Enhancement of Allergen-specific Suppressor Cell Activity by T-cell Growth Factor in House-dust Sensitive Asthmatic Children*

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SUMMARY The effect of T-cell growth factor (TCGF) on the generation of allergen-specific suppressor cell activity was studied in 18 newly diagnosed and 22 hyposensitised (> 1 year) asthmatic children and 12 normal school children. All the patients were house-dust (HD) sensitive in terms of positive skin test and RAST.

Peripheral blood mononuclear cells (MNCs) were obtained and cultured in the presence or absence of HD for five days, washed and incubated in the presence or absence of Jurkat cell line-derived crude TCGF for an additional two days. The effects of the generated suppressor activities upon phytohaemagglutinin (PHA)-induced and HD-induced lymphoproliferative responses were measured.

The presence of house dust was the prerequisite for the generation of HD-specific suppressor activity; moreover, TCGF was able to increase such activity from 44.5 ± 14.6 per cent to 67.7 ± 16.6 per cent ($p < 0.02$). This kind of suppressor activity in the untreated patients was 30.6 ± 36.5 per cent which was much lower than the 70.3 ± 18.8 per cent in the treated patients ($p < 0.001$). Interestingly, the normal subjects also possessed such activity (64.8 ± 11.2%, $p < 0.004$ when compared with untreated patients). However, there was no difference in the suppressor activity on PHA-induced lymphoproliferation among the three groups studied.

These findings support the hypothesis that allergen-specific suppressor activity is generated during hyposensitisation. Furthermore, this activity can be expanded by TCGF.

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In 1976, Morgan *et al*[1] first reported that T lymphocytes could be selectively grown and maintained for more than nine months when unfractionated normal human bone marrow cells were cultured with crude PHA-stimulated lymphocyte-conditioned media. The factor present in the conditioned media and responsible for the continuous growth of T lymphocytes has been well characterised and named T-cell growth factor (TCGF) or interleukin 2 (IL 2). By using TCGF, human antigen-specific cytotoxic[2,3] and helper T-cell lines,[4] and natural killer cell lines[5] have been established and cloned. Although suppressor T-cell clones of the inducer type[6] and the effector type[7] have been established in mice, reports on the long-term culture of human antigen-specific suppressor T-cell lines are still unavailable.

Most patients with allergic diseases are characterised by elevated serum IgE and several lines of evidence obtained from both animal experiments and human diseases strongly suggest that the augmented IgE production may result from a deficit of immunoregulatory (suppressor) T cells.[8-11]

In a previous study, we reported that after *in vitro* antigen exposure, both helper (Tμ) and suppressor (Tγ) T cells were increased in atopic patients but only Tγ was increased in normal subjects.[12] In another study we found that the lymphoproliferative response to house-dust was decreased after long-term hyposensitisation in house-dust-sensitive asthmatic children.[13] More recently, we further found that hyposensitisation was able to restore the proliferative capability to phytohaemagglutinin (PHA) and depress the sensitivity to specific allergen of OKT4 helper cells on the one hand and augment the proliferative responses to both PHA and the specific allergen of OKT8 suppressor cells on the other.[14] These studies argue for the generation of allergen-specific suppressor T cells after hyposensitisation, although decre-

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ased sensitivity of helper T cells to allergen may also contribute to the decreased allergen-specific lymphoproliferative response.

To the author's knowledge, there is still no report on the effect of TCGF on the allergen-specific suppressor T cells in allergic patients. This study was conducted to explore the possible role of TCGF in the treatment of allergic diseases. The experiment was designed to demonstrate the capability of TCGF in expanding *in vitro* the allergen-specific suppressor T-cell activity in asthmatic children receiving long-term hyposensitisation. It was conducted in the hope that in the near future human allergen-specific suppressor T-cell line(s) would be established and soluble suppressor molecules produced for the treatment of patients with allergic diseases.

MATERIALS AND METHODS

Study populations

Eighteen newly diagnosed asthmatic children, 22 patients who had been hyposensitised for one to three years (average 1.9 years) and 12 healthy school children were included in this study. The ranges of age and their means for the three groups were respectively 7-16 years and 10.8 years, 8-16 years and 11.2 years, and 8-15 years and 12.1 years. The male-to-female ratio was 2:1, 2.1:1 and 2:1, respectively; the mean total serum IgE was 1,465 ± 512 IU/ml, 1,624 ± 598 IU/ml and 155 ± 48 IU/ml, respectively. All the patients were house-dust sensitive in terms of positive skin test and a 2+ or greater radioallergosorbent test (RAST). All the patients treated were considered to have had a good response as judged from the decreased frequency and shortened duration of attacks and the diminished requirement for medications. No steroids had been given for at least two weeks and bronchodilators were withheld for at least six hours before blood samplings were taken.

