β2-adrenoceptor function in asthma

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Abstract

β 2-adrenoceptor agonists, often used in combination with corticosteroids, have been extensively used for the treatment of asthma. However, concerns have been raised regarding their adverse effects and safety including poor asthma control, life-threatening exacerbations, exacerbations that often require hospitalization, and asthma-related deaths. The question as to whether these adverse effects relate to the loss of their bronchoprotective action remains an interesting possibility. In the chapter, we will review the experimental evidence that describes the different potential factors and associated mechanisms that can blunt the therapeutic action of β 2-adrenoceptor agonists in asthma. We show here evidence that various key inflammatory cytokines, growth factors, some respiratory viruses, certain allergens, unknown factors present in serum from atopic asthmatics have the capacity to impair β 2-adrenoceptor function in airway smooth muscle, the main target of these drugs. More importantly we present our latest research describing the role played by mast cells in impairing β 2-adrenoceptor function. Although no definitive conclusion could be made regarding the implication of one single mechanism, receptor uncoupling or receptor desensitization due to phosphorylation represents the main inhibitory pathways associated with a loss of β 2-adrenoceptor function in airway smooth muscle. Targeting the pathways leading to β 2-adrenoceptor dysfunction will likely provide novel therapies to improve the efficacy of β 2-agonists in asthma.

1- Therapeutic actions of β_2 -adrenoceptor agonists in asthma

β_2 -adrenoceptor agonists are highly effective bronchodilator drugs used in the management of pulmonary diseases such as asthma and chronic obstructive pulmonary diseases (COPD). They provide rapid relief of symptoms via their potent bronchodilatory action by specifically promoting the relaxation of airway smooth muscle (ASM) (Giembycz & Newton, 2006). β_2 -agonists act on the β_2 -adrenoceptor which activates adenylyl cyclase via the stimulatory G-protein G_s , resulting in cyclic adenosine mono-phosphate (cAMP) production and protein kinase A (PKA) activation which has been described as the main mechanism driving relaxation of airway smooth muscle. The relaxant pathways are multiple and include a inhibition of the myosin light chain kinase (MCLK) activity leading to a reduction of calcium sensitivity of the contractile proteins, modulation of intracellular calcium levels through the blockade of calcium release from intracellular stores, and induction of membrane hyperpolarization through activation of large-conductance calcium-activated potassium channels (BK_{Ca}) (nicely reviewed in (Pera & Penn, 2016) and (Giembycz & Newton, 2006)). Inhibition of Rho pathways via the activation of cAMP-mediated exchange protein (Epac) has been also described as a mechanism for the beneficial effect of β_2 -agonists in ASM cells (Fogli et al., 2015). **Figure 1** summarizes the mechanisms leading to bronchodilation in response to β_2 -agonists.

The therapeutic value of combining long-acting β_2 -agonists with corticosteroids in the treatment of asthmatic patients is undeniable as evidenced by numerous reports showing better disease control, pulmonary function and reduced frequency of exacerbations (Newton & Giembycz, 2016). The mechanisms underlying the superior clinical benefits provided by the combination therapies have not been completely elucidated but several *in vivo* and *in vitro* studies have suggested that β_2 -agonists might possess anti-inflammatory properties in addition to their bronchoprotective effect. *In vivo* studies investigated whether several key markers of airway mucosal inflammation in asthmatic patients can be modulated by long-acting β_2 -adrenoceptor agonists. Levels of IL-8 and neutrophil number in the bronchoalveolar lavage fluids (BALFs) were reduced in patients with persistent asthma treated for 12 weeks with salmeterol (Reid et al., 2003). Patients with mild asthma treated with formoterol for 8 weeks had a significant reduction of number of eosinophils in both the submucosa and epithelium (EG2+) (Wallin, Sandstrom, Cioppa,

Holgate, & Wilson, 2002) as well as number of mast cells and eosinophils in the lung mucosa (Wallin et al., 1999). Salmeterol was also reported to decrease neutrophils number in the submucosa in mild asthmatics following a 6 weeks of treatment (Jeffery et al., 2002) while in children with asthma, short-term treatment with formoterol (4 weeks) decreased the number of eosinophil in the blood and as well as levels of eosinophil cationic protein and IL-4 (Stelmach et al., 2002). The anti-inflammatory efficacy of salmeterol was also demonstrated when given as an add-on therapy for 3 weeks to inhaled beclomethasone dipropionate which led to a reduction of levels of sputum eosinophil cationic protein (ECP) in mild and moderate asthmatics (Pinto Pereira et al., 2003). However, Roberts et al (1999) did not find any evidence of anti-inflammatory activity in bronchial biopsies or BAL when salmeterol was administered to steroid-naïve subjects for 6 weeks (Roberts et al., 1999). Furthermore, unlike budesonide, formoterol had no effect on the sputum eosinophil count in patients with poorly controlled asthma (Green et al., 2006). It is therefore unclear whether long-acting β_2 -agonists exert inhibitory actions on the accumulation of inflammatory cells and production of pro-asthmatic cytokines *in vivo*.

Parallel *in vitro* studies were also performed to investigate whether β_2 -agonists exert anti-inflammatory actions on different lung cells by studying their effect on the production of inflammatory mediators in response to various pro-asthmatic stimuli. In airway epithelial cells for example, salmeterol and formoterol inhibited production of CXCL-8 and VEGF induced by LPS (Chiu, Hsu, Fu, Chu, & Chi, 2007) and thymus and activation-regulated chemokine (TARC/CCL17) in response to $\text{TNF}\alpha$, IL-4 and $\text{IFN}\gamma$ (Hung et al., 2008) while albuterol suppressed IL-1 β / $\text{IFN}\gamma$ -induced GM-CSF production via iNOS pathways (Chorley, Li, Fang, Park, & Adler, 2006). IgE-mediated histamine release can be suppressed by SABAs and LABAs not only in basophils (Kleine-Tebbe, Frank, Josties, & Kunkel, 1994) but also in human lung mast cells via various mechanisms involving β_2 -adrenoceptor-dependent and -independent pathways (Chong, Cooper, Vardey, & Peachell, 1998; Lewis, Chachi, Newby, Amrani, & Bradding, 2016) or via the inhibition of KCa3.1 channels, which regulate cell membrane hyperpolarization and potentiation of both Ca^{2+} influx and mast cell degranulation (Duffy, Cruse, Lawley, & Bradding, 2005). Interesting observations were also made in studies using human ASM cells where combining short-acting (salbutamol) or long-acting (salmeterol, formoterol) β_2 -agonists with dexamethasone (or budesonide) results in

additive or synergistic inhibition of the production of various pro-inflammatory proteins (Ammit et al., 2002; Hallsworth, Twort, Lee, & Hirst, 2001; Kaur, Chivers, Giembycz, & Newton, 2008; Newton, 2014; Nie, Knox, & Pang, 2005; Pang & Knox, 2001; Pang & Knox, 2000). Among the inflammatory cytokines strongly repressed by the drug combination include various key players in asthma pathogenesis including the chemotactic proteins CCL11, CCL5, and CXCL8 known for the recruitment of eosinophils, T lymphocytes and neutrophils respectively (Castan, Magnan, & Bouchaud, 2017). Activation of β_2 -adrenoceptor, cAMP-dependent pathways leading to the induction of steroid-inducible MAP kinase phosphatase-1 (MKP-1) has been described as one mechanism explaining the augmented anti-inflammatory action of combining β_2 -agonists/corticosteroids in ASM cells (Kaur et al., 2008; Manetsch, Che, Seidel, Chen, & Ammit, 2012; Manetsch et al., 2013; Quante et al., 2008). The synergistic induction of the Regulator of G-protein signaling 2 (RGS2), a GTPase-activating protein that specifically switch-off G_q pathways, represent another mechanism of bronchoprotection that is induced by combining β_2 -agonists and corticosteroids (Holden et al., 2011). This hypothesis was later confirmed by the same authors who used knockout animals to demonstrate the bronchoprotective role of RGS2 in a murine model of house dust mite-induced allergic inflammation. Interestingly, the authors also found that the presence of RGS2 was required to suppress allergen-associated pulmonary inflammation including production of different chemokines such as CCL3, CCL11, CXCL9 and CXCL10 (George et al., 2017). Combined therapy also inhibits features of ASM remodelling where low doses of fluticasone synergized with fenoterol to suppress PDGF-induced proliferation and hypocontractility in bovine tracheal smooth muscle cells (Dekkers et al., 2012). Another study using precision cut lung slices (PCLS) showed that combining dexamethasone and albuterol prevented homologous desensitization of β_2 -receptor typically seen with chronic stimulation of the receptor (Cooper & Panettieri, 2008). The overall beneficial effects of β_2 -adrenoceptor agonists in asthma likely result from their bronchodilator effect by relaxing ASM but also on their ability to potentiate the anti-inflammatory and bronchoprotector actions of corticosteroids through their direct action on ASM cells and other lung cells. **Figure 2** summarizes the therapeutic actions of β_2 -agonists acting in ASM in asthma.

The following section will address the challenging question regarding the mechanisms behind the loss of bronchoprotection induced by β_2 -agonists often seen in patients with asthma.

