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Yong-Xiao Wang *Editor*

Lung Inflammation in Health and Disease, Volume II

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Lung Inflammation in Health and Disease, Volume II

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Preface

As previously discussed in Volume I, inflammation is a natural cellular process occurring in virtually all types of human body tissues, organs, and systems. This process can be acute or chronic. Acute inflammation is a healthy, immediate response to protect and repair the body from harmful stimuli. Usually it occurs within a couple of hours. Chronic inflammation is a lengthier cellular process that is not conducive to natural healing and may lead to pathological states including arthritis, asthma, and pulmonary hypertension.

Normally, inflammation can also be classified as systemic or localized. The former affects the entire human body, which is a pathogenetic component in numerous acute and chronic diseases including atherosclerosis, diabetes, sepsis, trauma, and others with a significant morbidity and mortality. The latter is localized as in a specific organ. For example, inflammation caused by asthma and pulmonary hypertension are localized in the lungs.

Lung diseases are very common and can also be very severe. It is well known that lung infections are the greatest single contributor to the overall global health burden. For instance, lung diseases are the most common causes of deaths of children under 5 years of age, which occur more than 9 million annually. Indeed, pneumonia is the leading killer for children worldwide. Asthma is the most common chronic disease, affecting about 14% of children globally and continuing to rise. Likewise, COPD is recognized to be the fourth leading cause of death in the world and the numbers are growing. The lung is not only the largest internal organ in the human body, but also the only internal organ that is exposed constantly to the external environment; as such, no other organ is more vital and vulnerable than the lung. This may explain the common morbidity and mortality of lung diseases.

Systemic inflammation may induce and even exacerbate local inflammatory diseases. Likewise, local inflammation can cause systemic inflammation. Indeed, there is increasing evidence of coexistence of systemic and local inflammation in patients with asthma, COPD, and other lung diseases. Moreover, the comorbidity of two and even multiple local inflammatory diseases often occurs. For instance, rheumatoid arthritis not only frequently happens together with but also promotes the development of pulmonary hypertension. The comorbidity of local and systemic as well as two or more inflammatory diseases significantly deteriorates the quality of life and may even exacerbate death in patients.

The current treatment options for lung diseases are neither always effective nor specific at all. The development of new therapeutics is earnestly needed. Equally desperately, the molecular mechanisms and physiological significance of lung diseases are still not fully understood. Apparently, this despondent fact is a major encumberment to creating new efficacious drugs in the treatment of lung diseases. This scenario is even worse in two and more lung diseases accompanied with other inflammatory diseases due to their complexity and diversity.

Despite the current state being unsatisfactory, great progresses have been made in many aspects of lung diseases from the molecular geneses to regulatory mechanisms to signaling pathways to cellular processes to basic and clinical technologies to new drug discoveries to clinical manifestations to laboratory and clinical diagnoses to treatment options to predictive prognosis. To the best of our knowledge, however, no one, cohesive book is available to present these state-of-the-art advances in the field. Thus, as one of the major aims, we compile this timely and much-needed book to provide a high-quality platform in which well-known scientists and emerging pioneers in basic, translational, and clinical settings can present their latest, exciting findings in the studies of lung inflammation in health and disease. The contents from multiple outstanding authors with unique expertise and skills of molecular and cell biology, biochemistry, physiology, pharmacology, biophysics, biotechnology, translational biomedicine, and medicine will provide new knowledge, concepts, and discoveries in the field. The second major aim is to help direct future research in lung diseases and other inflammatory diseases. The scope of the book includes nearly all new and important findings from very recent basic, translational, and clinical research in the studies of the molecular genesis, networks, microdomains, regulation, functions, elimination, and drug discoveries of inflammation in lung health and disease, which are involved in animal and human lung epithelial cells, smooth muscle cells, *endothelial* cells, adventitial cells, fibroblasts, neutrophils, *macrophages*, *lymphocytes*, and stem/progenitor cells. Lastly but importantly, the book will offer the latest and most promising results from clinical trials in terms of exploring interventions of local and systemic inflammation in the treatments of lung diseases.

This book features contributions from numerous basic, translational, and physician scientists in the fields of pulmonary vasculature redox signaling in health and disease, and as a result offers a widespread and comprehensive overview for academic and industrial scientists, postdoctoral fellows, and graduate students who are interested in redox signaling in health and disease and/or normal and pathological functions of the pulmonary vasculature. The book may also be valuable for clinicians, medical students, and allied health professionals.

We are sincerely grateful for the overwhelming support from leading scientists who contributed their expertise. Due to their contributions, we are pleased to share Volume II now. Similar to Volume I, the current volume is composed of 17 chapters from prominent investigators and clinicians covering novel fundamental roles and molecular mechanisms of inflammatory cellular responses in the development of acute respiratory distress syndrome,

asthma, pulmonary hypertension, sarcoidosis, and other lung illnesses. Several articles principally deal with the interactions among inflammatory signaling with reactive oxygen species, calcium, sex, and other vital intracellular molecular signaling in lung diseases. We also share articles focused on the innovative diagnostic approaches and therapeutic treatment options in the aforementioned lung disorders. We are confident these reports detailing the most important basic, translational, clinical, and drug discovery studies will not only enrich our current knowledge, but will also serve to direct and promote future research in the field.

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I want to express my wholehearted gratitude to all of the authors for their dedication and diligence in contributing book chapters, particularly during the challenging and unprecedented times of the global COVID-19 pandemic. Many of the authors in this book have not only performed exceptional roles as writer but also as reviewer. Their selfless contributions are sincerely appreciated. I also want to thank Ms. Alison Ball and Mr. Arjun Narayanan at Springer Nature for their assistance, patience, and enthusiasm in seeing this book to fruition.

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Can GPCRs Be Targeted to Control Inflammation in Asthma?

1

Pawan Sharma and Raymond B. Penn

Abstract

Historically, the drugs used to manage obstructive lung diseases (OLDs), asthma, and chronic obstructive pulmonary disease (COPD) either (1) directly regulate airway contraction by blocking or relaxing airway smooth muscle (ASM) contraction or (2) indirectly regulate ASM contraction by inhibiting the principal cause of ASM contraction/bronchoconstriction and airway inflammation. To date, these tasks have been respectively assigned to two diverse drug types: agonists/antagonists of G protein-coupled receptors (GPCRs) and inhaled or systemic steroids. These two types of drugs "stay in their lane" with respect to their actions and consequently require the addition of the other drug to effectively manage both inflammation and bronchoconstriction in OLDs. Indeed, it has been speculated that safety issues historically associated with beta-agonist use (beta-agonists activate the beta-2-adrenoceptor (β_2AR) on airway smooth muscle (ASM) to provide bronchoprotection/bronchorelaxation) are a function of pro-inflammatory actions of β_2AR agonism. Recently, however, previously unappreciated roles of various GPCRs on ASM contractility and on airway inflammation have been elucidated, raising the possibility that novel GPCR ligands targeting these GPCRs can be developed as anti-inflammatory therapeutics. Moreover, we now know that many GPCRs can be "tuned" and not just turned "off" or "on" to specifically activate the beneficial therapeutic signaling a receptor can transduce while avoiding detrimental signaling. Thus, the fledging field of *biased agonism pharmacology* has the potential to turn the β_2AR into an anti-inflammatory facilitator in asthma, possibly reducing or eliminating the need for steroids.

