

Soluble interleukin-2 receptor and cellular profiles in bronchoalveolar lavage fluid from patients with bronchial asthma

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Background: To elucidate the role of T-cell activation in the physiologic changes and cellular infiltrations in the bronchial tree of patients with symptomatic bronchial asthma, we measured the concentration of soluble interleukin-2 receptor (sIL-2R), cellular compositions and IL-2R expression on cells in bronchoalveolar lavage (BAL) fluid. We also measured physiologic parameters, including a provocation concentration of histamine required to reduce the forced expiratory volume in 1 second by 20% of the prechallenge baseline (PC₂₀).

Results: The concentration of sIL-2R and the ratio of IL-2R expression on CD3 positive lymphocytes were significantly higher in BAL fluid from 20 patients with symptomatic bronchial asthma than concentrations in fluid from nine healthy controls ($p < 0.01$) and six asymptomatic patients with bronchial asthma ($p < 0.05$). The concentration of sIL-2R correlated with the percentage of IL-2R expression on CD3 positive lymphocytes ($r_s = 0.709$, $p < 0.01$). There was no difference in sIL-2R concentration of BAL fluid between the healthy control and the patient with asymptomatic bronchial asthma. Among the inflammatory cells recovered in BAL fluid, the percentages of eosinophils showed significant positive correlation with the concentration of sIL-2R in BAL fluid in the patients with bronchial asthma. The concentration of sIL-2R significantly correlated with forced expiratory volume one second, peak expiratory flow rate, and PC₂₀ when observed in all study subjects, but not in the patients with bronchial asthma.

Conclusion: We conclude that T-cell activation within the bronchial tree appears to have a role in the infiltration of eosinophils into the bronchial tree, which may lead to enhanced bronchial hyperreactivity in patients with bronchial asthma. (*J ALLERGY CLIN IMMUNOL* 1993;91:623-33.)

Key words: Soluble interleukin-2 receptor, bronchial asthma, eosinophils, bronchoalveolar lavage, bronchial hyperreactivity

Bronchial asthma is a form of inflammatory airway disease.¹ The role of infiltrating inflammatory cells in the bronchial tree has received much attention with respect to their ability to induce inflammation: such

Abbreviations used

IL-2:	Interleukin-2
sIL-2R:	Soluble interleukin-2 receptor
BAL:	Bronchoalveolar lavage
PC ₂₀ :	Provocation concentration of histamine
FVC:	Forced vital capacity
FEV ₁ :	Forced expiratory volume 1 second
PEFR:	Peak expiratory flow rate
MAB:	Monoclonal antibody
FITC:	Fluorescein isothiocyanate

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inflammation may be the basis of bronchial hyperreactivity that is the hallmark of this disease.²⁻⁸

The mast cell is thought to be the major cell that secretes several mediators to recruit other inflamma-

TABLE I. Profiles of study subjects

	Age	Sex	FVC (%)	FEV ₁ (%)	PEFR (%)	PD ₂₀ (mg/ml)	Skin test	RAST	DP, DF	Medication
Healthy subject										
1	45	M	101	100	86	25	Negative	0	0	ND
2	35	F	96	93	85	13	Negative	0	0	ND
3	37	M	99	106	98	25	Negative	0	0	ND
4	26	M	103	103	95	21	Negative	0	0	ND
5	35	F	101	95	84	25	Negative	0	0	ND
6	35	F	95	81	92	16	Negative	0	0	ND
7	40	M	99	98	85	25	Negative	0	0	ND
8	42	M	96	102	100	25	Negative	0	0	ND
9	28	M	96	101	76	25	Negative	0	0	ND
mean	35.3		98.5	97.7	89.0	22.6				
SE	1.7		0.9	2.3	2.4	1.5				
Symptomatic bronchial asthma										
1	41	M	99 (89)	95 (72)	94 (76)	1.02	Negative	0	0	AMP, β_2
2	58	M	75 (60)	62 (42)	56 (28)	0.52	Negative	0	0	AMP, β_2
3*	59	F	70 (54)	60 (40)	68 (35)	ND	Negative	0	0	AMP, β_2 , SD
4	18	M	98 (89)	96 (63)	82 (53)	0.87	Negative	0	0	AMP, β_2
5	38	F	101 (95)	90 (65)	65 (33)	1.2	Negative	0	0	AMP, β_2
6	58	M	75 (59)	75 (50)	59 (34)	0.3	Negative	0	0	AMP, β_2
7	58	M	76 (54)	63 (42)	52 (26)	0.7	Negative	0	0	AMP, β_2 , SD
8*	59	F	74 (64)	61 (41)	67 (21)	0.025	Negative	0	0	AMP, β_2 , SD
9	48	F	95 (56)	61 (42)	65 (24)	0.2	Negative	0	0	AMP, β_2
10	41	M	99 (87)	86 (58)	56 (31)	3.9	Negative	0	0	AMP, β_2
11	59	F	100 (94)	99 (65)	73 (55)	1.1	HD, DF, DP	1	2	AMP, β_2
12	23	F	75 (61)	65 (40)	78 (54)	0.6	HD, DP	4	4	AMP, β_2
13*	58	F	65 (52)	60 (40)	67 (28)	ND	HD, DF, DP	3	3	AMP, β_2 , SD

RAST DP, RAST score to *Dermatophagoides pteronyssinus*; RAST DF, RAST score to *Dermatophagoides farinae*, HD, house dust; AMP, aminophylline; β_2 : β_2 -Stimulants; SD, steroid.