Preparation of house-dust allergen

The house-dust allergen was prepared by mixing house-dust materials vacuum-collected from houses of more than 10 asthmatic children and extracted according to the method of Phillips.[15] The allergen was able to elicit positive skin test in 75 per cent of asthmatic patients, detect circulating house-dust specific IgE antibody in 72.1 per cent of patients and trigger asthmatic attacks in 19.1 per cent of patients during the course of hyposensitisation.[16] The same batch was used for skin tests, preparations of allergen discs and hyposensitisation.

Source of TCGF

The TCGF-producing human leukaemia T-cell line, Jurkat-FHCRC, was a gift of Dr. R. Ting (Biotech Research Inc., Rockville, Maryland). The production of TCGF was done according to the method of Gillis *et al.*[17] The cells were maintained in complete culture medium (RPMI-1640 supplemented with 10% heat-inactivated foetal calf serum, 100 units penicillin/ml, 100 μg streptomycin/ml, 2mM L-glutamine/ml, GIBCO). For TCGF production, cells at a concentration of 1×10^6 cells/ml in RPMI-1640 containing 0.25% bovine serum albumin, 1% PHA, L-glutamine and antibiotics were cultured in a 37°C, 5% CO_2, humidified incubator for 24 hours. At the end of culture, the supernatants were collected by centrifugation for 30 minutes at 10,000 x G at 4°C. The supernatants were aliquoted and stored at -20°C in the presence of 0.1% polyethylene glycol (MW 6,000, Wako, Japan) until use.

Generation of allergen-specific, TCGF-expanded suppressor cells

The peripheral blood mononuclear cells (MNCs) were isolated by the method of Boyum.[18] Four 50-ml culture flasks (Nunclon, Denmark), each containing 10 ml of MNCs at a concentration of 1×10^6 cells/ml in complete culture medium were cultured in the presence (2 flasks) or absence (2 flasks) of house-dust allergen (50x final dilution, W/V) for five days in a 37°C, 5% CO_2, humidified incubator. (The reason why a 50x-dilution of house-dust was used in this study was because in our previous study, this magnitude of dilution was found to possess the optimal capability for stimulating the proliferation of MNCs of house-dust-sensitive asthmatic children.)[13] At the end of culture, both the house-dust stimulated and unstimulated cells were washed and resuspended in 10 ml of complete culture medium (1 flask) or 10 ml of complete culture medium containing 50% TCGF (V/V) (1 flask) and cultured for an additional two days. Finally, the viable cells in each flask were counted by the trypan blue exclusion test and then resuspended at a concentration of 1.5×10^6 viable cells/ml. The percents of cells carrying OKT3 (pan-T), OKT4 (inducer/helper T), OKT8 (cytotoxic/suppressor T) or OKIal (B cells and monocytes) antigens were counted as suggested (Orthoclone Pharmaceutic Corp., Raritan, N.J.) and had been detailed in the previous report.[19]

Assay of suppressor cell activity

Two-tenths ml of fresh autologous MNCs (1.5×10^6 cells/ml) or variously activated lymphocytes were cultured for three days (for PHA, 1%) or five days (for house-dust, 50x final dilution) in a 37°C, 5% CO_2, humidified incubator. For assay of suppressor activity, 0.1 ml of fresh autologous MNCs and 0.1 ml of activated lymphocytes were mixed and co-cultured under the same conditions described above. At the end of culture, 20 μl of RPMI-1640 containing 2 μCi of ^{3}H-thymidine (6.7 Ci/mmol, New England Nuclear, Boston, Mass.) were added. The cells were harvested the next morning by an automatic harvester (Micro-harvester, Bellco) and the level

of radioactivity was counted. Both the controls and stimulated cultures were done in triplicate. The result was calculated by the following formula:

$$\% \text{ suppression} = \frac{X-Y}{X} \times 100$$

where, X=1/2 (counts per minute (cpm) of fresh autologous MNCs + cpm of activated lymphocytes), Y = cpm of co-cultivated fresh autologous MNCs and activated lymphocytes. Therefore, a positive value means suppression and a negative value means enhancement.