Keywords

GPCR · Beta-2 agonists · Asthma · Inflammation · Bronchodilator · Obstructive lung disease · COPD · Biased agonism

Abbreviations

AHR	Airway hyperresponsiveness
ASM	Airway smooth muscle
AERD	Aspirin-exacerbated respiratory disease

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COPD	Chronic obstructive pulmonary disease
CaSR	Calcium-sensing receptor
CysLT	Cysteinyl leukotriene
IL	Interleukin
GPCR	G protein-coupled receptor
OLD	Obstructive lung diseases
mAChR	Muscarinic acetylcholine receptor
mPGES-1	microsomal prostaglandin E synthase-1
LABA	Long-acting beta-2 agonist
PG	Prostaglandin
LPS	Lipopolysaccharide
PAR-2	Protease-activated receptor-2
PMNT	Phenylethanolamine-N-mthyltransferase
PKA	Protein kinase A
SABA	Short-acting beta-2 agonist
TGFβ1	Transforming growth factor beta 1
TAS2R	Type II taste receptors
TNF-α	Tumor necrosis factor alpha
TRPV4	Transient receptor potential vanilloid 4
β_2AR	beta-2 adrenoceptor

The management of obstructive lung diseases (OLDs), asthma, and chronic obstructive pulmonary disease (COPD) is predicated on the importance of controlling excessive bronchoconstriction that increases airway resistance. Increased airway resistance manifests in the inability to breathe, which is not only potentially fatal but also impacts the quality of life [1–3]. Accordingly, drugs managing OLDs either directly regulate airway constriction by blocking or relaxing airway smooth muscle (ASM) contraction or indirectly regulate ASM contraction by inhibiting the principal cause of ASM contraction/bronchoconstriction and airway inflammation. To date, these tasks have been respectively assigned to two diverse drug types: agonists/antagonists of G protein-coupled receptors (GPCRs) and inhaled or systemic steroids.

Although it is important to recognize that non-allergic/nonatopic asthma is also an important health concern, this review will focus on allergic asthma, which has a rich history of research and drug discovery efforts dedicated to understanding and managing the disease. Herein, we will review the logic underlying allergic asthma management, the approaches undertaken to date to manage the two major features of the disease (bronchoconstriction and airway inflammation), and the ability of current and future GPCR-targeting drugs to go beyond their ability to directly regulate ASM contraction and manage airway inflammation [4].

1.1 Allergic Asthma Pathobiology and Attempts to Manage It

It is widely recognized that asthma is a complex disease, often labeled a syndrome, in which various factors can result in an exaggerated immune response to an allergen in the lung that results in obstruction to airflow. Although an increase in airway mucus contributes to this increased obstruction, airway narrowing caused by ASM contraction can greatly impede airflow, and the use of rapidly acting bronchodilators that work by relaxing ASM is usually sufficient to manage an acute asthmatic attack. Conceivably, preventing the *cause* of bronchoconstriction (airway inflammation) should be sufficient to manage asthma, but this is difficult to achieve in most asthmatics; thus, asthmatics are typically managed by either of the following: (1) prophylactic inhaled corticosteroids to control inflammation with the use of an inhaler of short-acting beta-agonist (SABA, acting on beta-2 adrenoceptors (β_2ARs) on ASM) bronchodilator to reverse bronchospasm when needed (mild asthmatics) or (2) daily prophylactic inhaled corticosteroids in combination with a long-acting beta-agonist (LABA) to help prevent (i.e., bronchoprotect)

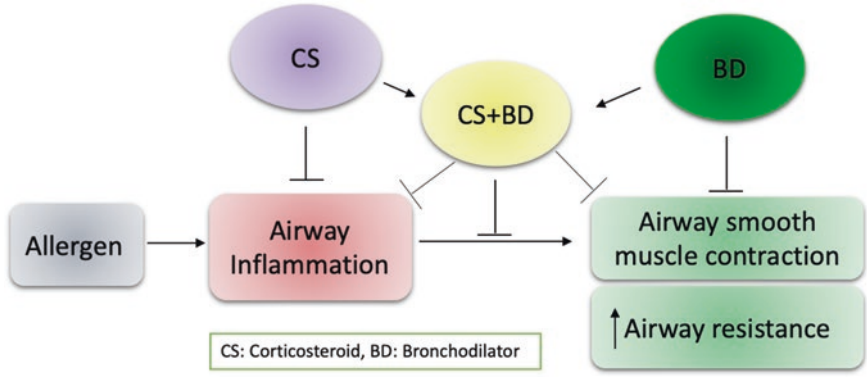

Fig. 1.1 Control of inflammation and bronchospasm in asthma. Corticosteroids (CS) when given alone can block allergic inflammation, while their effectiveness in asthma control is increased by concomitant administration of a bronchodilator (BD) such as β_2AR agonists which alone are not efficacious in controlling inflammation but are highly effective in preventing bronchospasm by targeting ASM. Of note, a modest cooperative effect in controlling inflammation and bronchospasm has been asserted by various studies

bronchospasm (mild, moderate, and some severe asthmatics). Schematic illustrating control of inflammation and bronchospasm by these drugs is depicted in Fig. 1.1.

