

Effects of inhaled corticosteroids on the dual asthmatic response

Zdenek Pelikan, M.D., Ph.D.

ABSTRACT

Bronchial asthma patients develop various asthmatic response types to allergen challenge, such as immediate asthmatic response (IAR), late asthmatic response (LAR), or dual asthmatic response (DAR), the latter being a combination of an early phase (IAR) and a late phase (LAR). This study was designed to investigate (1) the features of the DAR thus identifying it as either a genuine two-phase compact clinical entity or a simultaneous appearance of two independent asthmatic response types, IAR and LAR, and (2) the protective effects of inhaled budesonide (BUD) on the DAR. Two protection tests (PTs) with BUD and a placebo (PL), in a single dose of 800 micrograms, were performed on 48 DAR patients, divided into four groups. Each test consisted of two treatments, one given 30 minutes before and the other at 1, 2, 3, or 4 hours after the bronchial challenge with allergen. The study design was randomized, double-blind, double-dummy, placebo-matched, crossover. A single dose of inhaled BUD did not affect the early phase (IAR) when applied 30 minutes before the challenge ($p > 0.2$), whereas it significantly prevented the late phase (LAR) when administered either 30 minutes before ($p < 0.001$) or up to 4 hours after the allergen challenge ($p < 0.05$). The different protective effects of BUD on both of the phases of DAR would suggest that this response does not exist as a compact clinical entity, but it may be a manifestation of two independent simultaneous responses, IAR and LAR, because of different immunologic mechanisms. Inhaled corticosteroids in a single dose administered shortly before or up to 4 hours after the allergen exposure contribute significantly to the prevention of the LAR, whereas they are unable to affect the IAR.

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Different types of hypersensitivity and immunologic mechanisms in patients with allergic bronchial asthma may induce various types of asthmatic response to bronchial challenge with allergen, these being as immediate asthmatic response (IAR), late asthmatic response (LAR), dual asthmatic response (DAR), or a delayed asthmatic response.^{1–33}

The dual or dual LAR (DAR) is believed by some investigators to be a compact clinical phenomenon consisting of two integrated phases, an early and a late phase, being causally related,^{3,5,6,13,14,22,27} whereas other investigators would disagree with this hypothesis.^{8–10,16,17,19,28,29,34,35}

It has been shown that the early phase, which is similar to the isolated IAR, is caused by the IgE-mediated hypersensitivity type, upon involvement of mast cells, eosinophils, and Th₂ lymphocytes.^{1,3,6,7,9,11–16,18,19} The immunologic mechanism underlying the late

phase, being similar to the isolated LAR, is still insufficiently clarified and the opinions differ.^{1,3–10,12–20,22–39}

To date, the LAR has been considered a complex immunologic event, designated as an “inflammatory process,” upon involvement of various cell types producing and releasing a number of cytokines, chemokines, mediators, adhesion molecules, and other relevant compounds.^{1,3–9,14–17,20,22,26,27,29,37–39}

Similarly, data and findings pertaining to the pharmacologic modulation of IAR as well as LAR by various drugs, among others by glucocorticosteroid (GCS), also vary.^{1,3,4,9–14,16,17,20,26,36–39}

The purpose of this study, being a continuation of our previous studies,^{9–11,16,20,26,36} was to investigate (1) the existence of DAR as a compact two-phase clinical entity or as a simultaneous appearance of two independent asthmatic response types, an immediate (IAR) and a late response (LAR); (2) the existence of a possible causal relationship between the early and late phase; and (3) protective (blocking) effects of a single dose of inhaled budesonide (BUD) on the early phase (IAR) as well as the late phase (LAR) when administered before and at various time intervals after the allergen challenge, before the clinical manifestation of LAR.

METHODS

Patients

Forty-eight patients suffering from bronchial asthma, examined at our Department of Allergology and Immu-

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Address correspondence to Zdenek Pelikan, M.D., Ph.D., Allergy Research Foundation, Effenseweg 42, 4838BB Breda, The Netherlands
E-mail address: zpelikan@casema.nl
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Table 1 Characteristics of the patients and control subjects and the single diagnostic parameters

	Patients DAR (n = 48)	Control Subjects		
		Group I IAR (n = 14)	Group II LAR (n = 12)	Group III Healthy Subjects (n = 18)
Age (yr)	32 ± 8	34 ± 6	26 ± 5	29 ± 4
Sex (M/F)	21/27	5/9	6/6	8/10
Duration of BA (yr)	4.8 ± 1.4	3.3 ± 1.1	4.5 ± 1.8	0
Asthmatic attacks per month	3 ± 1	4 ± 1	3 ± 2	0
FEV ₁ (% predicted)	96.5 ± 4.1	94.2 ± 8.2	91.9 ± 7.3	101.0 ± 6.7
FVC (% predicted)	97.0 ± 7.8	99.5 ± 4.6	98.6 ± 4.6	98.6 ± 5.5
Blood leukocyte count (×10 ⁹ /L)*	8.2 ± 0.6	7.1 ± 0.3	8.3 ± 0.5	7.9 ± 0.4
Blood eosinophil count (×10 ⁶ /L)#	438 ± 36	391 ± 43	515 ± 69	219 ± 52
Bronchial Histamine PD ₂₀ §				
≤4 mg/mL	3	5	1	0
8–32 mg/mL	37	8	11	1
>32 mg/mL	8	1	1	17
BPT positive/negative¶	1/2	1/0	1/2	0/0
Positive skin response				
Immediate	27	12	6	1
Late	21	2	6	0
Increased total IgE in the serum**	5	6	1	0
Positive specific IgE in the serum##	22	9	3	0
Increased total IgG in the serum§§	11	0	7	0
Increased IgG subclasses in the serum¶¶				
IgG ₁	3	0	2	0
IgG ₂	0	1	0	0
IgG ₃	0	0	5	0
IgG ₄	9	0	8	0
Increased total IgM in the serum	0	0	1	0
Increased total IgA in the serum***	0	0	0	0

