Inhaled $\beta_2$ Adrenergic Agonists and Other cAMP-Elevating Agents: Therapeutics for Alveolar Injury and Acute Respiratory Disease Syndrome?

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Abstract——Inhaled long-acting β-adrenergic agonists (LABAs) and short-acting β-adrenergic agonists are approved for the treatment of obstructive lung disease via actions mediated by β2 adrenergic receptors (β2-ARs) that increase cellular cAMP synthesis. This review discusses the potential of β2-AR agonists, in particular LABAs, for the treatment of acute respiratory distress syndrome (ARDS). We emphasize ARDS induced by pneumonia and focus on the pathobiology of ARDS and actions of LABAs and cAMP on pulmonary and immune cell types. β2-AR agonists/cAMP have beneficial actions that include protection of epithelial and endothelial cells from injury, restoration of alveolar fluid clearance, and reduction of fibrotic remodeling. β2-AR agonists/cAMP also exert anti-inflammatory effects on the immune system by actions on several types of immune cells. Early administration is likely critical for optimizing efficacy of LABAs or other cAMP-elevating agents, such as agonists of other Gs-coupled G protein–coupled receptors or cyclic nucleotide phosphodiesterase inhibitors. Clinical studies that target lung injury early, prior to development of ARDS, are thus needed to further assess the use of inhaled LABAs, perhaps combined with inhaled corticosteroids and/or long-acting muscarinic cholinergic antagonists. Such agents may provide a multipronged, repurposing, and efficacious therapeutic approach while minimizing systemic toxicity.

Significance Statement——Acute respiratory distress syndrome (ARDS) after pulmonary alveolar injury (e.g., certain viral infections) is associated with ∼40% mortality and in need of new therapeutic approaches. This review summarizes the pathobiology of ARDS, focusing on contributions of pulmonary and immune cell types and potentially beneficial actions of β2 adrenergic receptors and cAMP. Early administration of inhaled β2 adrenergic agonists and perhaps other cAMP-elevating agents after alveolar injury may be a prophylactic approach to prevent development of ARDS.

I. Introduction

β-adrenergic agonists are part of the standard care for asthma and chronic obstructive pulmonary disease (COPD). Inhaled β2-adrenergic agonists include short-acting β-adrenergic agonists [SABAs; e.g., salbutamol (albuterol)], which are typically used to acutely relieve bronchoconstriction and associated symptoms, whereas long-acting β-adrenergic agonists (LABAs; e.g., salmeterol, formoterol, etc.) have a slower onset but longer actions (reviewed in the Guide to Pharmacology database: https://www.guidetopharmacology.org/; Altosar et al., 2019). Because of an association with exacerbations and symptom severity with isolated LABA administration, guidelines for their use in treating patients with asthma dictate that LABAs should be administered with an inhaled corticosteroid (ICS, e.g., budesonide, fluticasone, etc.) or long-acting muscarinic acetylcholine receptor antagonists [long-acting muscarinic antagonist (LAMAs), e.g., tiotropium] (Cloutier et al., 2020; Global Initiative for Asthma, 2020). When used according to current guidelines, inhaled LABAs (and their combinations with ICS/LAMAs) have an excellent safety profile (Kew et al., 2013, Maqsood et al., 2019), suggesting their suitability for repurposing to treat other indications. Here, we review information related to pulmonary and immune cell types with respect to actions of β2-adrenergic receptors (β2-ARs) and cAMP, the primary cellular signaling molecule that increases in response to β-adrenergic receptor (β-AR) agonism. We focus on actions of β2-ARs and cAMP and their impact on the pathobiology of injury to pulmonary alveolae, with particular emphasis on acute respiratory distress syndrome (ARDS), especially when induced by pneumonia.

A large amount of information exists regarding β2-ARs and cAMP, but their role in pulmonary inflammation and injury, in particular their impact on cell types involved in alveolar injury, has not been recently reviewed. We thus sought to provide an up-to-date, comprehensive compilation of data and concepts regarding these topics. Our analysis reveals novel therapeutic opportunities that may improve the outcome of patients with lung injury and ARDS. We propose the hypothesis that inhaled LABAs represent...
an underexplored therapeutic option for the early treatment of ARDS, especially in the setting of respiratory infection. Ex vivo findings and in vivo data from animal studies and clinical investigations in humans suggest that administration of inhaled LABAs (perhaps in combination with other approved drugs, such as ICS) early in disease progression may be protective against ARDS. Pharmacological and mechanistic data discussed in this review imply that additional cAMP-elevating agents, such as agonists for other Gs-coupled G protein–coupled receptors (GPCRs) or cyclic nucleotide phosphodiesterase (PDE) inhibitors may also be useful for treating/preventing ARDS.

