

Mechanisms of allergy

β -Adrenergic modulation of human type-1/type-2 cytokine balance

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Background: Neuroendocrine mediators are increasingly recognized as immunomodulatory agents. Lymphocytes and monocytes express receptors for a variety of neuroendocrine mediators, including catecholamines. It has been reported that β -adrenergic agonists decrease IFN- γ production, with varying effects on IL-4 and IL-5 production.

Objective: Our purpose was to determine the effects of catecholamines (including β -adrenergic agonists) on the type-1/type-2 cytokine balance in tetanus-stimulated human PBMCs.

Method: Human PBMCs were stimulated with tetanus in the presence of epinephrine (EPI), norepinephrine, or terbutaline. IFN- γ , IL-4, IL-5, and IL-10 levels in the supernatants were determined by ELISA.

Results: PBMCs stimulated in the presence of EPI produced decreased levels of IFN- γ and increased levels of IL-10, IL-4, and IL-5. A small decrease in IFN- γ production and an increase in IL-10, IL-4, and IL-5 production were also observed with norepinephrine. Terbutaline induced similar alterations in the type-1/type-2 cytokine balance compared with EPI, indicating that the β_2 -adrenergic receptor is involved in these cytokine alterations. Furthermore, these cytokine alterations were blocked by propranolol. Finally, IL-12p70 prevented the cytokine alterations, suggesting that the mechanism of β -adrenergic-induced cytokine alterations involves a decrease in IL-12.

Conclusion: β -Adrenergic agonists induce a shift in the human type-1/type-2 cytokine balance toward a type-2 response.

These data provide a potential mechanism to explain the paradoxical increase in asthma morbidity and mortality associated with the chronic use of scheduled dosing of short-acting β -adrenergic agonists. (*J Allergy Clin Immunol* 1999;104:91-8.)

Key words: Type-1/type-2 cytokines, adrenergic agonist, asthma

The balance of cellular and humoral immunity by type-1 (IL-12, IFN- γ) and type-2 (IL-4, IL-5, IL-10, IL-13) cytokines is critical in the maintenance of health and the development of immune-based diseases including

Abbreviations used

APC:	Antigen-presenting cell
AR:	Adrenergic receptor
EPI:	Epinephrine
GC:	Glucocorticoid
NOR:	Norepinephrine
PROP:	Propranolol
rh:	Recombinant human
TERB:	Terbutaline

autoimmune, asthmatic, allergic, and infectious diseases.¹ This balance is largely determined by the cytokine microenvironment.² IL-12 production by antigen-presenting cells (APC) promotes the development of a type-1 response and supports cell-mediated immunity as well as complement-fixing antibodies.¹ In contrast, IL-4 promotes the development of a type-2 response and supports humoral immunity, including IgE.¹ Recent data have suggested that neuroendocrine mediators regulate the type-1/type-2 cytokine balance.³ Immune cells express receptors specific for several neuroendocrine mediators, including glucocorticoids (GC) and catecholamines.⁴ Activation of these receptors alters various aspects of the immune response, including cytokine production. For example, physiologic concentrations of GC decrease in vitro IFN- γ and increase IL-10 and IL-4 production by human PBMCs.⁵

Inhaled GC and β_2 -adrenergic receptor (β_2 AR) agonists are commonly used in the treatment of moderate to severe asthma. GC are used for their potent anti-inflammatory properties and have been reported to decrease the in situ type-2 cytokine response relative to the type-1 cytokine response in GC-sensitive patients.⁶ β_2 AR agonists are used for their bronchodilating effects and are effective for symptomatic relief of acute asthmatic attacks. However, the scheduled use of short-acting β_2 AR agonists has been challenged by reports demonstrating an increased morbidity and mortality of asthma in patients using scheduled rather than as-needed short-acting β_2 AR agonists.⁷⁻⁹ The mechanism responsible for this worsening of asthma is unknown but may involve changes in bronchiolar smooth muscle β_2 AR expression and sensitivity. Additionally, the long-term use of short-acting β_2 AR agonists may also exacerbate allergic inflammation.

