

Interactive effects of steroids and β -agonists on accumulation of type 2 T cells

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Background: Airway inflammation associated with asthma has been reported to be either unaffected or slightly increased by inhaled β -agonist monotherapy, and concerns regarding disease exacerbations with continuous long-acting β -agonist monotherapy have prompted the recommendation of concomitant steroid treatment.

Objectives: Using peripheral blood lymphocytes from asthmatic subjects, we aimed to determine (1) whether short- or long-acting β -agonists increase IL-13-producing (IL-13⁺) or IFN- γ -producing (IFN- γ ⁺) T-cell numbers and (2) the ability of the corticosteroid budesonide to reverse these effects.

Methods: Peripheral blood lymphocytes from asthmatic subjects were cultured 6 days *ex vivo* with IL-2 and various concentrations of albuterol, formoterol, and budesonide. Numbers of IL-13⁺ and IFN- γ ⁺ T cells were determined by means of flow cytometric analysis.

Results: Both albuterol and formoterol increased IL-2-stimulated accumulation of IL-13⁺ T cells, and this increase was highest at concentrations approximating the dissociation constant of each β -agonist for the β_2 -adrenergic receptor. Budesonide at greater than 1 nmol/L reversed the augmenting effects of β -agonists on IL-13⁺ T-cell accumulation, and budesonide at greater than 10 nmol/L inhibited increases in IL-13⁺ T cells stimulated by IL-2. Budesonide decreased, whereas β -agonist did not affect, numbers of total and IFN- γ ⁺ T cells in IL-2-stimulated cultures.

Conclusion: β -Agonists at physiologically and clinically relevant concentrations stimulate increased antigen-independent, cytokine-stimulated accumulation, specifically of type 2 T cells from asthmatic subjects. The corticosteroid budesonide potently reverses this effect.

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Key words: Human, T cells, β -adrenergic receptor, β -agonist, asthma, IL-13, glucocorticosteroid, steroid, IL-2

The β_2 -adrenergic receptor (β_2 AR) mediates the effects of inhaled β -agonists and regulates numerous functions in multiple airway cell types. The most obvious and well-characterized effects of β_2 AR activation through inhaled β -agonists are on airway smooth muscle contraction because β -agonists are prophylactics (conferring bronchoprotection) and treatments (bronchorelaxation) for asthma.¹ Effects of β -agonists on other airway cell types, including inflammatory cells, have also been characterized, but their effects on asthma pathogenesis are poorly understood.^{2,3}

We recently reported that human type 2 T cells (which include both CD4⁺ T_H2 and CD8⁺ T_C2 subsets) express β_2 ARs capable of regulating numerous type 2 cell functions.⁴ We subsequently discovered that β -agonists augment the accumulation (ie, increase in number) of type 2 T cells in human peripheral blood lymphocyte (PBL) cultures subjected to bystander stimuli.⁵ This finding suggests a mechanism by which β -agonist therapy could promote type 2/type 1 T-cell skewing, which is believed to contribute to asthma pathogenesis. The idea that β -agonist therapy does not improve inflammation in asthma has been debated for years. Whereas some studies looking at clinical indices of airway inflammation have suggested some anti-inflammatory properties of β -agonists (reviewed by Remington et al⁶), other studies to the contrary exist. A provocative study by Sears et al⁷ in 1990 stimulated investigation into the role of β -agonist therapy in promoting deterioration of asthma control. Numerous clinical studies have since suggested that chronic dosing of β -agonists promotes asthma exacerbations and subtle deterioration of asthma control.⁸⁻¹¹ Concerns over the use of salmeterol prompted by the premature termination of the Salmeterol Multicenter Asthma Research Trial¹² have also further promoted the idea that β -agonist use is not an anti-inflammatory therapy, and it is clear that the effects of β -agonists on airway inflammation in asthma remain poorly understood. Several studies have reported various *in vivo* indices of inflammation associated with asthma to be unaffected or increased by inhaled β -agonist therapy,¹³⁻²⁴ and concerns regarding disease exacerbations with continuous long-acting β -agonist (LABA) monotherapy have prompted the recommendation of concomitant steroid treatment.

