS	0.15	0.3	0.9	0.6	0.3	0.17	0.2	0.15	0.4
Lymphocytes									
DYAR	2.7	1.3	2.5	3.3	4.5 ^h	4.9 ^h	5.1 ^h	1.2	1.4
PBS	2.4	3.0	3.1	2.8	2.7	3.2	2.2	2.5	2.1
Erythrocyte sedimentation ^f									
DYAR	11	10	12	10	9	13	10	10	8
PBS	7	5	10	9	12	8	5	4	5
Th1/Th2 ratio, %									
DYAR	8.2 (2.0)	8.7 (2.9)	9.5 (4.3)	12.9 (4.5) ^g	14.9 (4.6) ^h	17.7 (4.2) ^h	17.5 (5.4) ^h	15.9 (4.7) ^h	10.4 (5.0)
PBS	7.4 (3.1)	8.2 (3.5)	9.2 (5.1)	8.7 (4.1)	8.9 (4.2)	9.5 (4.7)	10.7 (4.2)	9.4 (5.3)	8.9 (4.7)
IFN- γ , pg/mL									
DYAR	192 (107)	185 (95)	211 (127)	200 (117)	398 (114) ^h	486 (147) ^h	479 (131) ^h	399 (121) ^h	236 (110)
PBS	171 (119)	210 (116)	199 (118)	183 (120)	209 (123)	174 (109)	183 (122)	169 (100)	182 (119)
IL-4, pg/mL									
DYAR	22.1 (2.5)	24.9 (3.1)	28.6 (5.0)	29.4 (4.5)	21.5 (5.6)	26.2 (5.7)	23.8 (3.1)	28.3 (5.5)	26.8 (4.7)
PBS	25.8 (5.0)	22.5 (4.4)	24.7 (3.3)	27.9 (5.2)	23.4 (5.1)	25.7 (3.5)	24.2 (4.5)	26.0 (3.4)	23.9 (5.5)

Abbreviations: DYAR, delayed asthmatic response; IFN- γ , interferon γ ; IL-4, interleukin 4; PBS, phosphate-buffered saline.^a Reference range, 4.0 to 10.0 $\times 10^6$ /L.^b Reference range, less than 300 $\times 10^6$ /L.^c Reference range, 2.0 to 7.2 $\times 10^9$ /L.^d Reference range, 150 to 400 $\times 10^9$ /L.^e Reference range, basophils, less than 0.2 $\times 10^9$ /L; eosinophils, less than 0.50 $\times 10^9$ /L; neutrophils, 2.0 to 7.2 $\times 10^9$ /L; monocytes, 0.2 to 1.0 $\times 10^9$ /L; lymphocytes, 1.0 to 4.0 $\times 10^9$ /L.^f Reference range, less than 20 mm/1 h.^g $P \leq .05$ compared with prechallenge values.^h $P < .05$ compared with prechallenge values.

cells ($P < .01$) at 24, 36, 48, and 56 hours and significantly increased intracellular concentrations of IFN- γ ($P < .05$) at 24, 36, 48, and 56 hours but not of IL-4 ($P > .05$) (Table 6 and Fig 2A).²⁸

The prechallenge cell adhesion molecule values in the serum of DYAR patients did not differ significantly from the healthy subjects ($P > .10$), whereas they differed only slightly ($P \leq .05$) from the values measured in other control patients (Table 5).