2- β_2 -adrenoceptor dysfunction in asthmatics

2.1- Evidence from asthmatic patients

The hypothesis of a reduced β_2 -adrenergic function in asthma was first demonstrated in animal models of allergic asthma and non-specific airway hyper-responsiveness (Barnes, Dollery, & MacDermot, 1980; C. Emala, Black, Curry, Levine, & Hirshman, 1993). In the basenji-greyhound dog model of airway hyperresponsiveness, Emala and colleagues found a close correlation between impaired β_2 -adrenoceptor responses and the decreased production of cAMP in response to isoproterenol in isolated tracheal muscle preparations of these animals (C. Emala et al., 1993). The existence of altered β_2 -receptor function has been suggested by the observed decreased receptor expression and cAMP production seen in mononuclear cells in patients with asthma (Sato, Bewtra, Hopp, Nair, & Townley, 1990). Allergen exposure was found to be a critical factor involved in driving by more than 21% the reduction of β_2 -receptor number on lymphocytes in patients with allergic asthma (Meurs, Koeter, de Vries, & Kauffman, 1982). Trian and colleagues found the cAMP responses to both short- and long-acting β_2 -agonists (i.e., isoproterenol and formoterol) were reduced by more than 50% in ASM cells derived from asthmatic subjects compared to non-asthmatics (Trian et al., 2011). A recent report also found that the coupling of adenylyl cyclase and cAMP production to β_2 -receptor was markedly reduced in ASM cells derived from patients who had died from fatal asthma. Increased activity of PI3K leading to the prevention of β_2 -receptor resensitization was found to be the main mechanism of β_2 -receptor dysfunction in these patients (Gupta et al., 2015). The relationship between the loss of β_2 -adrenergic function and the severity of asthma was also suggested with the observed reduced response to β_2 -agonists therapy experienced by asthma patients during exacerbations (Reddel et al., 1999). Bronchial smooth muscle tissues derived from patients who died from fatal asthma also demonstrate reduced bronchodilator responses to β_2 -adrenergic agonists (Bai, 1991; Goldie, Spina, Henry, Lulich, & Paterson, 1986).

There is also evidence that in addition to impaired β_2 -adrenergic responses in asthma, β_2 -adrenergic agonists may make asthma worse, particularly in the absence of corticosteroid therapy. For example, regular β_2 -adrenoceptor agonist use may enhance airway hyperresponsiveness (Cockcroft, McParland, Britto, Swystun, & Rutherford, 1993; Swystun, Gordon, Davis, Zhang, & Cockcroft, 2000; I. K. Taylor et al., 1992; van Schayck et al., 1990) and eosinophilic airway inflammation (Aldridge et al., 2000; Green et al., 2006), accelerate lung function decline (van Schayck et al., 1991), reduce asthma control (Nelson et al., 2006; D. R. Taylor et al., 1993; D. R. Taylor et al., 1998), and potentially contribute to asthma deaths in both short-acting and long-acting form (Johnston & Edwards, 2009; Nelson et al., 2006; D. R. Taylor et al., 1993).

The mechanism(s) behind the reduced response to therapy and potential adverse effects on asthma pathophysiology are poorly understood, and mechanisms such as agonist-evoked receptor desensitization (tachyphylaxis) and downregulation have been suggested as possible causes (Johnston & Edwards, 2009). It is likely that targeting the pathways leading to poor β_2 -agonist response would have the potential to enhance both their efficacy and safety in many patients. A large body of evidence demonstrates that numerous factors known to be associated with airway inflammation and remodelling in asthma can alter ASM cells responsiveness to β_2 -agonists through different mechanisms (**summarized in Table 1**).

2.2- Pro-asthmatic factors affecting β_2 -adrenergic function

A major clue for the mechanisms explaining the poor bronchodilator response to β_2 -adrenoceptor agonists seen in asthma has derived from *ex vivo* studies that have used isolated airway preparations isolated from different animal species. The bronchorelaxant responses of isolated rabbit tracheal smooth muscle to isoproterenol (both maximum (E_{max}) and potency (EC_{50})) were dramatically reduced in tissues pre-incubated for 24 hours with serum derived from atopic asthmatic patients (Hakonarson, Herrick, Serrano, & Grunstein, 1997; Hakonarson, Maskeri, Carter, Chuang, & Grunstein, 1999). Interestingly, the authors found that this loss of β_2 -adrenoceptor function by atopic serum was due to an increased activation of the muscarinic M2 receptor/ G_i protein-coupled pathways, leading to the attenuation of cAMP production. These *ex vivo* studies were among the first to highlight the

existence of soluble mediators in the serum of allergic patients that could interfere with the responsiveness of airway smooth muscle to β_2 -receptor agonists. Follow-up studies have identified a number of mediators that are capable of modulating β_2 -receptor function in different airway smooth muscle models including isolated tracheal/bronchial tissues or in cultured cells. The section below summarizes all known factors present in asthma that have the capacity of blunting β_2 -receptor function in airway smooth muscle.

2.2.1. Pro-asthmatic cytokines.

A number of reports have shown that TNF α is an important player in the pathogenesis of asthma by acting on different lung structural cells including ASM (C. Brightling, Berry, & Amrani, 2008). Our group provided the initial evidence that murine tracheal rings treated for 24 hours with TNF α have a 60% reduction in relaxant responses to isoproterenol (Chen, Tliba, Van Besien, Panettieri, & Amrani, 2003), an effect not due to a decreased β -adrenoceptor affinity as no change in EC₅₀ could be demonstrated. A similar observation of reduced isoproterenol response was observed in guinea-pig tracheal tissues treated with TNF α (Wills-Karp et al., 1993), suggesting that the effect of TNF α can be seen in different animal species. This attenuated relaxant response could be due to reduced β_2 -receptor activity as studies performed using cultured human ASM cells clearly demonstrated that a decreased β_2 -receptor coupling in TNF α -treated cells as evidenced by the reduced adenylyl cyclase activity (C. W. Emala, Kuhl, Hungerford, & Hirshman, 1997) and cAMP-dependent gene expression (Lahiri et al., 2002) in response to isoproterenol stimulation. Interestingly, the relaxant response induced by other stimuli including PGE₂ or forskolin, a non-receptor-dependent relaxant agent that directly activates adenylate cyclase, were not affected by TNF α . These studies suggest a specific effect of TNF α on the bronchorelaxant responses driven by the β_2 -receptor via mechanisms involving a receptor uncoupling to downstream signalling pathways (Chen et al., 2003).

Extensive work has also been done around the basic mechanisms by which another pro-asthmatic cytokine, IL-1 β , impaired β_2 -receptor responsiveness in airway smooth muscle. This hypothesis was initially described by studies performed in both isolated tracheal rings from guinea-pigs and rabbits reporting that IL-1 β caused a reduction of the maximal bronchorelaxant responses to isoproterenol (Wills-Karp et al., 1993) or right shift of the dose-response relaxation curve (Hakonarson, Herrick, Serrano, & Grunstein, 1996). In

the latter study, the authors demonstrated this effect was mediated via the negative cross-talk between muscarinic M_2 receptors and β_2 -receptors, resulting in reduced cAMP accumulation. The impact of IL-1 β has been also demonstrated in studies assessing ASM cell responsiveness to β_2 -agonists by their ability to block cell stiffness measured by magnetic twisting cytometry as readout of cell responsiveness. It was reported that ASM responsiveness to isoproterenol was markedly suppressed by IL-1 β in a concentration- and time-dependent manner, via a mechanism involving β_2 -receptor uncoupling to its downstream signalling pathways as revealed by the impaired Gs-adenylyl cyclase activation (Shore, Schwartzman, Le Blanc, Murthy, & Doerschuk, 2001), and decreased CRE-inducible gene expression (Lahiri et al., 2002). Several groups found that IL-1 β -induced β_2 -receptor hypo-responsiveness resulted in part from the heterologous desensitization of the receptor caused by the autocrine action of COX-2-derived prostanoids including PGE₂ (J. D. Laporte et al., 1998; Pang, Holland, & Knox, 1998). Interestingly, this synergistic induction COX-2-dependent mechanism appears to explain the augmented β_2 -receptor hypo-responsiveness in cells treated with a combination of TNF α and IL-1 β (Moore et al., 2001) or TNF α and IL-17A (Rumzhum, Patel, et al., 2016). It is interesting to note that IL-1 β -induced β_2 -receptor dysfunction could be prevented by the presence of dexamethasone which inhibited COX-2 induction and PGE₂ production (Moore et al., 1999). The restoration of the β_2 -agonist-induced relaxation by corticosteroids was suggested as one potential mechanism explaining their beneficial action in asthma.

Like TNF α , IL-13 has been also involved in the pathogenesis of allergic asthma in part by acting on different lung structural cells including airway smooth muscle cells and airway epithelial cells (Wills-Karp, 2001). In different reports, IL-13 was shown to alter β_2 -receptor function although the underlying mechanisms are still controversial due to the use of different experimental models. In cultured human ASM cells, overnight incubation with IL-13 can blunt the ability of isoproterenol to reduce cell stiffness by involving mechanisms that are dependent on ERK pathways (J. C. Laporte et al., 2001). In isolated rabbit tracheal rings, the reduced bronchochorelaxant response of isoproterenol induced by IL-13 occurred indirectly via the autocrine action of another TH2 cytokine IL-5 (Grunstein et al., 2002). An interesting recent report confirmed that IL-13 could also impair β_2 -agonist-induced cAMP generation in human airway epithelial cells (HAECs) via a mechanism involving G protein-

coupled receptor kinase (GRK2)-dependent phosphorylation of the β_2 -receptor (Albano et al., 2015). A similar mechanism involving both GRK2/PI3K pathways seems to be involved in the suppression isoproterenol-induced alveolar epithelial fluid transport induced by the chemokine IL-8 and TGF β (Roux et al., 2013; Wagener, Roux, Carles, & Pittet, 2015). Together, these studies show that TH2 cytokines are capable of blunting β_2 -receptor responsiveness in key lung structural cells via multiple direct and indirect mechanisms affecting β_2 -receptor phosphorylation status, and coupling to downstream signalling pathways.