Values = mean ± SD.

*Blood leukocyte count (normal value) = 4.0–10.0 × 10⁹/L.

#Blood eosinophil count (normal value) = <300 × 10⁶/L.

§Normal value of bronchial threshold = >32.0 mg/mL.

¶BPT = bronchial provocation tests with allergens per patient.

||Intracutaneous (intradermal) skin test.

**Total IgE in the serum (PRIST) – normal value = <500 IU/mL.

##Positive allergen-specific IgE in the serum (ImmunoCAP) = >0.70 U/mL (= more than class 1).

§§Total IgG in the serum (single radial immunodiffusion and ELISA) – normal value = <15.0 g/L.

¶¶IgG₁ < 5.0 g/L; IgG₂ < 2.6 g/L; IgG₃ < 0.4 g/L; IgG₄ < 0.5 g/L.

|||IgM = <3.8 g/L.

***IgA = <3.2 g/L.

BPT = bronchial provocation tests, DAR = dual late asthmatic response; IAR = isolated immediate asthmatic response; LAR = isolated late asthmatic response; BA = bronchial asthma; FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity.

nology, Institute of Medical Sciences “De Klokkenberg,” Breda, The Netherlands, developing a dual late asthmatic response to bronchial challenge with inhalant allergens (DAR), volunteered to participate in this study.

These patients, 23–51 years of age, showed reversible bronchial obstruction alternating with symptom-free

periods without any restrictive changes of their pulmonary function (Table 1). They did not suffer from airway infections and did not use oral corticosteroids or immunotherapy. They were examined using a routine diagnostic procedure (acting also as inclusion–exclusion criteria) consisting of (1) general part—dis-

Table 2 Allergens used for BPTs

Allergen	Standard Concentrations BU/mL (i.c./BPT)	Patients DAR (n = 48) 1:10/1:1	Total	Control groups			
				IAR (n = 14) 1:10/1:1	Total	LAR (n = 12) 1:10/1:1	Total
<i>Dermatophagoides pteronyssinus</i>	100/1000	3/6	9	1/4	5	1/2	3
<i>Dermatophagoides farinae</i>	100/1000	0/1	1		0		0
Dog*	300/3000	1/2	3	1/1	2	0/1	1
Cat*	200/2000	1/3	4	0/1	1	0/1	1
Guinea pig*	200/2000	0/1	1		0	0/1	1
Hamster*	200/2000	0/1	1		0		0
Horse*	200/2000	0/1	1		0		0
Parakeet feathers	300/3000	0/1	1		0		0
<i>Aspergillus fumigatus</i>	100/1000	1/0	1		0		0
Grass pollen mix I	100/1000	2/6	8	1/1	2	1/1	2
Grass pollen mix II	100/1000	1/3	4	0/1	1	0/1	1
Tree pollen mix	300/3000	1/1	2	0/1	1		0
Weed pollen mix	200/2000	1/1	2		0	0/1	1
Birch	100/1000	1/2	3	0/1	1	0/1	1
Willow	200/2000	0/1	1		0		0
Poplar	200/2000	0/1	1		0		0
Hazel	100/1000	1/1	2	0/1	1		0
Ragweed short	100/1000	0/1	1		0	0/1	1
Ragweed giant	100/1000	0/2	2		0		0

*Danders = hairs and squamae; grass pollen mix I = *Dactylis glomerata*, *Lolium perenne*, *Phleum pratensis*, and *Poa pratensis*; grass pollen mix II = *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*.

i.c. = intracutaneous (intradermal) tests; BPT = bronchial provocation tests with allergens; DAR = dual late asthmatic response; IAR = isolated immediate asthmatic response; LAR = isolated late asthmatic response.

ease history, physical examination, basic laboratory tests, x ray of the chest, lung function, and blood gases determination; (2) allergologic part—skin tests, bronchial histamine threshold,² and determination of the serum immunoglobulins (Table 1); and (3) bronchial provocation tests (BPTs) with inhalant allergens ($n = 107$) and control challenges with phosphate-buffered saline (PBS; $n = 48$). The allergens for BPT were chosen with respect to the disease history and skin tests (Table 2). The initial BPTs have always been performed with allergenic extracts diluted 1:10 and if they were negative, then BPTs with undiluted extracts (our standard) were performed (Table 2).

