

## Regulation of T cells in airway disease by beta-agonist

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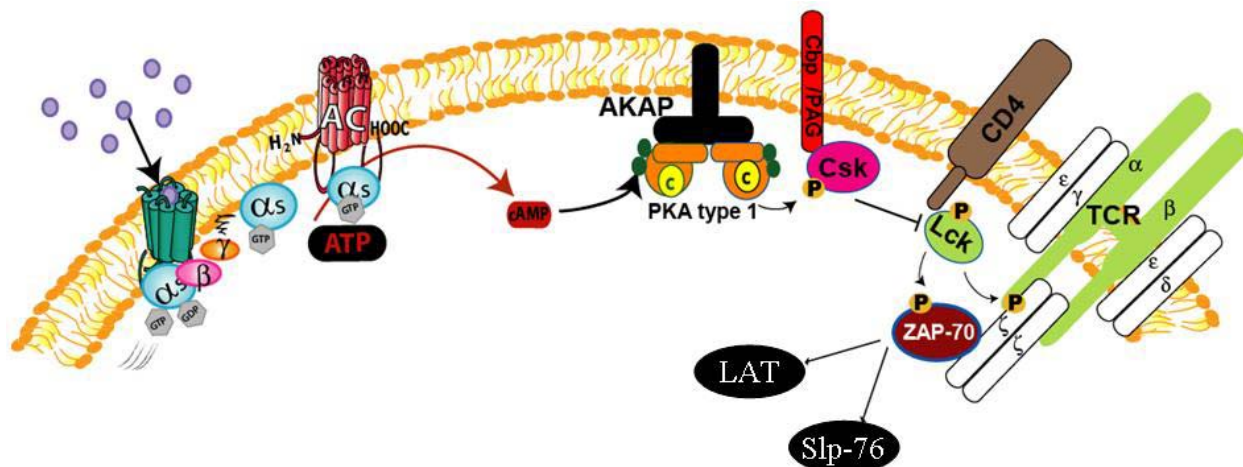
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## 1. ABSTRACT

It is widely recognized that Th2 cytokines derived from T cells play a major role in the development of allergic lung inflammation that causes most asthma. Beta-agonists are important rescue and maintenance therapies for asthma, yet our understanding of beta-agonist effects on T cell biology is surprisingly poor. Recent studies using both cell culture and more integrative models are beginning to reveal beta-agonist regulation of T cell signaling and function that may be important in the pathogenesis and treatment of asthma and possibly other inflammatory diseases. Here we provide a comprehensive review of the literature concerning beta-agonist effects on T cells, and discuss the relevance of emerging paradigms of beta-adrenergic receptor signaling to T cell function.

## 2. INTRODUCTION

T cells are a major focus of asthma research. It is widely recognized that Th2 cytokines derived from T cells are important if not required pathogenic agents of atopic asthma. Given the importance of beta-agonists in asthma therapy, one might suspect that the topic of beta-agonist-mediated regulation of T cell function in asthma would be exhaustively studied and well understood, but such is not the case. Most of the frustration in addressing this topic stems from the complex nature of those systems in which T cells are studied. Here we review the literature concerning beta-agonist effects on T cells that occur *in vivo* and in culture, and discuss the relevance of emerging paradigms of beta-adrenergic receptor signaling to T cell function.



**Figure 1.** Proximal TCR signaling and regulation by Gs-coupled receptors and PKA. Engagement of TCR with cognate peptide antigen presented by MHC molecules promotes the membrane recruitment and activation of the Src kinases Lck and Fyn (Fyn not shown), phosphorylation of immunoreceptor tyrosine-based activation motifs within the CD3 complex, and ultimate recruitment and activation of ZAP-70. Active ZAP-70 then leads to activation of multiple downstream signaling cascades via activation of LAT and SLP-76 (as depicted in Figure 2). Agonist-activated Gs-coupled receptors such as the Beta<sub>2</sub>AR promote adenylyl cyclase activity resulting in hydrolysis of ATP into cAMP. cAMP binds the regulatory subunits of the cAMP-dependent protein kinase (PKA, shown docking on an AKAP) which induce release and activation of PKA catalytic subunits. The Src kinase Csk phosphorylates Lck and inhibits its activity. Csk resides in lipid rafts in association with Cbp/PAG in resting T cells, but is transiently displaced to the cytosol upon T cell activation. PKA phosphorylates Csk on Ser 364 to increase Csk activity, which results in Lck inhibition, and inhibition of TCR zeta-chain phosphorylation, and proximal TCR signaling.