2.2.2 Respiratory viruses

There is clinical evidence showing that asthmatic patients with evidence of respiratory viral infection poorly respond to β_2 -agonists. Isolated leukocytes from two different cohorts of patients (mild and severe asthma) exhibit a reduced response to isoproterenol which was further decreased by the presence of respiratory infections believed to viral in origin based on the symptoms (Busse, 1977). Adult asthmatic patients experiencing virally-induced exacerbations have a reduced bronchodilator response to β_2 -agonists (Reddel et al., 1999). Similarly, asthmatic children presenting for acute asthma associated with symptoms of respiratory infection required more treatment with β_2 -agonists compared to children without any virus infection (Rueter et al., 2012). Whether respiratory viruses including rhinovirus (RV) directly interfere with the broncho-protective of β_2 -agonists has been suggested by multiple experimental studies. Hakonarson and colleagues reported that rhinovirus-exposed tracheal smooth muscle tissues present a reduced bronchodilatory response to isoproterenol, an effect mediated via IL-1 β (Hakonarson, Carter, Maskeri, Hodinka, & Grunstein, 1999). A direct effect of respiratory viruses on β_2 -receptor function was demonstrated by the failure of sulfoneterol to inhibit antigen-induced contraction of isolated guinea pig airway smooth muscle derived from animals exposed intranasally with to the parainfluenza 3 (Buckner et al., 1981). It was shown that loss of β_2 -receptor function could be induced in cultured human airway smooth muscle following exposure to conditioned media collected from lung epithelial cells infected with RV (Trian et al., 2010). The main mechanism was later shown to involve an autocrine effect of COX-2-dependent production of prostaglandins (PGE $_2$, PGF $_{2\alpha}$) by airway smooth muscle cells causing a heterologous desensitization of β_2 -receptors (Van Ly et al., 2013).

The ability of PGE₂ to heterologously desensitize β_2 -receptor in human airway smooth muscle has been well documented as a main mechanism driving poor responses to β_2 -agonists (Penn, Panettieri, & Benovic, 1998). Recently, two novel pro-asthmatic stimuli (TLR2 agonists or Sphingosine 1-Phosphate (S1-P)) were reported to cause a PGE₂-dependent heterologous desensitization of the β_2 -receptor responses (assessed by cAMP accumulation to salbutamol and formoterol) in human airway smooth muscle cells (Alkhouri et al., 2014; Rumzhum, Rahman, Oliver, & Ammit, 2016). Of interest, there is evidence that certain leukotrienes can also desensitize β_2 -receptor responses. LTD₄ for example was described to inhibit isoproterenol-induced responses in human airway smooth muscle cells via CysLT1R and PKC pathways (Rovati et al., 2006). This is an interesting hypothesis as some animal reports have described the therapeutic benefit of cysteine leukotriene antagonists in respiratory syncytial virus bronchiolitis (Fullmer et al., 2005). Moore and colleagues demonstrated that direct infection of airway smooth muscle with respiratory syncytial virus was associated with a profound inhibition of isoproterenol-induced cAMP production. The inhibitory effect was time- and concentration-dependent and was not seen with cells infected with ultraviolet-inactivated virus. This specific effect of the virus on isoproterenol response was possibly due to a downregulation of β_2 -receptor expression (Moore et al., 2006). These studies show the capacity of different respiratory viruses to interfere with the bronchodilator response to β_2 -agonist therapy via multiple mechanisms resulting in receptor desensitization.

2.2.3. Other pro-asthmatic stimuli

Because airway remodelling is an important feature in asthma (C. E. Brightling, Gupta, Gonem, & Siddiqui, 2011), some groups have questioned whether remodelled airway smooth muscle had an altered response to β_2 -agonists. Indeed, stimuli including the nature of extracellular matrix (ECM) environment on which the cells grow on were described to dramatically affect the response to β_2 -agonists. For example, airway smooth muscle cells grown on fibronectin significantly increased isoproterenol-induced cAMP production, while exposure to collagen V or laminin decreased response to isoproterenol (Freyer, Billington, Penn, & Hall, 2004). These studies show that the ECM environment trigger phenotypic changes to airway smooth muscle cells that could dramatically affect β_2 -receptor function. This is an interesting observation as accumulation of collagen V is a defining feature of

airway remodelling in asthma (Bergeron, Tulic, & Hamid, 2010), an observation that could explain why severe asthma patients with features of airway remodelling display a poor bronchodilator response (Goleva, Hauk, Boguniewicz, Martin, & Leung, 2007).

Human bronchial tissues sensitized with asthmatic serum challenged with the house dust mite allergen displayed a rightward shift of the dose-response curve to salbutamol. This effect was blocked by montelukast, suggesting a key role played by cysteinyl-leukotrienes in the desensitizing action induced by the allergen (Rovati et al., 2006). Whether this effect results from a direct activation of IgE receptor expressed on airway smooth muscle cells remains a possibility. Indeed, several reports demonstrated that activation of the low affinity receptor FC ϵ RII (CD23) has the capacity to alter the bronchorelaxant response of isoproterenol in isolated rabbit tracheal rings (Hakonarson & Grunstein, 1998). The presence of functional IgE receptors on airway smooth muscle has been confirmed by different groups. IgE receptor activation was reported to activate several signalling pathways including ERK_{1/2}, p38, JNK MAPK, and Akt kinases and to regulate airway smooth muscle cell proliferation via the transcription factor STAT3 (Redhu et al., 2013). CD23 upregulation by IL-4/GM-CSF was found to be closely associated with markers of ASM cells hypertrophy (Belleau, Gandhi, McPherson, & Lew, 2005) while CD23 was reported to be constitutively increased in cells from asthmatic compared to non-asthmatic subjects and to induce the production of IL-1 β (Hakonarson, Carter, Kim, & Grunstein, 1999), known to inhibit β_2 -receptor relaxation in ASM tissues (Hakonarson et al., 1996; Wills-Karp et al., 1993). Finally, another study concluded that the sensitization phase was not a requirement for the aeroallergen Derp 1 to blunt β_2 -agonist bronchorelaxant responses in rabbit bronchial rings. This nice study suggested that allergen, through its intrinsic cysteine protease activity, had a direct effect on β_2 -receptor function via the activation of MAPK pathways (Grunstein et al., 2005).

Finally, antigens extracted from *Candida albicans* can impair β_2 -receptor responses in peripheral human T cells. While isoproterenol suppressed IL-2 secretion induced by concavalin, it failed to suppress the IL-2 response by *Candida albicans* antigens (Aihara et al., 1999). Although this observation was not tested in the lungs, one can speculate that the reason why some fungal allergens are closely associated with asthma severity in some

patients (Castanhinha et al., 2015) may derive from their ability to directly or indirectly cause β_2 -receptor dysfunction in bronchial smooth muscle.

3- Cross-talk between mast cells and β_2 -receptor function in asthma

3.1. Mast cell infiltration of ASM is a key determinant of the asthmatic phenotype

Mast cells play a major role in asthma pathophysiology through their activation by both allergen and diverse non-immunological stimuli. Secretion of the autacoid mediators histamine, PGD₂ and LTC₄ induces bronchoconstriction, mucus secretion and mucosal oedema, and thus contributes to the symptoms of asthma. Mast cells also secrete pro-inflammatory cytokines including IL-4, IL-5, and IL-13, which regulate IgE synthesis and the development of eosinophilic inflammation (Cruse & Bradding, 2016). In addition, the mast cell-derived growth factors and neutral proteases including TGF β , basic FGF (FGF2), tryptase and chymase potentially contribute to airway wall remodelling (Zuyderduyn, Sukkar, Fust, Dhaliwal, & Burgess, 2008).

Mast cells infiltrate several important structures within asthmatic airways including the bronchial epithelium (Birring, Berry, Brightling, & Pavord, 2003), mucous glands (Carroll, Mutavdzic, & James, 2002) and airway smooth muscle (ASM) (C. E. Brightling et al., 2002). We provided the first demonstration that mast cell infiltration of the airway smooth muscle is a key determinant of the asthmatic phenotype (C. E. Brightling et al., 2002). This important observation was later confirmed by other groups (Amin, Janson, Boman, & Venge, 2005; Begueret et al., 2007; El-Shazly et al., 2006). The number of mast cells within the ASM bundles correlates with the severity of airway hyperresponsiveness suggesting that their presence is functionally relevant (C. E. Brightling et al., 2002; Siddiqui et al., 2008). Importantly, mast cells within the ASM demonstrate ongoing cytokine secretion (C. E. Brightling et al., 2003) and ultrastructural features of activation, with piecemeal degranulation evident using electron microscopy (Begueret et al., 2007). Since numerous mast cell mediators affect ASM function and vice versa (Arthur & Bradding, 2016), there is likely to be an intimate interaction between mast cells and ASM cells in vivo.

3.2 Bidirectional interactions between mast cells and ASM

There is no animal model which recapitulates the infiltration of ASM by mast cells that is present in patients with asthma. However, *our* co-culture studies have shown several important bi-directional interactions which occur in the absence of exogenous cell activators. Human lung mast cells (HLMC) adhere to airway smooth muscle cells, in part through the adhesion molecule, cell adhesion molecule-1 (CADM1) expressed on HLMC (Yang et al., 2006). Stem cell factor (SCF) which is an essential survival factor for HLMC is expressed by both airway smooth muscle cells and lung fibroblasts in both soluble and membrane-bound forms (Hollins et al., 2008; Kassel et al., 1999). Therefore mast cell-airway smooth muscle interactions also occur via the SCF receptor Kit (CD117) expressed on mast cells and membrane SCF on airway smooth muscle cells (Lewis et al., 2016). When mast cells are adherent to ASM cells, they survive in the absence of exogenous growth factors/cytokines, and proliferate rapidly. This is mediated through a co-operative interaction between CADM1, SCF, and soluble IL-6 (Hollins et al., 2008). CADM1 and c-kit co-precipitate and co-localise within HLMC, and we have proposed that CADM1-dependent adhesion presents Kit to membrane-bound SCF on the ASM membrane (Hollins et al., 2008).

