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Journal of Asthma

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597262>

Inhibitory Activity of Fenoterol on Dermatophagoides-, Parietaria-, Tetanus-Toxoid-, and Candida albicans-Stimulated Blood Mononuclear Cells: Differences in β_2 -Adrenoreceptor Stimulation but Not in Cell Apoptosis

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Online Publication Date: 01 May 2000

To cite this Article Silvestri, Michela, Oddera, Susanna, Scarso, Lucia, Pistoia, Vito, Tasso, Paola and Rossi, Giovanni A. (2000) 'Inhibitory Activity of Fenoterol on Dermatophagoides-, Parietaria-, Tetanus-Toxoid-, and Candida albicans-Stimulated Blood Mononuclear Cells: Differences in β_2 -Adrenoreceptor Stimulation but Not in Cell Apoptosis', *Journal of Asthma*, 37:3, 281 — 290

To link to this Article: DOI: 10.3109/02770900009055451

URL: <http://dx.doi.org/10.3109/02770900009055451>

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Inhibitory Activity of Fenoterol on *Dermatophagoides*-, *Parietaria*-, Tetanus-Toxoid-, and *Candida albicans*-Stimulated Blood Mononuclear Cells: Differences in β_2 - Adrenoreceptor Stimulation but Not in Cell Apoptosis

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Key words: Mononuclear cells; Fenoterol; Allergens; Cyclic AMP; Apoptosis

ABSTRACT

β_2 -adrenoreceptor agonists have the ability to downregulate in vitro the proliferative response of peripheral blood mononuclear cells (BMCs). This activity could be related to a variety of β_2 -adrenoreceptor-mediated functions, including induction of cell apoptosis in activated T-cells. To test this hypothesis, BMCs from atopic subjects, sensitized to house dust mites (*Dermatophagoides* [*Der p*]) and/or to *Parietaria* were incubated with fenoterol (10^{-8} – 10^{-5} M) in the presence of (a) purified allergen extracts (*Der p* [5 μ g/mL] or *Parietaria* [5 μ g/mL])

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or (b) antigens (tetanus toxoid [1 $\mu\text{g}/\text{mL}$] or *Candida albicans* [5×10^5 bodies/ mL]). The BMC proliferation was assessed by [^3H] thymidine incorporation and cell apoptosis was assessed by evaluating DNA fragmentation by a fluorescence technique, using propidium iodide. In cultures stimulated with *Der p* or with *Parietaria*, fenoterol induced a dose-dependent inhibition of BMC proliferation, significant also at the lowest concentration tested (10^{-8}M) ($p < 0.05$, each comparison). In contrast, the inhibitory activity of the drug on tetanus-toxoid-stimulated BMCs was significant only at the highest dose tested (10^{-5}M) ($p < 0.05$), whereas no effect was seen when BMCs were stimulated with *C. albicans* extract ($p > 0.05$). The different inhibitory efficacy of fenoterol appeared to be related to the degree of activation of β_2 -adrenoreceptors on the different BMC populations that responded to the different stimuli. Indeed, in the presence of fenoterol (10^{-6} and 10^{-5}M), a significant increase in cyclic adenosine monophosphate (cAMP) levels was seen in *Der p*- or *Parietaria*-stimulated cells ($p < 0.05$; each comparison), but not in cell cultures stimulated with tetanus toxoid or with *C. albicans* extracts ($p > 0.05$; each comparison). Finally, the percentage of cells with fragmented DNA was lower in cultures stimulated with *Der p* or *Parietaria* than in those stimulated with tetanus toxoid or *C. albicans*, and the presence of fenoterol did not modify cell apoptosis ($p > 0.05$; each comparison). Thus, the different inhibitory activity of fenoterol on BMCs activated by allergens (*Der p* or *Parietaria*) or by antigens (tetanus toxoid or *C. albicans*) seems to be related to differences in β_2 -adrenoreceptor expression and/or function in the different antigen-specific T-cell subsets, but it is not influenced by changes in cell apoptosis.