All BPTs were performed in a period without manifest bronchial complaints, outside the allergen-relevant season and during hospitalization under standard conditions. Inhaled corticosteroids ($n = 13$) and long-acting β_2 -sympathomimetics ($n = 5$) were withdrawn at 4 weeks; cromolyn ($n = 11$), nedocromil sodium ($n = 15$), and leukotriene modifiers ($n = 4$) at 2 weeks; and short-acting β_2 -sympathomimetics at 48 hours before the BPTs. A forced exoratory volume at 1 second

(FEV₁) decrease of 50% or more, with respect to the initial values, was treated with 200–400 μ g of salbutamol ($n = 3$). The local ethical committee of the Institute of Medical Sciences “De Klokkenberg”. approved this study and written informed consent was obtained from all study participants. The study was performed in accordance with the Declaration of the World Medical Association of Helsinki concerning the principles for medical research involving human subjects.

Allergens

Dialyzed and lyophilized allergen extracts (Allergopharma, Reinbek, Germany) diluted in PBS were used in concentrations of 100–500 BU/mL for skin tests and of 1000–3000 BU/mL for BPTs (Table 2). The manufacturer recommended concentrations of 500 BU/mL for skin tests and up to 5000 BU/mL for bronchoprovocation.

Skin Tests

Skin-prick tests with allergens in concentrations of 500 BU/mL were evaluated after 20 minutes. If these

were negative, intracutaneous tests were performed in concentrations of 100 and 500 BU/mL and then evaluated 20 minutes and 6, 12, 21, 36, 48, 72, and 96 hours after the injection. A skin wheal (>7.0 mm in diameter) appearing 20 minutes after the injection was considered to be a positive immediate skin response, the skin infiltration observed between 6 and 12 hours to be a positive late skin response, and the skin induration recorded later than 48 hours was designated as a delayed skin response (Table 1).^{8–11,16,21,36}

Bronchial Provocation Tests

The BPTs were performed by means of spirometry (Spirograph D-75 Lode; Lode, Groningen, The Netherlands), recording the forced vital capacity and FEV₁ values.^{2,8–11,16,21,50,51} The aerosolized allergenic extracts and PBS were administered by means of Wiesbadener Doppel-Inhalator (Wiesbadener Vertrieb, Wiesbaden, Germany) at an airflow of 10 L/min. The nebulizer output was 0.12–0.14 mL/min. The aerosol particles had a median mass diameter of 2.8–3.6 μ m. The BPTs were performed by the following schedule: (1) baseline values recorded at 0, 5, and 10 minutes; (2) PBS control values recorded at 0, 5, and 10 minutes after a 10-minute inhalation; (3) inhalation of the allergen aerosol for 2 $\times$ 5 minutes, with inserted spirometric measurement, followed by recording of spirometric parameters at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the 12th hour and every second hour during the 22nd to 38th and the 46th to 56th hour interval. The BPTs were evaluated according to the following criteria: (1) a decrease in FEV₁ of $<10\%$ with respect to the prechallenge values was considered as negative, from 10 to 20% as doubtful and $\geq 20\%$ as positive; (2) a decrease in FEV₁ values recorded at least three consecutive time intervals was considered to be a positive asthmatic response; (3) the asthmatic response appearing within 2 hours after the allergen challenge was considered to be an IAR, while that occurring between 6 and 12 hours to be an LAR. The BPTs were supplemented with a recording of a number of other diagnostic parameters (Tables 3 and 4).

Protection tests (PTs) with BUD and Placebo

BUD and the placebo (PL), provided by AB Draco, Sweden, were administered in the form of metered pressurized aerosols. Each administration consisted of four actuations of BUD or PL ($4 \times 200 = 800$ μ g) inhaled through the spacer-chamber within 2 minutes. PTs with BUD and PL were performed in the same manner, using the same allergens and according to the same schedule as that of the BPTs. An interval of 5–7 days was always allowed between the consecutive tests. The patients were randomly divided into four groups, each consisting of 12 subjects. Two PTs were

performed in each patient according to a reverse sequence of BUD and PL administration with respect to the allergen challenge (Fig. 1). Two treatments were given during each of the PTs. The first treatment (TH-I) was administered always 30 minutes before, whereas the second treatment (TH-II) was administered at various time points after the allergen challenge, varying per group: at 1 hour (T₁) in group I, at 2 hours (T₂) in group II, at 3 hours (T₃) in group III, and at 4 hours (T₄) in group IV. Each treatment consisted of two inhalations, either two PL inhalations or one PL and one BUD inhalation (Fig. 1). The study design was randomized, double-blind, double-dummy, placebo-matched, and subjected to crossover.

Control Groups

The 14 asthmatic patients presenting with an IAR, 12 asthmatic patients developing an LAR, and 18 healthy subjects volunteered to participate as controls (Table 1). In the asthmatic subjects the BPT with the same allergens and in the healthy subjects a BPT with PBS were repeated.