1.2 The Relationship Between Airway Inflammation and Airway Contraction

The exaggerated immune response to allergen in the lung of asthmatics results in the production of multiple factors that cause ASM contraction. These factors include GPCR agonists that directly act on ASM to either effect contraction or sensitize the ASM to pro-contractile agents. Multiple GPCRs that can mediate ASM contraction are expressed on the plasma membrane of ASM cells [5–10]. Among the best characterized GPCRs in airway/asthma biology are the m3 muscarinic acetylcholine receptor (m3mAChR), H1 histamine receptor (H1HR), and cysteinyl leukotriene type 1 receptor (CysLT1R). Cognate ligands (acetylcholine, histamine, and CysLTs, respectively) for each of these receptors tend to be upregulated in expression in the allergen-exposed airway of asthmatics. Numerous other well-established GPCR agonists, including, but not limited to, endothelin (acting on ET-1R on ASM to induce ASM contraction), thromboxane (TP receptor), prostaglandin E2 (PGE_2; acting on EP1, EP2, EP3, and EP4 receptor with variable effects), and adenosine (acting on A1, A2a, and A2b adenosine receptors with variable effects), are induced during allergic lung inflammation and act either directly or indirectly on ASM to cause bronchoconstriction [9, 11–15]. Moreover, recent studies in human lung suggest that, whereas EP2 receptors dominate mast cell stabilizing effects of PGE_2, EP4 receptors dominate bronchodilation [16]. Recent studies have also identified (in both murine and human ASM) various GPCRs on ASM with the capacity to regulate (either contract or relax) ASM cells, ASM tissue ex vivo, or airways in vivo [17]. Numerous GPCRs have been identified as capable of mediating relaxation of contracted ASM cells, with the β_2AR being the principal GPCR targeted in asthma management for over the last 50 years [9].

Additionally, certain GPCRs that have little or limited capacity to directly stimulate pro-contractile or pro-relaxant signaling can stimulate the production of autocrine or paracrine GPCR agonists that in turn directly regulate ASM contractile state. Some GPCR agonists acting on (non-ASM) resident or infiltrating airway cells can stimulate the local release of cytokines that regulate ASM contractile state. The complexity of this intercellular communication and regula-

tion can be evidenced in a recent study by Bonvini et al., in which trypsin-activated protease-activated receptor-2 (PAR-2) on ASM cells gates *t*ransient *r*eceptor *p*otential *v*anilloid 4 (TRPV4) channels to release ATP into the extracellular space and activate P2XY purinergic channels on mast cells, which in turn release CysLT to activate CysLT1Rs on ASM and cause contraction [18].

Many of the abovementioned GPCRs, stimulated by increased endogenous levels of their cognate ligands, likely contribute in some degree to the ASM contraction and airway narrowing caused by allergic inflammation in asthma. With the exception of the m3mAChR (therapeutically targeted in asthma/COPD with the m2/m3 mAChR antagonist tiotropium [19, 20]), however, pharmacological blockade of these receptors is not sufficient to reverse acute bronchoconstriction and manage an acute asthma attack [21].

Other inflammatory agents that are typically not GPCR agonists promote increased ASM contraction by sensitizing ASM to other more direct contractile stimuli. Such agents include cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β, as well as transforming growth factor beta 1 (TGFβ1), IL-4, IL-13, and IL-8 [22–26]. The mechanisms by which this sensitization occurs are well established and primarily involve increasing the ASM cell's ability to contract to a given level of intracellular calcium that is released in response to contractile GPCR activation (i.e., *calcium sensitization*) [27–36].

An additional mechanism by which inflammatory agents increase ASM contraction is via their enhancement of cholinergic discharge in the airway [37–39]. Physiological contraction of ASM occurs via the release of acetylcholine from sympathetic nerves innervating ASM, to activate m3mAChRs on ASM [40, 41]. In animal models of allergic inflammation, particularly rodent models, it is clear that increased cholinergic discharge contributes significantly to the airway hyperresponsiveness (AHR) associated with allergic lung inflammation [41–43]. In human asthmatics, the relative contribution of excessive cholinergic discharge to AHR is not well understood (and is not necessary for AHR to exist) but is likely important given the therapeutic effectiveness of m3mAChR antagonists in at least a subpopulation of asthmatics [19, 20].

1.3 Current Treatment of Acute Bronchoconstriction

The life-threatening nature of an acute asthmatic attack requires rapid reversal of airway narrowing via direct regulation of ASM contraction. To date, such treatment has been almost exclusively limited to short-acting beta-agonists (SABAs) targeting the β_2AR on ASM and to a lesser extent to m3mAChR antagonists. Because multiple procontractile agonists often exist in the inflamed, asthmatic airway, specific m3mAChR antagonism may not be sufficient to reverse bronchoconstriction; thus, SABAs are the drug of choice for an asthmatic attack. Beta-agonists relax contracted airway smooth primarily by antagonizing intracellular pathways that are stimulated by contractile agonists and their cognate receptors (see [44–46] for a comprehensive review). Thus, beta-agonists tend to be effective in reversing ASM contraction caused by variable, and multiple, contractile stimuli.

1.4 Prophylactic Management of Bronchoconstriction by Controlling Inflammation

Not surprisingly, limiting or preventing the airway inflammation that causes bronchoconstriction is an excellent, and preferred, approach to manage asthma. For decades, inhaled corticosteroids have been the primary drug of choice for the management of mild asthma (with SABA inhalation when needed) [47]. However, majority of asthmatics today are managed by prophylactic, maintenance drugs consisting of an inhaled GPCR ligand (β_2AR agonist or m3mAChR antagonist) that directly targets ASM contraction, in a formulation that combines an inhaled steroid. Initially, the combination of an inhaled

long-acting beta-agonist (LABA) salmeterol along with an inhaled steroid (fluticasone) dominated the market. However, over time, other combinations of LABAs plus steroids along with long-acting muscarinic receptor antagonists (LAMAs) combined with steroids emerged, although the latter combination was and is more applicable to COPD management. The prevention/mitigation of inflammation by steroids has remained the cornerstone of the combination approaches, while the addition of more direct bronchorelaxation agents represents an additional arm of prophylactic control that could translate into better disease management including reduced exacerbations [48–50].

1.5 Control of Allergic Airway Inflammation by Steroids: Why We Keep Using the Sledgehammer Instead of a Scalpel

For the majority of asthmatics, inhaled corticosteroids are effective in controlling lung inflammation, despite the fact that they are often administered during ongoing inflammation and not necessarily prophylactically [51]. Some asthmatics, including many severe asthmatics, are *steroid-resistant* (to both inhaled and oral steroids; the cause of which is a subject of intense research and debate) and consequently have difficulty managing their disease [52–54]. Notwithstanding steroid-resistant asthmatics, steroids are powerful in suppressing allergic inflammation based on their ability to greatly suppress the pro-inflammatory function of multiple resident and infiltrating cell types in the lung and halt the progression of inflammation at multiple steps [47, 55–57]. The principal mechanism of these actions of corticosteroids involves the glucocorticoid receptor-mediated suppression of inflammatory mediator genes and to a lesser extent glucocorticoid receptor-mediated induction of other genes [47, 56, 58–65]. However, although this "sledgehammer" effect on gene regulation and multiple cell functions typically results in effective control of allergic lung inflammation, the effects of steroids are not exclusively anti-inflammatory. Numerous "off-target" effects of steroids exist, contributing to various clinical side effects associated with both inhaled and oral steroid use [53, 65]. The complement of steroid actions on inflammatory processes includes antithetical/counterproductive actions that limit therapeutic efficacy (i.e., certain anti-inflammatory processes maybe inhibited, along with pro-inflammatory processes being stimulated) [66, 67]. Ideally, a more refined approach that avoids off-target and antithetical effects would be preferable, *if* such targeting could *sufficiently* reduce inflammation to manage asthma features. Until recently, however, a more precise targeting of inflammatory mechanisms that drive asthma pathobiology did not exist. Even with the early promising results of certain biologics (typically antibodies targeting specific inflammatory mediators including IgE, IL-5, IL-5Rα, IL-4Rα, and IL-13) [68, 69], it is unclear whether targeting specific cytokines or inflammatory steps will be a superior or equivalent means of managing inflammation when compared with steroids for the majority of asthmatics. Moreover, the administration of biologics is both difficult (typically *s.c.* or *i.v.* injection) and expensive and justified only in severe asthmatics whose asthma is otherwise unmanageable [70, 71]. For now, the sledgehammer remains the most effective tool for the most asthmatics. Is there another solution?