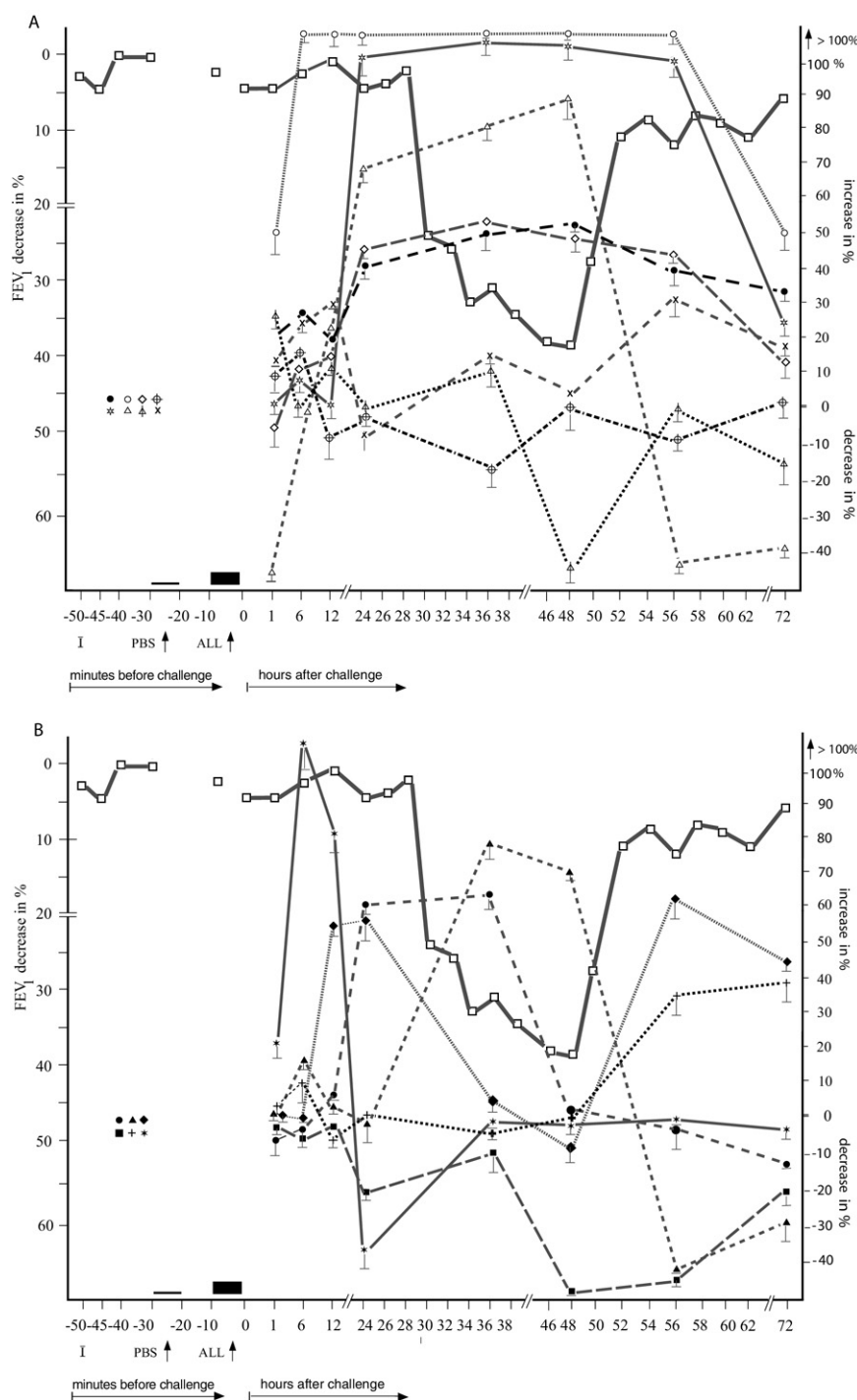


Figure 2. A, The repeated delayed asthmatic response (DYAR) (□) to allergen challenge and the mean percentage changes in the counts of the particular cell types and cytokine concentrations in peripheral blood with respect to their prechallenge values in 28 patients. ●, Total leukocyte counts; ⊕, basophil counts; △, eosinophil count; ○, neutrophil counts; ◇, monocyte counts; Δ, lymphocyte counts; ☆, interferon γ levels; x, interleukin 4 levels; I, initial (baseline) values; ALL, allergen challenge; PBS, phosphate-buffered saline (control). B, The repeated DYAR (□) and the mean percentage changes in the serum concentrations of adhesion molecules with respect to their prechallenge values in 28 patients. ★, soluble intercellular adhesion molecule 1; ●, soluble vascular cell adhesion molecule 1; ▲, soluble platelet endothelial cell adhesion molecule; ◆, soluble E-selectin; +, soluble L-selectin; ■, soluble E-cadherin; I, initial (baseline) values; ALL, allergen challenge; PBS, phosphate-buffered saline (control).

