

Total and Specific IgE in Serum, Bronchial Lavage and Bronchoalveolar Lavage of Asthmatic Patients

E. CRIMI, A. SCORDAMAGLIA, P. CRIMI, S. ZUPO and S. BAROCCI

Istituto di Medicina e Chirurgia Broncopolmonare dell'Università degli Studi di Genova, Italy

Total and specific IgE were assessed in serum, bronchial lavage (BL) and broncho-alveolar lavage (BAL) of allergic asthmatics and healthy controls. Serum total IgE were found to be correlated with total IgE in BAL but not in BL. Total IgE/K⁺ ratio in serum and BL was higher in asthmatics than in controls, while the total IgE/albumin ratio was significantly higher in asthmatics than in controls in serum but not in BL and BAL. The mean of specific IgE in serum and BL was significantly higher in the group of patients with positive specific bronchial provocation test (sBPT) than in the group with negative sBPT. Similar results were observed between specific IgE serum level and BL and prick tests (PT), which show that BL does not always reflect the total IgE level of serum; in asthmatics, albumin can not be used to determine the degree of dilution in the recovered fluids; as in the serum, there is agreement between specific IgE in BL and PT or sBPT results.

Key words: allergic asthma; bronchial lavage; bronchoalveolar lavage; IgE.

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Study of the local production of IgE may have clinical usefulness for two main reasons:

1. serum IgE levels do not always reflect the local immuno-allergic status (7, 8)
2. specific serum IgE levels do not necessarily correlate with specific (allergic) bronchial reactivity, perhaps because of inter-individual variability of non-specific bronchial reactivity (2, 11).

It is therefore difficult to state how much of the bronchial response in allergic patients is due to a local specific IgE-mediated reaction or to non-specific hypersensitivity. Until now, very few studies have been carried out to quantify the IgE at the broncho-alveolar and bronchial level in allergic asthmatic patients. Sputum may be contaminated by nasal secretions and saliva and thus not reflect the immunological status of bronchi.

The aim of this study has been to assess the local specific and non-specific IgE in bronchial

lavage (BL) and broncho-alveolar lavage (BAL) from allergic asthmatics and healthy controls using the bronchofiberscopy technique which allows uncontaminated fluids to be recovered at different levels of the bronchial tree. The results of these airways lavages were compared with IgE in serum, specific bronchial reactivity and skin sensitization.

MATERIAL AND METHODS

Subjects

The study comprised nine asthmatic patients (8 of whom were allergic) aged 18 to 32 years, and seven healthy controls, aged 20 to 51 years. One of the controls was an Egyptian man known to have high serum IgE level. Subjects with no history of rhinitis, asthma or allergic

diseases were chosen as controls on the basis of negative prick tests and low bronchial sensitivity. In these subjects the methacholine concentration causing a fall of the forced expiratory volume in 1 sec ($FEV_1 \geq 15\%$ of control solution) was higher than 10 mg/ml (methacholine PC15 $FEV_1 \geq 10$ mg/ml). Allergic patients were chosen on the basis of a clear-cut history of asthma during the pollen season or after exposure to house dust, prick tests with wheal diameter exceeding 0.5 cm for one or more allergen, and hypersensitivity to methacholine.

IgE determination. IgE in serum, BL, BAL were determined by Phadebas PRIST. Sera were diluted 10 times and BAL were concentrated 100 times using Minicon. All tests were performed in duplicate.

Specific IgE determination. In six patients and six controls specific IgE was also determined by Phadebas RAST, both in native serum and native BL. Results are reported as percent of the total radioactivity added.

Albumin and K^+ . In order to determine the degree of dilution recovered in the fluids compared with their original concentration in the lung, albumin was assayed by the radial immunodiffusion technique with middle and low level plates (Behringwerke – Scoppito, L'Aquila) in BAL according to the concentration stated above. The K^+ content of fluids was also measured by a flame photometer (Corning EEL 450).