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Abbreviations used

β_2 AR: β_2 -Adrenergic receptor
GM: Geometric mean
Kd: Dissociation constant
LABA: Long-acting β -agonist
PBL: Peripheral blood lymphocyte
SABA: Short-acting β -agonist

Regulation of IL-13–producing (IL-13⁺) type 2 T cells might explain, in part, increases in airway inflammation that occur in some asthmatic subjects treated continuously with inhaled β -agonists. The effects of β -agonists on IL-2–stimulated accumulation of type 2 T cells were previously determined by using PBLs from nonasthmatic subjects using a saturating concentration of the β -agonist isoproterenol.⁵ In the current study we examined the effects of the clinically relevant β -agonists albuterol and formoterol at physiologically relevant concentrations on *ex vivo* cytokine-mediated accumulation of T-cell subsets from asthmatic subjects. The capacity of the corticosteroid budesonide to inhibit T-cell accumulation and reverse the effects of β -agonists was also tested.

METHODS**Subject characterization**

Venous blood was obtained from atopic asthmatic subjects after informed consent was provided in accordance with a Wake Forest University School of Medicine Institutional Review Board–approved protocol and the Helsinki Declaration. Subjects met all of the following criteria to qualify as atopic asthmatic: (1) bronchial hyperreactivity, defined as a methacholine PC₂₀ value of less than 8 mg/mL, or reversibility of airway obstruction, defined as an inhaled albuterol-induced increase of greater than 12% in FEV₁; (2) symptoms consistent with asthma (eg, cough, wheeze, and shortness of breath); (3) a physician's diagnosis of asthma without a diagnosis of any other pulmonary disorder; (4) atopy determined on the basis of serum IgE measurements (>50 IU/mL) and skin prick tests to a panel of allergens (>3-mm wheal for at least 1 allergen); and (5) nonsmoking status (>12 months nonsmoking) and less than 3 pack-years cigarette exposure (Pack-years = Years smoking $\times$ Average number of packs smoked daily). Measurements and interpretations of these criteria are as previously reported.^{25,26} All subjects refrained from taking asthma control medications at least 12 hours before blood draw. Demographic information and asthma/atopy phenotype measurements for asthmatic subjects are presented in Table E1 in the Online Repository at www.jacionline.org.

Culture and analysis of cytokine production

PBLs, isolated by using standard procedures (Ficoll-Histopaque density gradient centrifugation and nonadherence to plastics yielding >98% lymphocytes, as determined by means of light-scatter flow cytometric characteristics)²⁷ at a density of 2×10^6 /mL per well, were cultured in RPMI-1640 media supplemented with 5% pooled human plasma, 50 U/mL IL-2, IL-12–neutralizing mAb (included in all conditions, except when noted, to eliminate confounding effects of variable levels of IL-12 produced by the few, if any, contaminating monocytes), and the indicated concentrations of racemic formoterol, racemic albuterol, and budesonide (all from Sigma, St Louis, Mo). Based on the kinetics of proliferation of IL-13⁺ T cells in response to IL-2,²⁸ 6 days is the optimum time in culture for detection of the maximum increase in IL-13⁺ T cells without further addition of IL-2. After 6 days in culture, cells were collected, viable cells were counted with an automated cell counter (ViCell; Beckman Coulter, Miami, Fla), and the remaining cells were washed and then stimulated for cytokine production (for 5 hours with 2 nmol/L PMA plus 0.2 μ g/mL calcimycin plus 5 μ mol/L monensin; all reagents from Sigma, St Louis, Mo). Intracellular IL-13 and IFN- γ accumulation by T cells was detected by means of immunofluorescence/flow cytometry, as previously described,^{4,29} by using phycoerythrin-