INTRODUCTION

β -adrenergic activity plays an important role in the pathophysiology of asthma and in its pharmacological treatment (1,2). In addition to bronchorelaxation, β_2 -agonists mediate a variety of nonbronchodilator activities including inhibition of mediator release by a variety of inflammatory cells, enhancement of vascular integrity, and downregulation of cholinergic neurotransmission (1–5). Because β_2 -adrenoreceptors have been identified on many cells in the lung, including monocytes (6) and B- and T-lymphocytes (7,8), it is not surprising that these compounds may downregulate a variety of inflammatory and immuno-effector cell functions such as cytokine release by mononuclear cells, cell surface molecule expression, and T-cell proliferative response to mitogens or antigens (7–11). The mechanisms involved in β_2 -adrenoreceptor-mediated downregulation of T-cell proliferation are only partially understood but are likely to include suppression of cytokine and growth factor release, inhibition of the expression of surface

receptors involved in antigen presentation, and cell activation (8–11).

An additional mechanism by which β_2 -adrenoreceptor agonists may influence the proliferative response of T-lymphocytes is cell apoptosis or programmed cell death (12). This is a physiologic process of selective cell deletion involving endogenous endonuclease activation and differs from pathologic cell death, necrosis, by its suicidal nature and complex regulation by physiologic stimuli (13,14). Apoptosis occurs during normal cell turnover, effector cell-mediated cytotoxicity, and tumor regression, and is induced by glucocorticoid hormones, Ca^{2+} ionophores, and stimulation of the CD3/T-cell receptor (TcR) complex (12–14). This latter phenomenon has been proposed as a potential mechanism underlying the deletion of self-reactive clones during tolerance induction in the thymus (14). More recently it has been shown that agents can elevate cyclic adenosine monophosphate (cAMP) levels, induce proliferative nonresponsiveness in mature T-lymphocytes, stimulate endogenous endonuclease activation in thymocytes,

and cause DNA fragmentation (15). It has been suggested that this cAMP-mediated T-cell apoptosis could be an additional mechanism leading to T- and B-cell tolerance (16).

With this background, the present study was designed to evaluate in vitro whether the inhibitory activity of fenoterol, a short acting β_2 -adrenoceptor agonist, on the proliferative response of mononuclear cells could be associated with elevation of cAMP and enhancement of cell apoptosis. Blood mononuclear cells isolated from atopic subjects were stimulated with allergens (*Dermatophagoides* or *Parietaria*) or with tetanus toxoid or *Candida albicans* in the presence of fenoterol, and the activity of the drug on cell proliferation, cAMP production, and DNA fragmentation was measured.

MATERIALS AND METHODS

Study Population

Forty-nine atopic individuals (24 ± 3 years of age, 24 females and 25 males) were evaluated. The inclusion criteria were (a) perennial asthma, with or without rhinitis, for the last 2 years or longer; (b) sensitization to *Dermatophagoides pteronyssinus* (*Der p*) and/or *Parietaria* but not to birch pollen, demonstrated by skin prick test and radioallergosorbent test; (c) asymptomatic status at the time of the study; (d) no evidence of respiratory infections for the previous 4 weeks. None of the patients had been treated with specific immunotherapy; none was currently under antiasthmatic treatment other than β_2 -adrenoceptor agonists on an "as necessary" basis, and subjects did not take any medication in the 24 hr preceding study entry. All of the subjects were informed of the nature and the purpose of the study and gave written consent.