HLMC in co-culture with airway smooth muscle cells also become activated with evidence of increased constitutive histamine release and reduced histamine content (Hollins et al., 2008). They nevertheless maintain their ability to respond to Fc ϵ RI cross-linking. This is not an artefact of cell culture as the opposite is seen when HLMC are co-cultured with airway epithelial cells (Yang et al., 2006). The mechanism behind this requires further investigation but the findings are compatible with the *in vivo* observations of mast cell piecemeal degranulation within the ASM in asthma (Begueret et al., 2007).

3.3. Cross-talk between mast cell-ASM leads to β ₂-adrenoceptor dysfunction

We have shown that within 2 minutes of SCF exposure, there is uncoupling of the β ₂-receptor in HLMC leading to impaired inhibition of both histamine release and KCa3.1 ion channel modulation, followed by later β ₂-receptor internalisation (Cruse et al., 2010). This effect was associated with phosphorylation of the β ₂-receptor at specific tyrosine residues including Tyr₃₅₀ but not Tyr₁₄₁. Remarkably, in the presence of IgE and SCF, salbutamol actually increased histamine release in a dose-dependent manner (Cruse et al., 2010), although the reasons for the enhanced mast cell responses by salbutamol remain to be

determined. This receptor cross-talk between receptor tyrosine kinases (RTKs) and β_2 -receptor has also been reported in other cell types including pancreatic β cells, DDT1MF-2 smooth muscle cells or Chinese hamster ovary cells stably overexpressing β_2 -receptor. It was found that phosphorylation of the β_2 -receptor at Tyr_{350/354}, Tyr₁₄₁, Tyr₃₆₀ can be induced by insulin or insulin-like growth factor and was associated with β_2 -receptor uncoupling and internalisation (Doronin, Wang Hy, & Malbon, 2002; Karoor, Baltensperger, Paul, Czech, & Malbon, 1995; Karoor & Malbon, 1996; Karoor, Wang, Wang, & Malbon, 1998). In addition the β_2 -receptor can also be phosphorylated by insulin at Ser_{345/346} residues through the phosphatidylinositol-3-kinase (PI3K)-dependent activation of Akt (Doronin, Shumay, Wang, & Malbon, 2002). Our studies show that phosphorylation of the β_2 -receptor at Tyr350 residues by the Kit RTK can result in receptor desensitization and internalization and loss in cell response to β_2 -agonists.

In keeping with the findings that exogenously added SCF leads to β_2 -receptor dysfunction in HLMCs, co-culture of HLMCs with human airway smooth muscle cells produced very similar results. Thus co-culture increased “constitutive” HLMC histamine release, and this was increased further by the addition of β_2 -adrenoceptor agonists (Lewis et al., 2016). Inhibition of Fc ϵ RI-dependent HLMC mediator release by β_2 -agonists was also greatly reduced in HLMC–HASMCM co-culture. These effects were again associated with phosphorylation of Tyr350 on the β_2 -receptor, and were reversed by neutralization of SCF or CADM1.

We also made the interesting finding that formoterol failed to inhibit airway smooth muscle cell contraction when cells were co-cultured with HLMC (Lewis et al., 2016). Another study also found that culturing airway smooth muscle with activated T cells led to an attenuation of β -adrenoceptor-mediated airway relaxation (Hakonarson, Kim, Whelan, Campbell, & Grunstein, 2001), although the hypothesis of a change in receptor phosphorylation was not investigated. Our study showed that the loss of airway smooth muscle responsiveness to formoterol in co-culture was also associated with the rapid phosphorylation of the β_2 -receptor at tyrosine 350 residues (Lewis et al., 2016). This effect was again dependent on both SCF and CADM-1 suggesting that cell-cell interactions played an important role in driving β_2 -receptor dysfunction. Whether airway smooth muscle β_2 -receptor phosphorylation also occurred indirectly via the release of a mast cell mediator

with receptor tyrosine kinase activity remains a strong possibility. Indeed a number of RTK mediators such as VEGF, FGF2, TGF and NGF have been shown to trigger various cellular responses in airway smooth muscle cells (Bosse & Rola-Pleszczynski, 2008; Freund-Michel & Frossard, 2008; Kazi et al., 2004; Kumawat et al., 2016). We have now evidence to suggest that at least FGF and TGF can desensitize β_2 -receptors in airway smooth muscle cells via receptor phosphorylation on tyrosine residues (manuscript in preparation) (all mechanisms are summarized in **Figure 3**).

4. Bitter taste receptor agonists: an alternative bronchodilatory strategy

Because of the loss of the therapeutic benefits of β_2 -agonists in asthma, there is an unmet need for a better understanding of the mechanisms that prevent the drugs from working properly in patients. Recently, an alternative therapeutic strategy to provide bronchodilation has also come under scrutiny.

4.1- Bronchodilator activity

In 2010 Deshpande and colleagues made the surprising discovery that activation of bitter taste receptors (TAS2Rs) by using the different agonists (saccharin, chloroquine and denatonium) evoked marked relaxation in isolated murine and human bronchial tissues despite triggering calcium responses (Deshpande et al., 2010). This finding has fuelled a lot of enthusiasm as TAS2R signalling and effects were different from those initiated by β_2 -receptors. TAS2R activation leads to a stronger bronchodilatory action when compared to isoproterenol, a response that was not dependent on the classical cAMP dependent manner. Of strong clinical interest was the observation that activation of TAS2Rs showed a relatively modest degree of agonist-promoted receptor desensitization ranging from 31% (Robinett, Deshpande, Malone, & Liggett, 2011) to a nonsignificant 11% (An et al., 2012), and did not affect the therapeutic responses to β_2 -agonists (An et al., 2012). Although the precise mechanisms by which TAS2R agonists trigger bronchorelaxation have not been completely elucidated, it was reported that chloroquine or quinine can both inhibit contractile agonist-evoked calcium oscillations and sensitivity in murine precision cut lung slices (Tan & Sanderson, 2014). It is interesting to note that the relaxant responses of TAS2Rs were still preserved in asthmatic ASM cells or was not affected by IL-13 (Robinett et al., 2014). These observations describe the interesting therapeutic profile of TAS2Rs with

their unique mechanism of relaxation and lack of promoting heterologous desensitization of β_2 -receptors.

4.2- Other therapeutic benefits of bitter taste receptors

Emerging studies have been performed to determine the preclinical value of TAS2R agonists in animal models of allergic asthma. Recently, it was reported that aerosol administration of TAS2R agonists chloroquine or quinine can reverse house dust mite-associated key allergic responses including features of airway remodelling (i.e., airway smooth muscle mass, lung fibrosis and mucus accumulation), airway inflammation, and airway hyper-responsiveness (Sharma et al., 2017). The striking effect of TAS2R agonists on ASM remodelling seen in the animal study could result from their direct cytotoxic action through changes in mitochondrial structure and function recently reported in cultured ASM cells (Pan, Sharma, Shah, & Deshpande, 2017). Combined together, these exciting studies suggest that TAS2R agonists represent a potential novel bronchodilatory strategy with additional anti-allergic properties.

Future clinical trials are needed to determine whether TAS2R agonists represent an additional bronchodilator therapy in the treatment of lung diseases, especially in patients who are not adequately responding to traditional drugs.

Concluding remarks

A wealth of clinical and experimental evidence has suggested that β_2 -receptors may be dysfunctional in certain groups of patients with asthma who lose their broncho-protection to β_2 -agonists, and who may experience decreased asthma control with chronic β_2 -agonist exposure. These patients include those suffering from a severe form of the disease, those experiencing exacerbations caused by a viral infection and those who died from asthma. Both “constitutive” and “inducible” alterations of β_2 -receptors have been reported in asthmatics not only in the ASM tissues/cells, the main tissue target of agonists, but also in various key inflammatory cells (mast cells, lymphocytes) and lung structural cells (epithelial cells). Different studies including those using different experimental models to study bronchorelaxation have identified some of the key pro-asthmatic factors and associated mechanisms that promote this status of hypo-responsiveness to β_2 -agonists seen

in asthma. Soluble factors including pro-asthmatic cytokines (both Th1 and Th2), growth factors, respiratory viruses, certain allergens, and activation of IgE receptors can interfere with β_2 -receptors at different levels. The changes in β_2 -receptor function susceptible of blunting cell responsiveness to agonists include a reduction receptor density, uncoupling of the receptor to downstream signalling pathways, heterologous desensitization of the receptor (growth factors, PGE2 receptors), and receptor phosphorylation by RTKs or GRK2. Our group has shown that cell-cell interactions between mast cells and airway smooth muscle could play a key role in driving β_2 -receptor dysfunction in asthma. Additional studies are required to determine whether targeting these different pathways could restore the bronchoprotective action of β_2 -agonists. A promising bronchodilator strategy was recently uncovered with the discovery of functional bitter taste receptors on ASM. Activation of TAS2Rs led to strong bronchorelaxant responses in both mouse and human isolated airway preparations, was not associated with marked agonist-evoked receptor desensitization and was not perturbed in asthmatic cells. The pathways inhibiting β_2 -receptor function and TAS2Rs represents therefore novel therapeutic targets for the design of novel bronchodilator drugs to treat lung diseases.