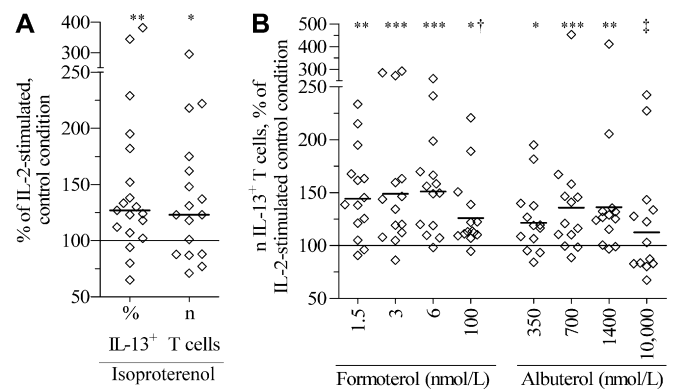


FIG 1. Effects of β -agonists on IL-13⁺ T-cell accumulation. PBLs from asthmatic subjects were cultured for 6 days with IL-2 and anti-IL-12 without (control condition) or with added β -agonist: **A**, 1 μ mol/L isoproterenol; **B**, indicated concentrations of formoterol or albuterol (*x*-axis). In Fig 1, **A**, the percentage and number of IL-13⁺ T cells after culture with isoproterenol (*x*-axis) and in Fig 1, **B**, number of IL-13⁺ T cells after culture with added formoterol/albuterol is expressed as a percentage of the value observed in the IL-2–stimulated control (ie, no β -agonist) condition (*y*-axis) for each subject tested. * $P < .05$, ** $P < .01$, and *** $P < .001$ for the difference in the median (Fig 1, **A**) or GM (Fig 1, **B**) from the IL-2–stimulated control condition. † $P < .05$ for the difference between 6 and 100 nmol/L formoterol. ‡ $P < .01/.05$ for the difference in GM between 700/1400 nmol/L and 10,000 nmol/L albuterol.

labeled anti-human IL-13, allophycocyanin-labeled anti-human IFN- γ , and fluorescein isothiocyanate-labeled anti-human CD3 and CD5 mAb (all mAbs from BioLegends, San Diego, Calif, and Caltag-Invitrogen, Carlsbad, Calif). Although important for asthma pathogenesis, IL-4⁺ and IL-5⁺ T-cell subsets were not included in the analyses because (1) they are subsets within the larger IL-13⁺ T-cell population and (2) they might have exceedingly low proportions in PBLs (often <0.5% for IL-4⁺ T cells and lower for IL-5⁺ T cells) that compromise the ability to accurately assess their changes in number.

Data presentation and statistical analyses

Among individuals, there is variability in the absolute numbers of cells in the starting (day 0) population and also the extent of IL-2–stimulated changes in numbers of cells, resulting in substantial interindividual variability in the numbers of cells observed after 6 days of culture with IL-2 (see the Results section and also see Fig E1 in the Online Repository at www.jacionline.org).^{5,28} Numbers of IL-13⁺, IFN- γ ⁺, and total T cells in the β -agonist/budesonide conditions are presented as a percentage of the values for IL-2–stimulated control condition (control condition = 100%) or starting population (day 0 condition = 100%) to graphically represent the specific effects of β -agonists and budesonide independently of the interindividual variations in absolute cell numbers in the control IL-2–stimulated condition. All statistical analyses for significance of differences were performed on the absolute, nonnormalized cell numbers by means of repeated-measures 2-way ANOVA with planned Bonferroni multiple comparison tests, except for the analysis of Fig 1, **A**, for which the Wilcoxon signed-rank test was used (1-sample test for nonnormal distribution of data). The 2 factor levels for the 2-way ANOVA analyses were as follows: for Fig 1, **B**, β -agonist (formoterol/albuterol) versus β -agonist concentration (relative to dissociation constant [Kd] for β_2 AR); for Fig 2, β -agonist (formoterol/albuterol) versus anti-IL-12 (–/+); for Fig 3, β -agonist (formoterol/albuterol) versus T-cell subset (CD4⁺/CD8⁺); and for Figs 4 and 5, β -agonist concentration versus budesonide concentration. Statistical analyses were performed with GraphPad Prism software (4.0.3; GraphPad, San Diego Calif; www.graphpad.com).

RESULTS**Effects of β -agonists on IL-13⁺ T cells**

Type 2 IL-13⁺ T cells increase in both number and proportion when stimulated with IL-2 and increase only in number but not

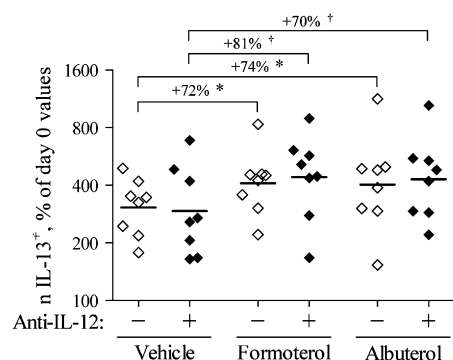


FIG 2. Effects of β -agonists in the absence of IL-12 neutralization. PBLs were analyzed for IL-13 production before (day 0) and after 6 days of culture with IL-2 without (–) or with (+) added anti-IL-12 mAb and vehicle, 3 nmol/L formoterol, or 700 nmol/L albuterol. Results are plotted as a percentage of day 0 values. Numbers above plots are the percentage increase in IL-13⁺ T cells relative to the respective vehicle condition (without or with added anti-IL-12 mAb). * $P < .01$. † $P < .001$.