RAST DP and DF were expressed as semiquantitative scores.

FVC, FEV₁, and PEFR are expressed as percent predicted.

Data in parenthesis represent the values on acute asthmatic attack before medication.

*Chronic persistent type of bronchial asthma.

All data are expressed as mean $\pm$ SE.

†p value < 0.01 vs after treatment in symptomatic bronchial asthma.

‡p value < 0.05 vs FEV₁ of normal subject

§p value < 0.01 vs PEFR of normal subject and < 0.05 vs PEFR of asymptomatic bronchial asthma.

||p value < 0.01 vs PD₂₀ of normal subject

tory cells, including eosinophils.⁹⁻¹¹ However, T lymphocytes, when activated by mitogens and antigens, release a variety of cytokines to amplify the immune and inflammatory responses.^{12,13} Some cytokines have influence on the traffic of inflammatory cells into the bronchial tree, possibly leading to bronchial hyper-reactivity.

Although the role of lymphocytes is not clearly defined in bronchial asthma, some evidence of T-cell activation exists in the patients with bronchial asthma. The lymphocytes are prominent among the inflammatory cells infiltrating the subepithelial tissue of asth-

matic airways,¹⁴ and infiltrating lymphocytes have been shown to express activation markers including HLA-DR antigen and interleukin-2R (IL-2R).¹⁵ The synthesis of IL-2 and interferon-gamma (INF- γ), as well as expression and secretion of activation markers including IL-2R by peripheral blood lymphocytes, is known to be increased in a state of a status asthmatic attack.^{16,17}

The sIL-2R, an extracytoplasmic domain of cell-bound IL-2R, effectively binds IL-2 and, therefore, can be used as a marker of T-cell activation.^{18,19} We have previously observed the presence of T-cell ac-

TABLE I. cont.

	Age	Sex	FVC (%)	FEV ₁ (%)	PEFR (%)	PD ₂₀ (mg/ml)	Skin test	RAST	DP, DF	Medication
14	18	M	101 (88)	96 (63)	99 (74)	3.6	DF, cat	2	2	AMP, β_2
15	20	M	101 (89)	70 (50)	73 (56)	0.35	DP, DF	1	2	AMP, β_2
16	55	M	76 (50)	65 (44)	63 (35)	0.25	DP, DF, cat	1	1	AMP, β_2
17	49	F	100 (93)	66 (43)	91 (66)	0.7	DP, DF	1	1	AMP, β_2
18	54	M	74 (64)	64 (43)	63 (28)	0.8	DP, DF, dog	2	2	AMP, β_2
19	18	M	98 (91)	93 (60)	62 (45)	0.4	DP, DF, HD	1	1	AMP, β_2
20	59	M	78 (62)	62 (41)	63 (24)	0.4	DP, DF, HD	2	1	AMP, β_2
mean	44.6		86.3 (72.6†)	73.9‡ (50.3†)	69.8§ (41.3†)	0.95				
SE	3.6		2.9 (3.8)	3.3 (2.3)	2.8 (3.8)	0.23				
Asymptomatic bronchial asthma										
1	49	M	109	104	81	0.68	Negative	0	0	ND
2	33	M	80	89	73	ND	Negative	0	0	ND
3	48	F	110	106	85	0.87	Negative	0	0	ND
4	47	M	110	86	79	ND	Negative	0	0	ND
5	35	M	77	74	77	1.2	Negative	0	0	ND
6	24	F	106	78	68	0.5	DP, DF, cat	2	3	ND
mean	39.3		98.7	89.5	77.2	0.81				
SE	3.8		5.9	4.9	2.2	0.13				

tivation in the bronchial tree of patients with bronchial asthma as evidenced by an elevated concentration of sIL-2R in bronchoalveolar lavage (BAL) fluid obtained from the patients with bronchial asthma during the recovery phase from asthmatic attack.²⁰

In this study we compared the concentrations of sIL-2R in BAL fluid and IL-2R expression on BAL cells between the patients with symptomatic and asymptomatic bronchial asthma to elucidate the role of T-lymphocyte activation in the development of asthmatic symptoms. In addition, we compared the concentration of sIL-2R with the physiologic changes and cell profiles in BAL fluid of the patients with bronchial asthma and control subjects to search for the effect T-lymphocyte activation on physiologic changes and cellular infiltration into the bronchial tree of patients with bronchial asthma.

MATERIAL AND METHODS

This study was approved by the Soonchunghang University Human Subject Committee. All subjects gave informed consent before participation.

Study populations

The study subjects included 11 patients with extrinsic bronchial asthma, 15 with intrinsic bronchial asthma, nine healthy volunteers, and eight patients with other lung diseases (two cases of bronchiectasis, three cases of solitary pulmonary nodule, two cases of bronchitis, and one case of miliary tuberculosis). The presence of allergy was de-

termined by the presence of immediate positive response to more than one of 48 common aeroallergen extracts in Korea on skin prick test. Specific IgE to allergens was checked by radioallergosorbent test (RAST) kit (Pharmacia Fine Chemicals, Uppsala, Sweden). All patients with extrinsic bronchial asthma had positive specific IgE to one of three allergens: house dust, *Dermatophagoides farinae*, and *D. pteronyssinus*. The criteria for definition of intrinsic bronchial asthma were the absence of immediate positive response on skin prick test and family history of atopy.