Figure legends

Figure 1: Molecular mechanisms driving the bronchodilator action of β 2-agonists. β 2-agonists directly activate β 2-receptor coupled to Gs on airway smooth muscle to augment the activity of adenylyl cyclase (AC) leading to cAMP generation, activation of cAMP-dependent protein kinase (PKA) and ASM relaxation and bronchodilation. Other pathways may also be involved including the activation of cAMP-mediated exchange protein (Epac) which inhibits the activation of the Rho pathways or the opening of the large conductance calcium-activated potassium channels (BKCa) which causes membrane hyperpolarization. It is believed that cAMP-dependent pathways represent the main mechanism driving ASM relaxation by β 2-agonists.

Figure 2: Therapeutic actions of β 2-agonists in ASM that are enhanced by corticosteroids. β 2-agonists stimulate different therapeutic responses in ASM that have been shown to be enhanced by corticosteroids. Corticosteroids have been shown to enhance bronchodilator action of β 2-agonists by i) increasing levels of Regulator of G-protein signalling 2 (RGS2) which interferes with agonist-evoked ASM contraction or ii) reducing receptor homologous desensitization. Corticosteroids also enhance the inhibitory action of β 2-agonists on production of various inflammatory mediators which may explain, at least in part, their impact on influx of inflammatory cells within the airways seen in some studies. Finally, β 2-agonists have been shown to reduce key features of ASM remodelling.

Figure 3: Mechanisms of β 2-receptor dysfunction in ASM by mast cells. Mast cells can impair ASM responsiveness to β 2-agonists via heterologous receptor desensitization which occurs via two main mechanisms. The first one is a direct mechanism where cell-cell physical interactions requiring SCF on ASM and c-kit on mast cells (and CADM-1) drive β 2-receptor phosphorylation at tyrosine residues. The second mechanism of β 2-receptor dysfunction is indirect and mediated via the paracrine action of mediators released by activated mast cells such as growth factors which also stimulate β 2-receptor phosphorylation at tyrosine residues. These mast cell mediators could also act on mast cells in autocrine manner and desensitize the anti-inflammatory action of β 2-agonists via β 2-receptor phosphorylation thus preventing mast cell activation and degranulation.

References

- Aihara, M., Dobashi, K., Horie, T., Iizuka, K., Nakazawa, T., & Mori, M. (1999). Effects of isoproterenol on IL-2 and cAMP production in peripheral T cells from asthmatic and non-asthmatic subjects sensitive to *Candida*. *Biol Cell*, 91(7), 525-531.
- Albano, G. D., Zhao, J., Etling, E. B., Park, S. Y., Hu, H., Trudeau, J. B., . . . Wenzel, S. E. (2015). IL-13 desensitizes beta2-adrenergic receptors in human airway epithelial cells through a 15-lipoxygenase/G protein receptor kinase 2 mechanism. *J Allergy Clin Immunol*, 135(5), 1144-1153 e1141-1149.
- Aldridge, R. E., Hancox, R. J., Robin Taylor, D., Cowan, J. O., Winn, M. C., Frampton, C. M., & Town, G. I. (2000). Effects of terbutaline and budesonide on sputum cells and bronchial hyperresponsiveness in asthma. *Am J Respir Crit Care Med*, 161(5), 1459-1464.
- Alkhouri, H., Rumzhum, N. N., Rahman, M. M., FitzPatrick, M., de Pedro, M., Oliver, B. G., . . . Ammit, A. J. (2014). TLR2 activation causes tachyphylaxis to beta2 -agonists in vitro and ex vivo: modelling bacterial exacerbation. *Allergy*, 69(9), 1215-1222.
- Amin, K., Janson, C., Boman, G., & Venge, P. (2005). The extracellular deposition of mast cell products is increased in hypertrophic airways smooth muscles in allergic asthma but not in nonallergic asthma. *Allergy*, 60(10), 1241-1247.
- Ammit, A. J., Lazaar, A. L., Irani, C., O'Neill, G. M., Gordon, N. D., Amrani, Y., . . . Panettieri, R. A., Jr. (2002). Tumor Necrosis Factor-alpha -Induced Secretion of RANTES and Interleukin-6 from Human Airway Smooth Muscle Cells . Modulation by Glucocorticoids and beta -Agonists. *Am J Respir Cell Mol Biol*, 26(4), 465-474.
- An, S. S., Wang, W. C., Koziol-White, C. J., Ahn, K., Lee, D. Y., Kurten, R. C., . . . Liggett, S. B. (2012). TAS2R activation promotes airway smooth muscle relaxation despite beta(2)-adrenergic receptor tachyphylaxis. *Am J Physiol Lung Cell Mol Physiol*, 303(4), L304-311.
- Arthur, G., & Bradding, P. (2016). New Developments in Mast Cell Biology: Clinical Implications. [Review]. *Chest*, 150(3), 680-693.
- Bai, T. R. (1991). Abnormalities in airway smooth muscle in fatal asthma. A comparison between trachea and bronchus. *Am Rev Respir Dis*, 143(2), 441-443.
- Barnes, P. J., Dollery, C. T., & MacDermot, J. (1980). Increased pulmonary alpha-adrenergic and reduced beta-adrenergic receptors in experimental asthma. *Nature*, 285(5766), 569-571.
- Begueret, H., Berger, P., Vernejoux, J. M., Dubuisson, L., Marthan, R., & Tunon-de-Lara, J. M. (2007). Inflammation of bronchial smooth muscle in allergic asthma. *Thorax*, 62(1), 8-15.
- Belleau, J. T., Gandhi, R. K., McPherson, H. M., & Lew, D. B. (2005). Research upregulation of CD23 (FcepsilonRII) expression in human airway smooth muscle cells (huASMC) in response to IL-4, GM-CSF, and IL-4/GM-CSF. *Clin Mol Allergy*, 3, 6.
- Bergeron, C., Tulic, M. K., & Hamid, Q. (2010). Airway remodelling in asthma: from benchside to clinical practice. *Can Respir J*, 17(4), e85-93.
- Birring, S. S., Berry, M., Brightling, C. E., & Pavord, I. D. (2003). Eosinophilic bronchitis: clinical features, management and pathogenesis. *Am J Respir Med*, 2(2), 169-173.

- Bosse, Y., & Rola-Pleszczynski, M. (2008). FGF2 in asthmatic airway-smooth-muscle-cell hyperplasia. [Review]. *Trends Mol Med*, 14(1), 3-11.
- Brightling, C., Berry, M., & Amrani, Y. (2008). Targeting TNF-alpha: a novel therapeutic approach for asthma. *J Allergy Clin Immunol*, 121(1), 5-10; quiz 11-12.
- Brightling, C. E., Bradding, P., Symon, F. A., Holgate, S. T., Wardlaw, A. J., & Pavord, I. D. (2002). Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med*, 346(22), 1699-1705.
- Brightling, C. E., Gupta, S., Gonem, S., & Siddiqui, S. (2011). Lung damage and airway remodelling in severe asthma. *Clin Exp Allergy*. doi: 10.1111/j.1365-2222.2011.03917.x
- Brightling, C. E., Symon, F. A., Holgate, S. T., Wardlaw, A. J., Pavord, I. D., & Bradding, P. (2003). Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin Exp Allergy*, 33(12), 1711-1716.
- Buckner, C. K., Clayton, D. E., Ain-Shoka, A. A., Busse, W. W., Dick, E. C., & Shult, P. (1981). Parainfluenza 3 infection blocks the ability of a beta adrenergic receptor agonist to inhibit antigen-induced contraction of guinea pig isolated airway smooth muscle. *J Clin Invest*, 67(2), 376-384.
- Busse, W. W. (1977). Decreased granulocyte response to isoproterenol in asthma during upper respiratory infections. *Am Rev Respir Dis*, 115(5), 783-791.
- Carroll, N. G., Mutavdzic, S., & James, A. L. (2002). Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax*, 57(8), 677-682.
- Castan, L., Magnan, A., & Bouchaud, G. (2017). Chemokine receptors in allergic diseases. [Review]. *Allergy*, 72(5), 682-690.
- Castanhinha, S., Sherburn, R., Walker, S., Gupta, A., Bossley, C. J., Buckley, J., . . . Saglani, S. (2015). Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33. *J Allergy Clin Immunol*, 136(2), 312-322 e317.
- Chen, H., Tliba, O., Van Besien, C. R., Panettieri, R. A., Jr., & Amrani, Y. (2003). Selected Contribution: TNF-{alpha} modulates murine tracheal rings responsiveness to G-protein-coupled receptor agonists and KCl. *J Appl Physiol*, 95(2), 864-872.
- Chiu, J. C., Hsu, J. Y., Fu, L. S., Chu, J. J., & Chi, C. S. (2007). Comparison of the effects of two long-acting beta2-agonists on cytokine secretion by human airway epithelial cells. *J Microbiol Immunol Infect*, 40(5), 388-394.
- Chong, L. K., Cooper, E., Vardey, C. J., & Peachell, P. T. (1998). Salmeterol inhibition of mediator release from human lung mast cells by beta-adrenoceptor-dependent and independent mechanisms. *Br J Pharmacol*, 123(5), 1009-1015.
- Chorley, B. N., Li, Y., Fang, S., Park, J. A., & Adler, K. B. (2006). (R)-albuterol elicits antiinflammatory effects in human airway epithelial cells via iNOS. *Am J Respir Cell Mol Biol*, 34(1), 119-127.
- Cockcroft, D. W., McParland, C. P., Britto, S. A., Swystun, V. A., & Rutherford, B. C. (1993). Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet*, 342(8875), 833-837.

