◎原 著

Activated blood T cells in patients with bronchial asthma. Relationship to asthmatic cycle.

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Abstract : The number of $CD4^+$ T-lymphocytes, CD4/CD8 ratio, and the number of $IL2R^+$ T-lymphocytes (CD25) were examined in 14 patients with bronchial asthma, and the results were compared in three different asthma stages : symptom-free stage, wheeze s tage, and attack stage.

1. The proportion of blood $CD4^+$ T-lymphocytes was more decreased in patients with asthma attacks than in those without symptoms. The CD4/CD8 ratio was also more decreased in patients with attacks than in those without symptoms. However, these differences were not significant.

2. The proportion of blood activated T cells ($IL2R^+$ cells, CD25) was significantly increased in patients with attacks than in those without symptoms. The proportion of activated T cells in 5 asthma patients was 2.9±0.8% in symptom-free stage and 6.6± 1.1% in attack stage ($p<0.001$).

These findings show that numbers of activated T-lymphocytes in peripheral blood were increased during asthma attacks, and this increase suggests the participation of activated T cells in the pathogenesis of asthma.

Key words : $CD4^+$ T cells, CD4/CD8 ratio, $IL2R^+$ T cells, bronchial asthma

Introduction

Mechanisms causing asthma attacks are divided into two major phases : early humoral phase in which release of chemical mediators play an important role (1,2), and late cellular phase in which inflammatory cell infiltration in airways is regarded as a major factor (3−7). These two major phases can be observed as immediate asthmatic reactions (IAR) and late asthmatic reaction (LAR) after bronchial allergen challenge (8,9). Thus,

the roles of chemical mediators such as histamine and leukotrienes in the IAR and inflammatory cells such as lymphocytes, neutrophils, eosinophils, and basophils in the LAR have been extensively studied by many investigators.

Of these inflammatory cells, increasing attension has been focused on the the roles of lymphocytes and eosinophils in airways. An increased number of lymphocytes (10－12) and eosinophils (13,14) in bronchoalveolar lavage (BAL) fluid has been observed in patients with asthma, and the increase in number of lymphocytes is confined to the T cell population (15). It has been reported that increased numbers of activated blood T cells are found during acute exacerbations of bronchial asthma (16,17). It has been also found that $CD4^+$ T-lymphocytes are depleted in peripheral blood and sequestered in the lung (18,19). Moreover, a close correlation has been found between numbers of BAL CD 4^+ $IL2R^+$ T cells and numbers of eosinophils (20).

In the present study, numbers of $CD4^+$ T-lymphocytes, CD4／CD8 ratio, and numbers of $IL2R^+$ T-lymphocytes in peripheral blood were examined in patients with bronchial asthma in relation to asthmatic cycle.

Subjects and Methods

The subjects of this study were 14 patients with bronchial asthma (7 females and 7 males, mean age 60.1 years, range 46－77 years). The mean level of serum IgE was 355 IU／mℓ (range 8－2700 IU／mℓ). All the subjects were outpatients. Asthma condition of patients was divided into three stages according to their symptoms : 1) symptom-free stage, 2) wheeze stage in which patients had occasional wheezing without dyspnea, and 3) attack stage in which patients had occasional dyspnea, although they had never required any urgent treatment.

Analysis of lymphocyt subsets was performed by observing specific binding of monoclonal antibodies against CD4 (T helper／inducer), CD8 (T suppressor／cytotoxic), and CD25 (IL2R)(20). Serum samples for analysis of lymphocyte subsets were taken in different conditions of asthma, and the results were compared among three asthma stages : symptom-free stage, wheeze stage, and attack stage.

Serum levels of IgG, IgA and IgM were measured by turbidometric immunoassay. Serum IgE levels were estimated by radioimmunosorbent test (RIST), and the results were expressed as IU／mℓ.

Statistically significant differences of the mean were assessed using Student't unpaired t test. The levels of significance were expresssed as p value.

Results

The level of serum IgE in the subjects was generally low, and low serum IgE level of less than 100 IU／mℓ was found in 9 of the 14 (64.3%) patients. The level of serum IgG was also generally low : 8 of the 14 (57.18%) patients showed low serum IgG level less than 1000 mg／dl (Table 1).

The proportion of $CD4^+$ T-lymphocytes in peripheral blood was highest in patients without symptoms (41.3±8.1%) (±SD) and lowest in those with asthma attacks (35.2± 4.3%), suggesting that the proportion of CD 4^+ T-lymphocytes were decreased as symptoms of asthma were severe. However, these differences were not significant among the three different asthma stages (Fig. 1).