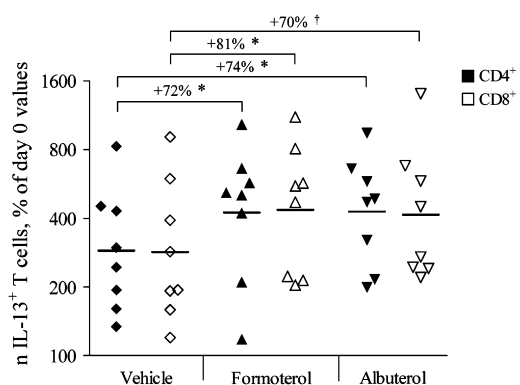


FIG 3. Effects of β -agonists on CD4⁺ and CD8⁺ T-cell subsets. PBLs were analyzed for IL-13 production before (day 0) and after 6 days of culture with IL-2 plus anti-IL-12 plus vehicle, 3 nmol/L formoterol, or 700 nmol/L albuterol. Results are plotted as a percentage of day 0 values for CD4⁺ and CD8⁺ T-cell subsets. Numbers above plots are the percentage increase in IL-13⁺ T cells relative to the respective vehicle condition. * $P < .01$ and † $P < .05$.

proportion when stimulated through CD3 + CD28.^{5,28,30} The prototypical T_H2-inducing cytokine IL-4 is not required for this IL-2- and CD3⁺CD28-stimulated accumulation of IL-13⁺ T cells.^{5,28} We recently reported that in healthy adults the β -agonist isoproterenol increases IL-2-stimulated accumulation of type 2 IL-13⁺ T cells.⁵ In our adult atopic asthma cohort, after 6 days of culture with IL-2, stimulated increases in IL-13⁺ T-cell numbers were compared with the starting day 0 numbers (geometric mean [GM], 295% of day 0 numbers; 95% CI, 190% to 450%; see Fig E1 in the Online Repository at www.jacionline.org), with IL-13⁺ cells representing $0.9\% \pm 0.3\%$ and $2.5\% \pm 1.1\%$ of T-cell populations before and after culture. Because of the importance of β -agonists in the treatment of asthma and potentially asthma development and pathology, the capacity of β -agonists to similarly affect type 2 T-cell accumulation in asthmatic subjects was tested in cultures of freshly isolated PBLs from asthmatic subjects. A saturating concentration of isoproterenol (1 μ mol/L) had a significant median effect of increasing IL-2-stimulated accumulation of IL-13⁺ T cells (Fig 1, A), which is similar to the effects observed in control subjects.⁵