### 3. AT THE CELLULAR/MOLECULAR LEVEL: T CELL SIGNALING

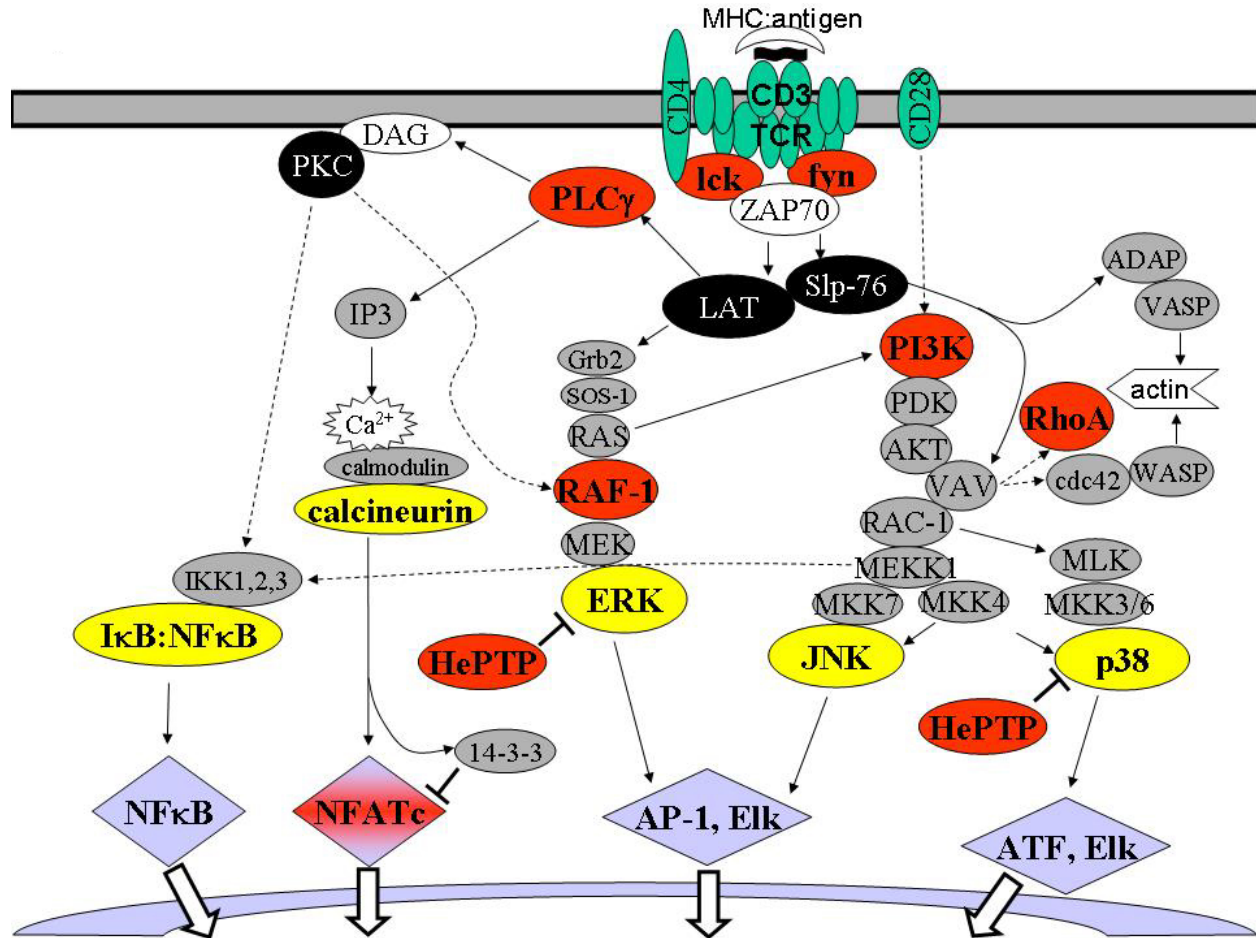
#### 3.1. Antigen-dependent signaling

Many important T cell functions, such as proliferation, survival, and cytokine production, are regulated by signaling via the T cell receptor (TCR)/CD3 complex, which is activated naturally by antigenic peptides presented by major histocompatibility complexes (MHCs). Experimentally, agonistic antibodies to CD3 (and usually also the co-stimulatory molecule CD28) or mitogens that agglutinate the TCR/CD3 complex, such as phytohemagglutinin-L (PHA) are frequently used to simulate antigenic stimulation. Such non-specific stimulations are often required in the human system to control for the diverse cognate antigenic repertoire of the T cell populations among individuals.

Provided below is a brief summary of the salient features of antigen-dependent TCR signaling (for a more detailed description, refer to (1-3), and references therein). The T cell receptor is actually a complex comprised of two TCR chains (TCRalpha and TCRbeta, or TCRgamma and TCRdelta), which recognize antigenic peptides presented by MHC molecules, and the CD3 subunits (gamma, delta, epsilon, eta/zeta), which are required to transduce the signals to the cytoplasm when the TCR engages its cognate antigenic peptide. Co-receptor molecules (CD4 or CD8, depending on the T cell subset) and co-stimulatory molecules (e.g., CD28) also may be present in the complex during or after initial engagement of the TCR with peptide/MHC. Proximal TCR signaling (Figure 1) involves the TCR “recognizing” its cognate peptide antigen

presented by MHC molecules. When the TCR binds its cognate antigenic peptide, this “recognition” is sensed by CD3 complex molecules, leading to recruitment and auto-activation of the Src family members Lck and Fyn. These two proteins activate CD3zeta/eta subunits, which in turn recruit zeta-chain-associated protein kinase 70 (ZAP-70) via their immunoreceptor tyrosine-based activation motifs, allowing Lck to phosphorylate and activate ZAP-70.

