

β 2-agonist exerts differential effects on the development of cord blood T cells but not on peripheral blood T cells

Huang M-T, Yang Y-H, Lin Y-T, Lu M-Y, Wang L-H, Tsai M-J, Chiang B-L. β 2-agonist exerts differential effects on the development of cord blood T cells but not on peripheral blood T cells.

Pediatr Allergy Immunol 2001; 12: 17–20. © Munksgaard, 2001

Asthma is a chronic inflammatory disease characterized by reversible airway obstruction caused by edematous airway lining, thickened mucosal secretions, and smooth muscle constriction. β 2-adrenoceptor agonists are widely used in the treatment of bronchial asthma because of their ability to induce relaxation of airway smooth muscle. Evidence indicates that desensitization and down-regulation of β -adrenoceptors occurs in long-term β 2-agonist therapy, and these medications were thought to cause increased severity of, and mortality in, asthma. The purpose of this study was to delineate further the potential adverse effects of β 2-agonists on the development of T lymphocytes. T cells isolated from umbilical cord blood and adult peripheral blood were cultured in the presence of salbutamol. Intracellular staining with fluorescence-labeled antibodies was used to differentiate the frequency of type 1 T-helper (Th1) and type 2 T-helper (Th2) cells. The results showed a statistically significant inverse relationship between the concentration of salbutamol and the ratio of Th1 over Th2 on cord blood T cells. However, this trend was not observed in adult peripheral blood T cells. The data revealed another potential adverse effect in which chronic β 2-agonist exposure predisposed differentiation of T lymphocytes towards Th2 while that of Th1 was relatively suppressed, especially in cord blood T cells. Hence, β 2-agonists, despite their effect in symptomatic rescue in asthma, should not be used indiscriminately as long-term therapeutic agents.

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Key words: asthma; β 2-agonist;
 β -adrenoceptor; helper T cells; type 1
T-helper cells (Th1); type 2 T-helper cells
(Th2); pediatric

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Accepted 20 September 2000

Allergen-specific immunoglobulin E (IgE) and mast cells are the effector molecules and cells that trigger inflammatory responses in allergic diseases, but they are under the overall control of allergen-specific T cells and their cytokines (1). Both *in vivo* and *in vitro* evidence suggest that cytokines such as interleukin (IL)-4 are important for the immunoglobulin class switch from immunoglobulin M (IgM) to IgE, and IL-5 is critical for eosinophilia, which causes a greater inflammatory response during the later stages of the disease. Allergen-specific T cells derived from atopic patients are of type 2 T-helper (Th2) cell pattern; in contrast, allergen-specific T cells derived from non-atopic normals are predominantly type 1 T-helper (Th1) cells (2).

The use of adrenoceptor agonists, especially those with selective β 2 effect, have a clear role in the management of asthma. However, several reports have pointed to the side-effects brought about by their long-term usage. Most of these studies focused on the adrenoceptors and concluded that sub-sensitization, down-regulation or tachyphylaxis of the receptors can occur (3–5). A variety of circulating and tissue leucocytes were found to possess β -adrenoceptors (β -AR) on their membrane. Furthermore, an increasing number of studies suggest the existence of differential sensitivities to cAMP-mediated inhibition in Th1- and Th2-associated lymphokines. The common messenger, cAMP, participates in the shift to

Th2-like responses in individual cells, selectively blocking IL-2 induction without affecting the production of IL-4 (6,7). There is thus far no consensual explanation for the selective modulation of Th1/Th2-cell cytokine production by cAMP. Different signaling pathways between subsets with involvement of promoter/enhancer regions, and a substantially higher level of β_2 -AR expression on Th1 cells but not on Th2 cells, are both rational mechanisms (8). This study investigates in greater detail the potential adverse effect of prolonged β_2 -agonist exposure on the development of T lymphocytes.

Materials and methods

Patients, and sample collection

Mononuclear cells were isolated from the cord blood of 18 pregnancies, and from the peripheral blood of nine male and one female adults, by using a Ficoll–Hypaque gradient. None of the controls or mothers of the newborns had histories of asthma, and none was taking drugs containing catecholamines during the study. Informed consent was obtained from all study subjects. Salbutamol (Ventolin) was chosen as the β_2 -agonist in the study and was prepared at concentrations of 10^{-4} , 10^{-5} , 10^{-7} , and 10^{-9} m. IL-2, 20 ng/ml, and phytohemagglutinin (PHA), 1 μ g/ml, were added to RPMI medium supplemented with 2% human AB type serum/2% TCH (human serum replacement; Celox Laboratories Inc., Hopkins, MN, USA) for cell growth throughout the experiment. Peripheral blood mononuclear cells (PBMCs), irradiated with 4000 cGy, were used as the antigen-presenting cells (APCs).