Collection of Peripheral Blood Mononuclear Cells (BMCs)

Heparinized blood samples were diluted with Hank's balanced salt solution (ICN Biomedicals SrL, Irvine, CA), with 0.35 g/L sodium bicarbonate and without calcium and magnesium. BMCs were isolated by Ficoll

density gradient (ICN Biomedicals), washed, counted, and resuspended at the concentration of 2×10^6 cells/mL in complete medium (RPMI-1640, supplemented with 2 g/L NaHCO_3 and 50 IU/mL penicillin plus 50 μg /mL streptomycin [ICN Biomedicals], 2 mM L-glutamine [ICN Biomedicals], 1% nonessential aminoacids [ICN Biomedicals], and 5 mM 2-mercaptoethanol [Sigma Chemical Co., St. Louis, MO] containing 10% autologous serum). Cell viability was determined by Trypan blue exclusion test (ICN Biomedicals) and was found to be >90% in all of the experimental conditions (17).

Der p and *Parietaria* Allergen Extracts

The allergen extracts used in this study were purified *Der p* or *Parietaria* powder preparation (Lopharma s.r.l., Milan, Italy) containing multiple allergenic determinants, as identified by polyacrylamide gel electrophoresis. The choice to use this mixture, instead of more purified allergen extracts containing single determinants, was due to the consideration that the repertoire of allergen-specific T-lymphocytes of each atopic patient includes different cell clones that are likely specific to different allergen determinants (18). Preliminary data established that the best allergen concentration to obtain significant and reproducible BMC proliferation to *Der p* or *Parietaria* extract was 5 μg /mL.

Preparation of *C. albicans* Extract

An overnight culture of *C. albicans* ATCC 26426 (Rockville, MD), grown in Sabouraud broth (Difco Laboratories, Detroit, MI) at 37°C in 5% CO_2 was killed by autoclaving. Preliminary dose-response curve tests demonstrated that the optimal proliferative response of BMCs to the *C. albicans* extract was obtained using the antigen at the concentration of 5×10^5 bodies/mL.

BMC Proliferation Assay

BMCs (2×10^5 cells/well) were incubated in 96-well plates at 37°C in 5% CO_2 with or without the different stimuli: (a) *Der p* extract (5 μg /mL); (b) *Parietaria* extract (5 μg /mL); (c)

tetanus toxoid (1 $\mu\text{g}/\text{mL}$); (d) *C. albicans* extract (5×10^5 bodies/ mL). Negative control was performed using purified birch extract (5 $\mu\text{g}/\text{mL}$) (Lopharma s.r.l.). To test the ability of fenoterol to inhibit BMC proliferation in response to the different allergens/antigens, the BMC cultures were studied in the presence of different concentrations of the drug (from 10^{-8} to 10^{-5} M). Fenoterol was generously provided by Boehringer Ingelheim Italia S.p.A. In preliminary sets of experiments we established that BMCs require 5–6 days to proliferate in response to *Der p*, *Parietaria*, tetanus toxoid, or *C. albicans* (data not shown). Therefore, proliferation was assessed on day 6, after the addition of tritiated [^3H] thymidine (0.5 $\mu\text{Ci}/\text{well}$) for the last 18 hr of culture. The cells were harvested with a Skatron cell harvester, and BMC proliferation was assessed by evaluation of [^3H] thymidine incorporation using a β -counter (17). Each experimental condition was set up in triplicate. Unstimulated control cultures included BMCs in complete medium.

Identification of Lymphocyte Subpopulations and Evaluation of Surface Antigen Expression by BMCs

Phenotypic characterization of T-lymphocyte subpopulations (CD4+, i.e., helper/inducer and CD8+, i.e., suppressor/cytotoxic) and the expression of IL-2 receptors (CD25) and of human leucocyte antigen-DR (HLA-DR) (major histocompatibility complex [MHC] class II antigens) surface molecules on BMCs were evaluated by specific antibody staining and flow cytometry analysis in cell cultures of 49 patients. The cells were cultured in 24-well plates as previously described; control cultures were set up. After incubation time, BMCs were trypsinized, washed, and resuspended in phosphate buffered saline (PBS, ICN Biomedicals) supplemented with 1% fetal calf serum and 0.2% sodium azide. The cells were then stained with 5 mL of different fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (mabs) recognizing (a) CD4 or CD8 surface antigens, (b) human low-affinity IL-2r (CD25), or (c) L-243 (DR) (Becton Dickinson, Mountain View, CA). In