1.6 Limitations of GPCR Ligands in the Control of Asthma and Inflammation

Arguably, the optimal asthma drug would be able to both prevent and neutralize inflammation while also directly inhibiting ASM contraction. To date, this drug does not exist, although as discussed recent studies have demonstrated the ability of certain GPCRs to regulate both ASM contraction and inflammation in murine models of asthma. CysLT1R antagonists have the capacity to both inhibit ASM contraction and airway inflammation, but their efficacy as a direct bronchodilator is minimal and as an anti-inflammatory agent is lim-

ited, perhaps being best in those patients whose lung inflammation is driven significantly by CysLT generation in the airway. Given the absence of any GPCR possessing efficacy as both a bronchodilator and anti-inflammatory, combination therapies of (GPCR ligand bronchodilator) LABAs/LAMAs and steroids are currently required for sufficient asthma control of both inflammation and bronchospasm and are overwhelming prescribed for most asthmatics.

However, this does not mean that inflammation control in asthma by GPCR pharmacology is not possible. Because multiple GPCRs participate in inflammation development, including many only recently appreciated receptors and others with but a nascent pharmacology, it is conceivable that the targeting of certain GPCRs may be sufficient to manage allergic lung inflammation. In addition, unlike biologics, GPCR agonists are small molecular drugs with inherent properties favorable for drug development, administration, and patient adherence [4, 72].

1.7 Recently Appreciated GPCRs Whose Targeting May Help in Managing Allergic Airway Inflammation

Advances in basic science capabilities in molecular and cell biology and receptor pharmacology have aided the discovery of previously unappreciated roles of various GPCRs in airway and asthma biology. Although many of these recent studies stemmed from attempts to find novel bronchodilators, a by-product of these studies has been the discovery of novel mechanisms by which GPCRs regulate allergic lung inflammation. Below, we discuss each of these GPCRs implicated in inflammation control and the potential of these receptors as asthma therapeutic targets.

1.8 Bitter Tastant Receptors

Evolutionarily bitter taste receptor signaling evolved as a mechanism to avoid potentially toxic food often bitter in taste. This function was primarily imparted by the bitter taste receptors (belonging to type II taste receptors, TAS2R), a family of seven transmembrane GPCRs expressed on the taste buds [73]. In the gastrointestinal system, these highly specialized chemosensory cells contribute to the innate host defense mechanism [74, 75]. It is now well established that TAS2Rs are expressed on variety of cell-types including ASM cells in the airways [76]. The first evidence to demonstrate the beneficial effect of bitter taste receptor signaling in providing effective bronchorelaxation was shown using human ASM cells where agonists of TAS2Rs were able to induce localized calcium and reverse airway obstruction to contractile agonists [76]. These observations were then verified in other species, and soon, it was established and recognized that TAS2R agonism may be a viable target to promote airway relaxation. Studies also demonstrated that the beneficial signaling activated by TAS2Rs in the airway smooth muscle was distinct and was not reliant on protein kinase A (PKA) activation, unlike β2 agonists [76–78]. Moreover, chronic treatment with TAS2R ligands does not lead to receptor desensitization in ASM, thereby preserving the beneficial bronchorelaxation effect [79]. Since the original characterization of bitter tastant receptors in the lung and ASM, it has been established that bitter tastants can provide effective bronchodilatory effects by promoting relaxation of ASM in multiple species and in animal models of asthma [76, 79–83]

It is also now apparent that bitter tastant receptor ligands can also mitigate other pathological features of asthma such as airway inflammation and airway remodeling, thereby providing a comprehensive asthma control [83]. The beneficial effects of bitter ligands have been shown in both prophylactic and treatment models where these agents acted on multiple levels in asthma pathology and prevented allergen-induced influx of immune cells into the airways and blocked key inflammatory cytokines that drive asthma pathogenesis that leads to airway remodeling and AHR [83]. Though bitter tastants are potentially an effective alternative to beta-agonists in terms of their bronchodilatory effects, it still remains to be seen whether these agents will be safe and equally

effective in the clinic as the biggest challenge in their development is the identification of a specific TAS2R subtype that is highly relevant in asthma and translation of preclinical studies to humans [84].

1.9 Calcium-Sensing Receptor

The calcium-sensing receptor (CaSR) is best known for its role in regulating calcium homeostasis in the body. CaSRs on the parathyroid gland survey circulating calcium levels, which involves calcium binding to and activating the CaSR which initiates intracellular signals that suppress the release of parathyroid hormone. Interestingly, the CaSR can be activated by numerous other molecules, including polyvalent cations, amino acids, and virus elements, and is expressed on multiple cell types, including those in the lung. In Yarova et al., a prominent role of CaSRs in mediating the development of the asthma phenotype was revealed. The capacity of CaSRs to promote ASM contraction was demonstrated by a loss of CaSR-stimulated contraction in ASM cells and tissue in which the CaSR gene was ablated and by pretreatment with CaSR antagonists known as calcilytics. Importantly, calcilytics could also reverse the hyperresponsiveness and inflammation induced in vivo in a mixed allergen model of murine asthma. Relevance of CaSRs to human asthma was suggested by data demonstrating expression of CaSR in human ASM, with greater levels observed in ASM from asthmatics. The ability of CaSRs to regulate inflammation is likely due to its expression on invading inflammatory cells (eosinophils, macrophages). Interestingly, inflammation was shown to increase CaSR expression in both human and mouse tissues [85].