Table 7. Mean (SEM) Serum Concentrations of Adhesion Molecules Recorded During DYAR and PBS Control Tests in 28 Patients

	Before the challenge	After the challenge, h							
		1	6	12	24	36	48	56	72
sICAM-1									
DYAR	110.4 (15.3)	136.9 (17.1)	242.7 (28.8) ^a	203.5 (14.4) ^a	71.3 (9.5) ^a	108.4 (11.9)	114.1 (13.5)	108.0 (12.6)	103.2 (9.9)
PBS	117.2 (12.4)	115.5 (11.6)	111.8 (10.2)	120.3 (15.8)	116.2 (12.9)	103.6 (8.8)	109.5 (10.1)	114.3 (13.7)	109.0 (10.1)
sVCAM-1									
DYAR	567.3 (44.9)	532.1 (38.8)	547.2 (35.0)	594.7 (33.2)	876.5 (57.3) ^a	919.0 (61.0) ^a	552.5 (33.7)	541.5 (28.8)	513.6 (25.9)
PBS	549.0 (37.1)	518.3 (16.4)	535.6 (23.7)	555.0 (31.9)	521.6 (28.0)	559.5 (43.3)	530.0 (20.9)	508.1 (25.8)	549.0 (22.3)
sPECAM-1									
DYAR	16.9 (2.6)	15.4 (3.7)	19.1 (3.5)	17.4 (3.1)	16.2 (2.8)	29.8 (4.2) ^a	28.6 (3.4) ^a	10.1 (3.3) ^a	11.9 (2.7) ^b
PBS	18.0 (3.1)	17.1 (2.5)	14.8 (2.4)	15.5 (2.9)	15.9 (3.0)	14.7 (2.3)	16.1 (3.0)	15.4 (2.5)	15.0 (2.0)
sE-selectin									
DYAR	30.4 (2.4)	31.0 (2.0)	28.9 (2.7)	46.9 (3.0) ^b	48.1 (3.3) ^b	32.3 (2.6)	27.8 (2.9)	49.5 (3.1) ^a	44.0 (3.2) ^b
PBS	34.1 (2.8)	29.0 (2.3)	30.1 (2.9)	28.5 (3.4)	29.3 (2.8)	33.0 (3.1)	33.6 (2.5)	32.0 (2.2)	31.1 (2.7)
sL-selectin									
DYAR	655.0 (67.3)	704.0 (48.3)	671.5 (54.0)	633.8 (39.1)	650.9 (47.0)	643.2 (39.8)	661.0 (69.2)	885.5 (44.0) ^b	898.3 (35.1) ^a
PBS	648.5 (42.9)	627.3 (54.0)	635.0 (43.8)	651.0 (39.1)	619.0 (23.4)	625.7 (35.1)	644.7 (50.0)	663.9 (41.0)	682.0 (41.4)
sP-selectin									
DYAR	40.7 (7.0)	42.3 (2.1)	47.6 (3.3)	45.5 (2.9)	38.7 (3.5)	39.6 (5.1)	43.8 (4.3)	44.5 (3.9)	41.4 (4.7)
PBS	41.9 (5.8)	37.9 (4.8)	39.2 (4.1)	46.8 (5.1)	42.3 (4.0)	46.0 (4.9)	40.1 (3.8)	42.2 (3.5)	43.0 (3.1)
sE-cadherin									
DYAR	6.4 (1.9)	6.0 (2.2)	6.1 (1.7)	5.8 (1.4)	5.5 (1.6)	5.9 (2.0)	1.3 (2.1) ^a	2.0 (1.8) ^a	5.5 (2.3)
PBS	5.5 (2.7)	5.7 (2.0)	5.6 (1.9)	6.1 (2.5)	6.3 (2.0)	6.2 (1.7)	5.8 (1.5)	5.5 (1.3)	5.9 (1.6)

Abbreviations: DYAR, delayed asthmatic response; IFN- γ , interferon γ ; IL-4, interleukin 4; PBS, phosphate-buffered saline; s, soluble; sICAM-1, soluble intercellular adhesion molecule 1; sPECAM-1, soluble platelet endothelial cell adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

^a $P < .05$ compared with prechallenge values.