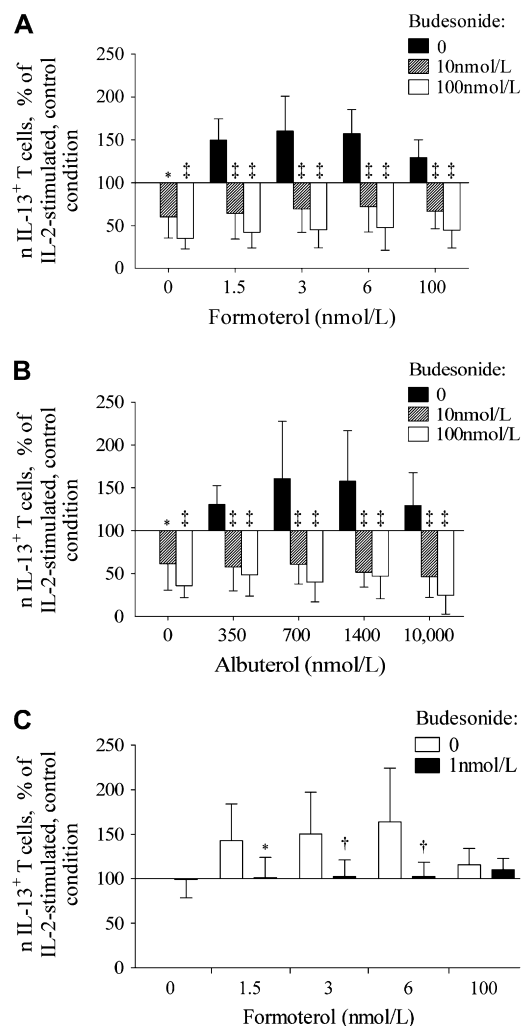


FIG 4. Interactive effects of β -agonist and budesonide. Relative number of IL-13⁺ T cells after culture of PBLs from asthmatic subjects with IL-2, anti-IL-12, and the indicated concentrations of budesonide and formoterol (A and C) or albuterol (B). * $P < .05$, † $P < .01$, and ‡ $P < .001$ for the difference in mean from the indicated β -agonist condition without budesonide ($n = 10$ asthmatic subjects [Fig 4, A and B]; $n = 5$ subjects in an independent asthmatic population [Fig 4, C]).

Albuterol and formoterol are a short-acting β -agonist and a LABA, respectively, that are commonly used, through inhalation, for rescue from acute bronchoconstriction (albuterol and formoterol) and for long-term control of asthma symptoms (formoterol), respectively. A saturating concentration (100 nmol/L) of formoterol increased accumulation of IL-13⁺ T cells in PBLs from asthmatic subjects (GM, 26% increase; Fig 1, B; absolute cell numbers are presented in Fig E1 in the Online Repository at www.jacionline.org). At concentrations of formoterol ranging from 0.5 to 2 times its K_d for the β_2 AR (K_d = 3 nmol/L), an even greater increase in IL-2-stimulated accumulation of IL-13⁺ T cells was observed (3-fold vs approximately 4.5-fold of starting day 0 numbers). The increase in accumulation was greatest at 3 to 6 nmol/L formoterol (GM, 50% increase), which is a significantly greater increase than that observed with 100 nmol/L formoterol. Albuterol increased IL-13⁺ T-cell accumulation at 0.5 to 2 times its K_d for the β_2 AR (350 nmol/L to 1.4 μ mol/L). Albuterol, 700 nmol/L and 1.4 μ mol/L, increased accumulation

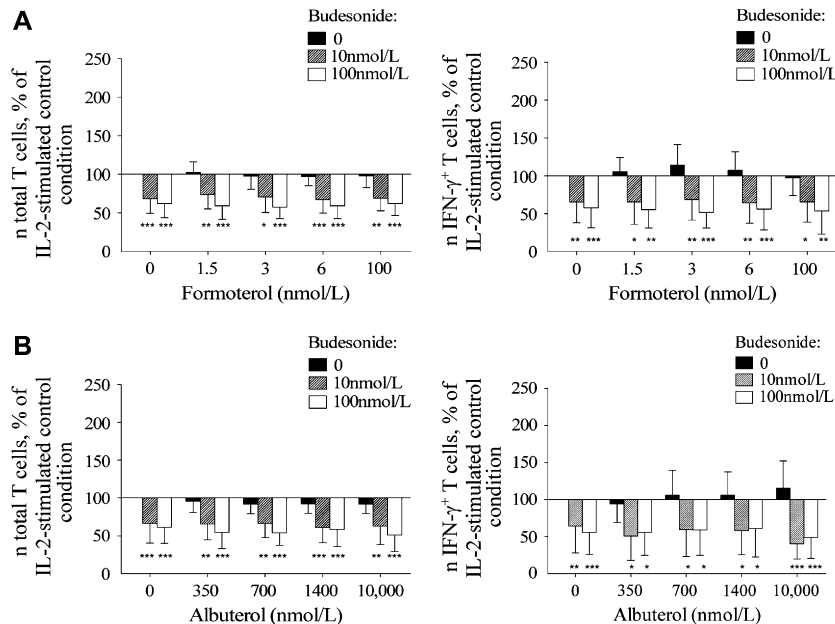


FIG 5. Effects of β -agonists and budesonide on total and IFN- γ^+ T cells. The relative number of total (left) and IFN- γ^+ (right) T cells after culture of PBLs from asthmatic subjects with IL-2, anti-IL-12, and the indicated concentrations of budesonide and formoterol (A) or albuterol (B) is shown. * $P < .05$, ** $P < .01$, and *** $P < .001$ for the difference in mean from the indicated β -agonist condition without budesonide ($n = 9$ -10 asthma subjects).