From ZAP-70, multiple downstream effector signaling pathways are activated, including p42/p44 mitogen-activated protein kinase (MAPK), p38 MAPK, c-Jun N-terminal kinase (JNK), phosphoinositide 3-kinase (PI3K), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB), and Ca<sup>2+</sup>/nuclear factor for activated T-cells (NF-AT) pathways, as illustrated in Figure 2. ZAP-70 activates LAT (Linker for activation of T cells), which is responsible for activating the Grb2/SOS complex and phospholipase C (PLC) –gamma. The first complex leads to activation of Ras and the downstream p42/p44 MAPK pathway, as well as connecting to the PI3K pathway. PLC-gamma releases diacylglyceride (DAG) and inositol-triphosphate (IP<sub>3</sub>) from phosphoinositol-diphosphate. DAG activates protein kinase C (PKC) theta, which transduces activating signals to the NF-kappaB and MAPK pathways. IP<sub>3</sub> release leads to elevation of cytoplasmic Ca<sup>2+</sup> levels. Ca<sup>2+</sup>-bound calmodulin stimulates calcineurin’s phosphatase activity, which activates the transcription factor NF-AT via dephosphorylation of its regulatory domain. ZAP-70 also activates SH2 domain containing leukocyte protein of 76kDa (SLP-76). SLP-76 mediates activation of Vav, which via Rac1, leads to activation of the p38 MAPK and JNK pathways. SLP-76



**Figure 2.** Downstream T-cell signaling events and impact of PKA. Major downstream signaling cascades resulting from MHC:cognate peptide stimulation of TCR:CD3 complex are depicted. Critical signaling molecules transmitting upstream signals from ZAP-70 to multiple major effector signaling pathways (e.g., MAPK, NF-kappaB, Ca<sup>2+</sup>/NF-AT pathways) are represented by black shading. Intermediate signaling moieties and adaptors are shaded gray. Terminal effector molecules (or complexes), which are responsible for the activation/nuclear translocation of transcription factors, are shaded yellow. Select transcription factors activated as a result of TCR/CD3-mediated stimulation are shaded blue. Inhibition events are indicated by T. Signaling molecules reported to be inhibited by catalytically active PKA are shaded red. This schematic is intended to present major signaling events in antigen-activated T cells, and may not include all signaling molecules, adaptors, and transcription factors involved in this complex system.

also connects to the actin reorganization machinery via Vav/Nek for Cdc42/Wiskott- Aldrich syndrome protein-mediated actin reorganization and TCR clustering. SLP-76 and Fyn stimulate degranulation promoting adaptor protein (ADAP) to recruit VASP, which directs actin-dependent clustering of integrins.

The co-receptors CD4 and CD8 enhance activation of downstream signaling pathways by recruiting and activating Lck to enhance ZAP-70 activation. Co-stimulatory molecules, such as CD28 and inducible costimulatory protein (ICOS), mainly contribute by strongly activating the PI3K pathway, dependent upon TCR/CD3- and co-receptor- activated Lck. Overall, the downstream effector signaling from the TCR/CD3 complex leads to activation and nuclear translocation of numerous transcription factors (including NF-κB, NF-AT, AP1

(fos/jun), Atf-1, Elk-1, 3'-5'-cyclic adenosine monophosphate (cAMP) response element binding, and c-Myc, among others), and reorganization of the actin network.

### 3.2. Antigen-independent signaling

Conversely, “antigen-independent” signaling, mediated by agents such as IL-2 and IL-15, may also regulate T cell functions under various conditions. Interleukin (IL) -2 or IL-15 preferentially induces proliferation of IL-13<sup>+</sup> T cells, relative to IL-13 non-producers, in peripheral blood lymphocytes. The major source of IL-2 is antigen-stimulated T cells. Sources of IL-15 are monocytes, macrophages, and dendritic cells, which produce IL-15 upon activation by a variety of stimuli including pathogens (e.g., virus, bacteria) and tissue damage (e.g., products of necrotic cells, endothelins

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released by endothelia or epithelia, platelet-derived factors). A comprehensive review of antigen-independent signaling, and associated functional consequences, is provided in Loza *et al.* (4).