II. \(\beta_2\)-Adrenergic Receptor Signaling and Pharmacology

\(\beta\)-ARs are arguably the best studied GPCRs. The three \(\beta\)-AR subtypes (\(\beta_1\)-AR, \(\beta_2\)-AR, and \(\beta_3\)-AR) are class A/rhodopsin-like GPCRs that are differentially expressed in tissues and cells in humans and many other species. In humans, \(\beta_1\)-ARs are preferentially expressed in the heart and adipose tissue, whereas \(\beta_2\)-ARs are expressed in smooth muscle cells and many other cell types (including immune, epithelial, skeletal muscle, and glandular cells). \(\beta_3\)-ARs are preferentially expressed in adipose tissue and the urinary bladder.

Figure 1 shows expression of \(\beta_2\)-AR mRNA (the \(ADRB2\) gene) in human tissues, based on data from the Genotype-Tissue Expression project (Lonsdale et al., 2013), curated by the Human Protein Atlas (http://www.proteinatlas.org; Uhlén et al., 2015). \(\beta_2\)-AR expression in the lung is among the highest of all tissues. \(\beta\)-ARs are expressed in multiple regions of the lung, including airway and alveolar epithelia, smooth muscle, and endothelia. Autoradiographic quantification of \(\beta\)-ARs and of \(\beta_1\- and \(\beta_2\)-ARs (Carstairs et al., 1985) (Fig. 2) indicate that \(\beta\)-AR expression is most abundant in alveoli and that most of these receptors are \(\beta_2\)-ARs. Subsequent work has shown that type 1 and type 2 alveolar epithelial cells (AECs) highly express \(\beta_2\)-ARs (Liebler et al., 2004; Mutlu and Factor, 2008).

Norepinephrine (noradrenaline) and epinephrine (adrenaline) are the physiologic agonists of \(\beta\)-ARs. Norepinephrine is primarily produced by neurons in the central nervous system and postsynaptic neurons of the sympathetic nervous system in peripheral tissues, whereas epinephrine is predominantly synthesized by chromaffin (neuroendocrine) cells (e.g., in the adrenal medulla) and is primarily a circulating hormone. Norepinephrine has much greater affinity for \(\beta_1\)-ARs than \(\beta_2\)-ARs and regulates effector cells innervated by postsynaptic sympathetic neurons. By contrast, as a circulating hormone, epinephrine can activate \(\beta_1\)-ARs, \(\beta_2\)-Ars, and \(\beta_3\)-ARs on target cells.

\(\beta\)-AR activation (by norepinephrine, epinephrine, or pharmacological agonists) activates the heterotrimeric (\(\alpha_\beta\gamma\)) GTP binding protein Gs, which stimulates adenylyl cyclase (AC) activity and promotes the synthesis of cAMP (Fig. 3). Although \(\beta\)-ARs, as 7-transmembrane GPCRs, were thought to be activated exclusively in the plasma membrane, work in recent years has provided evidence for receptor signaling at intracellular sites (Irannejad et al., 2013; Tsvetanova and von Zastrow, 2014; Irannejad et al., 2017; Calebiro et al., 2021). \(\beta\)-AR–stimulated increase in cellular cAMP concentration is blunted by the hydrolysis of cAMP to 5\(\alpha\)-AMP by PDEs and extrusion of cAMP from cells by multidrug resistance proteins 4 and 5 (ABCC4/5) (Sassi et al., 2012). The \(\beta\)-AR–promoted increase in cAMP regulates cellular physiology by activation of one more or at least three effector proteins: 1) protein kinase A (PKA) and PKA-mediated phosphorylation of protein targets; 2) exchange proteins activated by cAMP (EPACs), which are nucleotide exchange factors for the Rap subfamily of low molecular weight GTP binding proteins; and 3) cyclic

![Fig. 1. Expression of ADRB2 mRNA in human tissues. mRNA expression [normalized as transcripts per million (TPM)] from RNA sequencing studies in the Genotype-Tissue Expression database (Lonsdale et al., 2013) and were downloaded from the Human Protein Atlas (Uhlén et al., 2015).](image-url)
nucleotide-gated (CNG) ion channels. Those canonical signaling mechanisms alter many cellular functions via changes in activities of proteins/enzymes, ion channels, and gene expression (Fig. 3). In addition to activation of Gs, β-ARs can also regulate cellular function via β-arrestins, which facilitate endocytosis of activated/phosphorylated β-ARs and can serve as a platform for intracellular signaling (Ippolito and Benovic, 2021).