Proliferative assay

Mononuclear cells, isolated from cord blood and peripheral blood of adults, were cultured at a concentration of 2×10^5 cells/well in 96-well plates in the presence of different concentrations of salbutamol (0 , 10^{-4} , 10^{-5} , 10^{-7} , and 10^{-9} m), with or without PHA stimulation. After 2 days, cell cultures were pulsed with 1 μ Ci of [^{3} H]thymidine and harvested 18 h later (Parkard Instrument Co., Meriden, CT, USA). The stimulation indexes [SI=c.p.m. of mononuclear cells after PHA stimulation $\div$ c.p.m. of mononuclear cells with PHA stimulation (c.p.m.=counts per minute)] of cell cultures in different concentrations of salbutamol were compared.

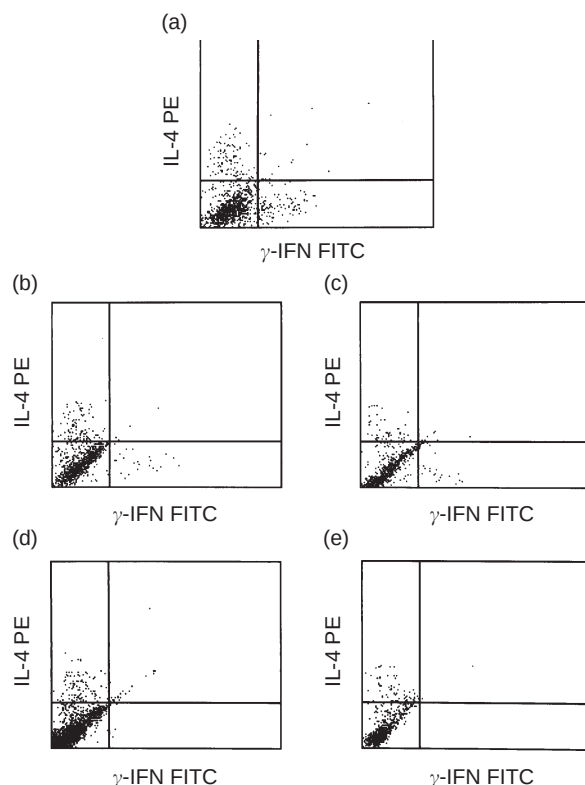


Fig. 1. The data demonstrate the distribution of T-helper 1 (Th1) and T-helper 2 (Th2) cells of cord blood T cells after culture with different concentrations of salbutamol. The concentrations of salbutamol used were as follows: (a) 0; (b) 10^{-9} m; (c) 10^{-7} m; (d) 10^{-5} m; and (e) 10^{-4} m. The percentage of Th1 cells decreased with the increase in concentration of salbutamol.

Lymphocyte culture and intracellular staining

PBMCs at a concentration of 1×10^6 /well were stimulated with PHA and IL-2 in the presence of different concentrations of salbutamol. The cells were re-stimulated after 10–14 days. At the end of the second cycle, the cells were harvested and stimulated for 12 h with phorbol 12-myristate 13-acetate (PMA) 20 ng/ml, ionomycin 1 μ m, and monensin 4 nm. These cells were then washed with phosphate-buffered saline, stained with Cy-chrome-conjugated anti-human CD4 antibody, and then fixed with 4% paraformaldehyde and permeabilized with saponin. Fluorescein isothiocyanate-conjugated anti-human IFN- γ (IFN- γ -FITC) and phycoerythrin-conjugated anti-human IL-4 (IL-4-PE) were added for staining of intracellular cytokines. The cells were suspended in 1% formaldehyde and the data were analyzed with a flow cytometer (FACScan; Becton-Dickinson, San Jose, CA, USA).