Non-specific bronchial sensitivity was assessed through methacholine bronchial provocation test (mBPT). Each patient inhaled control solution and then increasing concentrations of methacholine (0.05 to 50 mg/ml, 2-fold increments). Aerosol was generated by a DeVilbiss 646 at a flow of 12 l/min. The subjects, while breathing at tidal volume (0.5–1 l) were given aerosolized methacholine for 2 min and 30 sec corresponding to 0.5 ml of solution. The concentration causing the FEV_1 to fall below 15% (PC15 FEV_1) was chosen as the threshold value.

Specific bronchial sensitivity. Specific bronchial provocation tests (sBPT) were performed with the same threshold value (PC15 FEV_1) using allergens dosed in RAST inhibition arbitrary units (AU). Allergens were administered from 8 to 2000 AU with 2-fold increments (Lofarma, Milan).

Prick tests. These were performed with glycerinated allergens (Lofarma, Milan. Titrations of extracts were: 10000 AU/ml for pollens, 5000 AU/ml for *Dermatophagoides*. Results were judged positive when the wheal diameter exceeded 1 cm, doubtful when between 1 and 0.5 cm, and negative when below 0.5 cm.

Bronchial lavage and broncho-alveolar lavage. Patients were premedicated with 40 mg prednisolone $\times 2$ i.m. in the evening and 2 h prior to bronchofiberscopy. Patients and controls received atropine 0.5 mg plus promethazine 50 mg i.m. half an hour prior to the examination, and oxybuprocaine hydrochloride (0.4%) for local anesthesia of the tracheo-bronchial tree. During bronchofiberscopy, the asthmatic patients received continuous intravenous theophylline in normal saline. While the subjects were lying in the supine position the bronchofiberscope (BFS) was introduced through the nose or mouth. The tip of the BFS was introduced into the left stem bronchus 1 cm beyond the carina. For BL, normal saline (20 ml at 37°C) was injected, and after 2 sec suction was performed in the stem bronchus and at the orifices of the left lobar bronchi.

The BFS was then passed into the right bronchial tree for BAL and the tip was wedged into the lateral segmental bronchus of the middle lobe. 100 ml of normal saline at 37°C were infused twice into this broncho-pulmonary segment. After 30 sec, the effluent lavage fluid was collected by light aspiration following each infusion of saline. The fluid recovered from BL ranged from 5–10 ml (25–50%) and that recovered from BAL 60–120 ml (30–60%).

All patients were informed about the procedure and consented to be investigated.

Statistical analysis

Correlation coefficients were calculated between:

- Serum IgE/albumin ratio and BL IgE/albumin ratio.
- Serum IgE/albumin ratio and BAL IgE/albumin ratio.
- Serum IgE/K⁺ ratio and BL IgE/K⁺ ratio
- Serum IgE/K⁺ ratio and BAL IgE/K⁺ ratio.

Serum BL and BAL IgE of patients were compared with those of controls by means of Student's *t* test. The specific IgE levels in serum and BL of patients with positive sBPT or positive PT were compared with those of patients with negative sBPT or negative PT by means of Student's *t* test.

RESULTS

Serum total IgE/albumin ratio was significantly correlated with the BAL total IgE/albumin ratio ($r = 0.876$; $P < 0.001$) and with the BAL total IgE/K⁺ ratio ($r = 0.769$; $P < 0.001$) (Tables 1 and 2).

Neither total IgE/albumin ratio nor total IgE/K⁺ in serum correlated with those of BL ($r = 0.003$; $P = \text{NS}$ and $r = 0.179$; $P = \text{NS}$, respectively) (Tables 1 and 2).

The mean of the total IgE/albumin ratios in the allergic patients was significantly higher than that in controls with respect to serum levels but not in BL and BAL.

The mean of the total IgE/K⁺ ratios in the allergic patients was significantly higher than in controls in both serum and BL but not in BAL (Table 3). The albumin/K⁺ ratio (mg% / mEq/l) in BL of patients was five times higher than in controls (54.6 and 11.1, respectively) even though not statistically significant.

Table 4 shows the means and standard deviations expressed as per cent of total radioactivity added (TRA) of RAST in serum and BL of controls. Mean + 3 SD in serum was 2.3% and in BL 1.6%. Allergens tested were grass (*Lolium perenne*), *Parietaria officinalis* and *Dermatophagoides pteronyssinus* for a total of 18 assays.