- Cooper, P. R., & Panettieri, R. A., Jr. (2008). Steroids completely reverse albuterol-induced beta(2)-adrenergic receptor tolerance in human small airways. *J Allergy Clin Immunol*. doi: S0091-6749(08)01375-4
- Cruse, G., & Bradding, P. (2016). Mast cells in airway diseases and interstitial lung disease. *Eur J Pharmacol*, 778, 125-138.
- Cruse, G., Yang, W., Duffy, S. M., Chachi, L., Leyland, M., Amrani, Y., & Bradding, P. (2010). Counterregulation of beta(2)-adrenoceptor function in human mast cells by stem cell factor. *J Allergy Clin Immunol*, 125(1), 257-263 e251-255.
- Dekkers, B. G., Pehlic, A., Mariani, R., Bos, I. S., Meurs, H., & Zaagsma, J. (2012). Glucocorticosteroids and beta(2)-adrenoceptor agonists synergize to inhibit airway smooth muscle remodeling. *J Pharmacol Exp Ther*, 342(3), 780-787.
- Deshpande, D. A., Wang, W. C., McIlmoyle, E. L., Robinett, K. S., Schillinger, R. M., An, S. S., . . . Liggett, S. B. (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med*, 16(11), 1299-1304.
- Doronin, S., Shumay, E., Wang, H. Y., & Malbon, C. C. (2002). Akt mediates sequestration of the beta(2)-adrenergic receptor in response to insulin. *J Biol Chem*, 277(17), 15124-15131.
- Doronin, S., Wang Hy, H. Y., & Malbon, C. C. (2002). Insulin stimulates phosphorylation of the beta 2-adrenergic receptor by the insulin receptor, creating a potent feedback inhibitor of its tyrosine kinase. *J Biol Chem*, 277(12), 10698-10703.
- Duffy, S. M., Cruse, G., Lawley, W. J., & Bradding, P. (2005). Beta2-adrenoceptor regulation of the K+ channel iKCa1 in human mast cells. *FASEB J*, 19(8), 1006-1008.
- El-Shazly, A., Berger, P., Girodet, P. O., Ousova, O., Fayon, M., Vernejoux, J. M., . . . Tunon-de-Lara, J. M. (2006). Fraktalkine produced by airway smooth muscle cells contributes to mast cell recruitment in asthma. *J Immunol*, 176(3), 1860-1868.
- Emala, C., Black, C., Curry, C., Levine, M. A., & Hirshman, C. A. (1993). Impaired beta-adrenergic receptor activation of adenylyl cyclase in airway smooth muscle in the basenji-greyhound dog model of airway hyperresponsiveness. *Am J Respir Cell Mol Biol*, 8(6), 668-675.
- Emala, C. W., Kuhl, J., Hungerford, C. L., & Hirshman, C. A. (1997). TNF-alpha inhibits isoproterenol-stimulated adenylyl cyclase activity in cultured airway smooth muscle cells. *Am J Physiol*, 272(4 Pt 1), L644-650.
- Fogli, S., Stefanelli, F., Battolla, B., Bianchi, F., Breschi, M. C., & Mattii, L. (2015). Salbutamol inhibits RhoA activation in normal but not in desensitized bronchial smooth muscle cells. *J Pharm Pharmacol*, 67(10), 1416-1420.
- Freund-Michel, V., & Frossard, N. (2008). Overexpression of functional TrkA receptors after internalisation in human airway smooth muscle cells. *Biochim Biophys Acta*, 1783(10), 1964-1971.
- Freyer, A. M., Billington, C. K., Penn, R. B., & Hall, I. P. (2004). Extracellular matrix modulates beta2-adrenergic receptor signaling in human airway smooth muscle cells. *Am J Respir Cell Mol Biol*, 31(4), 440-445.

- Fullmer, J. J., Khan, A. M., Elidemir, O., Chiappetta, C., Stark, J. M., & Colasurdo, G. N. (2005). Role of cysteinyl leukotrienes in airway inflammation and responsiveness following RSV infection in BALB/c mice. *Pediatr Allergy Immunol*, 16(7), 593-601.
- George, T., Bell, M., Chakraborty, M., Siderovski, D. P., Giembycz, M. A., & Newton, R. (2017). Protective Roles for RGS2 in a Mouse Model of House Dust Mite-Induced Airway Inflammation. *PLoS One*, 12(1), e0170269.
- Giembycz, M. A., & Newton, R. (2006). Beyond the dogma: novel beta2-adrenoceptor signalling in the airways. *Eur Respir J*, 27(6), 1286-1306.
- Goldie, R. G., Spina, D., Henry, P. J., Lulich, K. M., & Paterson, J. W. (1986). In vitro responsiveness of human asthmatic bronchus to carbachol, histamine, beta-adrenoceptor agonists and theophylline. *Br J Clin Pharmacol*, 22(6), 669-676.
- Goleva, E., Hauk, P. J., Boguniewicz, J., Martin, R. J., & Leung, D. Y. (2007). Airway remodeling and lack of bronchodilator response in steroid-resistant asthma. *J Allergy Clin Immunol*, 120(5), 1065-1072.
- Green, R. H., Brightling, C. E., McKenna, S., Hargadon, B., Neale, N., Parker, D., . . . Pavord, I. D. (2006). Comparison of asthma treatment given in addition to inhaled corticosteroids on airway inflammation and responsiveness. *Eur Respir J*, 27(6), 1144-1151.
- Grunstein, M. M., Hakonarson, H., Leiter, J., Chen, M., Whelan, R., Grunstein, J. S., & Chuang, S. (2002). IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol*, 282(3), L520-528.
- Grunstein, M. M., Veler, H., Shan, X., Larson, J., Grunstein, J. S., & Chuang, S. (2005). Proasthmatic effects and mechanisms of action of the dust mite allergen, Der p 1, in airway smooth muscle. *J Allergy Clin Immunol*, 116(1), 94-101.
- Gupta, M. K., Asosingh, K., Aronica, M., Comhair, S., Cao, G., Erzurum, S., . . . Naga Prasad, S. V. (2015). Defective Resensitization in Human Airway Smooth Muscle Cells Evokes beta-Adrenergic Receptor Dysfunction in Severe Asthma. *PLoS One*, 10(5), e0125803.
- Hakonarson, H., Carter, C., Kim, C., & Grunstein, M. M. (1999). Altered expression and action of the low-affinity IgE receptor FcepsilonRII (CD23) in asthmatic airway smooth muscle. *J Allergy Clin Immunol*, 104(3 Pt 1), 575-584.
- Hakonarson, H., Carter, C., Maskeri, N., Hodinka, R., & Grunstein, M. M. (1999). Rhinovirus-mediated changes in airway smooth muscle responsiveness: induced autocrine role of interleukin-1beta. *Am J Physiol*, 277(1 Pt 1), L13-21.
- Hakonarson, H., & Grunstein, M. M. (1998). Autologously up-regulated Fc receptor expression and action in airway smooth muscle mediates its altered responsiveness in the atopic asthmatic sensitized state. *Proc Natl Acad Sci U S A*, 95(9), 5257-5262.
- Hakonarson, H., Herrick, D. J., Serrano, P. G., & Grunstein, M. M. (1996). Mechanism of cytokine-induced modulation of beta-adrenoceptor responsiveness in airway smooth muscle. *J Clin Invest*, 97(11), 2593-2600.

- Hakonarson, H., Herrick, D. J., Serrano, P. G., & Grunstein, M. M. (1997). Autocrine role of interleukin 1beta in altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest*, 99(1), 117-124.
- Hakonarson, H., Kim, C., Whelan, R., Campbell, D., & Grunstein, M. (2001). Bi-directional activation between human airway smooth muscle cells and T lymphocytes: role in induction of altered airway responsiveness. *J Immunol* 2001, 166(1), 293-303.
- Hakonarson, H., Maskeri, N., Carter, C., Chuang, S., & Grunstein, M. M. (1999). Autocrine interaction between IL-5 and IL-1beta mediates altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest*, 104(5), 657-667.
- Hallsworth, M. P., Twort, C. H., Lee, T. H., & Hirst, S. J. (2001). beta(2)-adrenoceptor agonists inhibit release of eosinophil-activating cytokines from human airway smooth muscle cells. *Br J Pharmacol*, 132(3), 729-741.
- Holden, N. S., Bell, M. J., Rider, C. F., King, E. M., Gaunt, D. D., Leigh, R., . . . Newton, R. (2011). beta2-Adrenoceptor agonist-induced RGS2 expression is a genomic mechanism of bronchoprotection that is enhanced by glucocorticoids. *Proc Natl Acad Sci U S A*, 108(49), 19713-19718.
- Hollins, F., Kaur, D., Yang, W., Cruse, G., Saunders, R., Sutcliffe, A., . . . Bradding, P. (2008). Human airway smooth muscle promotes human lung mast cell survival, proliferation, and constitutive activation: cooperative roles for CADM1, stem cell factor, and IL-6. *J Immunol*, 181(4), 2772-2780.
- Hung, C. H., Chu, Y. T., Hua, Y. M., Hsu, S. H., Lin, C. S., Chang, H. C., . . . Jong, Y. J. (2008). Effects of formoterol and salmeterol on the production of Th1- and Th2-related chemokines by monocytes and bronchial epithelial cells. *Eur Respir J*, 31(6), 1313-1321.
- Jeffery, P. K., Venge, P., Gizycki, M. J., Egerod, I., Dahl, R., & Faurschou, P. (2002). Effects of salmeterol on mucosal inflammation in asthma: a placebo-controlled study. *Eur Respir J*, 20(6), 1378-1385.
- Johnston, S. L., & Edwards, M. R. (2009). Mechanisms of adverse effects of {beta}-agonists in asthma. *Thorax*, 64(9), 739-741.
- Karoor, V., Baltensperger, K., Paul, H., Czech, M. P., & Malbon, C. C. (1995). Phosphorylation of tyrosyl residues 350/354 of the beta-adrenergic receptor is obligatory for counterregulatory effects of insulin. *J Biol Chem*, 270(43), 25305-25308.
- Karoor, V., & Malbon, C. C. (1996). Insulin-like growth factor receptor-1 stimulates phosphorylation of the beta2-adrenergic receptor in vivo on sites distinct from those phosphorylated in response to insulin. *J Biol Chem*, 271(46), 29347-29352.
- Karoor, V., Wang, L., Wang, H. Y., & Malbon, C. C. (1998). Insulin stimulates sequestration of beta-adrenergic receptors and enhanced association of beta-adrenergic receptors with Grb2 via tyrosine 350. *J Biol Chem*, 273(49), 33035-33041.
- Kassel, O., Schmidlin, F., Duvernelle, C., Gasser, B., Massard, G., & Frossard, N. (1999). Human bronchial smooth muscle cells in culture produce stem cell factor. *Eur Respir J*, 13(5), 951-954.