Type-2 cytokines play an important role in the devel-

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opment of the inflammation associated with asthma. Type-2 cytokines, including IL-4, IL-5, and IL-13, are elevated in asthmatic subjects,^{10,11} promote the isotype switch to IgE,¹² and promote the growth and differentiation of B cells, mast cells, and eosinophils.^{12,13} β 2AR agonists have been reported to alter immune function.⁴ Several reports have demonstrated a suppressive effect of adrenergic agonists on type-1 cytokines.¹⁴⁻¹⁷ Studies on the effects of adrenergic agonists on IL-4 and IL-5 production have had varied results,^{15,17} whereas β 2AR have been consistently reported to increase the monocyte production of IL-10.^{15,18} Finally, other investigators have reported that β 2AR can enhance IgE and Fc ϵ RII expression.¹⁹ These latter reports suggest that adrenergic agonists not only influence type-1 cytokine production but may also enhance type-2 cytokine responses, thereby increasing allergic inflammation and exacerbating asthma symptoms.

To our knowledge, the ability of β -adrenergic receptor (β AR) agonists to induce a shift in the type-1/type-2 cytokine balance toward a type-2 response has not been clearly defined in human PBMCs. Therefore in the current investigation we characterized the effects of several β AR agonists on the human type-1/type-2 cytokine balance.

METHODS

Reagents

Epinephrine (EPI), norepinephrine (NOR), terbutaline (TERB), and propranolol (PROP) (Sigma, St Louis, Mo) were resuspended in RPMI-1640 (Life Technologies, Gaithersburg, Md). Recombinant human (rh) IL-12p70 (R&D Systems, Minneapolis, Minn) was resuspended in PBS. All reagents were frozen at -70°C . Fresh dilutions were made on the day of each experiment.

Isolation of PBMCs

Venous blood was obtained from healthy human volunteers (21 to 45 years old). The study was approved by the Committee for the Protection of Human Subjects at the University of Texas Houston Medical School. Informed consent was obtained before enrollment. PBMCs were isolated with use of Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation, washed twice with $\text{Ca}^{++}/\text{Mg}^{++}$ -free HBSS (Life Technologies), and resuspended in RPMI 1640 (Life Technologies) supplemented with 10% human AB serum (Biocell, Rancho Dominguez, Calif), 90 U/mL penicillin, 90 $\mu\text{g}/\text{mL}$ streptomycin, and 2 mmol/L L-glutamine (Sigma).

Tetanus cultures

PBMCs were stimulated with tetanus toxoid as previously described.⁵ Cultures of 1×10^6 PBMCs were stimulated with 0.5 $\mu\text{g}/\text{mL}$ tetanus (Pasteur Merieux Connaught Laboratories, Swiftwater, Pa) and 10 IU/mL rhIL-2 for 5 days at 37°C and 5% carbon dioxide. On day 5, 0.5 mL of supernatant was removed, replaced with media and 20 IU/mL rhIL-2 for expansion of cells, and incubated for 5 days. On day 10, 0.5 mL of supernatant was removed and replaced with media containing 10 $\mu\text{g}/\text{mL}$ PHA and 1 ng/mL phorbol myristate acetate for 24 hours. Supernatants were harvested and frozen at -70°C .

LPS cultures

Cultures of 1×10^6 PBMCs in 1 mL of media were stimulated with 5 $\mu\text{g}/\text{mL}$ LPS (Sigma) for 24 hours at 37°C and 5% carbon dioxide.⁵ Supernatants were harvested and frozen at -70°C .

Cytokine analyses

Cytokine levels in culture supernatants were determined with use of ELISA (Pharmingen, San Jose, Calif). Concentrations were calculated on the basis of standard curves with use of recombinant human cytokines (Pharmingen).

Statistical analyses

All data are displayed as mean $\pm$ SEM. Statistical analysis was performed with use of the Friedman nonparametric repeated-measures ANOVA.

RESULTS

Adrenergic agonists shift the type-1/type-2 cytokine balance toward a type-2 response

To determine the effect of adrenergic agonists on PBMC production of type-1/type-2 cytokine production, PBMCs were stimulated with tetanus in the presence or absence of EPI (10^{-9} to 10^{-6} mol/L). EPI caused a dose-dependent decrease in IFN- γ production ($P = .001$) (Fig 1). PBMCs stimulated in the presence of EPI produced elevated levels of the type-2 cytokines IL-10 ($P = .0004$), IL-4 ($P = .0004$), and IL-5 ($P = .0034$). The decrease in the type-1/type-2 cytokine ratio²⁰ (IFN- γ /IL-10, IFN- γ /IL-4, IFN- γ /IL-5) further demonstrates a shift in the type-1/type-2 cytokine balance toward a predominant type-2 response by EPI (Table I).