Statistical Analysis

The nonpretreated as well as the pretreated asthmatic responses were statistically analyzed by means of fitting polynomials to the mean curves over time: 8 time points within 120 minutes and 14 time points up to 24 hours after the allergen challenge. The hypotheses were tested by means of the modified generalized multivariate analysis of variance (MANOVA computerized system).⁵⁰

RESULTS

Forty-eight patients developed positive dual LAR (DAR), a combination of an IAR and an LAR, to bronchial challenge with inhalant allergens. The IAR or “early phase” began within 10 minutes, reached its maximum within 30–45 minutes, and resolved within 90–120 minutes after the allergen challenge (Fig. 2 A). The LAR or “late phase” appeared at 4–5 hours, reached its maximum within 6–10 hours, and resolved mostly within 12 hours after the allergen challenge (Fig. 2 A). The “early phase” as well as the “late phase” were statistically highly significant both in a comparison of the postchallenge with the prechallenge FEV₁ values ($p < 0.001$ and $p < 0.001$, respectively) and as compared with the PBS control values ($p < 0.01$ and $p < 0.001$, respectively). No statistically significant changes were found in the FEV₁ or forced vital capacity values during the PBS control tests ($p > 0.2$). No significant differences were observed in the appearance of IAR or LAR with respect to the individual allergens ($p > 0.2$).

The nonpretreated IAR was accompanied by wheezing, dyspnea, dry cough, and increased blood eosino-

Table 3 Survey of the response-related variables in nonpretreated patients during the DLAR/DAR

Patients (n = 48)	Before the Challenge	After the Challenge (hr)					
		½	1	2	6	12	24
Bronchial complaints							
Dyspnea	0	+++§	+	±	++++§	++*	0
Wheezing	0	++++§	±	±	+++§	+	0
Cough	0	+	0	0	+	0	0
Expectoration	0	±	0	0	0	0	0
General complaints							
Pressure on the chest	0	0	0	0	+	0	0
Chills	0	0	0	0	0	0	0
Tiredness	0	0	0	0	0	+	0
Headache	0	0	0	0	0	+	0
Increased body temperature¶	0	0	0	0	0	0	0
Blood pressure							
Increase (systol/dyastol)	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Decrease (systol/dyastol)	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pulse rate							
Tachycardia	0	0	0	0	0	0	0
Bradycardia	0	0	0	0	0	0	0
Increased total IgE in the serum¶¶	5	—	5	—	4	—	4
Positive specific IgE in the serum**	22	—	29	—	15	—	15
Increased total IgG in the serum##	11	—	11	—	18	—	17
Blood leukocyte count ($\times 10^9/L$)§§	8.1 ± 0.9	8.6 ± 0.5	7.5 ± 0.3	7.9 ± 0.6	11.3 ± 0.7^D	10.7 ± 0.5^D	8.3 ± 0.6
Blood eosinophil count ($\times 10^6/L$)	395 ± 37^D	$445 \pm 29^\#$	$483 \pm 45^\#$	301 ± 29	$507 \pm 32^\S$	391 ± 28^D	315 ± 31
Blood neutrophil count ($\times 10^9/L$ ***	6.2 ± 0.9	5.9 ± 1.1	6.5 ± 1.4	7.3 ± 0.7	$7.9 \pm 1.1^+$	6.9 ± 0.6	6.5 ± 1.3
Blood differential count ($\times 10^9/L$)¶¶¶							
Basophils	0.08	0.07	0.05	0.09	0.03	0.05	0.06
Eosinophils	0.6	0.9#	1.05#	0.5	1.4#	0.8*	0.5
Neutrophils	5.8	6.3	6.2	7.0	8.5*	6.5	6.1
Monocytes	0.3	1.0	0.7	0.4	0.9	0.3	0.4
Lymphocytes	1.3	1.3	2.1	2.0	1.8	1.1	1.5
Erythrocyte sedimentation rate###	9	10	8	11	7	10	9

Values = mean $\pm$ SD; 0 = normal (negative, no change); — = not determined.

* $p \leq 0.05$.

$p < 0.05$.

§ $p < 0.01$.

¶More than $37^\circ\text{C} = 98.6^\circ\text{F}$ axillary.

¶¶Normal value <500 IU/mL.

**Normal value <0.70 U/mL.

##Normal value <15.0 g/L.

§§Normal value $4.0\text{--}10.0 \times 10^9/L$.

¶¶¶Normal values ($\times 10^9/L$): B = <0.2 , E = <0.5 , N = $2.0\text{--}7.0$, M = $0.2\text{--}1.0$, and L = $1.0\text{--}3.0$.

|||Normal value $<300 \times 10^6/L$.

***Normal value $2.0\text{--}7.2 \times 10^9/L$.

###Normal value <20 mm/1 h.r

DLAR/DAR = dual late asthmatic response.

phil count, whereas the nonpretreated LAR was associated with dyspnea, wheezing, increased blood eosinophil count, and slightly increased total leukocyte and neutrophil count (Table 3).