Antigen-independent signaling bypasses the TCR complex to transduce mitogenic and pro-survival signaling. For example, IL-2-mediated signaling feeds into the canonical TCR signaling pathways at the levels of Grb2 and PI3K. IL-2Rbeta chain activates Janus kinase (JAK) -3, which leads to activation of Grb2 via Shc. As discussed above for TCR/CD3-mediated signaling, Grb2/Son of sevenless protein activates Ras and the downstream p42/p44 MAPK pathway. JAK1 is also activated by IL-2Rbeta and transduces signaling to the PI3K/Akt pathway, contributing to IL-2-dependent increases in cell survival.

### 3.3. Beta-agonist effects on T cell signaling

The effects of beta-agonist (and other Gs-coupled receptor agonists such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)) on both TCR and antigen-independent signaling are presumed to be mediated via the cAMP-dependent protein kinase (PKA)(5;6). Previous studies have asserted PKA-dependent effects in T cells by either assessing agonist-stimulated PKA activity in an *in vitro* assay or demonstrating actions of pharmacologic PKA inhibitors and activators (7-14). Gs-coupled G protein-coupled receptors (GPCRs) such as the beta-2-adrenergic receptor (Beta<sub>2</sub>AR) and the EP2 receptor activate adenylyl cyclase, which hydrolyzes adenosine-5'-triphosphate (ATP) into cAMP, and cAMP in turn activates PKA. cAMP-independent and cAMP-dependent/PKA-independent actions of the Beta<sub>2</sub>AR have also been identified in some cells types, but not in T cells (15). In fact, the Rap1 guanine exchange factor Exchange Protein directly Activated by Cyclic AMP (EPAC), which can be activated by cAMP in both a PKA -dependent and -independent manner, does not appear to be expressed in T cells (unpublished observations and (16)).

Junctures at which Beta<sub>2</sub>AR-mediated PKA activation can regulate T cell signaling are depicted in both Figures 1 and 2. Regulation of Csk appears to be the principal means by which PKA regulates proximal TCR signaling. Csk is a Src family kinase that phosphorylates Lck at Tyr-505 to inhibit Lck activity, TCR zeta-chain phosphorylation, and TCR-mediated signaling. PKA type 1 phosphorylates Csk at Ser-364 and increases its activity 2-4 fold (17). This phosphorylation/activation is facilitated by the formation of a complex of PKA type 1, Csk, sodium-hydrogen exchanger regulatory factor-1, and Csk-binding protein/phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG). The A Kinase Anchoring Protein (AKAP) Ezrin (18) serves as a scaffold to coordinate the assembly of this complex, thus enabling efficient targeting of Csk to its substrate Lck.

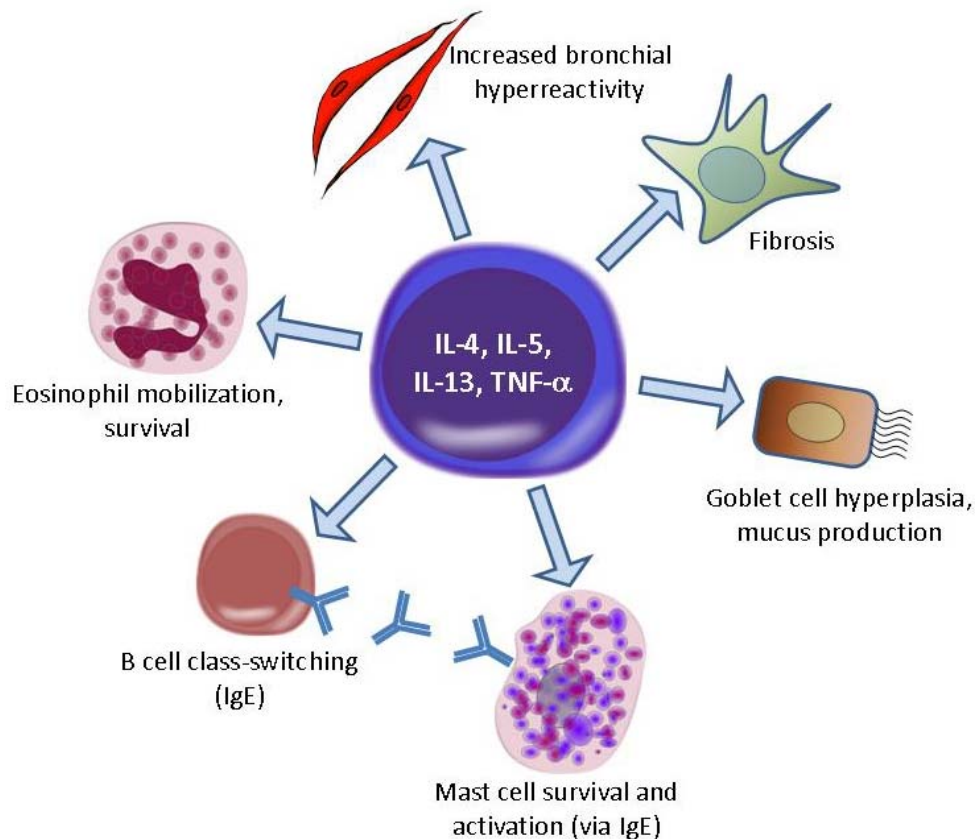
Numerous downstream intermediates are also regulated by PKA. PKA phosphorylation of Ser-43, Ser-233, and Ser-259 on Raf-1 disrupts Ras-Raf-1 association to inhibit downstream activation of p42/p44 (19). Conversely, Ser-23 phosphorylation of the hematopoietic protein-tyrosine phosphatase (HePTP) causes release of HePTP