by 36% (GM), which is significantly more than seen with 10 μ mol/L albuterol. Significant differences were not observed between formoterol and albuterol when compared at concentrations matched for their K_d for β_2 AR. These results indicate that enhanced IL-2-stimulated accumulation of type 2 T cells is a class effect of β -agonists, and concentrations of β -agonists around their K_d for the β_2 AR are most effective in enhancing accumulation of type 2 T cells.

Both our current and previous characterizations of antigen-independent, IL-2-stimulated increases in IL-13 $^+$ T cells use an IL-12-neutralizing mAb (anti-IL-12) during culture to avoid potential confounding effects of IL-12 produced by the few, if any, contaminating monocytes. Experiments were repeated without anti-IL-12 to assess whether IL-12 neutralization qualitatively affected our results. Results depicted in Fig 2 demonstrate that IL-2-stimulated increases in IL-13 $^+$ T cells and the augmenting effects of both formoterol and albuterol similarly occur in the absence of IL-12 neutralization. The percentage increase in IL-13 $^+$ cells relative to the starting (day 0) numbers was similar in both CD4 $^+$ and CD8 $^+$ T-cell subsets, and both β -agonists also similarly augment this increase in both subsets (Fig 3).

Inhibition of β -agonist effects on IL-13 $^+$ T cells by budesonide

LABAs that are used for asthma control therapy are most frequently used in combination with inhaled corticosteroids, in part because of concerns over lack of control of inflammation with a β -agonist alone. Various concentrations of budesonide were added to PBL cultures stimulated with IL-2 plus β -agonist to examine the ability of corticosteroids to inhibit β -agonist-mediated augmentation of type 2 T-cell accumulation (Fig 4, A and B). In the absence of a β -agonist, 10 and 100 nmol/L budesonide decreased (by 40% $\pm$ 32% and 65% $\pm$ 16%, respectively) IL-13 $^+$

T-cell numbers accumulated in culture with IL-2 ($n = 9$ asthmatic subjects). Budesonide inhibited the enhancing effects of both formoterol and albuterol on IL-13 $^+$ T-cell accumulation, with IL-13 $^+$ T-cell numbers decreasing to the same levels as in cultures without β -agonists. The same effects of formoterol and albuterol in enhancing IL-13 $^+$ T-cell accumulation were observed in PBLs from nonasthmatic control subjects, and budesonide similarly inhibited IL-13 $^+$ T-cell accumulation with or without added β -agonist (see Fig E2 in the Online Repository at www.jacionline.org).

Budesonide, 1 nmol/L, did not significantly inhibit IL-13 $^+$ T-cell accumulation stimulated by IL-2 (0.1% $\pm$ 19% change in number, Fig 4, C). However, 1 nmol/L budesonide prevented the enhancing effect of formoterol on accumulation.

Effects on total and IFN- γ^+ T cells

In our asthma cohort 6 days of culture with IL-2 did not significantly affect total T-cell numbers (median, 12% decrease; interquartile range, 28% decrease to 2% increase). However, IL-2 stimulated an increase in IFN- γ^+ T-cell numbers (median, 131% increase; interquartile range, 3% to 903% increase). Neither formoterol nor albuterol at any concentration demonstrated a significant mean effect on the effect of IL-2 on numbers of total and IFN- γ^+ T cells in PBLs from asthmatic subjects (Fig 5). Budesonide at 10 nmol/L and 100 nmol/L similarly decreased total T-cell numbers by 32% $\pm$ 25% and 38% $\pm$ 24% relative to the IL-2-stimulated control culture condition, and neither formoterol nor albuterol reversed this loss of T cells. IFN- γ^+ T-cell numbers were decreased relative to the control condition, indicating that budesonide inhibited the IL-2-stimulated increases in IFN- γ^+ T cells. Neither β -agonist reversed this budesonide-mediated inhibition (Fig 4). Similar results were observed in PBLs from control nonasthmatic subjects (data not shown). Other cytokines, such as GM-CSF, TNF- α , IL-2, and IL-17, were not included in these

analyses because either they do not define discrete subsets or, for IL-17⁺ cells, their numbers are not consistently regulated by IL-2 (unpublished data).