The onset of bronchial asthma varied from 1 to 23 years ago. Five patients with intrinsic and one patient with extrinsic bronchial asthma had no symptoms of bronchial asthma, including cough, wheeze, or dyspnea during the 4 weeks before the study. Twenty patients had apparent symptoms before the study. The pattern of asthmatic attack was "acute intermittent" in all symptomatic patients except two patients with intrinsic asthma and one with extrinsic asthma who had chronic persistent pattern of bronchospastic attack. Acute intermittent pattern was defined as having the episodes of shortness of breath and wheezing without persistent symptoms between episodes, and "chronic pattern" was described as having the persistent symptoms or derangement of lung function test even between the episodes of acute asthmatic attack. No patients showed evidence of respiratory bacterial infection within 4 weeks of the study.

Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), and peak expiratory flow rate (PEFR) were measured with Fukuda-300 (Fukuda Denshi Co., Ltd., Tokyo, Japan). Provocation concentration of histamine required to reduce the FEV₁ by 20% of the prechallenge baseline (PC₂₀) was measured by Cockcroft's²¹ method from the

concentration of 0.025 mg/ml to 25 mg/ml of histamine 2 days before the procedure of BAL without medication for the past 12 hours before lung function measurement.

The profiles of study subjects are summarized in Table I. The mean age was higher in the patients with symptomatic bronchial asthma than in patients with asymptomatic bronchial asthma and healthy controls; however this difference was not statistically significant. FVC, FEV₁, and PEFr were significantly lower in the patients with symptomatic bronchial asthma before treatment than in healthy controls and patients with asymptomatic bronchial asthma. After treatment of acute asthmatic attack in symptomatic patients, physiologic parameters were again evaluated 2 days before the procedure of BAL. FEV₁ and PEFr were significantly improved more than 20% in all patients with symptomatic bronchial asthma; however, FEV₁ and PEFr were still lower in the patients with symptomatic bronchial asthma when compared with those of healthy subjects. In the symptomatic patients, FVC, FEV₁, PEFr, and duration of disease were not different between intrinsic and extrinsic bronchial asthma.

PC₂₀ measured in 22 patients with bronchial asthma ranged from 0.025 mg/ml to 3.9 mg/ml with geometric means of 0.81 mg/ml in the patients with asymptomatic asthma and 0.95 mg/ml in those patients with symptomatic asthma, which were significantly lower than PC₂₀ of healthy controls. The minimum value of PC₂₀ was 13 mg/ml of histamine in the healthy subjects.

Bronchoalveolar lavage

In six asymptomatic patients, BAL was carried out without medication except two puffs of bronchodilator before the BAL procedure. In 20 of the apparently bronchospastic patients, BAL was carried out when the patients could tolerate bronchoscopy after treatment of the last acute bronchospastic attack for 2 weeks with aminophylline and beta-stimulants in all cases and steroids in four cases. Corticosteroids and beta-stimulants had been stopped at least 1 week before bronchoscopy. Aminophylline was also stopped at least 24 hours before the BAL procedure. None of the patients used disodium cromoglycate before the BAL procedure. All study subjects inhaled two puffs of bronchodilator, and atropine and diazepam were administered as premedication immediately before bronchoscopy, which was performed by one operator. No complications were experienced in any study subject during the BAL procedure.

After informed consent was obtained, the procedure was performed by instillation four times of 50 ml normal saline solution into the bronchoalveolar tree and gentle suction with the negative pressure below of 50 mm Hg under the fiberoptic bronchoscopy (Olympus B2-10; Olympus Optical Co., Ltd., Tokyo, Japan) as previously described.²¹ Cell pellets and supernatant were separated by centrifugation at 500 g for 5 minutes. Differential cell count up to 500 cells was done on the slide prepared by cytocentrifuge and Diff-Quick (Scientific Products, Gibbstowne, N.J.) stain. BAL fluid obtained was concentrated 10 times with YM-5 ultrafiltration membrane (Amicon, Division of W. R. Grace Co., Beverly, Mass.) for measurement of sIL-2R.

Measurement of soluble receptor for IL-2

The concentration of sIL-2R in BAL fluid was measured according to the method of Rubin et al.¹⁸ with cell-free IL-2 receptor test kit (T-cell Science, Cambridge, Mass.) at the same time. In brief, flat-bottomed 96-well microtiter plates were coated overnight with 100 µl of purified anti-Tac monoclonal antibody (MAb) at 1 µg/ml in carbonate buffer, pH 9.6, or carbonate buffer alone as a background control. After washing, 100 µl of various dilutions of standards and samples were incubated for 2 hours, washed, and 100 µl of horseradish peroxidase-conjugated anti-IL-2R antibody (7G7/B6) was incubated in all wells. After 1 hour of incubation, the plates were washed, and 100 µl of 0-phenylenediamine were added and incubated at room temperature for 30 minutes. The optical density of the well was determined at 490 nm with spectrophotometer (Beckman Co. Du-70; Beckman Instruments-Spinco Div., Palo Alto, Calif.) and microcuvette. The absorbance of the test wells was then compared with the standard curve and was converted to a numeric value. The detection limit is approximately 50 U/ml, and intrassay coefficient of variance was 4.3% and interassay coefficient of variance was 12.0%. Measured concentrations of sIL-2R were adjusted to original values by correction with the concentrating factors.