Table 5 summarizes the results of tests in atopic patients: PC15 FEV₁ of the mBPT

ranged between 0.074 and 0.5 mg/ml, K⁺ of BL ranged between 0.4 and 1.7 mEq/l. Albumin is not shown. All patients were sensitized by one or more of the three allergens mentioned above. Specific bronchial provocation test in one case showed only a late asthmatic reaction, in the remaining cases only early asthmatic reactions.

The agreement between serum- and BL-specific IgE with sBPT results is shown in Table 6, upper part. Specific BPT were grouped as positive or negative. The mean of serum-specific IgE of the positive sBPT group was significantly higher than that of the negative group ($P < 0.001$). The mean of BL-specific IgE of the positive sBPT group was higher than that of the negative group, although at a lower level of significance ($P < 0.05$).

Table 1

Correlation between serum and BL; serum and BAL (IgE U/ml / albumin mg%)

BAL	Serum $\times 10^{-3}$	BL
Δ 0.14	0.51	0.02
Δ 0.23	1.11	0.80
● 0.14	7.17	0.01
Δ 0.03	7.90	0.01
★ 0.48	16.09	0.13
Δ 0.16	22.22	0.10
● 1.26	31.73	0.09
Δ 1.48	32.99	0.07
● 0.04	37.77	0.05
Δ 1.50	56.47	0.02
● 2.96	102.04	0.02
● 2.40	127.66	0.04
● 6.25	157.44	16.00
● 4.58	204.08	0.13
Δ 18.67	259.74	0.31
● 37.50	333.33	0.01
$r = 0.876$		$r = 0.179$
$P < 0.001$		$P: \text{NS}$

Serum IgE = 0.009 BAL IgE + 0.04.

Δ Controls

● Atopic (asthmatics)

★ Non atopic (asthmatics)

Table 2
Correlation between serum and BL; serum and BAL
(IgE U/ml / K⁺ mEq/l)

BAL	Serum	BL
△ 0.26	0.55	0.63
△ 0.27	1.08	0.1
● 0.14	6.66	0.6
△ 0.15	8.50	0.23
△ 0.37	14.62	0.04
★ 0.26	14.79	4.5
● 1.66	31.73	5.28
△ 1.97	33.33	0.02
● 0.22	39.08	0.90
△ 3.19	47.52	0.27
● 7.27	98.81	0.80
● 3.75	125.00	3.50
● 5.31	154.16	1.66
● 2.71	187.61	0.53
△ 48.27	238.66	0.94
● 90.00	259.06	1.09
$r = 0.769$ $P < 0.001$		$r = 0.003$ $P: NS$

Serum IgE = 2.782 BAL IgE + 49.99.

△ Controls

● Atopic (asthmatics)

★ Non atopic (asthmatics)

Table 3
Total IgE in atopics and in controls

(IgE U/ml / K ⁺ mEq/l) m ± SE			
Subjects**	Serum	BL	BAL
Controls (n = 6)	17.6 ± 7.7	0.2 ± 0.1	1.0 ± 0.5
Atopics (n = 8)	112.6 ± 30.6	1.8 ± 0.6	13.9 ± 10.9
	$P < 0.05$	$P < 0.05$	$P: NS$
(IgE U/ml / alb. mg %) m ± SE			
	Serum*	BL	BAL
Controls (n = 6)			
Atopics (n = 8)			
	$P < 0.05$	$P: NS$	$P: NS$

* Albumin is measured in g percent ml

** The healthy Egyptian man was excluded from the controls due to his high serum total IgE level. If he was included only in BL the difference remains significant ($t = 2.2$; $P < 0.05$).