The IAR was not affected significantly either by BUD ($p > 0.1$) or by PL ($p > 0.2$), administered in a single dose at 30 minutes before the allergen challenge (Fig. 2 B). No significant differences were

Table 4 Parameters exhibiting changes during the non-treated DLAR/DAR compared with those recorded after the BUD pretreatment; ½ hr = early phase (IAR); 6 hours = late phase (LAR)

	Nontreated DAR (hr)		DAR Treated with BUD (hr)	
	½	6	½	6
Bronchial complaints				
Dyspnea	+++	++++	+++	±§
Wheezing	++++	+++	++#	0§
Cough	+	±	±	0
Expectoration	±	0	0	0
Blood leukocyte count ($\times 10^9/L$)¶	8.6 ± 0.5	11.3 ± 0.7	7.8 ± 0.5	$6.9 \pm 0.4\#$
Blood eosinophil count ($\times 10^6/L$)	445 ± 29	507 ± 32	$297 \pm 23\#$	$262 \pm 45§$
Blood neutrophil count ($\times 10^9/L$)**	5.9 ± 1.1	7.9 ± 1.1	6.3 ± 0.5	7.1 ± 0.9^D
Blood differential count ($\times 10^9/L$)##				
Basophils	0.07	0.03	0.04	0.06
Eosinophils	0.9	1.4	0.5#	0.3#
Neutrophils	6.3	8.5	5.5	5.7#
Monocytes	1.0	0.9	0.8	0.5
Lymphocytes	1.3	1.8	1.0	1.3*

Values = mean $\pm$ SD.

Statistical significance of BUD protective effects:

* $p \leq 0.05$.

$p < 0.05$.

§ $p < 0.01$.

¶Normal value $4.0-10.0 \times 10^9/L$.

||Normal value $<300 \times 10^6/L$.

**Normal value $2.0-7.2 \times 10^9/L$.

##Normal values ($\times 10^9/L$): B = <0.2 , E = <0.5 , N = $2.0-7.0$, M = $0.2-1.0$, and L = $1.0-3.0$.

Complaint degrees: 0 = absent; $\pm$ = very slight/incidental; + = slight; ++ = moderate; +++ = distinct; ++++ = pronounced; DLAR/DAR = dual late asthmatic response; IAR = isolated immediate asthmatic response; LAR = isolated late asthmatic response; BUD = budesonide.

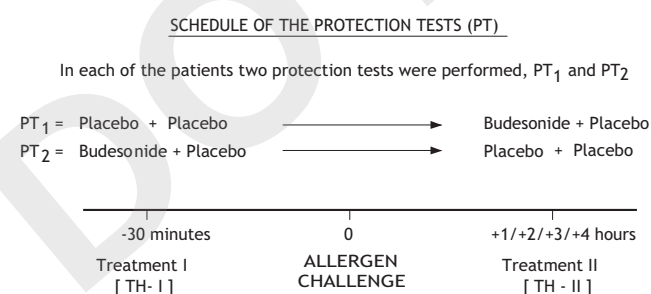


Figure 1. Schedule of the protection tests (PTs).

found between the effects of BUD and PL on the IAR ($p > 0.1$).

Administration of BUD both in a single dose at 30 minutes before the allergen challenge ($p < 0.01$) and at 1 hour ($p < 0.001$), 2 hours ($p < 0.001$), 3 hours ($p < 0.01$), or 4 hours ($p < 0.05$) after the challenge (Fig. 2 B) proved significantly effective in preventing the LAR. The PL showed no significant effect on the LAR at any time points ($p > 0.1$; Fig. 2 C).

Pretreatment with BUD attenuated also wheezing, coughing, expectoration, and increased blood eosinophil count, but not dyspnea associated with IAR (Table 4). The dyspnea, wheezing, coughing, increased total blood leukocyte, and eosinophil and neutrophil counts associated with LAR were decreased significantly by pretreatment with BUD (Table 4).

These results suggest that the DAR may not be a compact clinical entity consisting of two phases, but a manifestation of two independent responses, IAR and LAR, appearing simultaneously and caused by different immunologic mechanisms.

Control Subjects

The repeated IARs as well as LARs were significantly positive both when compared the post- with the pre-challenge FEV₁ values ($p < 0.001$ and $p < 0.01$, respectively) and when compared with the PBS control FEV₁ values ($p < 0.01$ and $p < 0.01$, respectively). No significant differences have been found between the initial and the repeated IAR ($p > 0.1$) or LAR ($p > 0.2$). The