DISCUSSION

In this study we demonstrate that type 2 T cells from asthmatic subjects accumulate to a greater extent when the clinically used β -agonists formoterol and albuterol are present during stimulation with IL-2. Importantly, the observed increase in accumulation is greatest at physiologically and clinically relevant concentrations approximating the K_d of each β -agonist for the β_2 AR. The effect of a β -agonist on increasing type 2 T-cell accumulation is a class effect observed with various β -agonists, including albuterol, formoterol, isoproterenol, and salmeterol (data not shown) and is independent of asthma disease status.

The corticosteroid budesonide inhibited the accumulation of type 2 T cells when present at concentrations of 10 or 100 nmol/L, and the extent of this inhibition was not affected by the presence of a β -agonist at any concentration tested. At a lower concentration of budesonide (1 nmol/L) that does not inhibit type 2 T-cell accumulation *per se*, the augmenting effect of β -agonists on accumulation is still inhibited. Because these results were obtained from *ex vivo* culture of lymphocytes, additional studies are warranted for investigating whether the observed effects can occur *in vivo*. At the present time, however, model systems of primary cultured T cells, by enabling control of numerous variables that confound *in vivo* studies, arguably represent the most useful experimental approach currently available for exploring fundamental questions regarding the regulatory effects of β -agonists and steroids on T-cell subtypes. Keeping the limitations of cell culture studies in mind, these results provide a framework for explaining how use of a β -agonist as monotherapy might increase airway inflammation and increase the risk for increases in the number and severity of exacerbations and how steroids prevent the proinflammatory effects of β -agonists.

The potential importance of antigen-independent, cytokine-mediated (bystander) stimulation of T cells in asthma has been recently reviewed.³¹ Antigen-independent proliferation of type 2 T cells could be induced by IL-2 and IL-15 produced by virus-stimulated T cells and monocytes/macrophages, respectively. Viral infections have been implicated in asthma pathogenesis and exacerbations by numerous epidemiologic studies, although limited mechanistic insight exists.³¹⁻³⁶ Bystander proliferation might explain allergen-independent sensitization in asthmatic subjects. For example, respiratory tract infections can promote IL-2- or IL-15-induced proliferation of resident memory, viral antigen-nonspecific, allergen-specific type 2 T cells. Another context in which IL-2-induced proliferation can occur would be when T cells reactive to a particular allergen produce IL-2, stimulating proliferation of type 2 T cells that are reactive to a different allergen.

Therefore, inhaled β -agonists taken during the course of a viral infection or allergen exposure might serve to further expand the type 2 T-cell pool in the airways and surrounding tissues. A larger type 2 T-cell pool can result in a greater sensitivity to subsequent viral or allergen exposure to increase the frequency and severity of exacerbations. Budesonide taken in combination with a β -agonist would inhibit this detrimental effect of the β -agonist, and importantly, the β -agonist would not diminish the inhibitory effect of what could be assumed to be concentrations of budesonide

effectively delivered to the airway. In addition, our results suggest that continuous use of not only formoterol but also of other therapeutic β -agonists has the potential to also promote airway inflammation through increased accumulation of type 2 T cells.

A recent meta-analysis of data from prospective clinical studies demonstrates an overall significant association with LABA use and subtle increases in asthma exacerbation-related hospitalizations and life-threatening events.³⁷ However, in another meta-analysis it was shown that in asthmatic subjects whose symptoms were not controlled with low-dose steroid monotherapy, a combination steroid/salmeterol therapy was significantly more efficacious in improving asthma control than doubling of steroid dose alone.³⁸ The Formoterol and Corticosteroids Establishing Therapy study demonstrated that budesonide/formoterol combination therapy significantly reduces asthma exacerbations compared with budesonide therapy alone.³⁹ These studies suggest the relative superiority of steroid plus LABA dual therapy. Our results demonstrating the inhibitory effect of budesonide on a potentially deleterious proinflammatory effect of β -agonists identifies one mechanism by which steroid/ β -agonist combination therapy might provide superior asthma control.

We thank the staff at the Cloverdale Clinical Research Center for their efforts in obtaining blood samples from asthmatic subjects for this project.