from p42/p44 and p38 to allow their activation by MAPK/ERK kinases (20;21). cAMP elevation has also been shown to inhibit IL-2-stimulated activation of PI3K and downstream, mammalian target of rapamycin-dependent phosphorylation of p70 s6 kinase in T cells (22).

PKA phosphorylates and inhibits PLC-gamma -1 and -2, causing a reduction of intracellular Ca<sup>2+</sup> mobilization and phosphatidylinositol hydrolysis (23;24), and thus possibly serving as a mechanism for inhibiting (Ca<sup>2+</sup>/calcineurin-mediated) nuclear translocation of the transcription factor NF-AT initiated by TCR signaling. However, a more direct effect of PKA on NF-AT occurs via direct NF-AT phosphorylation. Whereas desphosphorylation of NF-AT by calcineurin promotes dissociation of NF-AT from 14-3-3 protein and enables nuclear translocation, PKA phosphorylation of NF-AT promotes 14-3-3 binding and retention of NF-AT in the cytosol (25). This competitive regulation of NF-AT by calcineurin and PKA is believed to be important in the regulation of IL-2 gene expression (25-27).

PKA has been shown to regulate the activity of NF-kappaB via numerous mechanisms, many of which appear cell type specific. Numerous members of the NF-kappa B family can be phosphorylated by PKA, resulting in various effects: altered ability to retain heterodimer formation, promotion of nuclear translocation, DNA binding, and transcriptional activity. Zhong *et al.* (28) reported PKA phosphorylation of the p65 subunit promotes p65 interaction with the coactivator CBP/p300 to increase p65 transcriptional activity in Jurkat cells. Conversely, Neumann *et al.* (29) reported that PKA inhibits nuclear translocation of p65 in Jurkat cells by impairing degradation of inhibitory factor kappa B. Takahashi *et al.* reported PKA activation inhibits transcriptional activity of NF-kappaB in Jurkat cells via an effect involving the p65 C-terminal transactivation domain. These disparate results obtained in the same cell line are possibly explained by differences in experimental design, in particular the use of different reporter constructs as indicators of NF-kappaB activity. Of note, the majority of studies to date have demonstrated inhibitory effects of PKA on NF-kappaB-dependent gene expression.

In summary, activation of the TCR/CD3 complex promotes the stimulation of several major downstream effector signaling pathways. These pathways culminate in the activation and translocation of numerous transcription factors and activation of translation and cell cycle machinery. PKA has the capacity to block TCR/CD3-mediated signaling both at the most proximal (Lck/Fyn block by PKA-dependent localization of Csk) and at various downstream (including Raf-Ras interaction for the p42/p44 pathway, phosphorylation of NF-AT, and inactivation of PLC-gamma) points. Conversely, some degree of PKA activity may be necessary for the full potential of TCR/CD3-stimulated signaling (PKA-dependent release of inhibitory HePTP from p42/p44 and p38 MAPKs).



**Figure 3.** Impact of Th2-derived cytokines on airway inflammation and remodeling.

#### 4. ASTHMA, INFLAMMATION, AND T CELLS

Human asthma is typically associated with airway inflammation as evidenced by the large numbers of inflammatory cells, and their mediators, present in bronchoalveolar lavage (BAL) fluid (BALF) recovered from asthmatics. Although numerous inflammatory cell types probably contribute to the development of inflammation in asthma, most mechanistic studies of allergic lung inflammation have ascribed critical roles to eosinophils, mast cells, and T cells (reviewed in (30;31), Figure 3). T cells participate in allergic lung inflammation by driving allergen sensitization and B cell production of allergen-specific IgE. This critical step in allergic inflammation is mediated by production of type 2 (or Th2-like) cytokines, particularly IL-4 and IL-13, by Th2 cells. Allergen-activated Th2 cells provide “help” to allergen-reactive B cells to promote their survival, expansion, and, in conjunction with IL-4 and IL-13, immunoglobulin class-switching to IgE. After establishment of allergen sensitization, T cells further contribute to allergic inflammation by local production of type 2 cytokines. IL-4 and particularly IL-13 produced in airways lead to growth of airway epithelia and their transformation into mucus-producing goblet cells. IL-5 produced by Th2 cells is an important factor for eosinophil release from bone marrow and for their survival in the periphery.

