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Effect of Theophylline on the Proportion and Function of T-Suppressor Cells in Asthmatic Children

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T-cell subpopulations and their function were studied in asthmatic children with or without theophylline therapy. A decreased proportion and function of theophylline-sensitive T-suppressor cells was found in fresh peripheral blood of asthmatic children. Following peripheral blood mononuclear cells (PBMC) incubation in theophylline-free medium, the proportion of theophylline-sensitive T-cells in asthmatic children treated with theophylline was elevated to normal values. However, their suppressive effect on control lymphocyte proliferation was unchanged.

Key words: asthma; suppressor T-cells; theophylline.

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A disturbance in cell-mediated immunity, in particular with respect to T-suppressor cell activity, has been observed by several investigators in asthmatic children and adults (1, 4, 5, 12, 20). Others, however, could not detect any difference between asthmatics and healthy subjects (3, 6, 8). Theophylline, a bronchodilator widely used in the treatment of asthma, has been found to act as a modulator of immune responses, possibly via a rise in cAMP concentration (7, 11, 16) and consequent activation of T-suppressor cells (14, 17, 19).

In vitro exposure of PBMC to theophylline identifies two main T-cell subpopulations: 1) Theophylline sensitive cells, which upon exposure to theophylline, reversibly lose the characteristic ability of T-cells to form E rosettes and are considered suppressors of immune reactions (15, 25). 2) Theophylline-resistant cells, which preserve E rosette forming ability following exposure to this drug and are considered helpers of immune reactions (15, 25).

The purpose of this study was to elucidate the *in vivo* effects of theophylline administration on identification and function of T-lymphocyte subsets in asthmatic children.

PATIENTS AND METHODS

Patients

Asthmatic children between the ages of 3 and 15 years, who were healthy at the time of the test except for their atopic state, served as the study population. All patients had high levels of IgE and normal levels of IgG, IgM, and IgA. The first group comprised nine asthmatic children without theophylline treatment at the time of the test. None was on prophylactic treatment, either steroids or sodium cromoglycate, but several received occasional therapy with β_2-agonist inhalations, around the time of the test. The second group comprised 16 asthmatic children on prophylactic theophylline therapy. The duration of treatment at the time of the test was 10 to 60 days and theophylline serum levels ranged from 10-20 μg/ml. A third group of 15 nonatopic age-matched healthy children served

as controls. Informed consent was obtained from parents of all children participating in the study.

Mononuclear cell fractionation

PBMC were obtained by standard Ficoll-Hypaque (FH) density gradient separation (2).

E rosette formation

Fresh PBMC were divided into two halves. From one half of the cells T-cells were enumerated and separated by rosetting with alpha aminoethylsothinounomium bromide (AET)-treated sheep red blood cells (SRBC). Rosetted cells were isolated by centrifugation on FH gradient (18) and lysis of SRBC by hypotonic shock. The isolated T-cells were then incubated for 1 h with theophylline and the cells were again rosetted as before. Theophylline-resistant cells (which were E rosette positive following incubation with theophylline) were enumerated and separated on FH gradient from theophylline-sensitive T-cells (E rosette negative T-cells). T-cell subpopulations were further assayed. Half of the PBMC were incubated for for the night in theophylline-free medium. The next day, T-cells were separated by rosetting with SRBC, enumerated and further incubated for 1 h with theophylline. Theophylline-resistant and theophylline-sensitive T-cells were counted, isolated on FH and assayed.

Lymphocyte culture

Cocultures were performed essentially according to Kay et al. (13). Cells were cultured for 96 h in flat bottom microtiter plates (Costar, Cambridge, USA) at 37° C in an atmosphere containing 5 % CO_2 in air. The culture medium was PRMI-1640 (Beit Haemek, Israel) supplemented with 10 % FCS, 100 µg/ml penicillin and streptomycin. The cell population to be studied included fresh normal PBMC (10^6/ml) alone or combined with unseparated T-cells (T_l, 10^6/ml), theophylline-resistant T-cells (T_r, 10^6/ml) or theophylline-sensitive T-cells (T_s 10^6/ml). The unseparated T-cells (T_l) or their subpopulations (T_r and T_s) were prepared from allogeneic normal control PBMC, or from theophylline treated or untreated asthmatic children. The PBMC which served for T-cell separation were fresh or were incubated overnight in theophylline-free medium. Unseparated (T_l) and theophylline-resistant T-cells (T_r) from the three groups were treated with mitomycin C (50 µg/ml, 30 min in 37° C, 5 % CO_2) before addition to coculture. Cultures in a final volume of 0.2 ml/well were stimulated with 2 µg/PWM (Sigma). As controls, each individual cell population and each mixture was set up without mitogen and processed with the stimulated cultures throughout the experiment. Cultures were incubated for 72 h and then pulsed with 1 µci (^{3}H)-thymidine for another 24 h. Cells were then harvested on a "linca" (Israel) semi-automated harvester and counted in a Packard scintillation spectrometer. Results are reported as percent inhibition or enhancement of control PBMC proliferation by the various T-cell subsets.