Statistics

The Student's t test was used for statistical analysis throughout the study.

RESULTS

Table 1 shows the effects of house-dust and TCGF on the growth of lymphocytes in 10 house-dust-sensitive asthmatic children. At the end of one week of cultivation, the number of viable cells was greatest in the group of cells treated with both house-dust and TCGF. The two groups of patients without allergen stimulation had the lowest number of viable cells, no matter whether TCGF was added or not during the last two days of culture.

The lymphocyte subpopulations defined by monoclonal antibodies in the final cell suspensions are shown in Table 2. The group of cells not exposed to both house-dust and TCGF retained a proportion of OKT3, OKT4 and OKT8, and a T4/T8 ratio which were similar to that of normal peripheral blood. However, this group had the lowest number of viable cells. On the contrary, in the three other groups of cells, especially the group of cells exposed to both factors, not only was the number of OKT3 cells decreased, but the T4/T8 ratio was also markedly reversed. The number of OKIal (B) cells in all groups of patients was markedly decreased when compared with that of blood.

Table 3 shows the suppressor activity of variously treated lymphocytes on the house-dust induced lymphoproliferation of fresh autologous MNCs in six hyposensitised asthmatic children. Incubation of MNCs in the absence of allergen created no significant suppressor activity. On the contrary, the MNCs stimulated with only house-dust for five days were able to suppress the lymphoproliferation to a magnitude of 44.5 ± 14.6 per cent. Moreover, incubation of the house-dust activated lymphocytes with TCGF for an additional two days markedly increased their suppression capability (67.7 ± 16.0%, $p < 0.02$).

As the group of lymphocytes treated with both house-dust and TCGF not only had the greatest number of viable cells but also possessed the strongest suppressive effect, the suppressor activity of such treated lymphocytes from normal subjects, newly diagnosed and hyposensitised asthmatic children was studied; the results are presented in Table 4. When PHA was used as stimulator, the suppressor activity was not different among the three groups studied. In contrast, when house-dust induced blastogenesis was chosen as the indicator system, the suppressor activity of the new patients was much lower than that of the normal subjects (30.6 ± 36.5% vs 64.8 ± 10.6%, $P < 0.004$). However, the suppressor activity of the treated patients was much higher than that of untreated patients (70.3 ± 18.8% vs 30.6 ± 36.5%, $P < 0.001$) and even

Table 1 The effect of house-dust and TCGF on the growth of lymphocytes from house-dust sensitive asthmatic children

Group	House-dust	TCGF	No. of cells (x10^6)
I	–	–	5.43 ± 3.24*†
II	–	+	4.02 ± 2.48☆★
III	+	–	6.71 ± 4.14★
IV	+	+	9.37 ± 5.60†☆

*The starting number of cells was 10x10^6. Each figure represents the mean of 10 experiments (Mean ± SD).
† $p < 0.04$, ☆$p < 0.01$, ★$p < 0.05$

Table 2 The effects of house-dust and TCGF on the expression of T-cell surface antigens in 10 hyposensitised asthmatics

Group	House-dust	TCGF	OKT3	OKT4	OKT8	T4/T8	OKIal
I	–	–	54.9† ± 13.8	28.7 ± 3.2	20.0 ± 6.1	1.53* ± 0.51	5.8 ± 2.4
II	–	+	46.1 ± 11.1	20.5 ± 7.6	19.6 ± 5.1	1.02☆ ± 0.62	7.3 ± 3.2
III	+	–	31.0 ± 16.7	9.8 ± 5.6	12.3 ± 6.8	0.78* ± 0.40	7.2 ± 2.1
IV	+	+	35.4 ± 11.8	12.7 ± 6.9	17.8 ± 8.0	0.65*☆ ± 0.30	8.8 ± 4.1
Normal values in blood			69.0 ± 7.7	41.2 ± 6.1	23.8 ± 5.1	1.80 ± 0.41	15.6 ± 5.3

†Mean ± SD, *$p < 0.001$, ☆$p = 0.053$

Table 3 Suppressor activity of variously treated lymphocytes on the house-dust induced lymphoproliferation of fresh autologous MNCs in 6 hyposensitised asthmatic children