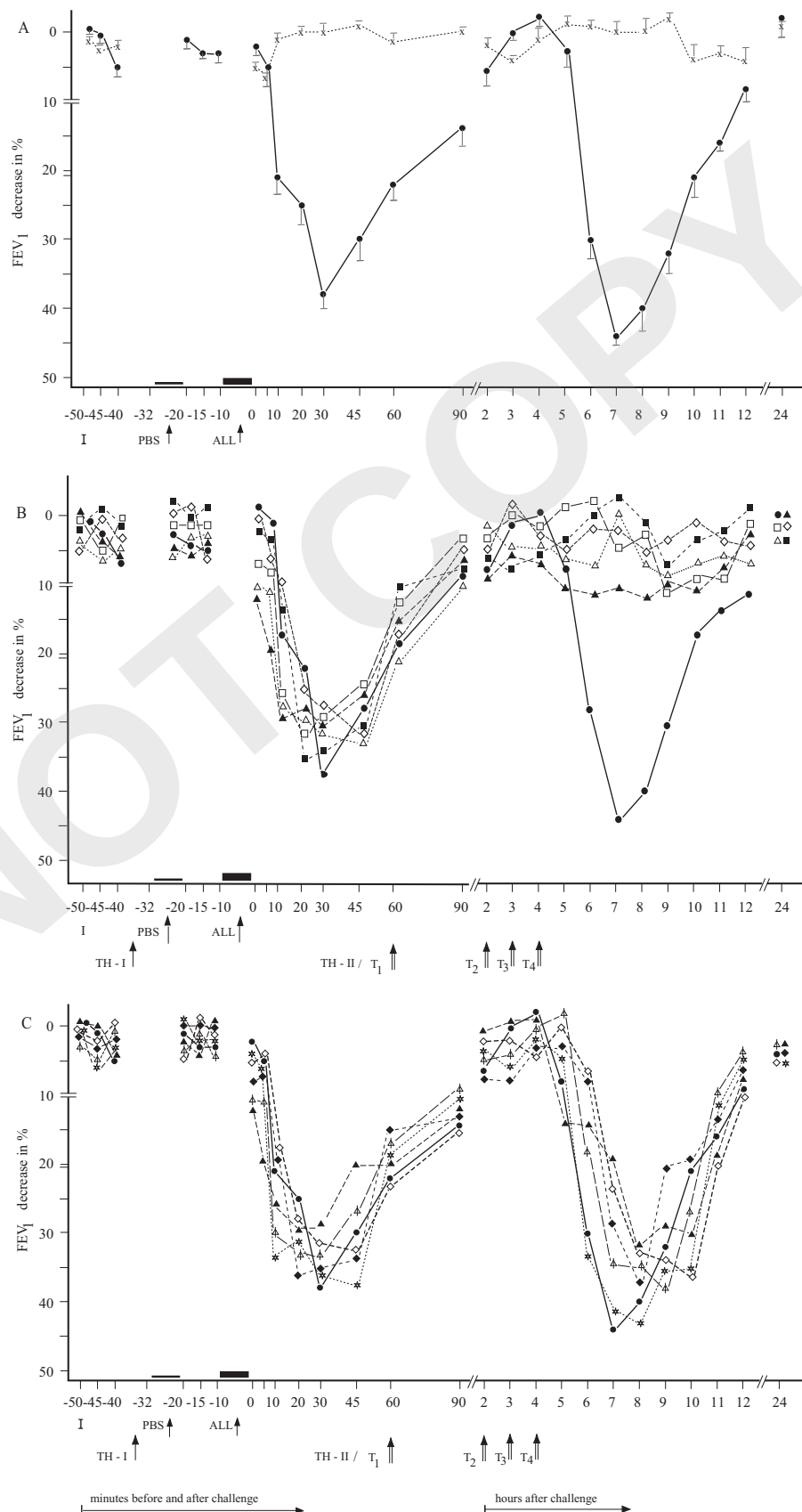


Figure 2. (A) The nonpretreated DAR to allergen challenge (●; $n = 48$) and PBS control tests (X; $n = 48$) bars = means $\pm$ SEM). (B) The nonpretreated DAR (●; $n = 48$) and responses treated with BUD at various time points: TH-I = 30 minutes before the allergen challenge (▲); TH-II/T₁ = 1 hour after the allergen challenge (■); T₂ = 2 hours after the challenge (□); T₃ = 3 hours after the challenge (◇); T₄ = 4 hours after the challenge (△). (C) The nonpretreated DAR (●; $n = 48$) and responses treated with PL at various time points: TH-I = 30 minutes before the allergen challenge (◇); TH-II/T₁ = 1 hour after the allergen challenge (*); T₂ = 2 hours after the challenge (◆); T₃ = 3 hours after the challenge (▲); T₄ = 4 hours after the challenge (△). DAR, dual late asthmatic response; I, initial (baseline) values; ALL, allergen challenge; PBS, phosphate-buffered saline; PL, placebo; BUD, budesonide.