One of the most intriguing aspects of CaSR in asthma is that (CaSR antagonists) calcilytics have real potential as asthma drugs. They are small molecules that are readily deliverable by inhalation, and their efficacy is favored by the ability to target multiple cell types and mechanisms that contribute to the asthma phenotype. Moreover, various calcilytics have already undergone clinical trials for safety and efficacy in diseases such as osteoporosis and autosomal dominant hypocalcemia (reviewed in [86]). Thus, despite the promiscuous nature of the CaSR, it might ultimately prove to be a useful asthma therapeutic target.

1.10 EP Receptors

The EP receptor family is activated by the ubiquitous inflammatory agent prostaglandin E2 and to a lesser extent other prostanoids [87]. The four members of the EP receptor family (EP1, EP2, EP3, and EP4) couple to different G proteins, signal to different pathways, and have variable expression in multiple cell types in the lung. With respect to allergic lung inflammation and asthma, PGE_2 through EP receptors thus regulates multiple cellular functions that serve different and often competing functions that control both inflammation and ASM contraction. For example, in humans, EP3 receptors in ASM cause contraction, whereas EP4 receptors mediate ASM relaxation [88]. Control of inflammation by EP receptors is complex. The net effect on PGE_2 activating multiple EP receptor subtypes in the allergen-challenged mice demonstrated that EP2, EP3, and EP4 agonists all could inhibit certain indices of allergen-induced inflammation in mice lacking mPGES [89]. In another study, employing three different airway disease models (including a more chronic ovalbumin (OVA) sensitization/challenge), in each of the EP knockout mice, demonstrated that the EP4R (and not EP1–EP3) was responsible for the anti-inflammatory effect of PGE_2 in each model [90]. Collectively, studies to date suggest that PGE_2 is largely beneficial, with the capacity to inhibit many pathological features of asthma. In both animal models and cell-based assays, PGE_2 inhibits multiple indices of allergic inflammation [89–93], inhibits proliferation of cultured ASM cells [94–99], and relaxes contracted airways ex vivo [100–103] while promoting bronchoprotection/bronchorelaxation in vivo [101, 103]. Moreover, these effects are conserved across

species and most importantly are evident in human subjects.

The *potential* of PGE_2 as an asthma therapeutic, at least with respect to its bronchorelaxant properties, has been recognized for years. The bronchodilator effects of PGE_2 have been demonstrated in a range of patients (normal, asthmatic, and chronic bronchitis) [104]. This effect has been shown in other studies using healthy and asthmatic subjects, respectively [105, 106]. PGE_2 also protects against exercise-induced [105] and aspirin-induced bronchoconstriction in subjects with aspirin-exacerbated respiratory disease (AERD) [107, 108]. PGE_2 also prevents early and late allergen-induced bronchoconstrictor responses when given before allergen challenge [109, 110] and is protective against bronchoconstrictors such as histamine and methacholine [106, 111]. With respect to inflammation, PGE_2 also blocks the recruitment of eosinophils and basophils to the bronchial mucosa during allergen-induced late-phase responses and attenuates the release of mast cell-derived products. Thus, PGE_2 has validated functions as an anti-inflammatory and bronchoprotective agent in asthmatics [110, 112] In that regard, it is most established among potential asthma therapeutics for its ability to directly bronchodilate and to suppress inflammation.

However, despite these benefits of inhaled PGE_2, the development of prostanoid agonists for the treatment of asthma has been hindered as inhaled PGE_2 has repeatedly been shown to produce reflex cough in humans [104, 112, 113]. PGE_2 has been shown to excite airway afferent nerves [114], which concurs with the cough seen in both healthy and asthmatic patients during studies with inhaled PGE_2. Recent studies using cell, tissue, and in vivo models strongly implicate the EP3 receptor subtype in mediating cough induced by PGE_2 across all species tested, including human [115–120].

Clearly, the answer to harnessing the pro-relaxant and anti-inflammatory properties of PGE_2 as an asthma treatment or prophylaxis relies on specific targeting of EP receptor subtypes, with a primary goal of avoiding EP3 receptor agonism. Unfortunately, the development of ligands with sufficiently high specificity for each of the EP subtypes has been difficult to date. Moreover, this solution first requires a clear understanding of the role of EP receptor subtypes in the many cell types participating in airway inflammation and pathobiology in asthma. In airway epithelial cells, PGE_2 modulates many functions including an increase in ciliary beat frequency [121] and Cl^- channel conductance [122], whereas both induction [123] and inhibition [124] of mucin production have been reported. In vivo administration of EP agonists has been shown to inhibit LPS- (EP2) and allergen-induced (EP2/EP3/EP4) mucous cell metaplasia in rat nasal epithelium [124, 125]. EP receptors also play an immunomodulatory role in the epithelium; EP2/EP4 agonists increase IL-6 release [126], whereas EP4 inhibits IL-8 release [127]. A recent report shows that human airway epithelial (HAE) cell migration is promoted by PGE_2 and selective EP agonists (EP1–EP4), but upon undergoing TGFβ-induced Epithelial mesenchymal transition (EMT), the response to PGE_2 and EP2 and EP4 agonists becomes inhibitory, indicating adaptation of EP responses to remodeling in the lung [128].

Human mast cells have been shown to express EP2, EP3, and EP4 receptors. PGE_2 suppressed the generation of cytokines and cysteinyl leukotrienes primarily by eliciting signaling through EP2 receptors (although a suppressive effect was evident at high doses) [91]. Others have noted regulatory effects of PGE_2 on other inflammatory cell types including eosinophils [129], T cells [130], T regs [131], alveolar macrophages [132], and neutrophils [133] using cells from various species. Whereas some of these studies have suggested that EP2 and EP4 are the principal EP subtypes capable of inhibiting the inflammatory functions of these cells, definitive insight has been limited [134] due the limitations of subtype selective ligands.

Collectively, the above studies all point to a high potential of EP receptor subtype targeting for regulating both bronchospasm and airway inflammation in asthma. One caveat is that most of our understanding of EP receptor subtype function in the lung and lung cells during allergic inflammation comes from animal studies, and (1) the nature of, and control of, of allergen-induced

inflammation in animals (particularly mice) differs (often significantly) from that of humans, and (2) species differences in cellular EP receptor subtype function have been identified, one striking difference being in the control of ASM contraction/relaxation [118]. However, the current pace of research in this area is encouraging, supported by ongoing industry efforts in EP subtype-selective drug discovery [135] aided by the increasing ability of both structural biology and receptor modeling science to enhance these efforts.