^b $P \leq .05$ compared with prechallenge values.

The DYAR was accompanied by significant changes in the serum concentrations of cell adhesion molecules as follows: an increase of sICAMs at 6 and 12 hours ($P < .05$ and $P < .05$, respectively) followed by its decrease at 24 hours ($P < .05$); an increase of sVCAM-1 at 24 and 36 hours ($P < .05$ and $P < .05$, respectively); an increase of sPECAM-1 at 36 and 48 hours ($P < .05$ and $P < .05$, respectively) followed by a decrease at 56 hours ($P < .05$); an increase of soluble E-selectin at 56 hours ($P < .05$); increased soluble L-selectin at 56 and 72 hours ($P < .05$ and $P < .05$, respectively); and a significant decrease of soluble E-cadherin at 48 and 56 hours ($P < .05$ and $P < .05$, respectively) after the bronchial challenge compared both with their prechallenge values and with the PBS control values (Table 7 and Fig 2B). No significant changes in the concentrations of any cell adhesion molecules were measured during the PBS controls ($P > .10$).

Compared with the healthy subjects, a statistically significant increase ($P < .05$) in the serum concentration of sVCAM-1 was recorded in the LAR patients, whereas no other significant changes in the serum concentrations of the cell adhesion molecules were found in the control patients (Table 5).

DISCUSSION

The adhesion of leukocytes and thrombocytes to vascular endothelium is considered a critical step in the immunologic

and inflammatory processes, leading to their migration from the vascular compartments into the airway tissues and the accumulation and expansion of their functions in the target tissue.^{5-9,14-26,34-40}

The adhesion mechanism is mediated by a number of cell adhesion molecules and their ligands expressed on the membrane of various circulating, epithelial, and vascular endothelial cell types, upon regulation of a number of cytokines, chemokines, CD molecules, and CD receptors.^{1,2,5-9,14-26,34-47} This multicascade process consists of several steps, such as tethering, rolling, and transmigration of the cells into the particular airway tissues and subsequently airway lumen.^{5-9,23-26,34-37}

The cell adhesion molecules are divided into particular superfamilies and their counterligands.^{5-8,23-26,36-46} The selectin gene superfamily includes E-, P-, and L-selectin; the integrin superfamily consists of heterodimers and is composed of α - and β -subunits (eg, very late antigens 1-6, lymphocyte function-associated antigen 1, membrane attack complex 1, and p150,95); the immunoglobulin gene superfamily includes ICAMs (ICAM-1 [CD54], ICAM-2 [CD102], and ICAM-3 [CD50]), sVCAM-1 [CD106], sPECAM-1, and sialic acid-binding immunoglobulin-like lectins; the cadherin superfamily consists of E-, N-, P-, T-, VE-, and transmembrane cadherins and procadherins and the galectin superfamily members.

The cell adhesion molecule functions include promotion of leukocytes to vascular endothelium, leukocyte-extracellular tissue matrix adhesion, leukocyte migration from vascular compartments into extravascular tissue, stimulation of antigen-specific recognition by T lymphocytes, acting as costimulatory signals for T-cell activation, stimulating effector mechanisms of activated lymphocytes, enhancing the cell proliferation, and regulating cell growth.^{5-9,23-26}

The occurrence and role of the cell adhesion molecules in the bronchial asthma has been intensively investigated in vitro and in vivo both in animal models and in asthma patients.^{1,2,5-9,14-26,34-57} In most of the human in vitro studies, the cell adhesion molecules were determined on the isolated cells^{14,17,19,42,53} or on the bronchial or lung tissue biopsy specimens.^{16,20,44,46,47,53} In most of the human in vivo studies, a single determination of soluble cellular adhesion molecules has been performed in the blood,^{22,43,44,49,51-54} sputum,^{56,57} or bronchoalveolar lavage fluid (BAL)⁵⁰ of the asthmatic patients. Relatively few studies deal with the determination of the soluble cell adhesion molecules in the blood, sputum, or BAL fluid during the IAR, LAR, or DLAR to the bronchial (BPT) or segmental challenge with an allergen.^{4-22,47}

In most of these studies, increased levels of sICAM,^{14,18,21,22,44,50-56} sVCAM,^{15,44,49,52-54} soluble E-selectin,^{21,43,49,51,53,54} and/or soluble L-selectin^{44,53,54} in the blood, sputum, or BAL fluid of asthmatic patients, as compared with control subjects, have been reported.