Table 1. Serum Immunoglobulin levels in patients with bronchial asthma

Name	Age (yr)	Sex	IgE*	IgG**	IgA**	IgM**
A.I.	45	M	78	1080	117	101
Y.K.	45	M	680	920	255	71
K.Y.	46	M	321	760	296	51
T.N.	50	F	90	1230	160	130
T.H.	54	F	120	770	331	101
H.K.	56	F	8	1200	230	66
K.O.	60	M	36	980	272	188
M.T.	61	F	14	1170	242	177
Y.H.	63	F	370	1630	187	169
K.N.	68	F	39	900	143	67
M.T.	71	M	38	870	218	138
A.S.	72	F	88	940	126	73
T.H.	74	M	63	1250	328	99
S.F.	77	M	2700	950	238	80

*Serum IgE levels, IU/ml. **Serum IgG, IgA and IgM levels, mg/dl.

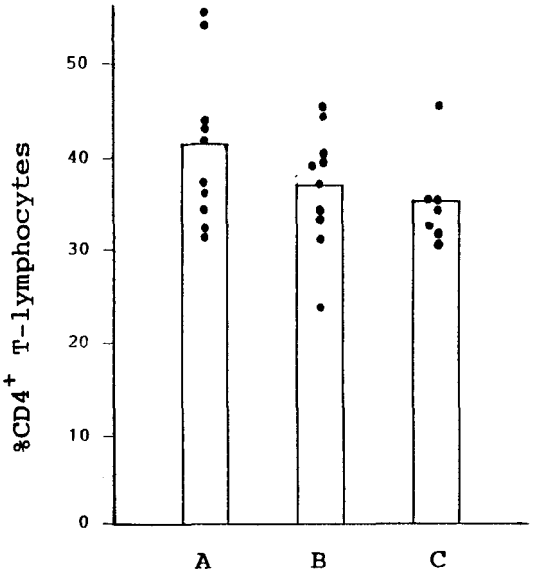

Fig. 1. The proportion of $CD4^+$ T-lymphocytes in peripheral blood of patients with bronchial asthma in relation to asthmatic cycle. Vertical columns represent the mean for each asthma stage. A : symptom-free stage ; B : wheeze stage ; C : attack stage.

The CD4/CD8 ratio was 2.4 ± 1.06 in patients without symptoms, 1.75 ± 0.69 in those with wheezes, and 1.59 ± 0.5 in those with attacks (dyspnea). The CD4/CD8 ratio was also decreased as asthma symptoms were severe, although these differences were not significant among the three stages (Fig. 2).

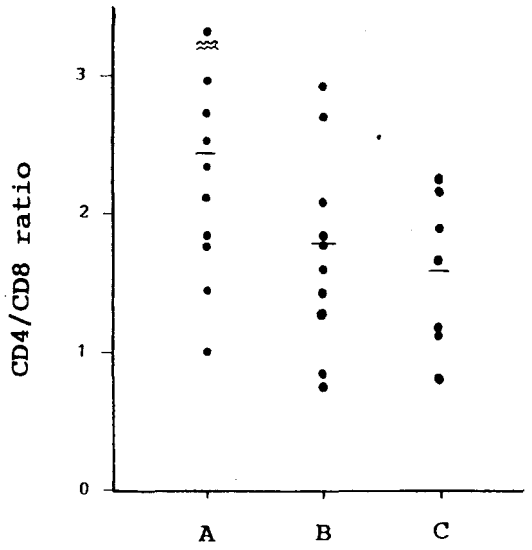

Fig. 2. CD4/CD8 ratio in patients with bronchial asthma in relation to asthmatic cycle. Vertical columns represent the mean for each asthma stage. A : symptom-free stage ; B : wheeze stage ; C : attack stage.

The proportion of $IL2R^+$ T-lymphocytes was $3.5\pm1.1\%$ in patients without symptoms, $3.8\pm1.1\%$ in those with wheezes, and $5.8\pm1.9\%$ in those with attacks, as shown in Fig. 3. The proportion of $IL2R^+$ T-lymphocytes was significantly higher in patients with attacks than in those without symptoms ($p<0.01$) or with wheezes ($p<0.02$). The proportion of $IL2R^+$T-lymphocytes was compared between the two different stages, symptom-free and attack stages of each subject with asthma. Blood samples in two different stages could be taken in 5 subjects. An increased proportion of $IL2R^+$ T cells was found in attack

stage of all 5 subjects. The proportion of IL2 R^+ T-lymphocytes in attack stage was 6.6±1. 1% in these 5 subjects, which was significantly higher than that in symptom-free stage (2.9±0.8%) ($p<0.001$) (Fig. 4).