1.11 Protease-Activated Receptor 2 (PAR2)

PAR2 signaling in the lung has complex effects reflecting the diverse functions of the various lung cell types that express PAR2. Initial studies demonstrated that airway delivery of anti-PAR2 antibodies, or a cell permeable peptide inhibitor of PAR2 signaling, prevented allergen-induced AHR and airway inflammation in mice [136]. Moreover, using a mouse OVA model for PAR(2)-modulated airway inflammation, genetic ablation of β-arrestin2 (βarr2) decreased leukocyte recruitment, cytokine production, and mucin production in OVA-treated mice, yet PAR(2)-mediated PGE_2 production and the associated and decreased baseline airway resistance were unaffected by βarr2 knockout. Subsequent studies using OVA, cockroach extract, or *Alternaria alternata* to induce lung inflammation further confirmed a protective effect of PAR2 activity acting via PGE_2-mediated relaxation of ASM and a pathogenic effect of PAR2 mediated through PAR2 activation (and dependent on β-arrestin2) most likely on inflammatory cells [137, 138]. Collectively, these studies suggest that PAR2 is a potential useful GPCR target for controlling allergic lung inflammation, and a strategy enabling specific targeting of PAR2-β-arrestin2 signaling would be optimal, enabling the protective effect of PAR2-PGE_2 signaling axis to be retained.

1.12 Any Finally … the β_2AR? How Biased Agonism Pharmacology May Turn a Problem into a Solution

The inability of current beta-agonists to control inflammation almost certainly underlies the need to treat asthmatics with steroids. Although a handful of studies examining the effects in cell-based models have attributed anti-inflammatory properties to beta-agonists [139–145], there is little if any evidence that beta-agonist use reduces allergic inflammation in the lung [146, 147]. The cooperative effect of LABA and steroids in managing asthma is likely a function of each drug addressing an individual disease feature: LABA prophylactic inhibition of airway constriction (bronchoprotection) and steroid prophylactic inhibition of inflammation. Certain studies have identified some interesting cooperative mechanisms at the cellular level [48, 58, 148–150], but the predominate means by which these two drugs work together well appears to be that beta-agonists directly manages ASM contraction, while steroids limit inflammation. Both the cause (inflammation) and the effect (bronchoconstriction) are addressed to the extent that synergy at the cellular level is probably not required for this combination to be effective in most asthmatics.

A long history of safety issues also suggests that beta-agonists alone are inadequate to manage the disease in many asthmatics, perhaps due to the inability to control inflammation. "Epidemics" in which beta-agonist use was associated with high levels of asthma mortality occurred in the 1960s with high dose of inhaled isoproterenol (a nonspecific beta-agonist) use in the United Kingdom, followed by the use of a potent SABA, fenoterol, which also increased asthma-related mortality in New Zealand [151–157]. In the USA, a statistically insignificant increase in asthma-related deaths and safety concerns were reported with the use of LABAs. It was later noted that these life-threatening adverse events with the use of LABA therapy were limited by suboptimal study designs. Follow-up prospective clinical trials and meta-analyses consistently demonstrated their effectiveness and safety, although these trials did not contain suffi-

cient power to address these safety concerns definitively [158, 159]. Moreover, clinical data further suggest that chronic beta-agonist use is associated with a reduction in bronchoprotective effect [160, 161], increase in AHR [162], and loss of asthma control [162–164]; whether this is due to a loss of effectiveness of beta-agonists caused by receptor desensitization or a failure to address the underlying cause (inflammation) of the disease is unclear. However, when the most recent (and highly publicized) beta-agonist safety issue arose during the SMART trial examining the efficacy of salmeterol monotherapy, it was widely speculated that the significant ability of salmeterol to relax ASM *masked* its inability to control airway inflammation [165, 166], thus leaving patients significantly at risk.

1.13 Evidence that Beta-Agonists and the β_2AR Actually Promote Inflammation and Asthma Development

There is a paucity of meaningful clinical data assessing the effects of beta-agonist use on inflammation in asthma. A handful of underpowered clinical studies suggest limited anti-inflammatory effects of beta-agonists in reducing IL-8, eosinophils, mast cells in Bronchoalveolar lavage (BAL), and airway mucosa samples after LABA therapy [167–169]. In contrast, a considerable amount of research into the effects of beta-agonists and the β_2AR in murine models of allergic lung inflammation exists. These studies were pioneered by the Bond lab, who initially proposed that, as in heart failure, a "βAR paradox" exists for asthma. With heart failure, a loss of pump function occurs as cardiac myocytes and their β_1ARs become less responsive to endogenous norepinephrine. Although, theoretically, the use of exogenous beta-agonists as therapy might help stimulate the hypodynamic heart, it turns out that βAR *blockers* are the more effective treatment. This is because, as ultimately determined, β_1AR activation is actually a pathogenic driver of heart failure, and preventing this β_1AR-driven pathogenesis with β_1AR antagonism proved to be therapeutically beneficial [170].

In a series of elegant studies, Bond and colleagues demonstrated (highlights summarized in Fig. 1.2) that pharmacological blockade, or genetic ablation of the β_2AR or systemic epinephrine (the only endogenous ligand for the β_2AR), improved the asthma phenotype and that agonist-induced activation of the β_2AR was necessary for full development of the lung inflammation and the asthma phenotype [171–177]. Moreover, results from a pilot clinical trial determined that, in 8 out of 10 mild asthmatics, 9 weeks of treatment with the "beta-blocker" nadolol produced a significant, dose-dependent increase in PC20 as well as a significant reduction in FEV1 [178, 179].

1.14 Was It as Simple as β_2AR Signaling Was Actually Bad and Fostered Asthma Development?

Not exactly. During this time, the GPCR biology field discovered that certain GPCRs, including β_2ARs, were capable of stimulating diverse and sometimes functionally antithetical signaling pathways, and most of these signaling events were dependent on arrestin proteins [180, 181]. Arrestins were originally identified as regulatory proteins that promote β_2AR desensitization but were subsequently found capable of mediated G protein-independent signaling by binding the β_2AR and helping form a distinct signalosome. Strategies that inhibited the ability of arrestin proteins to bind to the β_2AR (e.g., arrestin knockdown) could inhibit specific signaling (e.g., ERK1/ERK2 signaling) while preserving other signaling (cAMP/PKA) [182]. In addition, drugs classically known as βAR antagonists, based on their ability to block βAR-stimulated cAMP production, could cause arrestin recruitment to βARs and stimulate signals that appeared independent of G proteins [182, 183]. These studies ushered in the exciting new age of "biased ligand pharmacology," and the race was on to develop new drugs that could "tune" receptors to preferen-