addition, control staining with isotype MsIgG2a or MsIgG1 FITC-conjugated mabs was performed. After a 30-min incubation at 4°C , cells were washed three times with cold PBS, fixed with 0.5% paraformaldehyde, and analyzed by flow cytometry (FACS scan, Becton Dickinson) (10). Data were collected and stored in listmode files and analyzed with Lysis II software (Becton Dickinson). The expression of IL-2r and HLA-DR molecules was expressed as intensity of surface molecule expression (mfc). To compare the fluorescence intensities of different samples from the same experiments, the analyses were performed with identical settings of the logarithmic amplifier and listmode files were analyzed with Lysis II software as previously described (10,11).

Extraction and Evaluation of Intracellular cAMP Levels in BMC Cultures

Intracellular cAMP was extracted from cells by the liquid phase extraction method. The cells were cultured in 24-well plates as previously described, and after a 5–6-day incubation time, BMCs were trypsinized. Briefly, ice-cold ethanol (65%) was added to the cell suspension; after the suspension settled, the extracts were centrifuged at $2000 \times g$ for 15 min at 4°C and the supernatants were dried in a vacuum oven. The dried extracts were then dissolved in a suitable volume of assay buffer prior the measurement by enzyme immunoassay (EIA) system (Amersham International, Amersham, U.K.). The assay is based upon the competition between unlabeled cAMP and a fixed quantity of peroxidase-labeled cAMP for a limited number of binding sites on a cAMP-specific antibody. The amount of peroxidase-labeled cAMP bound to the antibody is determined by the addition of tetramethylbenzidine/hydrogen peroxide single pot substrate (19). The reaction is stopped by the addition of an acid solution, and the resultant color is read at 450 nm in a microtiter plate spectrophotometer. The sensitivity, defined as amount of cAMP needed to reduce zero dose binding by two standard deviations, is 14 pg/mL. The cross-reactivity, as determined by the concentration that produces 50% B/B_0 , is 100%. (B/B_0 is defined as [standard or sample

optical density – nonspecific binding optical density]/[zero standard optical density – nonspecific binding optical density]).

Analysis of DNA Content of Apoptotic Cells by Flow Cytometry

Briefly, cell cultures were set up in complete medium in a 24-well plate with or without the different stimuli and with or without fenoterol (from 10^{-8} to 10^{-5} M), as described above. After incubation, cells were harvested, washed, pelleted at $200 \times g$ for 5 min, and resuspended in 1.5 mL fluorochrome solution (propidium iodide [PI] 50 mg/L in 0.1% sodium citrate plus 0.1% Triton X-100) (20). After a 30-min incubation at room temperature in the dark, PI-stained cell suspensions were analyzed by flow cytometry.

Cellular apoptosis was expressed as percentage of apoptotic cells.

Statistical Analysis

Data on proliferation and cAMP levels are expressed as arithmetic mean $\pm$ standard error of the mean; data on apoptosis are given as median with lower and upper quartiles. Comparisons between values were made using Student's *t*-test or Mann-Whitney *U*-test. Analysis of variance (ANOVA) was used to compare data. The mean or median values were said to be statistically significant when the probability of the event was below 5% (i.e., $p < 0.05$).