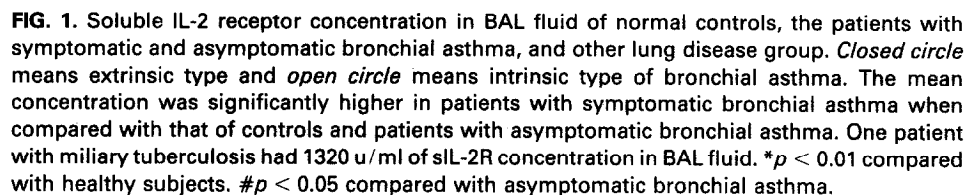
Measurement of IL-2R expression on BAL cells

BAL cells were washed twice with Ca⁺⁺ and Mg⁺⁺ free Hank's balanced salt solution and suspended in Hank's balanced salt solution containing 0.1% bovine serum albumin and 0.2% sodium azide. Specific binding of monoclonal antibodies (MAbs) was analyzed by dual immunofluorescence staining according to standard methods recommended by Becton-Dickinson Monoclonal Center (Mountain View, Calif.). Aliquots containing 1×10^6 cells were incubated for 30 minutes at 4° C in the presence of saturating concentration of fluorescein isothiocyanate (FITC) and phycoerythrin-conjugated MAbs. MAbs for CD3 (Leu-4), CD19 (Leu-12), and CD14 (Leu-M3) were obtained in conjugated form with FITC, and CD25 (anti-Tac) was obtained in conjugated form with phycoerythrin from Becton-Dickinson Co. The cells were washed in phosphate-buffered saline solution and fixed in 1 ml of 1% paraformaldehyde in phosphate-buffered saline solution.

Cytofluorometric analysis of the cells for the surface immunofluorescence was performed with the FACScan (Becton-Dickinson). The cells were excited with the 488 nm argon laser. The filter set used to separate the two signals was recommended by the manufacturer for FITC and phycoerythrin. For each sample 10000 cells were analyzed. The data were collected and analyzed on the program of Consort 40 (Becton-Dickinson).

Statistics

The differences between groups were compared with the nonparametric Kruskal-Wallis H test for continuous data, and if found significant, the Mann-Whitney test with Bonferroni's correction was applied to compare any two groups. Wilcoxon signed-rank test was used for paired samples. Chi-



The results are expressed as mean $\pm$ SE unless otherwise stated.

RESULTS

Concentration of sIL-2R in BAL fluid

The mean concentration of sIL-2R in BAL fluid was significantly higher in patients with symptomatic bronchial asthma than the mean concentration of healthy controls ($42.28 \text{ U/ml} \pm 11.44 \text{ U/ml}$ vs $1.07 \text{ U/ml} \pm 0.89 \text{ U/ml}$, $p < 0.01$) and asymptomatic bronchial asthma ($42.28 \text{ U/ml} \pm 11.44 \text{ U/ml}$ vs $8.61 \text{ U/ml} \pm 4.42 \text{ U/ml}$, $p < 0.05$). Among the symptomatic patients, sIL-2R concentration tended to

be higher in extrinsic asthma when compared with that of intrinsic bronchial asthma; however, this difference was insignificant (57.4 ± 12.4 U/ml vs 30.8 ± 10.8 U/ml, $p > 0.05$). Both groups with symptomatic bronchial asthma had significantly higher concentration of sIL-2R when compared with that of healthy controls (Fig. 1). Asymptomatic bronchial asthma tended to have greater concentration of sIL-2R in BAL fluid than that of healthy controls, but the difference was statistically insignificant (Fig. 1). Among the nine patients with other lung diseases, one patient with hemoptysis and one patient with miliary tuberculosis had sIL-2 concentration of 16.1 U/ml and 1320 U/ml, respectively, in their BAL fluids.

Relationship between sIL-2R concentration and physiologic parameters including PC₂₀ in bronchial asthma and controls

When observed in the entire study, including healthy patients and patients with bronchial asthma, sIL-2R concentration showed a significantly inverse correlation with FEV₁, PEFR (Fig. 2), and PC₂₀. Otherwise, correlations between sIL-2R concentration and each physiologic parameter became insignificant when observed only in the patients with bronchial asthma (Table II) and also when observed in intrinsic and extrinsic bronchial asthma separately.