In vitro *theophylline treatment*

Equal volumes of PBMC at 10^6 cells/ml and theophylline solution (5×10^{-3}M) were incubated for 1 h at 37° C and washed twice in cold PRMI-1640 medium prior to E rosette test. For testing recovery of E rosette formation following exposure to theophylline, treated cells were washed twice in cold PRMI-1640 and incubated for another 18 h with medium + 10 % FCS at 37° C and 5 % CO_2, 95 % air atmosphere, before E rosette assay. Aliquots of cells were further reincubated for 1 h with theophylline and then washed and examined for E rosette formation.

Statistical analysis

Two-way ANOVA with repeated measures was used to assess the significance of the data obtained.

RESULTS

The proportion of E rosette forming cells was measured in normals and asthmatics. Fig. 1

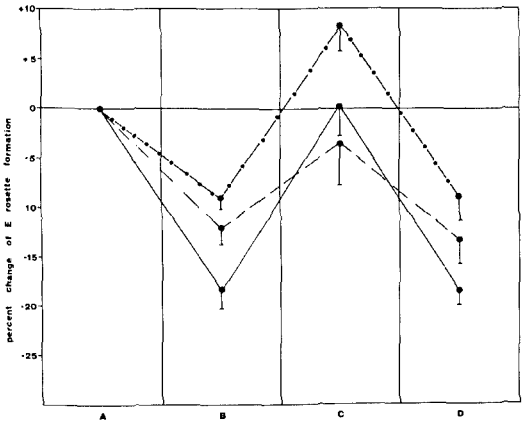

Fig. 1. E rosette formation following exposure of peripheral blood mononuclear cells to theophylline. Normal controls: ————————; asthmatic children not treated with theophylline: - - - - - - - -; asthmatic children treated with theophylline: -·-·-·-·-·-.

A: number of E rosettes in fresh peripheral mononuclear cells of controls and asthmatic children (considered 0) (rosettes of normal controls: 59 ± 2%; of asthmatic children not treated with theophylline: 57 ± 2.6%, and of asthmatic children treated with theophylline: 51.5 ± 8.3%). B: change (percent) in number of E rosettes following 1 h preincubation of mononuclear cells with theophylline (rosettes of normal controls: 40.8 ± 2.2%; of asthmatic children not treated with theophylline: 45 ± 2.8%; and of asthmatic children treated with theophylline: 44 ± 2.4%). C: change (percent) in number of E rosettes after overnight culturing of peripheral blood mononuclear cells (B) in theophylline-free medium (rosettes of normal controls: 58.3 ± 2.2%; of asthmatic children not treated with theophylline 53.1 ± 3.1%; and of asthmatic children treated with theophylline: 58.5 ± 1.7%). D: change (percent) in number of E rosettes following reincubation of cultured T-cells (C) with theophylline (rosettes for normal controls: 41.2 ± 1.4%; of asthmatic children not treated with theophylline: 43.9 ± 2.6%; and of asthmatic children treated with theophylline: 42.7 ± 1.9%).

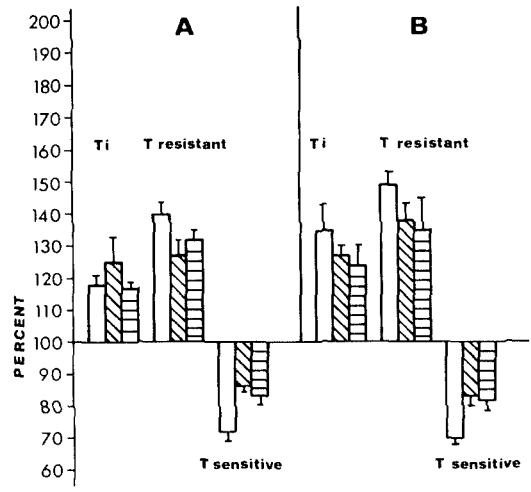

Fig. 2. Mean ± SEM% in DNA synthesis of PBMC following adddition of T-cell subgroups. T_I = unseparated T-cells, T_s = theophylline-sensitive T-cells. T_r = theophylline-resistant T-cells. Normal controls: ▭ . Asthmatics not treated with theophylline: ▨ . Asthmatics treated with theophylline: ▦ . A: T-cells derived from fresh PBMC. B: T-cells derived from overnight preincubated PBMC. The results presented are the mean ± SEM of five identical experiments. Proliferation values (in CPM) of one representative experiment are given below.