Table 4
Control subjects: negative PT, methacholine BPT PC 15 FEV₁ > 10 mg/ml

No.	Serum			BL			K ⁺ mEq/ml
	RAST % TRA						
	<i>Lolium perenne</i>	<i>Parietaria off.</i>	<i>Dermatophagoides</i>	<i>Lolium perenne</i>	<i>Parietaria off.</i>	<i>Dermatophagoides</i>	
1	0.6	0.6	0.4	0.6	0.5	0.4	1.0
2	0.3	0.9	0.6	1.1	0.7	0.9	2.5
3	1	1.3	0.8	1	1	0.7	2.2
4	1.6	1.2	0.8	1.1	0.9	0.7	4.6
5	1.2	1.8	1.4	0.7	1	0.8	1.5
6	1.1	1.1	1.4	1.3	1.1	1	2.3
Mean = 1.03				Mean = 0.87			
SD = 0.42				SD = 0.23			
Mean + 3 SD = 2.3				Mean + 3 SD = 1.6			

Agreement between serum and BL-specific IgE with PT results are shown in Table 6, lower part. The results are practically similar to those of sBPT.

DISCUSSION

Several reports have appeared suggesting that the diagnosis of type I hypersensitivity cannot

Table 5
Atopic patients

Diagnosis	Methacholine PC 15 FEV ₁ (mg/ml)	K ⁺ BL (mEq/l)	Allergen	Serum IgE (% TRA)	BL IgE (% TRA)	sBPT PC 15 FEV ₁ (AU)	Prick test
Rhinitis-Asthma	0.5	1.2	<i>Lolium perennis</i>	41.3	2.7	250	positive
			<i>Parietaria off.</i>	3.8	0.8	neg.	negative
			<i>Dermatophagoides</i>	3.7	0.7	neg.	negative
Asthma	0.3	3.6	<i>Lolium perennis</i>	1.6	1.1	neg.	negative
			<i>Parietaria off.</i>	1.8	1.4	neg.	negative
			<i>Dermatophagoides</i>	32	3	125	positive
Asthma	0.2	1.2	<i>Lolium perennis</i>	3.8	1.3	neg.	doubt
			<i>Parietaria off.</i>	7.1	1.1	500	doubt
			<i>Dermatophagoides</i>	25	3.1	250	positive
Asthma	0.1	1.4	<i>Lolium perennis</i>	8.3	0.9	ND	doubt
			<i>Parietaria off.</i>	7.4	1.2	ND	positive
			<i>Dermatophagoides</i>	28	1.7	100	positive
Asthma	0.5	0.4	<i>Lolium perennis</i>	1.3	1.5	neg.	negative
			<i>Parietaria off.</i>	1.6	1.2	neg.	negative
			<i>Dermatophagoides</i>	12	1.3	neg. LAR	doubt
Rhinitis-Asthma	0.8	1.7	<i>Lolium perennis</i>	33	1.3	830	positive
			<i>Parietaria off.</i>	36	1.2	425	positive
			<i>Dermatophagoides</i>	0.8	1.1	neg.	negative

AU = arbitrary units; LAR = late asthmatic reaction.

always be achieved without a direct study of samples recovered from the affected organ (10).

Most of this work was done on nasal secretions, sputa and tracheo-bronchial secretions (1, 4, 5, 9, 15). Because there are patients with rhinitis who have never experienced symptoms of asthma, and patients with asthma without rhinitis, it is important to be able to study samples of the affected organs (nose, bronchi) without reciprocal contamination of the recovered fluids.

Our technique in airways lavage by fiberoptic bronchoscopy is a relatively simple method for obtaining uncontaminated samples at different levels of the bronchial tree and from the alveolar spaces. By this method we can assess the local immunoglobulin production in the large bronchi, and in deep lung, without contamination from the upper airways (14). Undoubtedly, asthmatics run a high risk of bronchoscopic

complications. Endoscopists must therefore be well trained and equipped for any emergency. None of the patients in our group had to be intubated, or treated for hypoxemia, cardiac or other severe complications.

Why some patients have only rhinitis and not asthma, has yet been unanswered. Two major differences may exist between rhinitic and asthmatic patients. The first is that non-specific bronchial hyperreactivity is more pronounced in asthmatics than in rhinitics (2, 3, 13). Nevertheless, rhinitic patients often react to specific bronchial provocation tests but to a lesser extent than asthmatics (6, 12, 16). The second difference might be that patients with only rhinitis have a lower level of IgE on the surface of their bronchial mucosa than asthmatic subjects. Since we were interested in studying this second point, we carried out this pilot study in order to see if our technique proved adequate in establishing differences between total and specific