Statistical analysis

A non-parametric comparison using the Wilcoxon Signed-Ranks test was applied. A

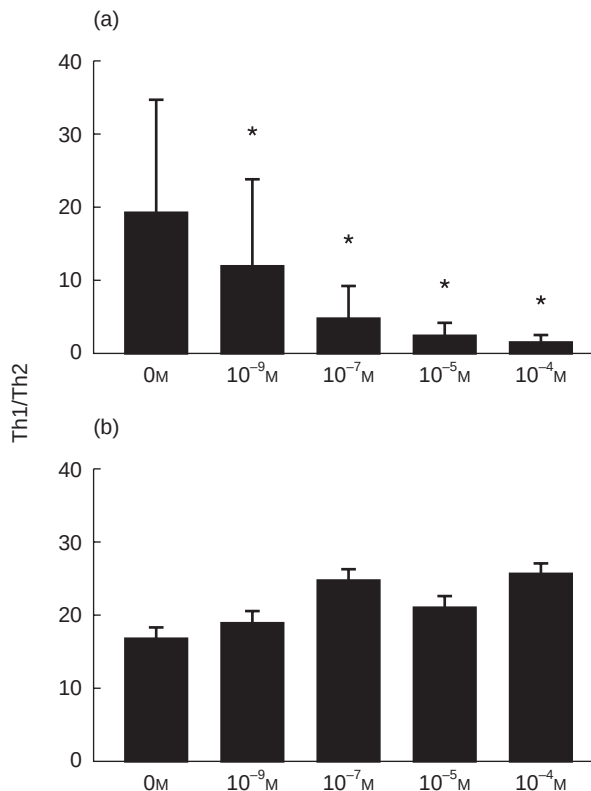


Fig. 2. The ratio of T-helper 1 (Th1)/T-helper 2 (Th2) cells after culture with different concentrations of salbutamol. (a) The presence of salbutamol affects the ratio of Th1/Th2 cells, compared with controls, in cord blood T cells; in addition, a dose-response is observed. (b) In healthy adults, salbutamol does not exert any significant effect on cultured T cells. Data is presented as mean $\pm$ SEM. * $p < 0.01$; Wilcoxon Signed-Ranks test.

p-value of less than 0.05 was considered to be statistically significant.

Results

Proliferative assay

The SI values were not statistically different between the cell cultures with different concentrations of salbutamol and those without salbutamol (cord blood, $p = 0.13$; adult peripheral blood, $p = 0.72$). The presence of salbutamol did not inhibit the response of PBMCs to stimulation by PHA.

Intracellular staining of mononuclear cells

For cord blood T cells, the ratio of Th1/Th2 cells was significantly decreased in the presence of salbutamol vs. that of T cells without salbutamol (Fig. 1). Furthermore, the effect of salbutamol on the differential development of cord blood T cells was dose dependent (Fig. 2a). However, the presence of salbutamol did not affect the ratio of

Th1/Th2 cells in adult peripheral blood T cells (Fig. 2b). Although further studies are warranted, these results suggest that β 2-agonist might have a critical effect on the naïve T cells of cord blood. Phenotypic analysis of cord blood mononuclear cells showed a predominant CD45 RA-positive staining. However, the frequency of type 0 T cells (Th0; IFN- γ^+ and IL-4 $^+$ T-helper cells) in cord blood mononuclear cells was not significantly increased (data not shown).

Discussion

The increasing prevalence of bronchial asthma has become a major health problem worldwide. The use of β 2-agonists to relieve bronchoconstriction during both acute and chronic stages of asthmatic attack has been extensively applied to asthmatic patients. However, only a few studies have been performed on the possible shortcomings of these β 2-agonists on immune effector cells. It has been demonstrated that sympathetic stimulation of the β 2-AR on lymphocytes exerted an anti-proliferative effect of these cells via activation of the paralytic pathway associated with adenylate cyclase (9–11), and sympathectomy enhances *in vitro* lymphocyte proliferation and IL-2 and IL-4 production following immunization in mice (12,13). Long-term use of β 2-agonists may thus affect the development of allergen-specific Th2 cells and result in a subsequently more severe inflammatory response.

Our data clearly demonstrated that cultured T cells from cord blood exposed to β -AR agonists fostered the development of Th2 cells, but not Th1 cells. This finding agrees with previous studies showing that activation of the β 2-AR on lymphocytes induced a shift towards a predominant Th2 pattern, and it indicates a possible risk of asthma aggravation as a result of prolonged β 2-agonist usage, by causing unbalanced Th2 activity. In the current study, the preferential effect for Th2 development was not observed in lymphocytes from healthy adults. This may reflect the fact that exposure to β 2-agonists exerts its effect more on the naive population of lymphocytes. This finding suggested that long-term use of β 2-agonist in asthmatic children, although it can relieve the symptoms of bronchoconstriction, may result in the development of an increased number of allergen-specific T cells. It will also be essential to determine whether long-term use of β 2-agonists in pregnant mothers could influence the T-cell cytokine profile of their fetus. Further studies are needed to clarify these important issues.

Despite the claim that long-acting β_2 -agonists have prophylactic and anti-inflammatory effects in the management of asthma (14), short-acting β_2 -agonists should not be over-used in situations other than acute asthmatic attacks, nor should they be prescribed for preventive purposes in the absence of adequate anti-inflammatory medication.

Acknowledgments

This study was supported by a grant from The C.-L. Chen Pediatric Research Scholarship Foundation.

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