RECORDING OF THE "DUAL ASTHMATIC RESPONSE" one dimensional lateral projection

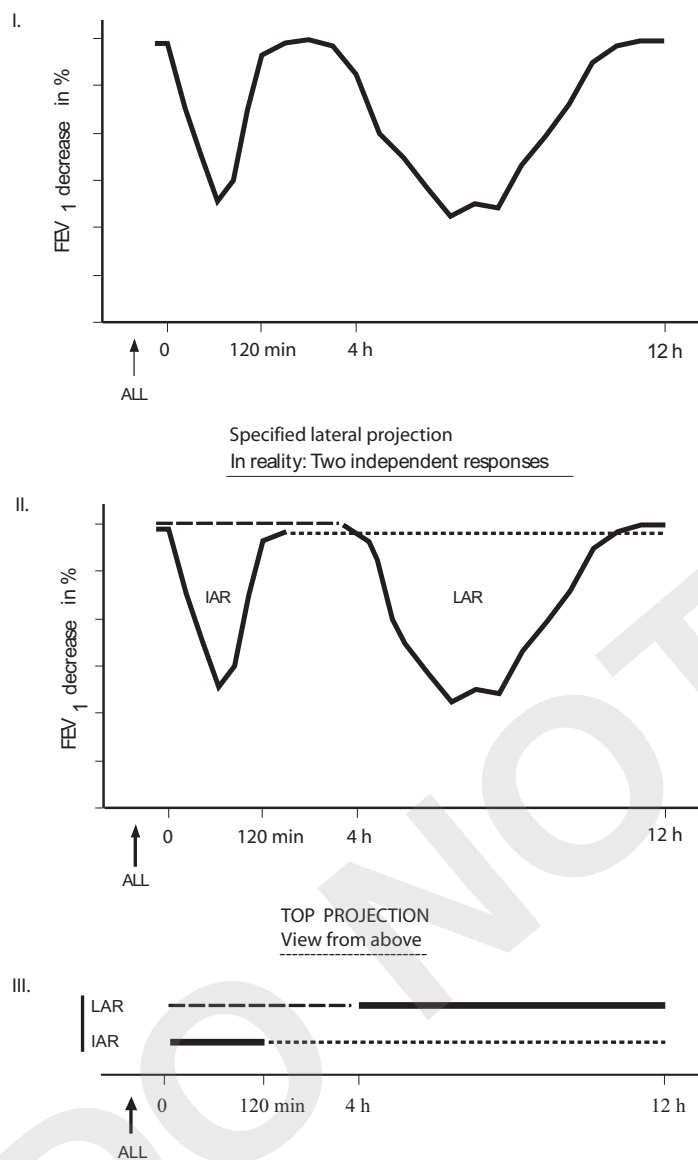


Figure 3. DAR is a presumed combination of an early/immediate and a late phase. (I) A front view on the recorded DAR; (II) the real composition of the DAR (two independent responses, IAR and LAR); (III) a view from above (LAR: dashed line = premanifestation stage of LAR, solid line = LAR. IAR: solid line = IAR, dotted line = postmanifestation stage of IAR.) DAR, dual late asthmatic response; IAR, immediate asthmatic response; LAR, late asthmatic response.

healthy subjects showed negative responses to PBS challenge ($p > 0.2$). These results confirm the excellent reproducibility of the BPT method used.

DISCUSSION

The results of this study, showing different protective effects of BUD, suggest that the so-called DAR does not exist as a compact clinical entity, consisting of an early and a late phase, but, in reality, it is an accidental appearance of two independent clinical events, IAR and LAR, caused by different immunologic mechanisms, having been initiated simultaneously at the same time by the same allergen (Fig. 3).^{8,9,16,20,44} This hypothesis is supported by existence of the LAR as well as IAR in an isolated form, reported in our previ-

ous articles^{8-11,16,20,36,44} and confirmed also by other authors.^{3-7,12-15,18,19,27-32,37,43} These results would also repute the previously proposed causal role of the early phase response in the development of the late-phase response of the "dual late asthmatic response" (DAR).^{3,13,14,22,37,42}

Undoubtedly, the IAR is caused by the immediate (IgE mediated) hypersensitivity, where the influence of IgE antibody, mast cells, eosinophils, and Th₂ lymphocytes is pivotal.^{1,4-6,9,11,14,18,23,34,38} The immunologic mechanisms underlying the LAR have not, thus far, been satisfactorily clarified.^{1,3-10,12-20,23,27-32,34-36} The opinions vary with regard to the specific immunologic mechanisms potentially involved in the LAR. Some investigators presumed involvement of the classically

understood "late (type III) hypersensitivity" mechanism(s) upon participation of "IgG or IgE immune complexes" and complement parts, especially C5 and C5a, in the LAR.^{8,20,33,53–61} Other authors assumed the principal role of IgE antibodies and mast cells in the pathogenesis of the LAR as well.^{3,14,24,25} Later, other investigators, unable to confirm the above mentioned hypotheses, proposed the possible involvement of the non-IgE or so-called IgE-independent immunologic mechanisms in the LAR, including participation of various circulating cells as well as cells resident in the bronchial tissue, producing a number of potent factors and bearing a high variety of membrane-bound receptors, CD molecules.^{1,3,5–9,12–16,23,32,35,38,41,62}

As observed in our previous results, the IAR was accompanied by increased eosinophil and basophil counts in peripheral blood, increased serum concentrations of histamine and ECP, decreased Th₁/Th₂ ratio in favor of Th₂, and increased intracellular IL-4.

The LAR was associated with increased counts of eosinophil and neutrophil in blood, unchanged Th₁/Th₂ ratio, and increased IL-4 and IL-5.^{8,9,11,20,21} However, the literature provides few data concerning the changes in the serum concentrations of various cytokines and chemokines during the IAR and the LAR.

Our preliminary, as yet unpublished, data indicate increased serum concentrations of IL-1 β , IL-4, IL-7, IL-13, and IL-12p40 and increased plasma concentrations of leukotriene C₄ (LTC₄) and prostaglandin F_{2 α} during the IAR, whereas increased serum concentrations of IL-4, IL-5, IL-8, IL-12p70, and granulocyte colony-stimulating factor and increased plasma concentrations of LTC₄, leukotriene B₄, prostaglandin E₂, and thromboxane B₂, during the LAR.