Unlike EPI, NOR has both α - and β -adrenergic agonist activity with a higher affinity for α receptors. To further characterize the ability of adrenergic agonists to modulate type-1/type-2 cytokines, PBMCs were stimulated with tetanus in the presence of NOR (10^{-9} to 10^{-6} mol/L). IFN- γ production was slightly decreased and IL-10 production was slightly increased, but neither was statistically significant (data not shown). PBMCs stimulated in the presence of NOR produced significantly increased levels of IL-4 ($P = .0084$) and IL-5 ($P = .0002$), thus decreasing the IFN- γ /IL-4 and IFN- γ /IL-5 ratios (Table I). Therefore NOR shifted in the type-1/type-2 cytokine balance toward a type-2 response primarily by increasing the production of type-2 cytokines.

PROP blocks the type-2 cytokine shift by EPI and NOR

To confirm that these cytokine alterations are mediated through the β AR, PBMCs were stimulated in the presence of EPI or NOR with and without 10^{-6} mol/L PROP, a β_1/β_2 -adrenergic antagonist. PROP was added to PBMCs 1 hour before the addition of EPI or NOR. PROP blocked the EPI-induced changes in IFN- γ , IL-10, IL-4, and IL-5 production (Fig 2). PROP also blocked the NOR-induced alterations in IL-4 and IL-5. These data indicate that the shift in the type-1/type-2 cytokine balance by EPI and NOR is mediated through the β AR.

TERB shifts the type-1/type-2 cytokine balance toward a type-2 response.

The predominant β AR expressed on lymphocytes and monocytes is the β_2 -adrenergic receptor.^{21,22} Therefore it was of interest to determine whether β 2AR agonists

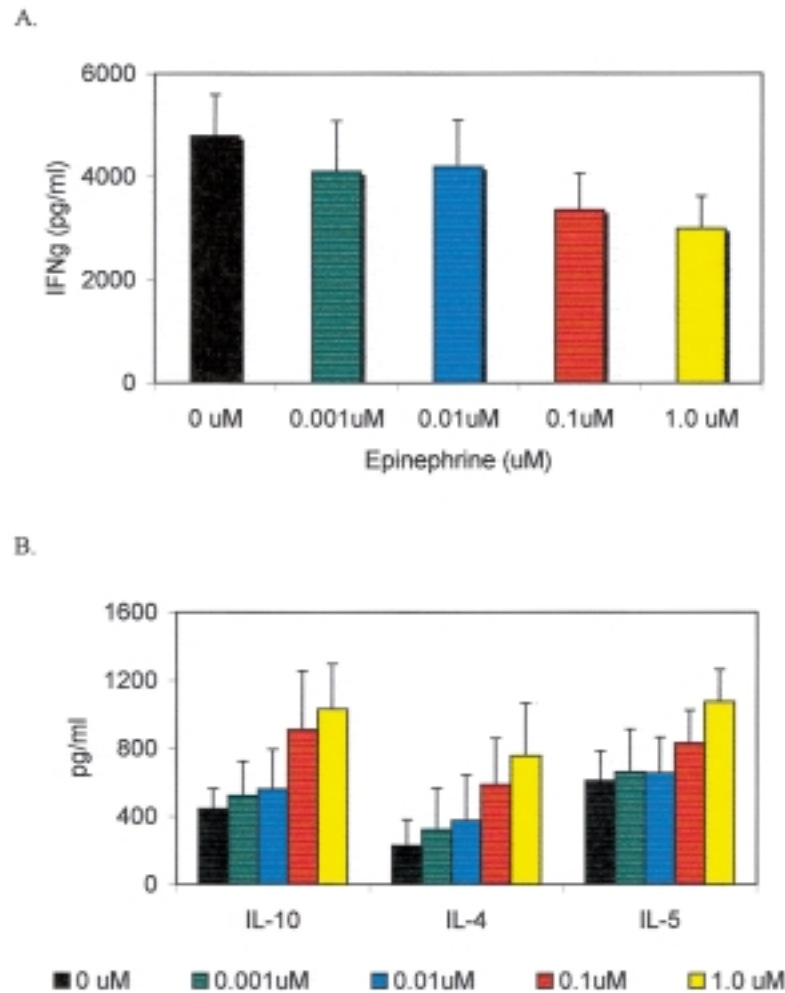


FIG 1. Effect of EPI on type-1/type-2 cytokine production. Human PBMCs were stimulated with tetanus with EPI. Stimulation of PBMCs with EPI (**A**) decreased IFN- γ production ($P = .001$) and (**B**) increased IL-10 ($P = .0004$), IL-4 ($P = .0004$), and IL-5 ($P = .003$); $n = 10$ subjects.