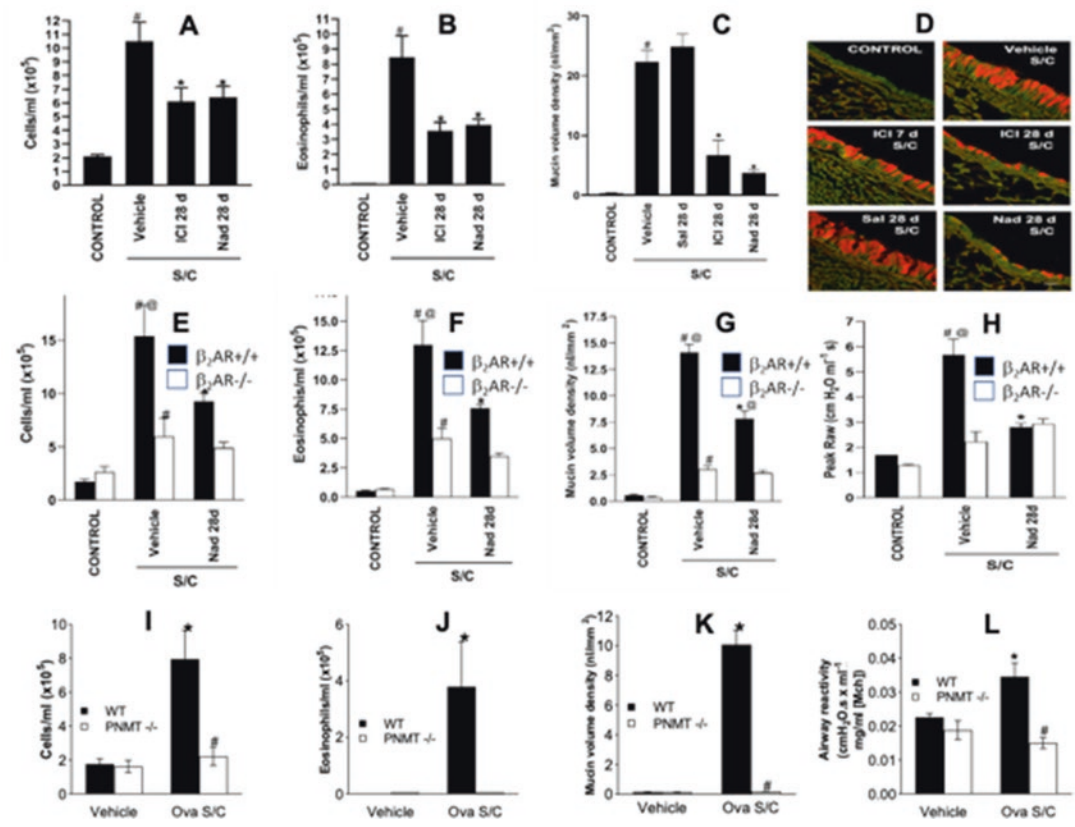

Fig. 1.2 Pharmacological, genetic inhibition of β_2AR signaling improves the asthma phenotype. *A-D*. In the classic ovalbumin (OVA) model of murine allergic lung inflammation, chronic co-administration of inverse agonists ICI118551 and nadolol, both of which block both canonical β_2AR signaling as well as arrestin binding to β_2AR, inhibits the OVA-induced increases in BALF total cellularity (**a**), eosinophils (**b**), and mucin (**c**). Moreover, chronic salbutamol treatment augments OVA-induced mucin (**c**). *D*. Periodic Acid-Schiff-stained airways from mice treated as per (**c**). *E-F*. Genetic ablation of the β_2AR similarly inhibits OVA-induced increases in lung cellularity (**e**), eosinophils (**f**), and mucin (**g**), as well as increases in methacholine-stimulated airway resistance (**h**). *I-J*. Genetic ablation of phenylethanolamine N-phenylethanolamine N-methyltransferase (PMNT, the enzyme catalyzing the final step in the synthesis of epinephrine) similarly inhibits OVA-induced increases in lung cellularity (**i**), eosinophils (**j**), and mucin (**k**), and methacholine-stimulated airway resistance (**h**). Data from Nguyen et al. *Am J Respir Cell Mol Biol* 2006 (**a**–**d**), Nguyen et al. Proc Natl Acad Sci USA 2009 (**e**–**h**), and Thanawala et al. *Am J Respir Cell Mol Biol* 2013 (**i**–**j**)

tially activate specific pathways, instead of simply turning receptors on or off.

Intrigued by this fledgling field of biased ligand pharmacology, Bond and colleagues considered whether the permissive effect of beta-agonists on asthma pathobiology was linked to arrestin effects on β_2ARs and possible arrestin-dependent signaling. The modest albeit encouraging effect of nadolol in the clinical pilot study led them to consider whether the use of nadolol, which blocks both G protein and arrestin-dependent signaling by the β_2AR, might be to some extent "throwing the baby out with the bath water." *Might a better strategy be to preserve the β_2AR-Gs-cAMP-PKA signaling while blocking arrestin-dependent effects?* They therefore launched a series of studies that demonstrated that certain "beta-blockers" (e.g., carvedilol, propranolol) that blocked β_2AR-Gs signaling yet failed to inhibit β_2AR-arrestin binding (and stimulated