The results of this study indicate that BUD, a typical representative of the GCS drugs, has the capacity to prevent significantly the development of LAR when administered in a single dose either before or up to 4 hours after an allergen challenge, but it failed to affect the IAR significantly when administered before the allergen challenge. These results are consistent with our earlier data,^{10,11,16,26,44} where beclomethasone dipropionate and BUD, as well as fluticasone administered in a single dose before the allergen challenge, inhibited significantly the LAR but not the IAR. This inability of the GCSs to prevent the IAR was observed even after a pretreatment for a longer period of time, such as 4⁴⁴ and 8 weeks.¹⁶ Our results are in agreement with other investigators' observations of inability of beclomethasone dipropionate, BUD, and fluticasone to attenuate the IAR, while being significantly effective in preventing the LAR.^{4,39,40,42,49} In contrast, our results would question the findings of some authors reporting inhibition of IAR by BUD or fluticasone after a pretreatment for 1–4 weeks preceding the bronchial allergen challenge.^{37,38,41,43}

The significant protective effects of GCSs on the LAR, administered even in a single dose 30 minutes before the allergen challenge, observed in our current as well as our previous studies,^{10,16,44} are in agreement with other investigators' results.^{4,37–39,40–43} No substantial differences in the effects of BUD, beclomethasone dipropionate or fluticasone, either on the IAR or on the LAR, were observed by us.^{10,11,16,44} This observation would not confirm the differences in effects of particular GCSs reported by some investigators.³⁷

Another interesting finding was the capacity of a single dose of BUD in preventing the development of LAR when administered at various time points from 30 minutes before and up to 4 hours after the allergen challenge. This finding suggests that the immunologic process leading to the development of LAR consists of a number of consecutive steps including participation of various cell types and factors. This event can be interrupted at any time between allergen exposure and clinical manifestation of LAR, which means ~4 hours after the allergen exposure.

The pretreatment with BUD reduced partly wheezing and blood eosinophilia, but did not affect dyspnea, accompanying the IAR, whereas it prevented almost fully all complaints associated with the LAR, such as pronounced dyspnea and wheezing, tiredness, headache and pressure on the chest, and increased blood leukocyte and eosinophil and neutrophil counts (Tables 3 and 4). These results, being consistent with other authors' observations,³⁸ would additionally support the assumption of involvement of different immunologic mechanisms in the IAR and LAR.

No simultaneous recording of the cytologic and immunologic features in the induced sputum or in bronchoalveolar lavage during the nonpretreated as well as pretreated BPTs were performed for technical reasons. This fact may be regarded as a certain deficit of the current study and will be included in additional studies.

The inability of BUD and, generally, of corticosteroids to affect the IAR to a significant degree, while completely blocking the LAR, may be explained by their pharmacologic properties and anti-inflammatory effects on various cell types, biochemical and immunologic processes, at various levels and stages.^{3,4,9–11,14,16,26,37–62}

The anti-inflammatory effects of corticosteroids can be summarized as follows: (1) inhibitory effects on synthesis and activation of arachidonic acid metabolites, vasoactive kinins, complement components, IgG and IgA, and release of various chemotactic factors, cytokines, chemokines, adhesion molecules expression, transcription of gene coding of some receptors (*e.g.*, natural killer 1 cells), cGMP action, cellular calcium channels, vascular permeability of capillary walls; and (2) direct effects such as inhibition of eosinophil proliferation and tissue accumulation, decrease of neutro-

phil chemotaxis, their adherence to vascular epithelium and production of their constituents, elevation of neutrophils in circulation, decrease of circulating basophils, eosinophils, monocytes, T lymphocytes (the Th₁ and Th₂ subsets and natural killer cells), increase of phagocytosis, inhibition of activation of T lymphocytes, monocytes and macrophages, suppression of B-lymphocyte activation and matured circulating plasma cells producing immunoglobulins, limitation of some processes in the epithelial cells, and antigen-presenting cells in the circulation and in the tissue (e.g., dendritic cells).^{9,12,14,26,37–39,51–62}

Despite the manifold anti-inflammatory effects of GCSs on various cell types, their direct inhibitory effects on the human mast cells (MC_T or MC_{TC}) and basophils have not been unequivocally confirmed.^{9,10,15,32,51,58} In human *in vivo* studies, topical GCSs reduced numbers of epithelial and mucosal mast cells after an allergen challenge in the nasal mucosa and in the bronchoalveolar lavage fluid, but they did not show inhibitory effects on the membrane-bound IgE receptors (e.g., FcεRI) on the human MC or on the release of their mediators.^{10,51} Unlike in the animals, such as rat or guinea pig, no specific membrane-bound receptors for GCSs have been confirmed on the human MC of any type (MC_T and MC_{TC}) thus far, whereas they have been shown on the membranes of other human cells, such as eosinophils, neutrophils, and T-helper lymphocytes (Th₁ and Th₂ subsets).^{10,26,47,48,51,52}

It can be concluded that (1) the DAR does not exist as a compact clinical phenomenon. It is a parallel manifestation of two separate asthmatic response types (IAR and LAR) due to different immunologic mechanisms, initiated simultaneously by the same allergen at the same time; (2) inhalant BUD is unable to affect the IAR significantly, whereas it shows significant effectiveness in preventing the LAR; and (3) the LAR can be prevented by BUD in a single dose of 800 μg administered either 30 minutes before or 4 hours after an allergen exposure.

Finally, the above mentioned study would argue that it is more suitable to designate these responses as simply IAR and LAR, suggesting the existence of two separate events instead of the terms early and late phase of DAR, implicating some relationship between both of the events.

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