RESULTS

BMC Proliferative Response, T-Cell Phenotype, and Activation Marker Expression

Each of the four allergens/antigens induced a significant proliferative response of peripheral blood BMCs in all of the individuals, as compared to unstimulated control cultures (1250 ± 225.7 cpm) or to cultures stimulated with a nonrelevant allergen, the purified birch allergen extract (1607.9 ± 425.7 cpm); $p < 0.01$, each comparison. The difference in

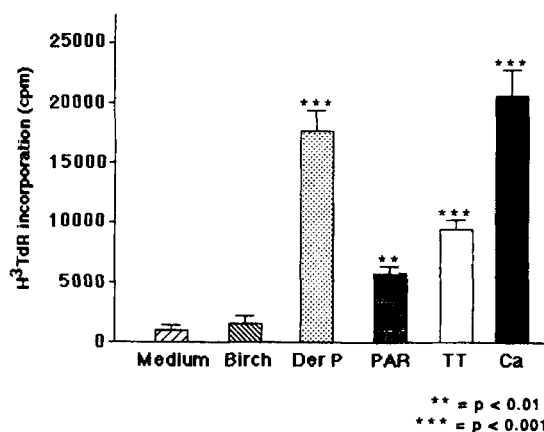


Figure 1. Proliferative response of peripheral BMCs cultured in medium alone (medium) or in the presence of birch allergen extract (Birch), *Dermatophagoides pteronyssinus* allergen extract (*Der p*), *Parietaria* (PAR), tetanus toxoid (TT), or *Candida albicans* (Ca). Mononuclear cell proliferation, measured as ^3H thymidine incorporation, is shown on the ordinate, whereas the different culture conditions are shown on the abscissa. The data represent the mean $\pm$ standard error of the experiments performed in 49 individuals. ** $p < 0.01$, *** $p < 0.001$.

the magnitude of the ^3H thymidine incorporation as compared to unstimulated control cultures was higher in cell cultures stimulated with *C. albicans* or *Der p* ($p < 0.001$), than in cell cultures stimulated with tetanus toxoid or *Parietaria* ($p < 0.01$) (Fig. 1).

Phenotypic characterization of T-cell subpopulations demonstrated that the stimulation with each of the allergens (*Der p* or *Parietaria*) or antigens (tetanus toxoid, *C. albicans*) did not change the ratio of CD4+ / CD8+ lymphocytes, suggesting that both helper/inducer and suppressor / cytotoxic lymphocytes proliferated (not shown). However, each of the stimuli significantly enhanced the activation marker expression of both IL-2r and HLA-DR on blood mononuclear cells (Table 1).

Effects of Fenoterol on BMC Proliferative Response, Phenotypic Characterization, and Activation Marker Expressions

In the cell cultures stimulated with *Der p* or *Parietaria* (the two allergen extracts), fenoterol (10^{-8} – 10^{-5} M) induced a dose-dependent inhibition of ^3H thymidine incorporation, which

Table 1. Activation Marker Expression in Allergen- or Antigen-Induced BMC Cultures

	IL-2r EXPRESSION	HLA-DR EXPRESSION
Control cultures	7.5 ± 0.1	4.0 ± 1.6
<i>Der p</i> -stimulated cultures	27.1 ± 2.2*	21.1 ± 8.2*
PAR-stimulated cultures	26.8 ± 4.9*	20.7 ± 4.1*
TT-stimulated cultures	25.5 ± 4.8*	17.8 ± 4.8*
Ca-stimulated cultures	32.5 ± 4.8*	16.8 ± 3.8*

* $p < 0.05$, as compared to control.

Der p: *Dermatophagoides pteronyssinus*.

PAR: *Parietaria*.

TT: tetanus toxoid.

Ca: *C. albicans*.

was statistically significant at all of the concentrations tested (Fig. 2). A similar dose-dependent inhibitory activity of the drug was observed in cultures stimulated with tetanus toxoid, but the statistical significance was seen only at the highest concentration tested. In contrast, no inhibition of BMC proliferation

was obtained when fenoterol was added to cell cultures stimulated with *C. albicans* ($p > 0.05$, each comparison). Finally, the CD4+/CD8+ T-cell ratios and the HLA-DR and IL-2r expression were not modified by the presence of fenoterol at all of the concentrations tested ($p > 0.05$, each comparison) (not shown). Cell viability, as assessed by trypan blue exclusion test, was not altered by any concentrations of fenoterol evaluated ($p > 0.05$) (not shown).