Exp.	MNCs	Treated lymphocytes	Co-cultivated Observed	Co-cultivated Theoretical	% change
Group I (–House-dust, – TCGF)					
1	26 532†	3 117	6 192	14 825	58
2	22 493	4 611	11 684	13 552	14
3	21 123	9 057	11 468	15 090	24
4	46 041	29 262	50 324	37 652	–34
5	27 062	3 821	11 272	15 442	27
6	27 977	4 085	17 634	16 031	–10
					13.2 ± 31.9☆
Group II (–House-dust, + TCGF)					
1	26 532	3 008	8 567	14 770	42
2	22 493	10 489	17 716	16 491	– 7
3	21 123	12 498	19 164	16 811	–14
4	46 041	18 750	26 240	32 396	19
5	27 062	2 485	11 228	14 774	24
6	27 977	5 611	13 267	16 794	21
					13.8 ± 21.3
Group III (+House-dust, – TCGF)					
1	26 532	2 626	6 704	14 579	54
2	22 493	4 621	4 338	13 557	68
3	21 123	1 258	7 364	11 191	34
4	46 041	29 374	24 217	37 708	36
5	27 062	2 317	10 429	14 689	29
6	27 977	2 807	8 312	15 392	46
					44.5 ± 14.6*
Group IV (+ House-dust, + TCGF)					
1	26 432	3 454	4 029	14 993	73
2	22 493	2 903	2 972	12 698	77
3	21 123	3 647	3 839	12 385	69
4	46 041	1 214	3 851	23 628	84
5	27 062	3 827	5 406	15 445	65
6	27 977	4 032	9 923	16 005	38
					67.7 ± 16.0*

†Counts per minute (cpm), ☆Mean ± SD, * $p < 0.02$,

Table 4 Comparison of suppressor activity generated by house-dust and TCGF treatment on house-dust-and PHA-induced lymphoproliferation of autologous MNCs among normal subjects, and newly diagnosed and hyposensitised asthmatic children

Subject	No. of cases	% suppression House-dust	% suppression PHA
Newly diagnosed	18	30.6 ± 36.5†*☆	54.8 ± 19.0
Hyposensitised	22	70.3 ± 18.8*	52.5 ± 25.7
Normal	12	64.8 ± 10.6☆	55.3 ± 15.6

† Mean ± SD, * $p < 0.001$, ☆$p < 0.004$

greater than that of the normal subjects although that difference was not significant.

DISCUSSION

Since the initial demonstration of the presence of TCGF in the supernatants of mitogen-stimulated human leukocyte cultures,[1] the cell origins and conditions required for the production of and responsiveness to TCGF have been studied extensively.[20] As with TCGF production, the activation of T cells to a TCGF-responsive state requires an initial signal supplied by mitogen/antigen-cell membrane binding and in the presence of macrophages (source of interleukin 1), resulting in the expression of TCGF receptors on the activated T-cell surfaces, the so-called TCGF-responder cells. The TCGF-responder cells then receive the second signal supplied by TCGF and continue to proliferate indefinitely. Lotze *et al*[21] clearly showed that in crude conditioned media (i.e. PHA-containing TCGF) but not partially purified media (PHA-depleted) TCGF was capable of promoting the growth of fresh human MNCs. Braciale *et al*[22] also reported the requirement for antigen stimulation to maintain the influenza virus-specific cytotoxic T-cell clones. The data shown in Table 1 (i.e. that only the lymphocytes which had been stimulated first with allergen and then cultured in the presence of TCGF could grow while the lymphocytes of other groups decreased to nearly one half of the initial number at the end of one week of culture) could be explained by the above mentioned studies.[20-22]

Rocklin *et al*[23] reported that MNCs from untreated ragweed hayfever patients were unable to exhibit suppressor activity *in vitro* after being incubated with AgE for 24-48 hours. But after the patients had been desensitised by injections of ragweed extract for 12 months, their MNCs could specifically suppress the AgE-induced lymphopro-