- Kaur, M., Chivers, J. E., Giembycz, M. A., & Newton, R. (2008). Long-acting beta2-adrenoceptor agonists synergistically enhance glucocorticoid-dependent transcription in human airway epithelial and smooth muscle cells. *Mol Pharmacol*, 73(1), 203-214.
- Kazi, A. S., Lotfi, S., Goncharova, E. A., Tliba, O., Amrani, Y., Krymskaya, V. P., & Lazaar, A. L. (2004). Vascular endothelial growth factor-induced secretion of fibronectin is ERK dependent. *Am J Physiol Lung Cell Mol Physiol*, 286(3), L539-545.
- Kleine-Tebbe, J., Frank, G., Josties, C., & Kunkel, G. (1994). Influence of salmeterol, a long-acting beta 2-adrenoceptor agonist, on IgE-mediated histamine release from human basophils. *J Investig Allergol Clin Immunol*, 4(1), 12-17.
- Kumawat, K., Koopmans, T., Menzen, M. H., Prins, A., Smit, M., Halayko, A. J., & Gosens, R. (2016). Cooperative signaling by TGF-beta1 and WNT-11 drives sm-alpha-actin expression in smooth muscle via Rho kinase-actin-MRTF-A signaling. *Am J Physiol Lung Cell Mol Physiol*, 311(3), L529-537.
- Lahiri, T., Moore, P. E., Baraldo, S., Whitehead, T. R., McKenna, M. D., Panettieri, R. A., Jr., & Shore, S. A. (2002). Effect of IL-1beta on CRE-dependent gene expression in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol*, 283(6), L1239-1246.
- Laporte, J. C., Moore, P. E., Baraldo, S., Jouvin, M. H., Church, T. L., Schwartzman, I. N., . . . Shore, S. A. (2001). Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. *Am J Respir Crit Care Med*, 164(1), 141-148.
- Laporte, J. D., Moore, P. E., Panettieri, R. A., Moeller, W., Heyder, J., & Shore, S. A. (1998). Prostanoids mediate IL-1beta-induced beta-adrenergic hyporesponsiveness in human airway smooth muscle cells. *Am J Physiol*, 275(3 Pt 1), L491-501.
- Lewis, R. J., Chachi, L., Newby, C., Amrani, Y., & Bradding, P. (2016). Bidirectional Counterregulation of Human Lung Mast Cell and Airway Smooth Muscle beta2 Adrenoceptors. *J Immunol*, 196(1), 55-63.
- Manetsch, M., Che, W., Seidel, P., Chen, Y., & Ammit, A. J. (2012). MKP-1: a negative feedback effector that represses MAPK-mediated pro-inflammatory signaling pathways and cytokine secretion in human airway smooth muscle cells. *Cell Signal*, 24(4), 907-913.
- Manetsch, M., Rahman, M. M., Patel, B. S., Ramsay, E. E., Rumzhum, N. N., Alkhouri, H., . . . Ammit, A. J. (2013). Long-acting beta2-agonists increase fluticasone propionate-induced mitogen-activated protein kinase phosphatase 1 (MKP-1) in airway smooth muscle cells. *PLoS One*, 8(3), e59635.
- Meurs, H., Koeter, G. H., de Vries, K., & Kauffman, H. F. (1982). The beta-adrenergic system and allergic bronchial asthma: changes in lymphocyte beta-adrenergic receptor number and adenylate cyclase activity after an allergen-induced asthmatic attack. *J Allergy Clin Immunol*, 70(4), 272-280.
- Moore, P. E., Cunningham, G., Calder, M. M., DeMatteo, A. D., Jr., Peeples, M. E., Summar, M. L., & Peebles, R. S., Jr. (2006). Respiratory syncytial virus infection reduces beta2-adrenergic responses in human airway smooth muscle. *Am J Respir Cell Mol Biol*, 35(5), 559-564.

- Moore, P. E., Lahiri, T., Laporte, J. D., Church, T., Panettieri, R. A., Jr., & Shore, S. A. (2001). Selected Contribution: Synergism between TNF-alpha and IL-1beta in airway smooth muscle cells: implications for beta-adrenergic responsiveness. *J Appl Physiol*, 91(3), 1467-1474.
- Moore, P. E., Laporte, J. D., Gonzalez, S., Moller, W., Heyder, J., Panettieri, R. A., Jr., & Shore, S. A. (1999). Glucocorticoids ablate IL-1beta-induced beta-adrenergic hyporesponsiveness in human airway smooth muscle cells. *Am J Physiol*, 277(5 Pt 1), L932-942.
- Nelson, H. S., Weiss, S. T., Bleecker, E. R., Yancey, S. W., Dorinsky, P. M., & Group, S. S. (2006). The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest*, 129(1), 15-26.
- Newton, R. (2014). Anti-inflammatory glucocorticoids: changing concepts. *Eur J Pharmacol*, 724, 231-236.
- Newton, R., & Giembycz, M. A. (2016). Understanding how long-acting beta2 -adrenoceptor agonists enhance the clinical efficacy of inhaled corticosteroids in asthma - an update. [Review]. *Br J Pharmacol*, 173(24), 3405-3430.
- Nie, M., Knox, A. J., & Pang, L. (2005). beta2-Adrenoceptor agonists, like glucocorticoids, repress eotaxin gene transcription by selective inhibition of histone H4 acetylation. *J Immunol*, 175(1), 478-486.
- Pan, S., Sharma, P., Shah, S. D., & Deshpande, D. A. (2017). Bitter Taste Receptor Agonists Alter Mitochondrial Function and Induce Autophagy in Airway Smooth Muscle Cells. *Am J Physiol Lung Cell Mol Physiol*, ajplung 00106 02017.
- Pang, L., Holland, E., & Knox, A. J. (1998). Role of cyclo-oxygenase-2 induction in interleukin-1beta induced attenuation of cultured human airway smooth muscle cell cyclic AMP generation in response to isoprenaline. *Br J Pharmacol*, 125(6), 1320-1328.
- Pang, L., & Knox, A. (2001). Regulation of TNF-alpha-induced eotaxin release from cultured human airway smooth muscle cells by beta2-agonists and corticosteroids. *FASEB J*, 15(1), 261-269.
- Pang, L., & Knox, A. J. (2000). Synergistic inhibition by beta(2)-agonists and corticosteroids on tumor necrosis factor-alpha-induced interleukin-8 release from cultured human airway smooth-muscle cells. *Am J Respir Cell Mol Biol*, 23(1), 79-85.
- Penn, R. B., Panettieri, R. A., Jr., & Benovic, J. L. (1998). Mechanisms of Acute Desensitization of the beta 2AR-Adenylyl Cyclase Pathway in Human Airway Smooth Muscle. *Am. J. Respir. Cell Mol. Biol.*, 19(2), 338-348.
- Pera, T., & Penn, R. B. (2016). Bronchoprotection and bronchorelaxation in asthma: New targets, and new ways to target the old ones. [Review]. *Pharmacol Ther*, 164, 82-96.
- Pinto Pereira, L. M., Clement, Y., Rouse, J., Matthew, J., Asgarali, Z., Ramoutar, D., & Teelucksingh, S. (2003). Salmeterol decreases eosinophilic cationic protein and rescue medication in patients inhaling beclomethasone dipropionate: preliminary study in mild and moderate asthma in Trinidad, West Indies. *Int J Clin Pharmacol Res*, 23(2-3), 69-74.