Normal PBMC: 37,588±7546

Normal PBMC added to:	T_I*	T_r*	T_s*
Control	43,719±6412	54,502±6302	26,311±4211
Theophylline-untreated	46,070±2115	48,107±9718	33,829±1079
Theophylline-treated	43.212±9085	48,928±7016	32,012±2580

*As there was no difference between the effects of fresh and incubated T_I, T_r and T_s, only the results of added fresh T-cells are given.

depicts changes in E rosette formation (A, B, C, D) following the different stages of the study. The initial values of E rosettes in fresh blood are shown in A and considered 0. Following 1 incubation with theophylline (B in Fig. 1) the three groups studied showed a decrease of E rosette formation. Percent E rosette reduction in the normal group was significantly more marked ($P < 0.01$) than that of the two asthmatic groups. Eighteen hours incubation of theophylline-treated lymphocytes in theophylline-free medium reversed this drug effect on E rosette formation in the three groups (C in Fig. 1). No difference was observed between rosetting ability of fresh vs. incubated lymphocytes from controls and untreated asthmatics, but percent E rosettes of theophylline-treated asthmatics increased significantly ($P < 0.01$) above the initial value. T-cells which were isolated after overnight incubation in theophylline-free medium were reincubated for 1 h with theophylline, and significant reduction of E rosette percent was observed in the three groups studied (D in Fig. 1). The reduction of E rosette formation in lymphocytes from theophylline-treated children was significantly more marked

($P < 0.05$) than in fresh blood, in contrast to the two other groups studied.

Functional studies of unseparated T-cells (T_t) and T-cell subpopulations (T_r, T_s) of the three groups were performed. Fig. 2 shows the mean percent change in proliferation of normal PBMC following the addition of unseparated, theophylline-resistant and theophylline-sensitive T-cells.

Unseparated T-cells and theophylline-resistant T-cells from the three groups supplied significant help ($P < 0.01$) to normal PBMC proliferation.

Normal T-cell addition caused higher PBMC proliferation than asthmatic T-cell addition, but the difference between the groups was not significant.

Theophylline-sensitive T-cells derived from all groups inhibited significantly ($P < 0.05$) normal PBMC proliferation. However, theophylline-sensitive T-cells of asthmatic children were significantly ($P < 0.01$) deficient in suppressor ability compared to those of normals.

Overnight incubation did not change the suppressor or helper ability of T-cells in the three groups.

DISCUSSION

We found a decreased proportion and number of suppressor T-cells (theophylline-sensitive) in peripheral blood of asthmatic children not treated with theophylline. In theophylline-treated asthmatics normal level of these cells was demonstrated.

Decreased proportion and number of theophylline-sensitive cells cannot be a proof of suppressor function deficiency, nor can the increment in their proportion be, by itself, an indicator for correction of suppressor activity. T-cells identified only by theophylline sensitivity, or any other single phenotypic marker, have been recently found to be heterogeneous with respect to their function (9, 23, 26). We, therefore, investigated the activity of theophylline-sensitive and theophylline-resistant T-cells of asthmatic children in a PBMC proliferation assay (13). We found significant deficient suppressor function of theophylline-sensitive T-cells in all asthmatic children. Help capability of theophylline-resistant T-cells was also reduced in asthmatic children, but the difference did not reach significant values.

Suppressor and helper T-lymphocytes were found by various authors to be affected by theophylline. Gupta demonstrated that its most pronounced effect is on Tμ cells, which are considered helpers (10, 11). Other investigators have reported an elevation of T-suppressor cell activity following theophylline treatment in renal allograft patients (22, 24) and experimental heart transplanted animals (21). In our assay, in spite of the elevation in theophylline-sensitive suppressors, there was no change in the deficient function of T-cell subpopulations derived from asthmatic children following theophylline therapy. Increase in the absolute number of suppressor cells could, however, induce *in vivo* greater overall suppression. Our data emphasize the need for functional tests in addition to phenotypic identification, as cell phenotypes do not invariably reflect their putative activity.

Another practical consequence of our findings is that *in vivo* administration of theophylline affects theophylline-sensitive T-cells, and their E rosette forming ability *in vitro* is reversibly inhibited. In order to estimate the "real" percent of E rosette forming T-cells in theophylline treated subjects, their fresh PBMC should be preincubated overnight in theophylline-free medium and then tested.

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