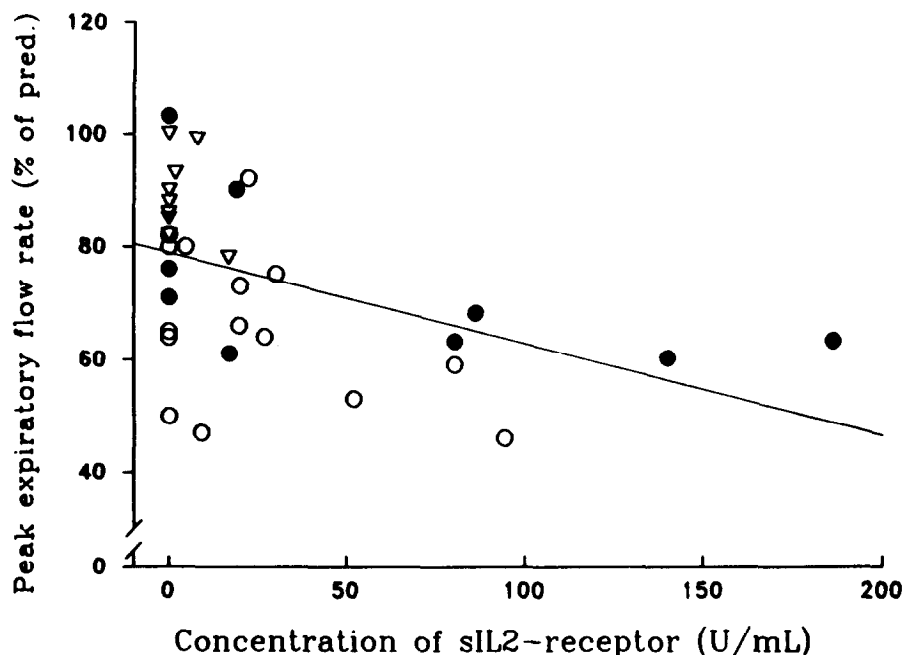


FIG. 2. Relationship between concentration of sIL-2R in BAL fluid and PEFR. sIL-2R concentration inversely correlated with PEFR in entire study subjects including normal controls (*triangle*), patients with intrinsic asthma (*open circle*) and patients with extrinsic asthma (*closed circle*) ($r_s = -0.37$, $p = 0.022$, $n = 35$). Correlation not significant when observed only in patients with bronchial asthma ($r_s = -0.25$, $p > 0.05$, $n = 26$).

TABLE II. Correlation matrix of sIL-2R concentration and physiologic parameters

	No.	FVC	FEV ₁	PEFR	PC ₂₀ *
Bronchial asthma and control	35	-0.27	-0.31*	-0.37†	-0.38†
Asymptomatic bronchial asthma	6	-0.15	-0.22	-0.20	-0.46
Symptomatic bronchial asthma	20	-0.45	-0.28	-0.26	-0.35
Total bronchial asthma	26	-0.25	-0.26	-0.25	-0.30

Physiologic parameters were measured 2 days before the BAL procedure

*The study number of PC₂₀ was four asymptomatic and 18 symptomatic patients in the group with bronchial asthma.

† p value was less than 0.05 when observed in bronchial asthma and control subject together, but greater than 0.05 in total bronchial asthma as well as in each group of bronchial asthma.

Cellular profiles and correlation with sIL-2R in BAL fluid

The recovery rate and total number of cells recovered in BAL fluid were not different between the patients with bronchial asthma and control patients. In cellular composition, the patients with symptomatic bronchial asthma had significantly greater percentages of lymphocytes, neutrophils, and eosinophils than did healthy controls. Otherwise, the percentage of macrophages was significantly lower in BAL fluid from the symptomatic and asymptomatic patients with bronchial asthma when compared with percentages from control patients (Table III).

Although the patients with symptomatic bronchial asthma tended to have greater percentage of lympho-

cytes than patients with asymptomatic bronchial asthma, this difference was not significant ($p > 0.05$) (Table III). However, the percentage of macrophages was lower, and that of neutrophils and eosinophils was significantly higher in patients with symptomatic bronchial asthma when compared with asymptomatic patients.

Among inflammatory cells in BAL fluid, percentages of eosinophils significantly correlated with sIL-2R concentrations in BAL fluid when observed in the patients with bronchial asthma (Fig. 3). However, the percentages of other cells did not correlate with sIL-2R concentrations in BAL fluid of these groups. The percentages of macrophages showed significantly inverse correlation with sIL-2R concentration when ob-

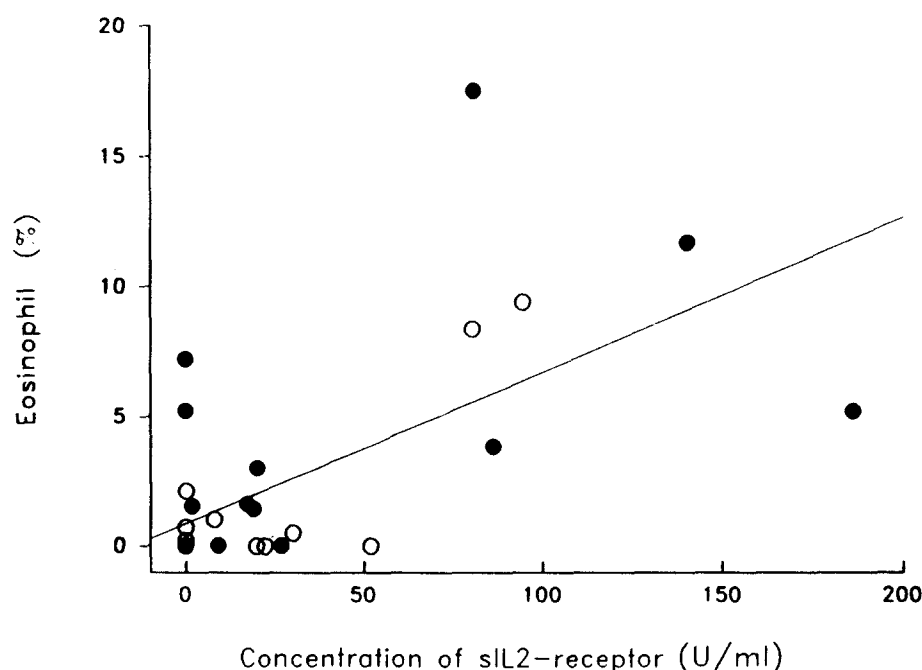


FIG. 3. Relationship between concentration of sIL-2R and percentage of eosinophils in BAL fluid. Correlation statistically significant when observed in patients with asymptomatic and symptomatic bronchial asthma ($r_s = 0.41$, $p < 0.05$, $n = 26$). Closed circle means extrinsic type and open circle means intrinsic type of bronchial asthma.