- Quante, T., Ng, Y. C., Ramsay, E. E., Henness, S., Allen, J. C., Parmentier, J., . . . Ammit, A. J. (2008). Corticosteroids reduce IL-6 in ASM cells via up-regulation of MKP-1. *Am J Respir Cell Mol Biol*, 39(2), 208-217.
- Reddel, H., Ware, S., Marks, G., Salome, C., Jenkins, C., & Woolcock, A. (1999). Differences between asthma exacerbations and poor asthma control. *Lancet*, 353(9150), 364-369.
- Redhu, N. S., Shan, L., Al-Subait, D., Ashdown, H. L., Movassagh, H., Lamkhioued, B., & Gounni, A. S. (2013). IgE induces proliferation in human airway smooth muscle cells: role of MAPK and STAT3 pathways. *Allergy Asthma Clin Immunol*, 9(1), 41.
- Reid, D. W., Ward, C., Wang, N., Zheng, L., Bish, R., Orsida, B., & Walters, E. H. (2003). Possible anti-inflammatory effect of salmeterol against interleukin-8 and neutrophil activation in asthma in vivo. *Eur Respir J*, 21(6), 994-999.
- Roberts, J. A., Bradding, P., Britten, K. M., Walls, A. F., Wilson, S., Gratziau, C., . . . Howarth, P. H. (1999). The long-acting beta2-agonist salmeterol xinafoate: effects on airway inflammation in asthma. *Eur Respir J*, 14(2), 275-282.
- Robinett, K. S., Deshpande, D. A., Malone, M. M., & Liggett, S. B. (2011). Agonist-promoted homologous desensitization of human airway smooth muscle bitter taste receptors. *Am J Respir Cell Mol Biol*, 45(5), 1069-1074.
- Robinett, K. S., Koziol-White, C. J., Akoluk, A., An, S. S., Panettieri, R. A., Jr., & Liggett, S. B. (2014). Bitter taste receptor function in asthmatic and nonasthmatic human airway smooth muscle cells. *Am J Respir Cell Mol Biol*, 50(4), 678-683.
- Roux, J., McNicholas, C. M., Carles, M., Goolaerts, A., Houseman, B. T., Dickinson, D. A., . . . Pittet, J. F. (2013). IL-8 inhibits cAMP-stimulated alveolar epithelial fluid transport via a GRK2/PI3K-dependent mechanism. *FASEB J*, 27(3), 1095-1106.
- Rovati, G. E., Baroffio, M., Citro, S., Brichetto, L., Ravasi, S., Milanese, M., . . . Brusasco, V. (2006). Cysteinyl-leukotrienes in the regulation of beta2-adrenoceptor function: an in vitro model of asthma. *Respir Res*, 7, 103. doi: 10.1186/1465-9921-7-103
- Rueter, K., Bizzintino, J., Martin, A. C., Zhang, G., Hayden, C. M., Geelhoed, G. C., . . . Le Souef, P. N. (2012). Symptomatic viral infection is associated with impaired response to treatment in children with acute asthma. *J Pediatr*, 160(1), 82-87.
- Rumzhum, N. N., Patel, B. S., Prabhala, P., Gelissen, I. C., Oliver, B. G., & Ammit, A. J. (2016). IL-17A increases TNF-alpha-induced COX-2 protein stability and augments PGE2 secretion from airway smooth muscle cells: impact on beta2 -adrenergic receptor desensitization. *Allergy*, 71(3), 387-396.
- Rumzhum, N. N., Rahman, M. M., Oliver, B. G., & Ammit, A. J. (2016). Effect of Sphingosine 1-Phosphate on Cyclo-Oxygenase-2 Expression, Prostaglandin E2 Secretion, and beta2-Adrenergic Receptor Desensitization. *Am J Respir Cell Mol Biol*, 54(1), 128-135.
- Sato, T., Bewtra, A. K., Hopp, R. J., Nair, N., & Townley, R. G. (1990). Alpha- and beta-adrenergic-receptor systems in bronchial asthma and in subjects without asthma: reduced mononuclear cell beta-receptors in bronchial asthma. *J Allergy Clin Immunol*, 86(6 Pt 1), 839-850.

- Sharma, P., Yi, R., Nayak, A. P., Wang, N., Tang, F., Knight, M. J., . . . Deshpande, D. A. (2017). Bitter Taste Receptor Agonists Mitigate Features of Allergic Asthma in Mice. *Sci Rep*, 7, 46166.
- Shore, S. A., Schwartzman, I. N., Le Blanc, B., Murthy, G. G., & Doerschuk, C. M. (2001). Tumor necrosis factor receptor 2 contributes to ozone-induced airway hyperresponsiveness in mice. *Am J Respir Crit Care Med*, 164(4), 602-607.
- Siddiqui, S., Mistry, V., Doe, C., Roach, K., Morgan, A., Wardlaw, A., . . . Brightling, C. (2008). Airway hyperresponsiveness is dissociated from airway wall structural remodeling. *J Allergy Clin Immunol*, 122(2), 335-341.
- Stelmach, I., Gorski, P., Jerzynska, J., Stelmach, W., Majak, P., & Kuna, P. (2002). A randomized, double-blind trial of the effect of treatment with formoterol on clinical and inflammatory parameters of asthma in children. *Ann Allergy Asthma Immunol*, 89(1), 67-73.
- Swystun, V. A., Gordon, J. R., Davis, E. B., Zhang, X., & Cockcroft, D. W. (2000). Mast cell tryptase release and asthmatic responses to allergen increase with regular use of salbutamol. *J Allergy Clin Immunol*, 106(1 Pt 1), 57-64.
- Tan, X., & Sanderson, M. J. (2014). Bitter tasting compounds dilate airways by inhibiting airway smooth muscle calcium oscillations and calcium sensitivity. *Br J Pharmacol*, 171(3), 646-662.
- Taylor, D. R., Sears, M. R., Herbison, G. P., Flannery, E. M., Print, C. G., Lake, D. C., . . . Li, Q. (1993). Regular inhaled beta agonist in asthma: effects on exacerbations and lung function. *Thorax*, 48(2), 134-138.
- Taylor, D. R., Town, G. I., Herbison, G. P., Boothman-Burrell, D., Flannery, E. M., Hancox, B., . . . Richards, G. (1998). Asthma control during long-term treatment with regular inhaled salbutamol and salmeterol. *Thorax*, 53(9), 744-752.
- Taylor, I. K., O'Shaughnessy, K. M., Choudry, N. B., Adachi, M., Palmer, J. B., & Fuller, R. W. (1992). A comparative study in atopic subjects with asthma of the effects of salmeterol and salbutamol on allergen-induced bronchoconstriction, increase in airway reactivity, and increase in urinary leukotriene E4 excretion. *J Allergy Clin Immunol*, 89(2), 575-583.
- Trian, T., Burgess, J. K., Niimi, K., Moir, L. M., Ge, Q., Berger, P., . . . Oliver, B. G. (2011). beta2-Agonist induced cAMP is decreased in asthmatic airway smooth muscle due to increased PDE4D. *PLoS One*, 6(5), e20000. doi: 10.1371/journal.pone.0020000
- Trian, T., Moir, L. M., Ge, Q., Burgess, J. K., Kuo, C., King, N. J., . . . McParland, B. E. (2010). Rhinovirus-induced exacerbations of asthma: How is the {beta}2-adrenoceptor implicated? *Am J Respir Cell Mol Biol*, 43(2), 227-233.
- Van Ly, D., Faiz, A., Jenkins, C., Crossett, B., Black, J. L., McParland, B., . . . Oliver, B. G. (2013). Characterising the mechanism of airway smooth muscle beta2 adrenoceptor desensitization by rhinovirus infected bronchial epithelial cells. *PLoS One*, 8(2), e56058.
- van Schayck, C. P., Dompeling, E., van Herwaarden, C. L., Folgering, H., Verbeek, A. L., van der Hoogen, H. J., & van Weel, C. (1991). Bronchodilator treatment in moderate asthma or chronic bronchitis: continuous or on demand? A randomised controlled study. *BMJ*, 303(6815), 1426-1431.

- van Schayck, C. P., Graafsma, S. J., Visch, M. B., Dompeling, E., van Weel, C., & van Herwaarden, C. L. (1990). Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsensitization to salbutamol. *J Allergy Clin Immunol*, 86(5), 793-800.
- Wagener, B. M., Roux, J., Carles, M., & Pittet, J. F. (2015). Synergistic Inhibition of beta2-adrenergic Receptor-mediated Alveolar Epithelial Fluid Transport by Interleukin-8 and Transforming Growth Factor-beta. *Anesthesiology*, 122(5), 1084-1092.
- Wallin, A., Sandstrom, T., Cioppa, G. D., Holgate, S., & Wilson, S. (2002). The effects of regular inhaled formoterol and budesonide on preformed Th-2 cytokines in mild asthmatics. *Respir Med*, 96(12), 1021-1025.
- Wallin, A., Sandstrom, T., Soderberg, M., Howarth, P., Lundback, B., Della-Cioppa, G., . . . Melander, B. (1999). The effects of regular inhaled formoterol, budesonide, and placebo on mucosal inflammation and clinical indices in mild asthma. *Am J Respir Crit Care Med*, 159(1), 79-86.
- Wills-Karp, M. (2001). IL-12/IL-13 axis in allergic asthma. *J Allergy Clin Immunol*, 107(1), 9-18.
- Wills-Karp, M., Uchida, Y., Lee, J. Y., Jinot, J., Hirata, A., & Hirata, F. (1993). Organ culture with proinflammatory cytokines reproduces impairment of the beta-adrenoceptor-mediated relaxation in tracheas of a guinea pig antigen model. *Am J Respir Cell Mol Biol*, 8(2), 153-159.
- Yang, W., Kaur, D., Okayama, Y., Ito, A., Wardlaw, A. J., Brightling, C. E., & Bradding, P. (2006). Human lung mast cells adhere to human airway smooth muscle, in part, via tumor suppressor in lung cancer-1. *J Immunol*, 176(2), 1238-1243.
- Zuyderduyn, S., Sukkar, M. B., Fust, A., Dhaliwal, S., & Burgess, J. K. (2008). Treating asthma means treating airway smooth muscle cells. [Review]. *Eur Respir J*, 32(2), 265-274.