IL-13, in addition to IL-4, has been shown to be a critical modulator of allergic inflammation. In murine models (reviewed by (32)), introduction of elevated levels of IL-13 in the airways via pulmonary delivery and transgenic expression has been shown to be sufficient to induce both airway inflammation and increased airway resistance and hyperresponsiveness (AHR). In contrast, targeted expression of IL-4 in the airways by transgenic expression leads to increased airway inflammation but not significant AHR. Ablation of the genes encoding IL-13, IL-4R $\alpha$ , or IL13Ralpha1 (but not the decoy/antagonistic IL-13Ralpha2) or blocking IL-13 by soluble IL-13Ralpha2-Fc largely inhibits the development of allergic inflammation, fibrosis, and AHR induced by allergen. Allergen-induced allergic inflammation and AHR are only weakly blocked, and fibrosis is unaffected, in *IL4* knockout mice. Deletion of both *IL4* and *IL13* genes results in a complete block in allergen-induced inflammation, fibrosis, and AHR. These results underscore the importance of IL-13 in the fibrosis and AHR components, in addition to its overlapping role with IL-4 in inflammation, in murine models of allergic inflammation/asthma.

In humans, IL-13 and IL-4 are elevated in BALF obtained from asthmatics, and segmental antigen challenge of airways of human asthmatics causes large increases in Th2 (IL-4, IL-5, and IL-13) as well as the Th1 cytokine

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interferon (IFN)-gamma. Overall, the cellular and cytokine profile observed in airways of human asthmatics and in animal models of allergic airway inflammation defines an exaggerated Th2 cytokine response, and has contributed to the “Th2:Th1 hypothesis” in asthma (33). The extent to which Th1 cytokine levels in the asthmatic airway are altered is subject to debate, as is the role of Th1 cytokines in airway inflammation and asthma (34). Although the exact dynamic between Th2 and Th1 T cells in asthma is not clear, it is likely that both subtypes are important in regulating the nature of airway inflammation in asthma.

T<sub>regs</sub>, a T cell subset critical for maintaining peripheral tolerance to self-antigen, are reported to be found in reduced numbers in airways of asthmatics, although their numbers in peripheral blood are normal. However, the role of T<sub>regs</sub> in the development of allergy and local allergic inflammation is still a matter of ongoing debate (35).

### 5. EFFECTS OF BETA-AGONISTS ON T CELLS

#### 5.1. Effects of beta-agonists in *in vivo* models of allergic lung inflammation

It is well accepted that inhaled beta-agonists are effective in reversing or preventing bronchoconstriction via their direct actions on airway smooth muscle (36). Whether effects on other cell types contribute to their therapeutic benefit is subject to debate. Although *in vitro* studies have identified various inflammatory cell functions inhibited by beta-agonists, results from *in vivo* studies are mixed and suggest little if any effect of inhaled beta-agonist on lung inflammation associated with asthma. In fact, several studies have indicated that beta-agonist therapy may actually worsen inflammation (37-41).

Because inflammation is a multi-cellular, multi-event, time-dependent process whose presentation is highly variable and is effected by numerous stimuli, integrative approaches attempting to understand T cell function and its regulation must be interpreted with extreme caution. Changes in airway T cell cytokine levels associated with beta-agonist therapy can reflect either direct or indirect effects of beta-agonist on T cells. In addition, analyses of direct effects of beta-agonist on T cells in models of allergic inflammation may be confounded by the capacity of inflammation to desensitize the Beta<sub>2</sub>AR on T cells, which has been suggested by both *in vivo* (42-44) and *in vitro* (45) studies.

A limited number of *in vivo* studies exist examining the effects of beta-agonists on various indices of inflammation including outcomes suggestive of T cell function. Several of these studies have provided comparisons among racemic and enantiomer formulations of albuterol. Using a guinea pig model, Westerhof *et al.* (46) noted inhalation of RS-, R-, or S- albuterol had no effect on inflammatory cell infiltration (including lymphocytes) induced by allergen challenge. In a murine model of ovalbumin (OVA)-induced allergic inflammation (47), both R- and S- albuterol delivered by a miniosmotic pump reduced airway eosinophil infiltration, yet neither

affected the OVA-induced increase in BAL levels of IL-5 or IL-13. In contrast, using a similar infusion protocol with C57BL/6 mice Ferrada *et al.* (48) found no effects of S-albuterol on various indices of inflammation. R- and RS-, but not S- albuterol inhibited OVA-induced increases in BAL eosinophils, and the compounds similarly affected serum IgE levels. Collectively, these *in vivo* studies suggest modest if any effects of beta-agonists on airway inflammation. Studies examining enantiomers are consistent with most of the literature on beta-agonist enantiomer pharmacology, i.e., R- enantiomers are the active component of racemic mixtures and S- enantiomers simply have a null effect.