Many approved drugs target β-ARs. Initial therapeutics were norepinephrine and epinephrine, which activate α-ARs in addition to β-ARs and thus can produce numerous adverse effects (AEs). Subsequent efforts led to the discovery and use of α-AR– and β-AR–selective agonists and antagonists and then β1-AR– and β2-AR–selective drugs. The recognition that inhalational delivery of β2-AR agonists preferentially targets the airways and lungs and minimizes systemic AEs was an important advance in the treatment of pulmonary disorders, especially asthma and COPD (Larsson and Svedmyr, 1977; Heel et al., 1978).

β2-AR agonists with relatively short duration of action (< 6 hours) were initially developed and

Fig. 2. Normalized expression of β-ARs in different regions of the human lung. The data (from tabulated values in Carstairs et al., 1985) are presented as the total abundance of β-ARs and relative proportion of β1- and β2-ARs, based on autoradiography using [125I]iodocyanopindolol to label β-ARs in lung sections and with β1- and β2-selective inhibitors to identify the β-AR subtypes.

Fig. 3. Signaling mechanisms/pathways through which β2-ARs regulate multiple aspects of cell physiology. CREM, cAMP responsive element modulator; Csk, C-Terminal Src Kinase; GSK-3β, Glycogen Synthase Kinase 3 Beta; ICER, Inducible cAMP early repressor; IP3R, Inositol 1,4,5-trisphosphate receptor; MRP4/5, multidrug resistance-associated protein; RAP1/2, Ras-related protein 1/2.
approved for administration to patients with bronchoconstriction, in particular asthma and COPD. These SABAs rapidly relax airway smooth muscle (bronchodilation) and improve symptoms. Other \( \beta\text{-AR} \) agonists were synthesized that had similar actions but longer half-lives (~12 hours) and thus were termed long-acting beta-agonists (LABAs). Use of LABAs was viewed as a way to decrease administration of corticosteroids in patients, especially those with mild to moderate asthma, to treat ongoing symptoms in patients with COPD and/or to prevent acute exacerbations in patients with asthma or COPD (e.g., Larj and Bleecker, 2004; Mapel et al., 2006). However, LABA administration as monotherapy is associated with an increased risk of fatal exacerbations in asthma (McMahon et al., 2011; Cates et al., 2012; Kersten et al., 2017). Hence, guidelines for treating patients with asthma dictate that LABAs should be administered together with an ICS [e.g., budesonide, fluticasone, etc., which are glucocorticoids (GCs)], to decrease inflammation and prevent sequelae of an LABA administered alone (Cloutier et al., 2020; Global Initiative for Asthma, 2020). LABA administration as monotherapy is associated with logical advantages over SABAs.

A pharmacological rationale exists for combining \( \beta\text{-AR} \) agonists with GCs (Barnes, 2002; Page et al., 2017) and/or LAMAs (Dale et al., 2014; Calzetta et al., 2018), and such combinations are approved as inhaled therapy for COPD (Calverley et al., 2017). LAMAs primarily inhibit M3 muscarinic receptors in the lungs but also block M1 and M2 receptors (depending on selectivity of the drugs) (Ejiofor and Turner, 2013; Alagha et al., 2014). M1 and M3 receptors couple to Gq/G11 signaling mechanisms [Inositol trisphosphate (IP3)-mediated calcium and protein kinase C–mediated responses], whereas M2 receptors couple to Gi/Go (reducing cAMP) (Guide to Pharmacology database; Alexander et al., 2019). Inhibition of M1–M3 receptors together with activation of \( \beta\text{-AR} \) receptors can yield additive or perhaps synergistic

<table>
<thead>
<tr>
<th>Drug</th>
<th>Action</th>
<th>Potency (pEC50)</th>
<th>Specificity at ( \beta\text{-AR} ) ( \beta/\beta/\beta )</th>
<th>Duration of effect</th>
<th>References</th>
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<tbody>
<tr>
<td>SABAs</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Salbutamol</td>
<td>Partial agonist</td>
<td>7.7</td>
<td>27:1</td>
<td>4-6 hours</td>
<td>Battram et al., 2006; Tashkin and Fabbri, 2010; Baker, 2010; Slack et al., 2013; Cazzola et al., 2013; Billington et al., 2017; Altsaar et al., 2019; Cazzola et al., 2019</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>Partial agonist</td>
<td>7.3</td>
<td>63:1</td>
<td>4-6 hours</td>
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<tr>
<td>LABAs</td>
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<tr>
<td>Salmeterol</td>
<td>Agonist</td>
<td>9.9</td>
<td>3000:1</td>
<td>12 hours</td>
<td></td>
</tr>
<tr>
<td>Formoterol</td>
<td>Agonist</td>
<td>10.1</td>
<td>150:1</td>
<td>12 hours</td>
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<tr>
<td>Olodaterol</td>
<td>Agonist</td>
<td>10.0</td>
<td>65:1</td>
<td>12 hours</td>
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<tr>
<td>Vilanterol</td>
<td>Agonist</td>
<td>9.4</td>
<td>2400:1</td>
<td>12 hours</td>
<td></td>
</tr>
<tr>
<td>Indacaterol</td>
<td>Agonist</td>
<td>6.8</td>
<td>16:1</td>
<td>24 hours</td>
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*pEC50, negative logarithm of EC50 (half maximal effective concentration).*
(i.e., more than additive) responses, such as in the relaxation of airway smooth muscle (Calzetta et al., 2018). Synergy between β2-ARs and GCs can also occur: GCs can reduce β2-AR desensitization and enhance β2-AR functional responses, whereas β2-AR signaling increases GC receptor translocation, transcriptional response, and receptor expression (Barnes, 2002; Johnson, 2004; Tamm et al., 2012, Newton and Giembycz, 2016; Page et al., 2017). In addition, as discussed below, GC signaling enhances actions/expression of ion channels regulated by β2-ARs in epithelial cells. LAMAs (Alagha et al., 2014; Beeh et al., 2017) and GCs (Cain and Cidlowski, 2017) also exert anti-inflammatory actions on immune cells, suggesting additive or perhaps synergistic effects when combined with LABAs.