TABLE I. Effect of adrenergic agonists on type-1/type-2 cytokine ratios of tetanus-stimulated PBMCs

		Control	10^{-9} mol/L	10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
EPI	IFN- γ /IL-10*	31 $\pm$ 13	35 $\pm$ 15	26 $\pm$ 11	9 $\pm$ 3	5 $\pm$ 1
	IFN- γ /IL-4*	110 $\pm$ 30	128 $\pm$ 58	118 $\pm$ 66	38 $\pm$ 18	15 $\pm$ 5
	IFN- γ /IL-5*	14 $\pm$ 5	14 $\pm$ 3	11 $\pm$ 3	6 $\pm$ 2	4 $\pm$ 1
NOR	IFN- γ /IL-10†	32 $\pm$ 15	27 $\pm$ 10	24 $\pm$ 12	37 $\pm$ 22	12 $\pm$ 4
	IFN- γ /IL-4‡	110 $\pm$ 34	143 $\pm$ 57	158 $\pm$ 82	112 $\pm$ 63	89 $\pm$ 62
	IFN- γ /IL-5‡	16 $\pm$ 5	15 $\pm$ 3	15 $\pm$ 3	12 $\pm$ 3	6 $\pm$ 2
TERB	IFN- γ /IL-10*	139 $\pm$ 40	77 $\pm$ 26	80 $\pm$ 24	53 $\pm$ 17	47 $\pm$ 20
	IFN- γ /IL-4†	201 $\pm$ 52	142 $\pm$ 56	147 $\pm$ 46	147 $\pm$ 71	118 $\pm$ 50
	IFN- γ /IL-5*	5276 $\pm$ 2798	2153 $\pm$ 1287	2301 $\pm$ 1242	1171 $\pm$ 796	1036 $\pm$ 808
	IL-12p40/IL-10§	0.73 $\pm$ 0.38	0.65 $\pm$ 0.26	0.46 $\pm$ 0.26	0.55 $\pm$ 0.3	0.25 $\pm$ 0.12

* $P < .0001$.

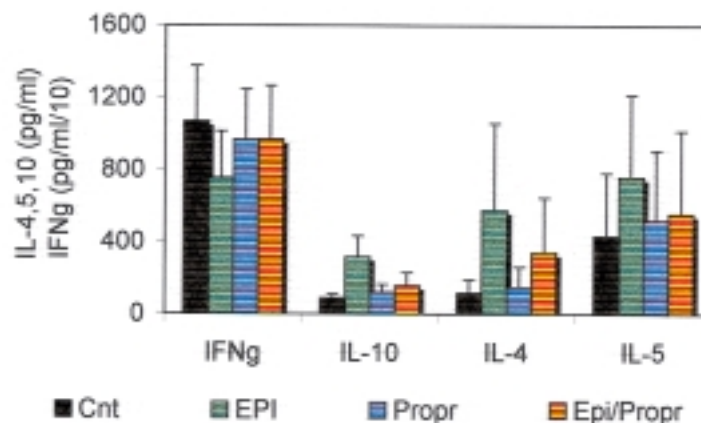
† $P < .01$.

‡ $P < 0.001$.

§ $P < .05$.

||LPS stimulation.

A.



B.

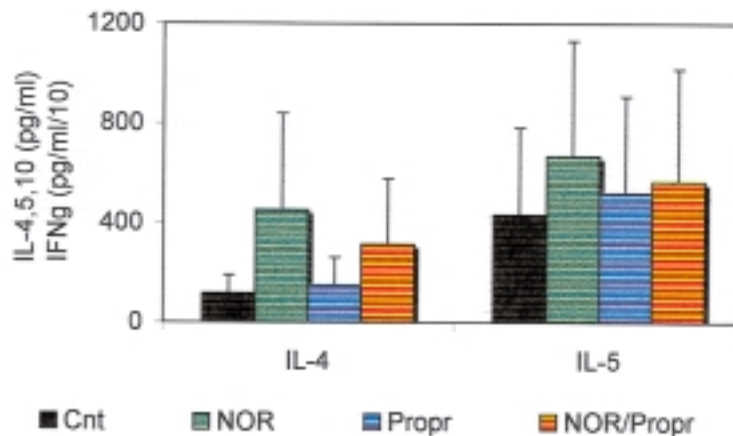


FIG 2. PROP blocks cytokine alterations by EPI and NOR. PBMCs were preincubated with 10^{-6} mol/L PROP for 1 hour and then stimulated with tetanus in presence of 10^{-6} mol/L EPI or 10^{-6} mol/L NOR. PROP blocked alterations in IFN- γ , IL-10, IL-4, and IL-5 induced by EPI and alterations in IL-4 and IL-5 induced by NOR. $n = 8$ subjects.