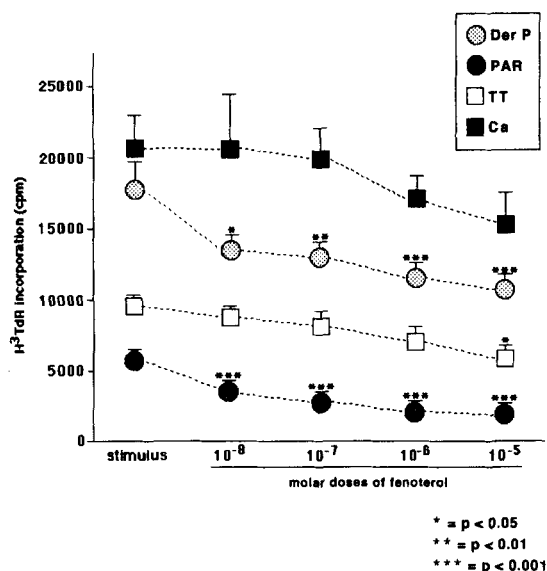


Figure 2. Effect of fenoterol on the proliferation of peripheral BMCs in response to different stimuli: *Dermatophagoides pteronyssinus* allergen extract (*Der p*), *Parietaria* (PAR), tetanus toxoid (TT), and *Candida albicans* (Ca). Mononuclear cell proliferation, measured as [³H] thymidine incorporation, is shown on the ordinate, whereas the different concentrations of fenoterol (from 10⁻⁸ to 10⁻⁵ M) are shown on the abscissa. The data represent the mean ± standard error of the experiments performed in 49 individuals. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Pharmacologic Effect on Intracellular cAMP Levels and on Cellular Apoptosis in BMC Cultures

Each of the stimuli induced a significant increase in the intracellular cAMP levels in BMC cultures, as compared to unstimulated cultures [Fig. 3(a)]. However the increase of cAMP levels as compared to unstimulated cultures was lower in cell cultures stimulated with *Parietaria* ($p < 0.05$) than in those stimulated with *Der p*, tetanus toxoid, or *C. albicans* ($p < 0.001$, each comparison). Fenoterol, at all of the concentrations tested, significantly upregulated the intracellular levels of cAMP in cultures stimulated by the allergens (i.e., *Der p* and *Parietaria*) ($p < 0.05$, each comparison), but not in those stimulated by the antigens (i.e., tetanus toxoid or *C. albicans*) ($p > 0.05$, each comparison) [Fig. 3(b)].

Finally, the percentage of cells with fragmented DNA, as an index of cell apoptosis, was lower in cultures stimulated with *Der p* or *Parietaria* than in cultures stimulated by tetanus