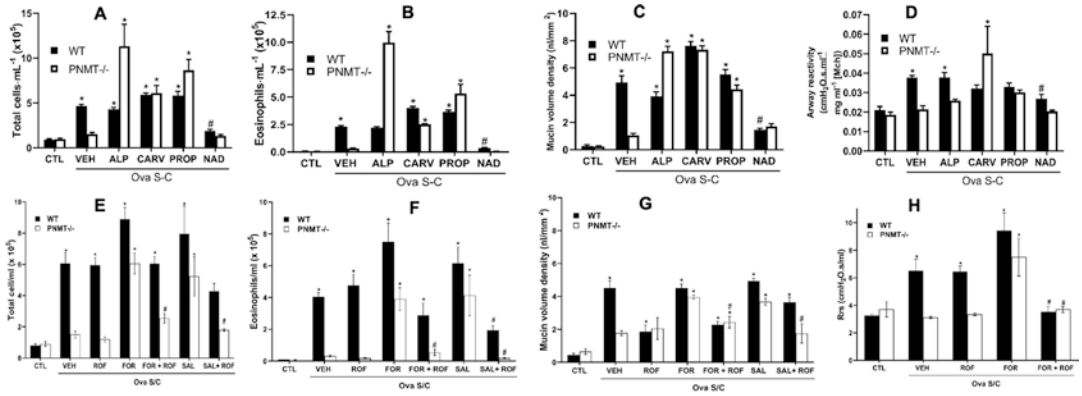

Fig. 1.3 Asthma pathology is influenced by the nature, degree of biased β_2AR signaling. *A-D*. Systemic depletion of epinephrine caused by PMNT deletion inhibits OVA-induced BALF total cellularity (**a**), eosinophils (**b**), mucin (**c**), and airway hyperresponsiveness (AHR) (**d**), while replacement of systemic epinephrine with a balanced β_2AR partial agonist alprenolol (ALP) or arrestin-biased carvedilol (CAR) or propranolol (PROP) helps restore each of these features of asthma. Conversely, nadolol (NAD), which blocks both β_2AR-Gs signaling as well as arrestin binding to the β_2AR does not. *E-F*. Replacement of systemic epinephrine with balanced β_2AR agonists formoterol (FOR) or salmeterol (SAL) in PMNT −/− mice restores OVA-induced increases in lung cellularity (**e**), eosinophils (**f**), and mucin (**g**), as well as increases in methacholine-stimulated airway resistance (**h**). Moreover, co-treatment with the PDE4 inhibitor roflumilast (ROF), which increases β_2AR-Gs-stimulated cAMP accumulation, partially reverses the deleterious effects of chronic treatment with balanced agonists formoterol and salmeterol on total lung cellularity, eosinophils, mucin, and AHR. Data from Thanawala et al. *Br J Pharmacol* 2015 (**a**–**d**) and Forkuo et al. *Am J Respir Cell Mel Biol* 2016 (**e**, **f**)

ERK1/ERK2 signaling in 293 cells as per [182]) (i.e., arrestin-biased ligands) were not effective in mitigating allergen-induced inflammation and AHR and in fact exacerbated the condition, especially in PMNT−/− (epinephrine-deficient) mice [177] (Fig. 1.3a–d). Without a Gs-biased β_2AR ligand in hand, Bond and colleagues biased signaling toward the Gs/cAMP/PKA pathway through use of phosphodiesterase (PDE) inhibitors which specifically increased intracellular cAMP in lung cells. Both PDE4 inhibitors rolipram (not shown) and roflumilast (Fig. 1.3e–h) significantly reversed the adverse effects of formoterol and salmeterol on allergen-induced bronchoalveolar lavage fluid (BALF) cellularity and eosinophils, mucin, and AHR in both wild-type and PMNT−/− mice [173].

Additional studies demonstrated that the loss of IL-13-induced asthma phenotype caused by global genetic ablation of β_2AR was rescued by transgenic expression of the β_2AR in only airway epithelia [184], suggesting that β_2AR-arrestin signaling regulates the immunomodulatory function of airway epithelia and is critical to the development of the asthma phenotype. Should future studies clarify this as true, the two critical questions that remain are as follows: (1) is this the case with human asthma and (2) can Gs-biased β_2AR agonists or allosteric modulators that are both effective and safe in asthmatics be developed? To date (unlike arrestin-biased β_2AR ligands), identifying or developing such drugs has been a challenge, but with the rapid advances in structural biology and computer modeling that enable drug development, it is only matter of time before such drugs are known or developed.

1.15 What the Future Holds: The Promise of Biased Agonism Pharmacology

To date, the failure to identify a GPCR target, and a therapeutic ligand for it, capable of successfully managing allergic lung inflammation in human stems from the limitations of GPCR biology and pharmacology in basic research and the limitations of drug discovery. GPCR biology and phar-

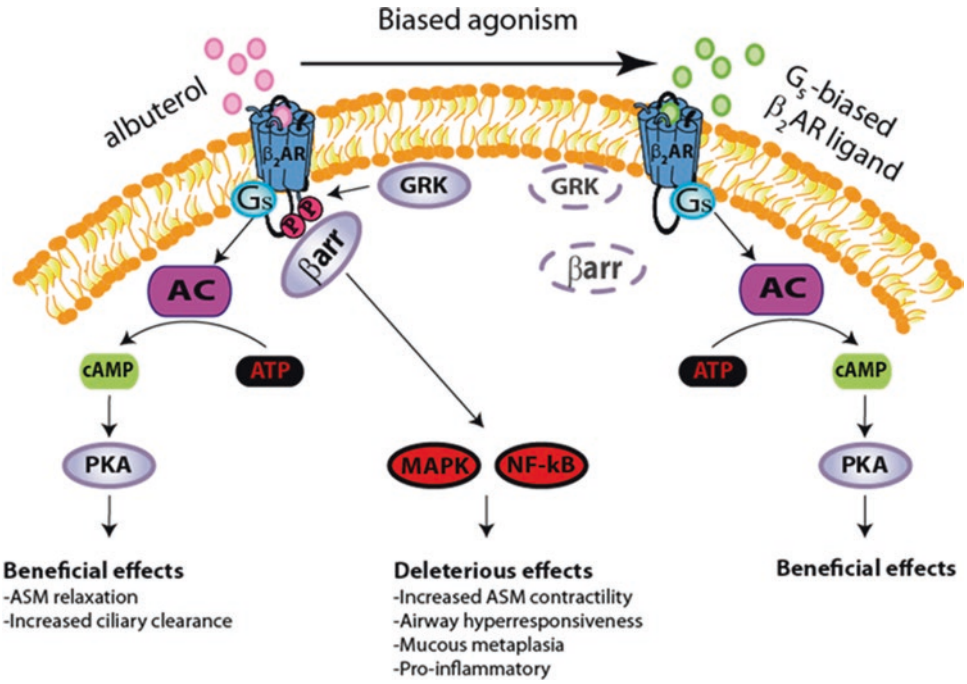

Fig. 1.4 Biased agonism pharmacology in ASM. Illustration of signaling mechanisms using principles of biased agonism to promote beneficial therapeutic effects in asthma

macology research in asthma is currently exploding, aided by increasing powerful genetic, molecular, cell biology, and computational tools and by the increasing rate of drug discovery, itself aided by advances in structural biology, computer modeling, and better screening tools and strategies. Moreover, the recent appreciation of qualitative signaling properties by GPCRs, and the realization that receptors can be "tuned" and not just turned "off" or "on" to specifically activate the beneficial therapeutic signaling a receptor can transduce, suggests that biased agonism pharmacology will develop the drugs we need to optimally control airway inflammation and asthma, as shown in Fig. 1.4 [185]. In addition, because GPCR ligands are small molecules and can be continuously refined to improve specificity of targeting while minimizing off-target effects, we should ultimately have an asthma therapy that supplants steroids and has a superior efficacy and safety profile.

1.16 Why Do We Care About Developing GPCR Ligands Capable of Managing Allergic Lung Inflammation?

As mentioned above, the properties of small-molecule GPCR ligands make them attractive therapies. In addition, it would be advantageous to control inflammation without the numerous side effects associated with corticosteroid treatment. The major issue remains whether control of inflammation by such drugs is truly sufficient to manage allergic inflammation and whether such a scalpel can do the job currently performed by a sledgehammer.

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