induce a shift toward a type-2 cytokine response similar to physiologic adrenergic agonists. PBMCs were stimulated with tetanus with and without TERB (10^{-9} to 10^{-6} mol/L), a selective β 2AR agonist. TERB induced a decrease in IFN- γ ($P = .002$) and an increase in IL-10 ($P = .0005$), IL-4 ($P = .048$), and IL-5 ($P = .011$) production (Fig 3). The decrease in the IFN- γ /IL-10,4,5 ratio further indicates a shift in the type-1/type-2 cytokine balance toward a type-2 response (Table I).

To confirm that TERB altered the type-1/type-2 cytokine balance through the β 2AR, PBMCs were stimulated in the presence of TERB with and without PROP (Fig 4). PROP prevented the TERB-induced alterations in both type-1 and type-2 cytokines. Therefore the TERB-induced shift in the type-1/type-2 cytokine balance is mediated through the β 2AR.

IL-12 prevents the β 2AR-induced changes in type-1/type-2 cytokines

The development of a type-1 response is highly dependent on the production of IL-12 by the APC.²³ Previous reports have demonstrated that β 2AR agonist inhibits IL-12 production by human monocytes.^{15,17} Consistent with previous reports, TERB decreased the production of IL-12p40 by LPS-stimulated PBMCs ($P = .0003$) (Fig 5). In addition, there was a slight increase in IL-10 at low concentrations of TERB but a slight decrease in IL-10 at higher doses ($P > .05$). The net effect on the type-1/type-2 cytokine ratio was a shift toward a type-2 cytokine response (Table I).

To determine whether the TERB-induced inhibition of IL-12 contributes to the β 2AR agonist-induced cytokine

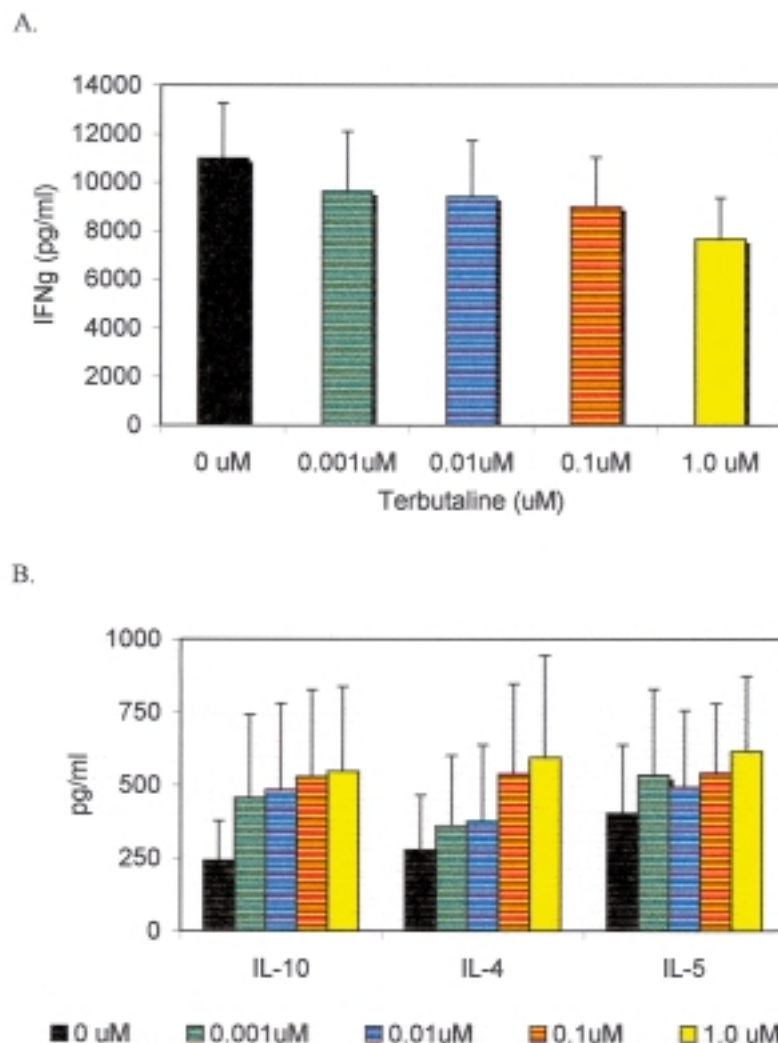


FIG 3. Effect of terbutaline on type-1/type-2 cytokine production. Human PBMCs were stimulated with tetanus with TERB. Stimulation of PBMCs with TERB (**A**) decreased IFN- γ production ($P = .002$) and (**B**) increased IL-10 ($P = .0005$), IL-4 ($P = .0048$), and IL-5 ($P = .011$). $n = 7$ subjects.