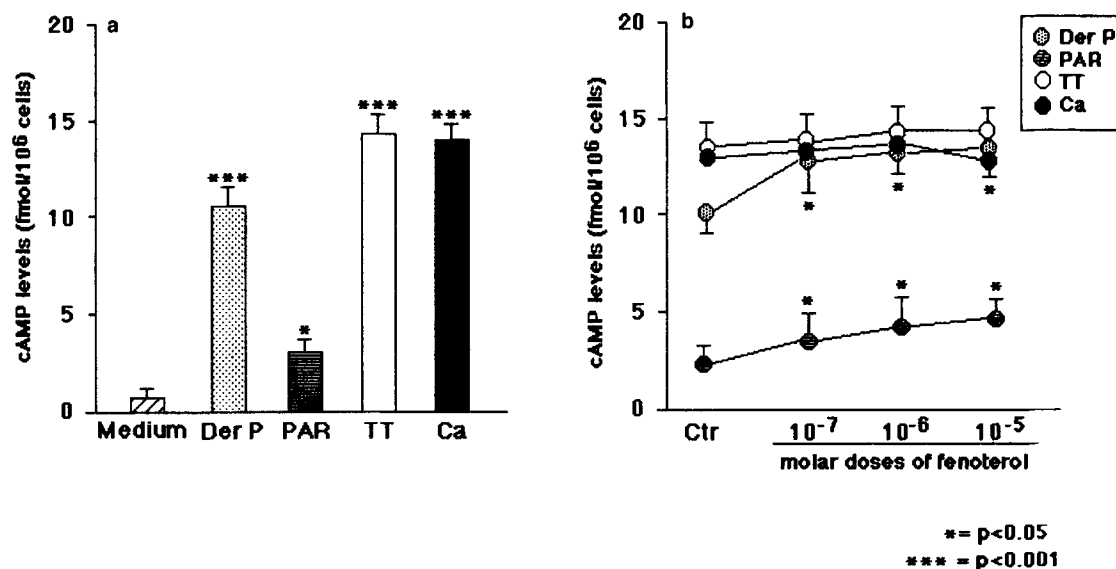


Figure 3. cAMP levels induced by different stimuli (a) and effect of fenoterol on cAMP levels in different culture conditions (b). *Der p*: *Dermatophagoides pteronyssinus* allergen extract, PAR: *Parietaria*, TT: tetanus toxoid, and Ca: *C. albicans*. cAMP levels, measured as fmol/10⁶ cells, are shown on the ordinate, whereas the different stimuli (a), or the different concentrations of fenoterol (from 10⁻⁷ to 10⁻⁵M) (b) are shown on the abscissa. The data represent the mean $\pm$ standard error of the experiments performed in 49 individuals. * $p < 0.05$, *** $p < 0.001$.

nus toxoid or *C. albicans* ($p < 0.05$, each comparison) (Table 2). However, cell apoptosis in each culture was not modified by the presence of fenoterol, even at the highest concentration used (10⁻⁵ M).

DISCUSSION

We evaluated in vitro mononuclear cells recovered from atopic patients and demonstrated a different inhibitory activity of a β_2 -adrenoreceptor agonist (fenoterol) on mononuclear cells stimulated with allergen extracts (*Der p* or *Parietaria*) or antigens (tetanus toxoid or *C. albicans*). The degree of inhibition on cell proliferation was associated to the magnitude of the β_2 -adrenoreceptor-induced increase in cAMP, but not to differences in cell apoptosis.

β_2 -adrenoceptor agonists act through interaction with specific receptors located in the plasma membranes of virtually all types of cells (1). Binding of agonists with the receptors activates signal transduction mechanisms, which produce cellular responses that may be different in different cell subpopulations (2,21,22). Although in the same subject, β_2 -ad-

renoceptors appear to be similar in the cells of different tissues (23), cellular responses are dependent on cell types and may also vary between asynchronous cells, at different stages of the cell cycle (24), or between cell subpopulations with different functions (1,2,10,24–26). In this context, there is evidence that different subpopulations of T-cells releasing different cytokines in response to the same stimulus show different sensitivity to the inhibitory activity of β_2 -adrenergic agonists (10). In the present study, we also demonstrated that mononuclear cells stimulated by *Der p* or *Parietaria* are more susceptible to the inhibitory activity of fenoterol as compared to mononuclear cells of the same subjects stimulated by tetanus toxoid or *C. albicans*.