TABLE III. Cellular profiles of BAL fluid in patients and controls

	No.	Recovery rate (%)	Total number (1×10^6)	Macrophage (%)	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)	Epithelial cell (%)
Normal	9	50.5 ± 7.9	15.9 ± 3.8	95.6 ± 0.9	2.5 ± 0.6	1.1 ± 0.4	0.5 ± 0.3	0.4 ± 0.3
Asymptomatic bronchial asthma	6	49.2 ± 3.8	20.5 ± 6.1	$90.7 \pm 1.5^*$	3.9 ± 0.9	1.1 ± 0.4	0.6 ± 0.5	$2.6 \pm 0.9^*$
Symptomatic bronchial asthma	19	43.7 ± 2.8	17.3 ± 0.7	$75.6 \pm 3.5^{\dagger\dagger}$	$7.9 \pm 1.8^{\ddagger}$	$8.8 \pm 3.0^{\ddagger\dagger}$	$4.1 \pm 1.2^{*\ddagger}$	$6.5 \pm 1.4^{\ddagger}$
Bronchial asthma	25	45.5 ± 2.2	18.1 ± 2.6	$74.8 \pm 3.1^*$	$6.9 \pm 1.5^*$	$6.6 \pm 1.1^{\dagger}$	$3.3 \pm 1.0^{\dagger}$	$5.5 \pm 1.2^{\dagger}$

Data presented as mean $\pm$ SE.

* $p < 0.05$ vs healthy controls.

$^{\dagger}p < 0.01$ vs healthy controls.

$^{\ddagger}p < 0.05$ vs asymptomatic bronchial asthma.

served in subjects including healthy control subjects and those with bronchial asthma (Table IV). PC_{20} was significantly correlated with the percentages of neutrophils ($r_s = -0.35$, $p < 0.05$), eosinophils ($r_s = -0.37$, $p < 0.05$), lymphocytes ($r_s = -0.32$, $p < 0.05$), and epithelial cells ($r_s = -0.45$, $p < 0.01$) when observed in study subjects including healthy controls and patients with bronchial asthma ($n = 30$); however, these correlations were insignif-

icant when observed in the patients with bronchial asthma as a whole as well as patients with intrinsic asthma and those with extrinsic asthma with bronchial asthma separately.

Expression of IL-2R on cells in BAL fluid

IL-2R expressed significantly higher on CD3 positive lymphocytes in BAL fluid of the symptomatic patients with bronchial asthma when compared with

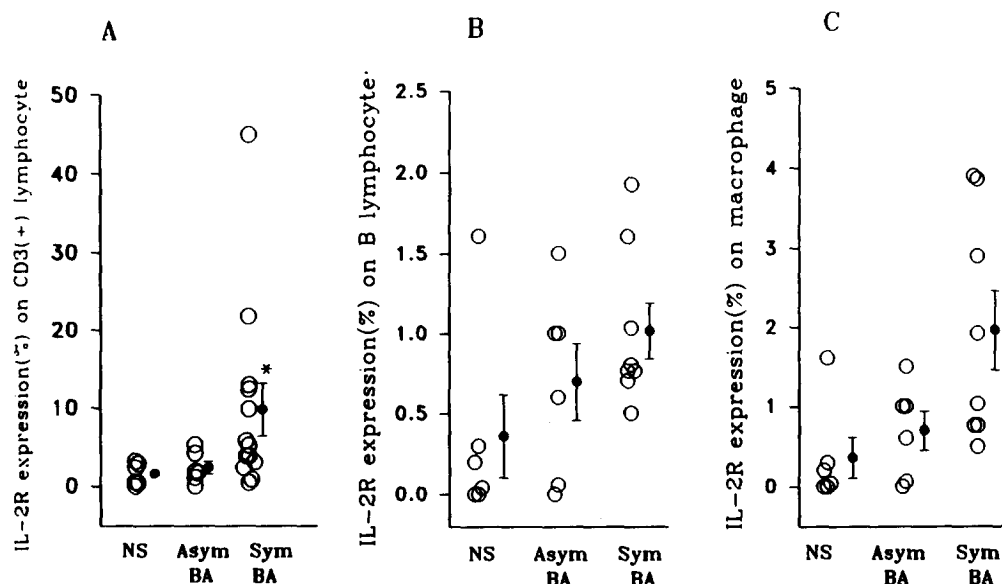


FIG. 4. Expression of IL-2 receptor on cells in BAL fluid. (A) IL-2R expressed significantly higher on CD3 positive lymphocytes in BAL fluid of symptomatic patients (Sym BA) with bronchial asthma when compared with those of normal (healthy) subject (NS) ($10.5\% \pm 1.9\%$ vs $1.9\% \pm 0.4\%$, $p < 0.01$) and the asymptomatic patients (Asym BA) with bronchial asthma ($10.5\% \pm 1.9\%$ vs $2.34\% \pm 0.74\%$, $p < 0.05$). On B lymphocytes (B) and macrophages (C) in BAL fluid there was no difference on IL-2R expression between NS (healthy subjects), the asymptomatic, and symptomatic patients with bronchial asthma.

