Transforming Growth Factor-β1: A Novel Cause of Resistance to Bronchodilators in Asthma?

Asthma is a chronic airway inflammatory disease of the airways characterized by reversible airflow obstruction and symptoms such as chest tightness, wheezing, and cough (1). The current gold standard treatment for bronchoconstriction in asthma is the use of β_2-adrenoceptor agonists (β_2-agonists), which are potent and effective bronchodilators that relax airway smooth muscle contraction regardless of stimulus (2). However, some patients with severe asthma have shown increased resistance to the effects of β_2-agonists (3), and interestingly, it also has been demonstrated that β_2-agonist therapy is less effective the higher the severity of airway hyperresponsiveness, or bronchoconstriction (4). The mechanisms behind this are not yet fully elucidated, and further understanding may help in the treatment of airway obstruction in patients with severe asthma.

In this issue of the *Journal* (pp. 209–218), Ojiaku and colleagues demonstrate a new way in which transforming growth factor (TGF)-β1, a profibrotic cytokine, may cause an attenuation of β_2-agonist–induced relaxation of airway smooth muscle (5). TGF-β1 has previously been implicated in the pathogenesis of asthma, with increased levels in the BAL fluid (6) together with increased expression in bronchial tissue samples of patients with asthma (7). In the context of airway smooth muscle function, Ojiaku and colleagues have previously shown that preincubation with TGF-β1 can increase muscarinic agonist–induced contractile responses in human airway smooth muscle (HASM) (8). It has also been shown that bronchoconstriction caused by the contractile agent methacholine can cause the release of TGF-β1 (9). This interplay highlights the key role that TGF-β1 may play in asthma pathogenesis.

In the context of asthma therapy rather than pathogenesis, TGF-β1 has also been shown to decrease β_2-adrenoreceptor agonist function via a reduction in receptor expression (10), suggesting a role for TGF-β1 in the development of resistance to the mainstay asthma therapeutic, such as that seen in patients with severe asthma. The key message of the article by Ojiaku and colleagues (9) is that there may be an additional mechanism for the reduction in β_2-agonist efficacy caused by TGF-β1, whereby the downstream cAMP signaling induced by β_2-agonists is attenuated by increased PDE4D expression.

In their study, the authors used magnetic twist cytometry, which measures dynamic changes in cell stiffness as a surrogate for contraction, and demonstrated that pretreatment with TGF-β1 decreased the β_2-adrenoreceptor agonist isoproterenol–induced relaxation in HASM cells precontracted with the muscarinic agonist carbachol. Although isoproterenol is a partial agonist of both β_1- and β_2-adrenoreceptors, HASM cells have been shown to express only the β_2-subtype (11), indicating a potential role for TGF-β1 in the β_2-agonist tolerance seen in many patients with asthma (5).

β_2-Agonists induce relaxation though activation of adenylyl cyclase and increases in cAMP to inhibit contractile stimuli. HASM cells that were precontracted with carbachol and then treated with isoproterenol demonstrated an increase in cAMP, which was inhibited after pretreatment with TGF-β1. This has also been indicated in previous publications in which TGF-β1 was shown to inhibit cAMP accumulation in response to isoproterenol in HASM through downregulation of β_2-receptor number and function (10). However, TGF-β1 had no effect on forskolin-induced cAMP elevation, indicating that TGF-β1 is not impacting the function of adenylyl cyclase itself (5).

The actions of cyclic nucleotides are counteracted by a group of PDE enzymes, which hydrolyze cAMP (or cGMP) to the inactive 5′-AMP (or 5′-GMP) (12). Ojiaku and colleagues demonstrate that the inhibitory effect of TGF-β1 is reversed after pretreatment with the pan-PDE inhibitor 3-isobutyl-1-methylxanthine, implicating PDEs as drivers of the response. Further investigation revealed that treatment with the selective PDE4 antagonist roflumilast reversed the blunted isoproterenol cAMP responses, as well as that TGF-β1 increased the expression of an isoform of PDE4 (PDE4D) in HASM cells (5). PDE4D5, a splice variant of PDE4D, has previously been shown using targeted siRNA knockdown to be the key physiological regulator of β_2-cAMP turnover within HASM (13).

The canonical TGF-β1 pathway involves phosphorylation of the intracellular signal transducers Smad2 and Smad3 after activation by TGF-β1 (14). Phosphorylated Smad2 and Smad3 then integrate with Smad4 and translocate to the nucleus, where the complex regulates gene transcription (15). Ojiaku and colleagues have shown that Smad2 and Smad3 knockdown in HASM cells reduced the increased PDE4D gene expression induced by TGF-β1, leading to the conclusion that TGF-β1 decreases β_2-agonist–induced relaxation in a Smad2/3-dependent manner (5) (Figure 1). This is a novel observation and may help in the therapeutic use of β_2-agonists in patients with severe asthma.

There are, of course, a number of questions that this work raises that we look forward to seeing examined in future studies. This includes determining whether TGF-β1 pretreatment affects relaxation responses in whole human tracheal tissues and whether TGF-β1 also attenuates the effectiveness of more clinically relevant β_2-agonists such as formoterol or salbutamol. Furthermore, it would be interesting to determine if this mechanism plays a role in the reduced β_2-agonist effectiveness in tracheal tissues from patients with severe asthma.

In summary, this highly interesting and novel study reveals a mechanism by which resistance to the effects of bronchodilators may develop in disease, implicating TGF-β1 as a key driver in the

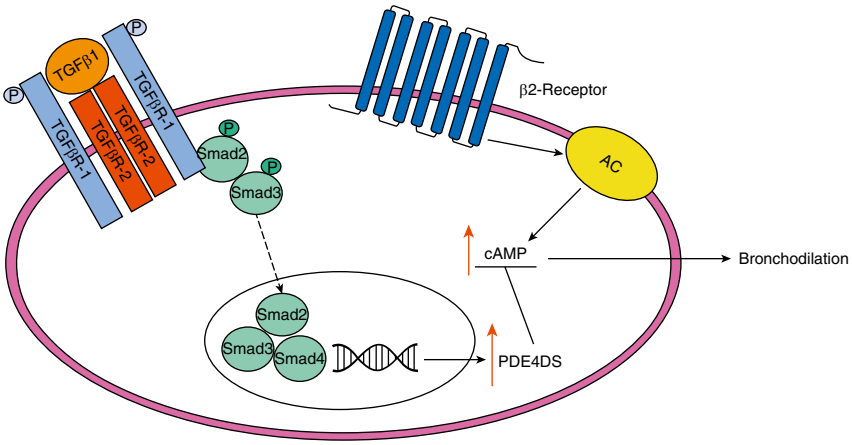

Figure 1. β_2-Agonists cause relaxation through activation of the β_2-adrenoceptor, which subsequently activates adenylyl cyclase (AC) to increase cAMP and cause bronchodilation. Ojiaku and colleagues suggest that the increased resistance to β_2-agonist–induced bronchodilation in asthmatics may be mediated by the effects of transforming growth factor (TGF)-β1. Activation of the TGF-β receptor by TGF-β1 causes phosphorylation of the transcription factors Smad2 and Smad3, which then translocate to the nucleus, where they form a complex with Smad4. This complex increases expression of the PDE isomer PDE4DS, which leads to greater breakdown of cAMP and thereby reduces the level of bronchodilation. P = phosphorylation.

resistance to β_2-agonist bronchodilator effects. If future research confirms these findings in the context of severe asthma, this mechanism could lead to the development of novel therapeutics to increase bronchodilator sensitivity. ■

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