#### 5.2. EFFECTS OF BETA-AGONISTS ON T CELLS USING CELL CULTURE MODELS

Unlike integrative models, studies using T cell cultures offer the opportunity to assess direct effects of beta-agonists on T cells, with the caveat that indirect effects on T cell subtypes are possible in populations of mixed cells (e.g., Peripheral Blood Mononuclear Cells (PBMC)). Beta-agonist activation of the Beta<sub>2</sub>AR has been reported to block TCR-mediated signaling to inhibit both cytokine production (IFN-gamma and IL-2) and proliferation of T cells (49;50). Although there appears to be a consensus regarding the ability of beta-agonists to directly regulate Th1 T cells, disparate results exist with respect to Th2 T cells. Two studies have reported beta-agonist-mediated inhibition of IL-4 and IL-13 production in freshly isolated human T cells (51;52). However, several studies examining various primary and clonal populations and polyclonal lines of T cells in murine and human systems have reported that beta-agonists do not directly affect type 2 T cells (53). Recently, to clarify this issue we analyzed beta-agonist-mediated signaling events at the single cell level (6). In both CD56<sup>+</sup> (Th1) and cells expressing Chemoattractant-receptor homologous molecule expressed on Th2 cells (CRTH2<sup>+</sup>) (Th2) in freshly isolated peripheral blood lymphocytes, and in a near homogenous population of Th2 cells (CD2<sup>-lo</sup> cultures (54)) beta-agonist induced the phosphorylation of the intracellular PKA substrate vasodilator-stimulated phosphoprotein (VASP). Moreover, in CRTH2<sup>+</sup> cells beta-agonist inhibited the induction of CD25, and this effect was reversed by inclusion of a Beta<sub>2</sub>AR-selective antagonist. Thus, both human Th1 and Th2 T cells appear to express functional Beta<sub>2</sub>ARs.

Regarding effects of beta-agonist enantiomers, Ferrada *et al.* (48) recently reported that after 12 h stimulation of the T cell line EL-4 or murine splenocytes with Con A and phorbol 12-myristate 13-acetate (PMA), treatment with R- but not S- albuterol reduced levels of induced IL-2 and IL-13 mRNA, and effects of RS-albuterol were similar to those of R- albuterol. In parallel experiments in EL-4 cells, R- and RS- but not S- albuterol inhibited the induction of Con A + PMA- stimulated NF-κB reporter activity.

Borish and colleagues have examined both albuterol (55) and formoterol (56) enantiomers in antigen-specific T cell lines generated by culture of

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PBMC in tetanus and IL-2. R- albuterol and RR formoterol inhibited whereas the S-/SS-enantiomers had no effect on PMA-induced proliferation. Inhibitory effects on cytokine production were also observed for R- albuterol and RR- formoterol. In these studies, however, racemic mixtures were less effective in inhibiting proliferation and cytokine production than were R-/RR- enantiomers.

### 6. WHAT'S NEXT: FUTURES STUDIES EXAMINING BETA-AGONIST EFFECTS ON T CELLS

Although recent studies have been helpful, numerous questions remain regarding the effects of beta-agonists on T cell signaling and function in inflammatory lung disease. Important topics include:

#### 6.1. The fundamental question of the relevance of beta-agonist regulation of T cells

Although *in vitro* studies suggest that beta-agonist can stimulate the Beta<sub>2</sub>AR on T cells to modulate T cell proliferation, survival, and cytokine production, the extent to which this occurs *in vivo* and whether it is of any physiological consequence is essentially unknown. Circulating catecholamines, especially when levels are altered such as with stress or heart failure, could conceivably affect T cell numbers and function and impact the pathogenesis of various inflammatory diseases. However, it is difficult to speculate how findings from *in vitro* studies relate to the effects of inhaled beta-agonist on infiltrating (and activated) T cells in the asthmatic lung. Complicating the issue is the need to address these questions in the *human* lung, in which experimental strategies for assessing T cell function are limited, and the mechanisms of allergic inflammation and T cell function are in many ways dissimilar to those in the mouse.

#### 6.2. Agonism properties of various beta-agonists

The majority of *in vitro* studies have noted a profound difference between beta-agonists and PGE<sub>2</sub> (both Gs-coupled receptor agonists) with respect to their ability to regulate T cell proliferation, survival, and cytokine production. We determined that in human type 2 T cells, beta-agonists are weak activators of PKA, whereas PGE<sub>2</sub> is relatively efficacious (6;57;58). This disparity is associated with differences in the inhibitory effect on IL-2 and IL-13 production, p38, p42/p44, and NF-kappaB activity, and cell proliferation, and in the ability of beta-agonists to promote increased survival of IL-2-stimulated type 2 T cells (6;57). These results suggest agonism is an important characteristic of beta-agonists, and that an increased ability to generate cAMP and stimulate PKA may enable beta-agonists to function more like PGE<sub>2</sub> and promote a greater anti-inflammatory effect. Such an increased ability may occur by relieving mechanisms of Beta<sub>2</sub>AR desensitization or by developing new beta-agonists with increased intrinsic activity or capacity for allosteric modulation (36;59). Of note is a recent study by Oostendorp *et al.* (60) in which differences in Beta<sub>2</sub>AR desensitization among cells expressing different ADRB2 gene haplotypes were

associated differences in beta-agonist inhibition of lymphocyte IFN-gamma and IL-5 production.