Clinical implications: Continuous use of β -agonists as asthma monotherapy has the potential to increase type 2 cytokine-mediated airway inflammation. Importantly, budesonide inhibits this proinflammatory effect of β -agonists, underscoring the importance of combined β -agonist/steroid therapy.

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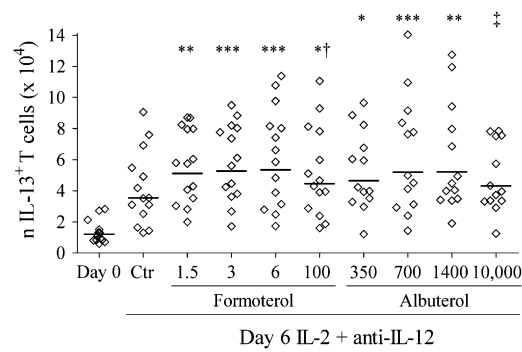


FIG E1. Effects of β -agonists on IL-13⁺ T-cell accumulation. Absolute numbers of IL-13⁺ T cells before (day 0) and after culture of 1×10^6 PBLs from asthmatic subjects with IL-2, anti-IL-12 alone (control condition [Ctr]), or the indicated concentrations of formoterol or albuterol. * $P < .05$, ** $P < .01$, and *** $P < .001$ for the difference in GM from IL-2-stimulated control condition. † $P < 0.05$ for the difference between 6 and 100 nmol/L formoterol. ‡ $P < .01/.05$ for the difference in GM between 700/1400 nmol/L and 10,000 nmol/L albuterol.

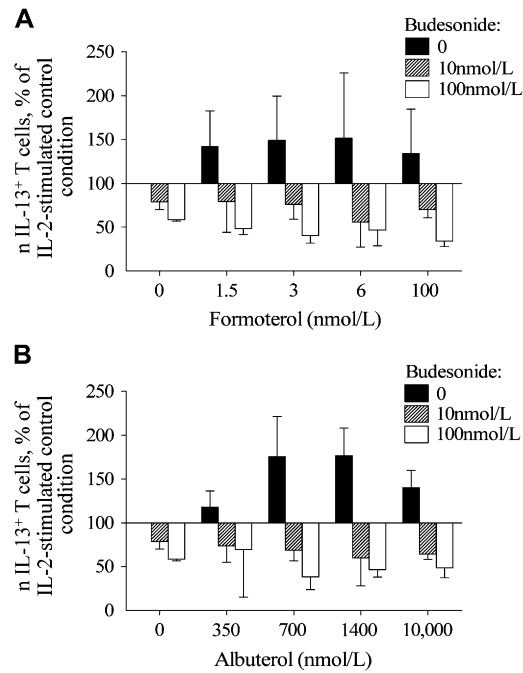


FIG E2. Effects of β -agonist and budesonide on IL-13⁺ T cells from control subjects. Relative numbers of IL-13⁺ T cells after culture of PBLs from control subjects with IL-2, anti-IL-12, and the indicated concentrations of budesonide and formoterol (**A**) or albuterol (**B**) are shown.

TABLE E1. Demographic and spirometric data for asthmatic subjects

Parameter	Statistic	Asthma cohort
Subjects	n	18
Sex	n: Male/female	6/12
Race	n: White/black	10/8
Age (y)	Mean $\pm$ SD (range)	32 $\pm$ 8 (18–46)
PC ₂₀	Geometric mean (range)	2.0 (0.01–8.0)
FEV ₁ (% predicted)	Mean $\pm$ SD (range)	78 $\pm$ 16 (26–101)
FVC (% predicted)	Mean $\pm$ SD (range)	86 $\pm$ 15 (46–107)
FEV ₁ /FVC	Mean $\pm$ SD (range)	0.75 $\pm$ 0.11 (0.45–0.88)
FEV ₁ reversal (%)	Median (range)	13 (–3 to 41)
No. of positive SPT responses	Mean $\pm$ SD (range)	5 $\pm$ 4 (1–11)
Albuterol	n: Yes/no	18/0
LABA	n: Yes/no	13/5
Inhaled steroid	n: Yes/no	8/10

FVC, Forced vital capacity; *FEV₁ reversal*, increase in FEV₁ above baseline induced by inhaled albuterol; *SPT*, skin prick test for reactivity to common inhaled allergens.