Table 6

Agreement between specific bronchial provocation tests (sBPT) and serum- or BL-specific IgE

	sBPT		
	Pos.	Neg.	
TRA % serum IgE	28.9 ± 10.9	2.3 ± 1.2	<i>t</i> 6.85 <i>P</i> < 0.001
TRA % BL IgE	2 ± 0.9	1.1 ± 0.3	<i>t</i> 2.66 <i>P</i> < 0.05

Agreement between prick test (PT) and serum- or BL-specific IgE			
	PT		
	Pos.	Neg.	
TRA % serum IgE	28.9 ± 10.9	2.1 ± 1.2	<i>t</i> 6.5 <i>P</i> < 0.001
TRA % BL IgE	2 ± 0.8	1.1 ± 0.3	<i>t</i> 2.69 <i>P</i> < 0.05

IgE are expressed as percent of the total radioactivity added (TRA %). sBPT with only late asthmatic reaction and doubtful PT are not included.

IgE in BL and BAL of healthy and asthmatic subjects. We found that total IgE levels are strongly correlated in serum and BAL but not in serum and BL, either when expressed as their ratio to albumin or K^+ .

We interpret this good correlation between serum and BAL to be due to an easy exchange of immunoglobulins from serum to broncho-alveolar spaces and vice versa, while the thickness of tissues separating bronchial spaces from blood may account for the lack of correlation between serum and BL. Thus, in the large bronchi the presence of IgE may not always be a consequence of IgE in the blood, but of a local immunological state. Supporting this interpretation is the high level of IgE found in the BAL of the Egyptian man. This high IgE serum level was probably due to parasitosis, the subject being healthy and RAST negative, while in BL his IgE level was closer to mean level of the controls than of the asthmatics.

As expected, we found significantly higher IgE levels in the serum of allergics than of

controls. In BL this applied to the IgE/ K^+ ratio but not to the IgE/albumin ratio. As the albumin/ K^+ ratio tended to be higher in allergic patients than in controls, we interpret this as the cause for the lack of difference between the two groups. The albumin increment in BL of allergic patients might be due to the allergic inflammation causing an increased permeability of bronchial mucosa. Therefore, the albumin value should not be used to calculate the dilution index of the recovered fluids when increased permeability is suspected and different groups of subjects are compared.

In BAL, even when the means of IgE/ K^+ ratio and IgE/albumin ratio in atopics were about 10 times higher than in controls, there was no statistically significant difference between the groups.

To distinguish between "positive" and "negative" subjects, we chose as the cutoff points the mean + 3 SD of controls' RASTs in serum and BL.

With regard to the serum, we found two positive RASTs corresponding with negative PT, and three positive RASTs corresponding with negative sBPT.

Doubtful PT results and sBPT with only a late asthmatic reaction were not considered.

With regard to BL we found three negative RASTs with corresponding positive PT, and three negative RASTs with corresponding positive sBPT. These data indicate that for the three allergens tested in this study serum RAST gives some false-positive results while BL RAST gives some false-negative results. Therefore, at this stage we need to improve the sensitivity of BL RAST for a better agreement with the clinical status.

Improvement of the low sensitivity of BL RAST can be obtained by concentrating the recovered fluid when the K^+ level is too low (< 1 mEq/l).

As expected, the serum RAST values were significantly higher ($P < 0.001$) in patients with positive PT and/or positive sBPT than in patients with negative PT and/or negative sBPT. Although at a lower level of statistical significance ($P < 0.05$) the means of BL RASTs showed the same findings.

In addition, negative results of BL RAST corresponding to positive sBPT were observed in the patients who had the higher PC15 FEV₁ even if the correlation between BL RAST and PC15 FEV₁ was not statistically significant.

In conclusion, this preliminary study shows that it is possible to measure IgE levels in BL and BAL by a relatively simple and safe technique. Since the local specific IgE level seems to reflect the allergic status of an asthmatic we believe this method will be useful to study whether the local immunological status of rhinitis is similar to that of asthmatics or to that of healthy subjects.

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Address:

Emanuele Crimi, M.D.

Istituto di Medicina e Chirurgia Broncopolmonare

Università degli Studi di Genova

Via F. Mosso, 8

16132 Genova

Italy