alterations in tetanus-stimulated PBMCs, rhIL-12p70 was added to tetanus cultures (Fig 6). The TERB-induced decrease in IFN- γ or increase in IL-4 and IL-5 was not observed in cultures containing both rhIL-12p70 and TERB. However, coincubation of TERB and rhIL-12p70 further increased IL-10 production compared with TERB alone and with control. This is likely a result of the ability of IL-12p70 to directly increase IL-10 production.²⁴ These data suggest that the β 2AR agonist-induced alterations in the type-1/type-2 cytokine balance are mediated by a decrease in IL-12.

DISCUSSION

In the current investigation we demonstrated that β 2AR agonists shift the *in vitro* type-1/type-2 cytokine balance of human PBMCs toward a predominant type-2 response. EPI, a mixed adrenergic agonist with a higher

affinity for β - compared with α -adrenergic receptors, decreased IFN- γ production and increased IL-10, IL-4, and IL-5 production by tetanus-stimulated PBMCs. NOR, a mixed adrenergic agonist with a higher affinity for α AR compared with β AR, increased IL-4 and IL-5 production with mild effects on IFN- γ and IL-10 production by T lymphocytes. The cytokine alterations could be blocked by PROP, indicating that these effects were mediated through β AR. Furthermore, TERB, a selective β 2AR agonist, decreased type-1 cytokine production and increased type-2 cytokine production by T-lymphocytes. Therefore the type-1/type-2 cytokine balance of PBMCs stimulated with tetanus, a monocyte-dependent T-lymphocyte stimulus, in the presence of adrenergic agonists is shifted toward a type-2 response primarily through the β 2AR. Furthermore, PBMCs stimulated with LPS, a monocyte stimulus, in the presence of TERB produced less IL-12p40. Finally, rhIL-12p70 prevented the

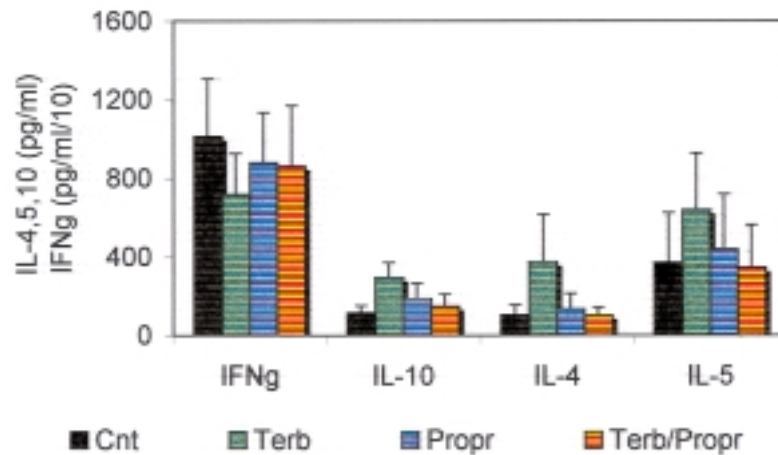


FIG 4. PROP blocks cytokine alterations by TERB. PBMCs were preincubated with 10^{-6} mol/L PROP for 1 hour and then stimulated with tetanus in presence of 10^{-6} mol/L TERB. PROP blocked alterations in IFN- γ , IL-10, IL-4, and IL-5 induced by TERB. $n = 10$ subjects.