The results of the present study demonstrating increased serum concentrations of sICAM-1, sVCAM-1, sPECAM-1, soluble E-selectin, soluble L-selectin, and soluble E-cadherin, together with increased counts of leukocytes, neutrophils, lymphocytes, and monocytes during the DYAR, would indicate their active role in this response type. Unfortunately, no reliable data concerning the changes of particular cell adhesion molecules in the serum during the IAR, LAR, and DLAR in control subjects, which means serial measurements of cell adhesion molecules for at least 48 or even 72 hours, are available. Our results cannot therefore be compared with the course of particular cell adhesion molecules in the serum during the other types of asthmatic response. This fact may be regarded as a certain deficit of this study.

The early increase in sICAM-1 and the biphased increase in soluble E-selectin concentrations, followed by increased sPECAM-1 and IFN- γ concentrations, early increased neutrophil and monocyte counts, and later increased lymphocyte counts, would suggest an almost simultaneous activation of neutrophils, monocytes, and Th1 cells (Table 6, Table 7, and Fig 2A and B). The later (second) increase peak of soluble E-selectin concentrations may be indicative of the transmigration, accumulation, and adhesion of neutrophils, lymphocytes, and monocytes to the activated endothelial and epithelial cells in the airway tissue (Fig 2B). The later increase in the soluble L-selectin concentrations, confirming the adhesion of activated neutrophils to the endothelium, is caused by its shedding from the cell surface and its subsequent release into the circulation (Fig 2B). Because ICAM-1 stimulates the

activation and recruitment of T cells, the activation of Th1 cells is associated with upregulation of ICAM-1, E-selectin, and RANTES. Additionally, the E-selectin potentiated by IFN- γ supports the adhesion of neutrophils and T lymphocytes to the endothelium and epithelium, and the increased concentrations of IFN- γ together with the shifting of the Th1/Th2 ratio in favor of Th1 cells, the key role of activated Th1 cells in the mechanism underlying the DYAR can be assumed. Moreover, some of our results would not support any evidence for any pivotal role of eosinophils and Th2 cells in the mechanism(s) leading to the development of the DYAR. These results include the nonchanged counts of eosinophils and basophils, the absence of significant concentration changes of sP-selectin and IL-4, and the increased concentrations of sICAM-1, sE-selectin, and sPECAM-1 (Tables 6 and 7, and Fig 2A and 2B). The significant late decrease in the sPECAM-1 and soluble E-cadherin concentrations would indicate their downregulation and abandonment from the circulation and their accumulation on the activated epithelial cells. The increased sVCAM-1 concentrations were a somewhat surprising finding for which we have not a reliable explanation at least at this time.

Our results are partly consistent with other investigators' findings of increased levels of sICAM-1, sVCAM-1, and soluble E-selectin and increased neutrophil counts in blood, BAL, or lung biopsy specimens^{14-16,18,20-22,26,43,47} at 8 to 24 hours after the bronchial or segmental allergen challenge and increased expression of ICAM-1 and E-selectin on CD4⁺ and CD8⁺ T lymphocytes.¹⁴ However, our results are not fully comparable with the other investigators' findings because (1) the DYAR differs from the IAR and LAR; (2) our patients were investigated during hospitalization upon exclusion of additional allergen exposure; (3) the blood concentrations of cell adhesion molecules were recorded during the whole course of DYAR up to 56 hours after the allergen challenge, in which period the IAR and the LAR are long resolved; (4) the postchallenge cell adhesion molecule values were evaluated by a double comparison, both with the baseline and with the corresponding PBS control values; and (5) the CAM levels were measured simultaneously with the differential counts of leukocytes, the Th1/Th2 ratio, and the intracellular concentrations of IFN- γ and IL-4. Nevertheless, further concurrent studies will be needed to clarify fully the mechanisms underlying the DYAR.

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