TABLE IV. Correlation matrix of sIL-2R concentration and percentages of inflammatory cells in BAL fluid

	No.	Lymphocyte	Macrophage	Epithelial cell	Neutrophil	Eosinophil
Bronchial asthma and control	34	-0.13	-0.35‡	0.26	0.29	0.46†
Asymptomatic bronchial asthma	6	-0.51	0.30	0.32	0.60	0.45
Symptomatic bronchial asthma	19	-0.36	-0.21	0.06	0.33	0.34
Total bronchial asthma	25	-0.26	-0.23	-0.14	0.27	0.41*

Percentages of eosinophils significantly correlated with sIL-2R concentrations in BAL fluid when observed in patients with bronchial asthma (* $p < 0.05$) as well as total study subjects including bronchial asthma and healthy subjects († $p < 0.01$). Percentages of macrophages showed significantly inverse correlation with sIL-2R concentration when observed in subjects including healthy controls and bronchial asthma (‡ $p < 0.05$).

those of healthy controls ($10.5\% \pm 1.9\%$ vs $1.9 \pm 0.4\%$, $p < 0.01$) and the asymptomatic patients with bronchial asthma ($10.5\% \pm 1.9\%$ vs $2.34\% \pm 0.74\%$, $p < 0.05$; Fig. 4).

IL-2R expression tended to increase on macrophages in BAL fluid of the symptomatic patients with bronchial asthma when compared with healthy con-

trols ($1.68\% \pm 0.30\%$ vs $0.87\% \pm 0.27\%$) and the asymptomatic patients with bronchial asthma ($1.68\% \pm 0.30\%$ vs $1.01\% \pm 0.24\%$), but these differences were statistically insignificant (Fig. 4).

IL-2R expression on B lymphocytes in BAL fluid of symptomatic patients with bronchial asthma was the same as those of healthy controls ($1.13\% \pm$

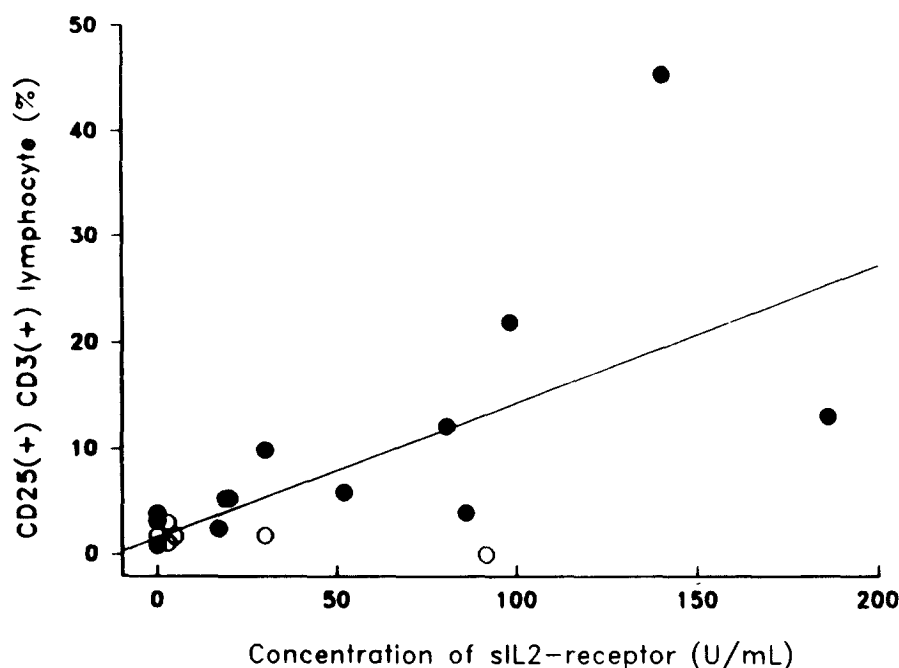


FIG. 5. Relationship between concentration of sIL-2R and percentages of IL-2R expression on T lymphocytes in BAL fluid. Concentration of sIL-2R in BAL fluid correlated with expression rate of IL-2R on CD3 positive lymphocytes when observed in patients with symptomatic bronchial asthma (closed circle) ($r_s = 0.658$, $p = 0.02$, $n = 13$) as well as all patients with bronchial asthma, including asymptomatic patients (open circle) ($r_s = 0.709$, $p = 0.007$, $n = 19$).

0.14% vs $0.88\% \pm 0.28\%$) and the asymptomatic patients with bronchial asthma ($1.13\% \pm 0.14\%$ vs $0.84\% \pm 0.28\%$; Fig. 4).

The concentration of sIL-2R in BAL fluid correlated with the expression rate of IL-2R on CD3 positive lymphocytes when observed in patients with symptomatic bronchial asthma ($r_s = 0.658$, $p = 0.02$, $n = 13$) as well as all patients with bronchial asthma, including asymptomatic patients ($r_s = 0.709$, $p = 0.007$, $n = 19$) (Fig. 5).