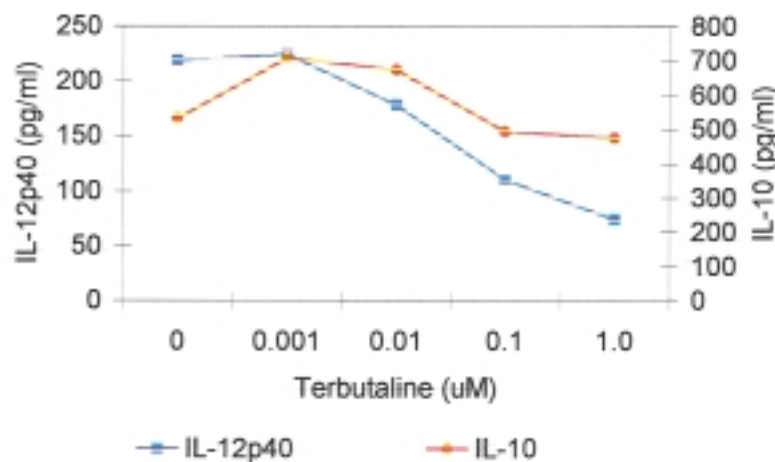


FIG 5. Effect of TERB LPS-stimulated cytokine production. Human PBMCs were stimulated with LPS with TERB. LPS stimulation of PBMCs with TERB decreased IL-12p40 production ($P = .0003$) and slightly decreased IL-10 ($P > .05$). $n = 5$ subjects.

cytokine alterations in tetanus-stimulated PBMCs, further delineating the mechanism to involve the APC.

These findings are consistent with previous reports demonstrating that the predominant adrenergic receptor on PBMCs is the β 2AR.^{21,22,25} Previous reports demonstrating a modulation of cytokine production by adrenergic agonists have also shown that the β 2AR, not the α AR, is the principal receptor involved.²⁶ This is supported by the data in which NOR induced a smaller change in cytokine production compared with EPI or TERB. These changes were also mediated through the β -adrenergic receptor because PROP blocked the increase in IL-4 and IL-5. The decreased type-2 cytokine shift is likely a result of the weaker affinity of NOR for the β AR agonist compared with EPI and TERB. However, α AR binding may partially prevent the β -adrenergic-mediated type-2 cytokine shift. The effect of α AR agonists on the type-1/type-2 cytokine balance is currently under investigation.

Previous reports have focused on the ability of adrenergic agonist to modulate human monokine (IL-12, IL-10) production.^{15,17} However, β 2ARs have been identified on both human lymphocytes and monocytes.⁴ With regard to T-cell cytokine production, adrenergic agonists suppress IFN- γ production and increase IL-10 production with varying effects on IL-4 and IL-5.^{14,16,17} Consistent with our data, adrenergic agonists have also been found to potentiate in vitro IL-4-stimulated IgE production as well as CD23 and Fc ϵ R2 expression.^{19,27,28} Our data add to those of previous reports¹⁷ by demonstrating that the type-2 cytokine shift is, at least in part, mediated through an inhibition of IL-12 production by APCs. Unlike our data, some reports have demonstrated either no direct effect on or a suppression of IL-4 and IL-5.¹⁴ This difference may be in part the result of the culture conditions. Previous reports used either short-term stimulation of PBMCs or T-cell clones. Our culture conditions are

designed to represent the physiologic activation of T cells and allows for better sensitivity to detect changes in type-2 cytokine production compared with short-term in vitro stimulation.^{29,30}

Binding of the β 2AR modulated the type-1/type-2 cytokine balance in human PBMCs stimulated by either a monocyte-dependent T-cell stimulus (tetanus) or a monocyte stimulus (LPS). The addition of IL-12p70 to tetanus-stimulated PBMC cultures prevented the TERB-induced type-1/type-2 cytokine alterations, suggesting that the effect of β 2AR agonists is, at least in part, mediated through inhibition of the ability of the monocyte to promote a type-1 cytokine production in the T cell. This is similar to what has previously been reported to occur in the GC-induced type-1/type-2 cytokine alterations.³¹ Therefore the interaction of the APC and the T cell is likely involved in these cytokine alterations. However, more recent reports have suggested that the β 2ARs are selectively expressed on T_H1 clones but not on T_H2 clones.^{22,26} Activation of β 2AR on T_H1 cells could directly inhibit type-1 cytokine production. Because type-1 cytokines directly inhibit type-2 cytokine production, the decrease in IFN- γ could allow unopposed cytokine production by type-2 cells. Whether these cytokine alterations are directly dependent on the decrease in T-cell IFN- γ production or the decrease in APC production of IL-12 deserves additional investigation.