liferative response to a degree of 48 per cent. They therefore concluded that antigen-specific suppressor cells were generated during desensitisation to allergy. However, no normal subjects were studied in their series. The results of this study are in line with their conclusion. As shown in Table 3, MNCs from treated house-dust sensitive asthmatics produced no significant suppressor activity when they were cultured in the absence of allergen for one week, no matter whether TCGF was added (group II) or not (group I). However, they were capable of suppressing the house-dust-induced lymphoproliferative response to a magnitude of 44.5 ± 14.6 per cent which is comparable to the figure of 48.0 per cent reported by Rocklin *et al.*[23] These two studies strongly indicate that the allergen-specific suppressor cells from hyposensitised patients should be activated first by allergen to express suppressor activity. More importantly, in this study we first demonstrated that the addition of TCGF to the allergen (house-dust)-stimulated lymphocytes not only increased the number of viable cells (group IV, Table 1), but also significantly enhanced their suppressor activity (increased from 44.5 ± 14.6% to 67.7 ± 16.0%, $P < 0.02$). This result strongly suggests that in our system house-dust specific suppressor cells can be expanded *in vitro* with TCGF and human allergen specific suppressor T-cell line(s) may be established by this method. Such studies have now been carried out in our laboratory.

Monoclonal antibodies (OKT series) were used to study the surface markers of activated lymphocytes (Table 2). In the population of allergen-activated and TCGF-expanded lymphocytes, not only did a majority of viable cells lose the OKT3 antigen, but the T4/T8 ratio was also markedly reversed. The relatively highly increased proportion of OKT8 cells may be used to explain the enhanced suppressor activity for this group of cells, but the cells without detectable markers very possibly also contributed to such activity. Moretta *et al*[24] found no precise correlation between surface markers and cell function because among their eight cloned human cytotoxic T-cell lines, only one expressed OKT8 antigen, three expressed OKT4 and the remaining four expressed neither OKT4 nor OKT8, although all of them were capable of forming E-rosettes with sheep RBCs. Therefore, it will be interesting and mandatory to study those activated lymphocytes by using more recently developed monoclonal antibodies such as TQ1,[25] OKT17,[26] and 3A1[27] for the OKT4 subset and 9.3[28] for the OKT8 subset after they become commercially available.

The suppressor activity of house-dust and TCGF-treated lymphocytes obtained from normal subjects, new and hyposensitised asthmatic children were compared (Table 4). When the non-specific PHA-stimulated lymphoproliferative response was used as the indicator system, no difference in suppressor activity was found among the three groups studied. Church *et al*[29] also reported that MNCs from both atopic and nonatopic individuals could be activated by *in vitro* exposure to allergen (grass pollen) to generate suppressor activity on PHA-induced blastogenesis and that there was no difference in the magnitude of suppression between these two groups. On the contrary, marked difference in suppressor activity among normal subjects, untreated and hyposensitised patients was found when house-dust-induced lymphoproliferative response was used as the indicator system. The suppressor activity for untreated children was only 30.6 ± 36.5 per cent which was much lower than the figure of 70.3 ± 18.8 per cent for the hyposensitised ones ($p<0.001$). Interestingly, normal subjects also possessed house-dust-specific suppressor activity which was comparable to that of the treated patients but much higher than that of untreated patients (64.8 ± 10.6% vs 30.6 ± 36.5%, $p<0.004$). Thus our study is the first report to demonstrate that normal individuals also possess allergen-specific suppressor activity. The presence of allergen-specific suppressor activity in normal subjects may be accounted for by the finding of our previous study[12] and that of Ong *et al*[30] that in normal subjects only suppressor ($T\gamma$) cells were activated *in vitro* by antigen. This fact may explain why normal subjects never develop an allergy although they are exposed to the same environmental stimulation as atopic patients.

Unfortunately, only the good responders to hyposensitisation could be included in this series because it was very difficult to follow up the poor responders for more than 12 months (although much effort has been made to collect data on such cases). Therefore, it was impossible for us, just as it was for Rocklin *et al*,[23] to correlate the generation of suppressor activity with the clinical response. Moreover, study of *in vitro* IgE biosynthesis, both total and allergen-specific, demonstrated no difference between MNCs cultured alone and those cultured with activated lymphocytes (data not shown). Although this may be explained by the finding that the suppressor cells regulating antibody production may be different from those regulating cellular immune response,[31] the clinical relevance of generation of suppressor activity after hyposensitisation needs further study.

In conclusion, the data obtained in this and our previous studies[12-14] strongly support the postulation that allergen-specific suppressor cells can be generated by hyposensitisation. Moreover, as TCGF was found to be capable of expanding *in vitro* the suppressor activity, it may be used to establish human allergen-specific suppressor T-cell lines and play a role in the near future in the treatment of allergic diseases.

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