Even if it is not clear, at present, whether inhibition of mononuclear cell activation mediated by β_2 -adrenoceptor agonists occurs only through interaction with their specific receptors on the cell surface (1,2,10), we found that fenoterol induced an increase in cAMP in cells stimulated with *Der p* or *Parietaria* (i.e., those whose proliferative response was inhibited by the drug), but not in cells stimulated by tetanus toxoid or *C. albicans*. This observation

Table 2. Cell Apoptosis in Different Culture Conditions

	PERCENT CELLS WITH FRAGMENTED DNA		<i>p</i> VALUE (COMPARISON WITH CONTROL)
	WITHOUT DRUG	WITH Fen 10 ⁻⁵ M	
Unstimulated cultures	19 (17.0–29.3)	—	—
<i>Der p</i> -stimulated cultures	25 (21.1–35.0)	24.5 (14.5–41.5)	>0.05
PAR-stimulated cultures	24 (20.0–33.1)	25.5 (19.5–32.5)	>0.05
TT-stimulated cultures	36 (29.8–47.5)	37 (30.3–54.0)	>0.05
Ca-stimulated cultures	43.5 (32.8–49.8)	47 (28.9–48.9)	>0.05

Der p: *Dermatophagoides pteronyssinus*.

PAR: *Parietaria*.

TT: tetanus toxoid.

Ca: *C. albicans*.

suggests that mononuclear cells activated by different stimuli may show a different β_2 -adrenoreceptor expression and/or function.

The mechanisms involved in β_2 -adrenoreceptor-mediated downregulation of T-cell proliferation are likely to include suppression of cytokine and growth factor release, inhibition of the expression of surface receptors involved in antigen presentation and cell activation, and possibly apoptosis (8–11). Apoptosis is a controlled biological strategy to remove unwanted cells from a given tissue, such as virus-infected or tumor cells and self-reacting hyperactivated immune cells (12). In most cellular systems apoptosis is associated with the activation of endonucleases, which cleave double-stranded DNA producing mono- and polynucleosomal DNA fragments, regarded as biochemical markers of apoptotic cells (12–14). Both intracellular and extracellular factors regulate the delicate balance between effectors and inhibitors of apoptosis with mechanisms that may be different in the different cell types. Apoptotic pathways may be activated by loss of environmental survival and growth factors or by substances activating cell surface receptors signaling cell death (14). In this context, it has been shown that agents able to elevate intracellular cAMP levels, such as

β_2 -adrenoreceptor agonists, may induce apoptotic cell death in a variety of cells, including human B- and T-lymphocytes. On T-cells, however, the effect of cAMP appears to be controversial.

In the thymus, apoptosis is involved in the selection process during T-cell maturation and differentiation to maintain self-tolerance (27). Indeed, immature thymocytes respond to β_2 -adrenergic stimulation with an increase in cAMP, which results in cell apoptosis. On the contrary, in some T-cell hybridomas, elevation of cAMP inhibits programmed cell death induced by stimulation of the T-cell receptor (28), and it was reported that β_2 -adrenergic stimulation of mature peripheral blood T-cells does not result in cell apoptosis (15). We have here shown that, despite the significant increase in cAMP levels, exposure to the β_2 -adrenoreceptor agonist fenoterol does not result in programmed cell death of peripheral blood T-cells stimulated with *Der p*, *Parietaria*, tetanus toxoid, or *C. albicans*.

In conclusion, the present report indicates that "tissue" levels of fenoterol may influence allergen-induced, but not antigen-induced, mononuclear cell activation, and that this different response to β_2 -adrenoreceptor agonists may be related to the different expression (or

function) of β_2 -receptors on the T-cell subpopulations.

The concentrations of fenoterol required to inhibit in vitro mononuclear cell proliferation can be achieved in humans at the airway level, when the drug is used by inhalation (29–32). Although these experimental results may not be clinically relevant to the regular treatment of asthmatic patients, they may possibly explain some acute clinical anti-inflammatory activity of β_2 -adrenoreceptor agonists (33,34) that is not confirmed with the regular prolonged use of these compounds (35,36).

ACKNOWLEDGMENT

This work was supported by a grant from Boheringer Ingelheim, Florence, Italy, and by a grant from "Ricerca Corrente," Ministero della Sanità, Rome, Italy.

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