DISCUSSION

We have demonstrated that the concentration of sIL-2R was higher in BAL fluid from the patients with symptomatic bronchial asthma than the concentration of healthy control subjects and the patients with asymptomatic bronchial asthma. The presence of atopy did not influence the concentration of sIL-2R in symptomatic patients. This finding suggests that the enhanced T-cell activation in the bronchial tree is related to the development of symptoms in the patients with intrinsic asthma as well as patients with extrinsic bronchial asthma.

These data support the other evidence of T-cell activation within the bronchial tree of patients with bronchial asthma; a selective increase in CD4 positive lymphocytes in BAL fluid after specific antigen chal-

lenge in subjects with a late-phase asthmatic reaction,²³ and selective loss of circulating CD4 positive lymphocytes and an increase in HLA-DR antigen-positive lymphocytes in peripheral blood after an asthmatic attack induced by antigen inhalation.²⁴

The persistent elevation of IL-2R on CD3 positive lymphocytes in our study is more evidence of T-cell activation in the bronchial tree even after treatment of acute bronchospastic reaction, because the expression of IL-2R appears transiently and disappears in the absence of continuous stimulation.²⁵

sIL-2R, as an extracytoplasmic domain of cell surface IL-2R, has been reported to be elevated in a variety of diseases associated with T-cell activation, such as sarcoidosis,²⁶ allograft rejection,²⁷ lymphoreticular malignancy,^{28, 29} and one case of miliary tuberculosis in our study.

In severe bronchospastic state, concentrations of sIL-2R in serum and the percentage of IL-2R positive lymphocytes have been reported to elevate and return to normal ranges when asthmatic symptoms improved.¹⁷ However, in our study, although sIL-2R was measured within 1 to 2 weeks after treatment for acute bronchospasm, its concentration was still elevated in BAL fluid of the symptomatic bronchial asthma. This suggests the possibility that T-cell activation may last longer within the bronchial tree than in the peripheral

blood of the patients with bronchial asthma. Another possibility is that IL-2R may be locally synthesized within the bronchial tree. This is suggested by our previous observation that the permeability index of sIL-2R into the bronchial tree, calculated as the ratio of BAL-sIL-2R to BAL-protein divided by the ratio of serum-IL-2R to serum-protein, is greater than one.²⁰ sIL-2R may be cleared later from the bronchial tree than peripheral blood, which remains to be solved.

Although the function and origin of sIL-2R is unknown in BAL fluid, most of sIL-2R in BAL fluid probably originates from BAL cell surface-bound IL-2R, since sIL-2R is an extracytoplasmic domain of cell surface IL-2R.³⁰ sIL-2R was detected in BAL fluid and released by bronchoalveolar cells from the patients with sarcoidosis.²⁶ However, T cells, B cells, and macrophages express IL-2R on their surfaces.³¹ The major source of sIL-2R in BAL fluid seems to be lymphocytes in the bronchial tree from our observation that its concentrations correlated with the expression of IL-2R on CD3 positive lymphocytes in BAL fluid and IL-2R expression elevated on T lymphocytes, but not on macrophages and B lymphocytes of the symptomatic patients with bronchial asthma. This evidence can be supported by the observations that IL-2R expression increased on the surface of lymphocytes in submucosa but not on the surface of macrophage,¹⁵ and infiltrating CD4 positive T lymphocytes expressed IL-2R in the allergen-induced cutaneous late-phase reaction,³² bronchial biopsy study,¹³ and BAL analysis in atopic patients.³³

The nature of antigens that T lymphocytes recognize and are activated by is unknown within the bronchial tree, especially in patients with intrinsic bronchial asthma, because sIL-2R was elevated in BAL fluid of the symptomatic patients with intrinsic bronchial asthma. It was suggested that persistence of inhaled antigens or alteration of mucosal structure provide the activation signals to infiltrating lymphocytes.¹⁷

We have also demonstrated that sIL-2R concentration correlated with the percentages of eosinophils in BAL fluid when observed in the patients with bronchial asthma. These findings suggest that T-cell activation may have an influence on the infiltration of eosinophils into the bronchial tree of the patients with bronchial asthma.

Physiologic changes and bronchial hyperreactivity has been proved to be related to eosinophil infiltration in the bronchial tree and to the amount of major basic protein recovered in BAL fluid in other studies.^{3, 5, 6, 8} However, it has not been ruled out that other mechanisms may also play a role in the infiltration of inflammatory cells, including neutrophils

into bronchial tree leading to physiologic changes, because sIL-2R concentration and percentages of inflammatory cells in BAL fluid did not significantly correlate with physiologic changes when observed only in the patients with bronchial asthma. This discrepancy might originate from the different time of BAL procedure because the BAL was done in recovery phase of bronchospastic reaction in our study.

Of the lymphokines, IL-5 has pronounced effects on eosinophils, including prolongation of survival, enhanced adherence, and activation to the hypodense phenotype.³⁴⁻³⁶ Enhanced survival of eosinophils locally in the mucosa may be one mechanism whereby these cells accumulate in the bronchial tree of patients with bronchial asthma. This might explain the finding that sIL-2R concentration correlated with the percentage of eosinophils in BAL fluid. Activated CD4 positive T lymphocytes could initiate and propagate a selective eosinophil-rich inflammatory response presumably by IL-5 released from IL-2 stimulated lymphocytes in the bronchial mucosa and thus bring about an exacerbation of asthma symptoms.³⁷

In conclusion, the results of this study suggest that T cells are activated within the bronchial tree and the T-cell activation may have a central role in eosinophil infiltration.

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