Alternatively, beta-agonists capable of qualitatively distinct signaling (functional selectivity or biased agonism (59)) properties with superior anti-inflammatory features may be developed (or identified). Such development will require a more expansive screening strategy for pipeline Beta<sub>2</sub>AR agonists that considers numerous, diverse outcomes of Beta<sub>2</sub>AR signaling in T cells.

It is also important to note the possibility that Beta<sub>2</sub>AR agonism per se in T cells promotes inflammation. A recent study by Bond and colleagues (61) demonstrated that either ADRB2 gene knockout, or treatment of mice with inverse agonists of the Beta<sub>2</sub>AR, attenuated both airway inflammation and AHR promoted by sensitization and challenge with antigen. Although the cells that participate in this effect of Beta<sub>2</sub>AR antagonism remain to be identified, T cells are an obvious candidate.

#### 6.3. Compartmentalization of Beta<sub>2</sub>AR signaling in T cells

Numerous studies have demonstrated that both T cell receptor and cAMP-mediated signaling in T cells is highly compartmentalized (reviewed in (62)). Proximal TCR signaling occurs within lipid rafts. cAMP-dependent signaling in T cell also initiates in lipid rafts and is further compartmentalized by AKAPs. In numerous elegant studies, Tasken and colleagues have demonstrated that both AKAPs and lipid rafts in T cells help to coordinate, dependent on the extracellular stimulus, the formation of specific signaling complexes into discrete microdomains for the purpose of localizing signaling events to either propagate signals or regulate feedback processes (15;17;18;63-67). Although expansion on these studies is beyond the scope of this review, AKAPs and compartmentalized signaling represent another layer of regulation of Gs-coupled receptors that might contribute to differential effects among Gs-coupled receptor ligands on T cells.

Clarification of the questions proposed above will be important to our understanding of T cell biology and have obvious therapeutic implications as well. A critical issue to current asthma therapy involves the limitations, and possible deleterious effects, of inhaled beta-agonists. It has been speculated that a failure of beta-agonists to treat inflammation underlies the adverse clinical outcomes associated with chronic beta-agonist therapy. Should this be the case, the possibility exists that new generations of beta-agonists (possessing distinct signaling capabilities) could regulate T cell or other inflammatory cell functions to promote positive therapeutic benefits. Alternatively, understanding the limitations or negative consequences of beta-agonists with respect to inflammation/inflammatory cells will assist in identifying necessary adjunct therapies.

## 7. ACKNOWLEDGEMENT

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**Abbreviations:** ADAP: degranulation promoting adaptor protein, AHR: airway hyperresponsiveness, AKAP: A kinase anchoring protein, ATP: adenosine-5'-triphosphate, Beta<sub>2</sub>AR: beta-2-adrenergic receptor, BAL: bronchoalveolar lavage, BALF: bronchoalveolar lavage fluid, cAMP: 3'-5'-cyclic adenosine monophosphate, CRTH2: Chemoattractant-receptor homologous molecule expressed on Th2 cells, DAG: diacylglyceride, EPAC: exchange protein directly activated by cyclic AMP, GPCR: G protein-coupled receptor, HePTP: hematopoietic protein-tyrosine phosphatase, IFN: interferon, IL: interleukin, IP<sub>3</sub>: inositol-triphosphate, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, LAT: Linker for activation of T cells, MAPK: mitogen-activated protein kinase, MHC: major histocompatibility complex, PAG: phosphoprotein

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associated with glycosphingolipid-enriched microdomains, PBMC: peripheral blood mononuclear cells, PHA: phytohemagglutinin-L, PGE<sub>2</sub>: prostaglandin E2, PI3K: phosphoinositide 3-kinase, PKA: cAMP-dependent protein kinase, PKC: protein kinase C, PLC: phospholipase C, PMA: phorbol 12-myristate 13-acetate, NF-AT: nuclear factor for activated T-cells, NF-kappaB: nuclear factor kappa-light-chain-enhancer of activated B cells, OVA: ovalbumin, SLP-76: SH2 domain containing leukocyte protein of 76kDa, TCR: T cell receptor, VASP: vasodilator-stimulated phosphoprotein, ZAP-70: zeta-chain-associated protein kinase 70

**Key Words:** beta-agonist, T cell, Asthma, Inflammation, Interferon, Interleukin, Airway Hyperresponsiveness, NF-kappaB, JAK, Beta-2-Adrenergic Receptor, Th1, Th2, receptor, AKAP, MAPK, NF-AT, ZAP-70, CD3, Bronchoalveolar Lavage, Prostaglandin, Review

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