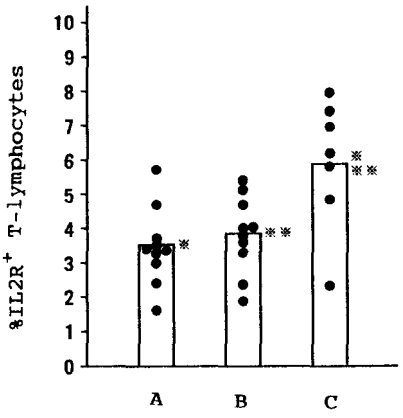

Fig. 3. The proportion of $IL2R^+$ T-lymphocytes in patients with bronchial asthma in relation to asthmatic cycle. Vertical columns represent the mean for each asthmsa stage. A : symptom-free stage ; B : wheeze stage ; C : attack stage. $^*P<0.01$; $^{**}P<0.02$.

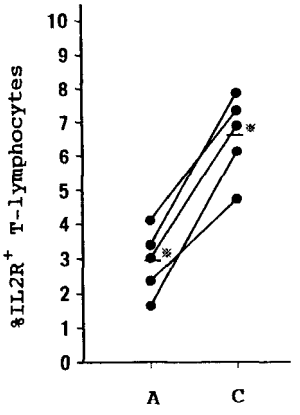

Fig. 4. The proportion of $IL2R^+$ T-lymphocytes in symptom-free stage and attack stage of each patient with asthma. A : symptom-free stage ; C : attack stage. $^*P<0.001$.

Discussion

Airway inflammation has been noted as a major factor related to the pathogenesis of asthma. The roles of various inflammatory cells have been studied by analyzing humoral an cellular events in bronchoalveolar lavage (BAL) fluid (3-7, 10-14, 21, 22). The roles of inflammatory cells can be evaluated by activation of these cells. Regarding lymphocytes in BAL fluid, increased numbers of BAL lymphocytes have been observed in patients with bronchial asthma (10-12), and this increase in BAL lymphocyte number is clarified to be due to T cell population (15). These findings suggest that T lymphocytes play an important role in the late cellular phase of asthma. Furthermore, Walker et al reproted that BAL lymphocytosis consisted of increased numbers of both $CD4^+$ and $CD8^+$ T cells, and that these T cell populations expressed elevated levels of T cell activation markers (interleukin-2 receptor : CD25, HLA-Dr, and very late activation antigen). They also concluded that there was a close correlation between numbers of BAL $CD4^+$ $IL2R^+$ T cells and numbers of BAL eosinophils, and that the numbers of activated T cells and eosinophils were related to the severity of asthma (20). The results show that activation of T lymphocytes is closely related to inflammatory cell infiltration.

Regarding blood lymphocytes, it has been reported that $CD4^+$ T-lymphocytes are decreased and sequestered in the lung after bronchial allergen challlenge (18, 19). In the present study, numbers of $CD4^+$ T-lymphocytes in peripheral blood were compared in three different asthma stages, symptom-free stage, wheeze stage, and attack stage. The results demonstrated that the proportion of

$CD4^+$ T-lymphocytes was more decreased in patients with attacks than in those without symptoms. The CD4/CD8 ratio was also decreased as the symptoms of asthma were severe. Decreased number of blood $CD4^+$ T-lymphocytes in patients with attacks seems to be in qgreement with the results reported by Gelblich et al (19). However, the difference in this study was not significant.

The proportion of activated T-lymphocytes ($IL2R^+$ cells) were significantly increased in patients with attacks than in those without symptoms. The results show that the proportion of activated T cells is increased during asthma ttacks, and this increase suggest that activated T cells participate in the pathogenesis of asthma.

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気管支喘息におげるActivated Tリンパ球について，喘息発作との関連

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気管支喘息14例を対象に，末梢血$CD4^+$リンパ球の頻度，CD4／CD8比および$IL2R^+$Tリンパ球の頻度について，喘息発作との関連のもとに検討を加えた。

1．$CD4^+$リンパ球の頻度は，非発作時の症例で最も高く，喘鳴のみの症例，発作の見られる症例へと順次低くなる傾向が見られたが，推計学的には有意の差は見られなかった。CD4／CD8比も同様に，非発作時に最も高く，発作を有する症例では非発作時に比べ低い値を示したが，推計学的には有意の差は見られなかった。

2．$IL2R^+$Tリンパ球（activated Tリンパ球）の頻度は，非発作時の症例で最も低く（3.5±1.1%），喘鳴のみをともなう症例（3.8±1.1%），発作の見られる症例（5.8±1.9%）へと順次高くなる傾向を示し，発作のある症例では，非発作および喘鳴のみの症例に比べ有意に高い値を示した。また,非発作時と発作のある時期に$IL2R^+$Tリンパ球を観察し得た５症例では，非発作時2.9±0.8%，発作のある時期6.6±1.1%であり，発作のある時期に有意の高値を示した（$P<0.001$）。

これらの結果は，発作のある時期にはactivated Tリンパ球の頻度が増加すること，そして，この増加はactivated Tリンパ球が喘息発作の病態と密接な関連を有していることを示唆するものと考えられた。

キーワード：$CD4^+$リンパ球，CD4／CD8比，$IL2R^+$Tリンパ球，気管支喘息