Current guidelines for the therapy of asthma recommend that short-acting β 2AR agonists should be used as an as-needed supplement to inhaled GC therapy. Acute asthma symptoms usually respond well to short-acting β 2AR agonists, but their use to control the disease is not recommended. The long-term use of short-acting β 2AR agonists has been associated with increased morbidity and mortality.^{8,9} The mechanisms of this paradoxical increase in asthma severity are unknown but are believed to involve changes in smooth muscle β 2AR expression and sensitivity. Our data suggest that β 2AR agonists can promote type-2 cytokine production and thus may be an additional mechanism contributing to the deterioration of asthma control associated with the long-term use of short-acting β 2AR agonists. Type-2 cytokines are important in the development of the inflammatory response associated with asthma. PBMCs,¹⁰ bronchial biopsy specimens,¹⁰ and bronchial alveolar lavage fluid¹¹ from asthmatic subjects all demonstrate increased expression of IL-4, IL-5, and IL-13. IL-4 is an important B-cell and mast cell growth factor, promotes the isotype switch to IgE, and increases expression of proinflammatory adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and very late activation antigen-4.¹² IL-5 is important in the development of eosinophilia, serving to recruit and differentiate eosinophils.¹³ Finally, treatment of GC-sensitive asthma with inhaled GC decreases the levels of type-2 cytokines expressed in BAL.⁶ Therefore the observed increase in IL-4 and IL-5 production resulting from β 2AR agonists may result in an exacerbation allergic inflammation by

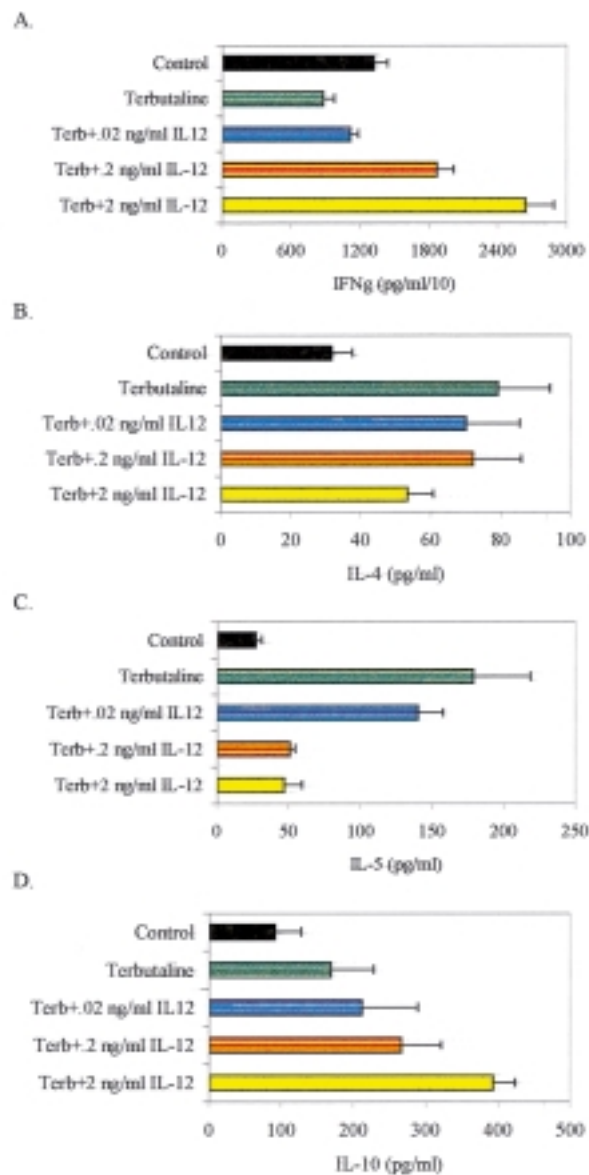


FIG 6. IL-12 prevents cytokine alterations by TERB. PBMCs were stimulated with tetanus in presence of 10^{-6} mol/L TERB $\pm$ rhIL-12p70 (0.02-2.0 ng/mL). IL-12p70 prevented TERB-induced decrease in IFN- γ (A) and increase in IL-4 (B) and IL-5 (C). However, IL-12p70 did not prevent TERB-induced increase in IL-10 (D). n = 4 subjects.

increasing IgE production, mast cell activity, eosinophil recruitment, and activity, as well as adhesion molecule expression on inflammatory and endothelial cells. These changes could contribute to the deterioration of asthma control because of long-term use of scheduled short-acting β 2AR agonists, particularly as a single agent.

In summary, these data demonstrate that adrenergic agonists, EPI, NOR, and TERB, induce a shift in the type-1/type-2 cytokine balance toward a type-2 response. These effects are mediated through β 2AR and are prevented by coincubation with rhIL-12p70. Similar cytokine alterations may in part contribute to the exacer-

bation of asthma observed in some patients taking long-term short-acting β_2 -adrenergic agonists. Future studies will seek to establish the in vivo relevance of these findings in patients with asthma and to determine whether the β_2 AR-mediated type-1/type-2 cytokine alterations are involved in other immune-based diseases and physiologic states.

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