III. Acute Lung Injury/Acute Respiratory Distress Syndrome: An Overview

ARDS is a syndrome defined by acute onset hypoxic respiratory failure with bilateral pulmonary edema that is not due to heart failure and that has an identifiable provocation. (Matthay et al., 2019). The revised Berlin criteria stratify ARDS by the ratio of partial pressure of arterial oxygen to fraction of inhaled oxygen (P/F). Severe disease has a P/F <100, moderate disease 100–200, and mild disease <300 (ARDS Definition Task Force et al., 2012). More recently other stratification has been proposed: severe disease, P/F <150; nonsevere disease, P/F 150–300 (Maiole et al., 2018). Acute lung injury (ALI) was historically used to categorize patients with a P/F ratio of 200–300 (Johnson and Matthay, 2010) and to describe pathologic injury of ARDS when not referring to the clinical syndrome. ALI is no longer commonly used in the clinical literature, but studies of animal models of ARDS still frequently use ALI as an alternative for ARDS. In this review, when discussing ALI/ARDS, we refer to a spectrum of pulmonary pathology (including edema and decreased lung function) in animal studies that is consistent with mild-to-severe ARDS in humans, per the Berlin definition (ARDS Definition Task Force et al., 2012; Ferguson et al., 2012).

ARDS develops in numerous clinical settings, including pneumonia, sepsis, trauma, aspiration, pancreatitis, and after exposure to various medications or toxins. Approximately 40% of patients with ARDS die (Bellani et al., 2016). The lungs of such patients typically have increased stiffness, resulting from diffuse alveolar damage that involves airway epithelial cells and the pulmonary endothelia with edematous fluid in the alveolar space. ARDS is heterogeneous; new classifications of the subphenotypes of patients may help advance understanding of ARDS and improve its outcomes (e.g., Standiford and Ward, 2016; Carla et al., 2020). The necessity to better understand ARDS pathobiology has been underscored by the coronavirus disease 2019 (COVID-19) pandemic: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can result in alveolar injury that progresses to ARDS in severe cases, which can lead to cytokine storm and systemic organ failure (Sriram and Insel 2020a; Sriram and Insel 2021; Batah and Fabro 2021; Hasan, 2021).

ARDS arising from COVID-19 has also underscored the importance of coagulopathy and thromboinflammation (i.e., a pathologic combination of excessive inflammation and thrombosis) in driving lung injury (reviewed in Sriram and Insel 2021). Numerous strategies have been proposed to tackle these aspects of COVID-19, including a range of agents that target aspects of coagulation, inhibit cytokine signaling [e.g., antibodies for cytokines such as interleukin (IL)-6] (Liu et al., 2020), or reduce inflammation more generally, including corticosteroids (Scavone et al., 2020; Zhang et al., 2020). The RECOVERY trial (RECOVERY Collaborative Group et al., 2021) revealed beneficial effects of systemic dexamethasone treatment of patients receiving oxygen or on mechanical ventilation. In addition, recent data indicate benefits using ICS (Ramakrishnan et al., 2021), suggesting the possible utility of combining these drugs with inhaled LABAs; this topic is discussed further in subsequent sections.

Current therapy of ARDS emphasizes the need to treat the underlying condition and to employ appropriate use of ventilators (in particular, with positive end-expiratory pressure), prone positioning, management of fluids, and supportive care. Neuromuscular blocking agents, typically nicotinic cholinergic blocking agents, have been employed to aid in the use of ventilators, but a recent systematic review questioned the impact of such agents on patient-important outcomes (Tarazan et al., 2020). “Rescue therapies” to treat patients with progressive clinical deterioration include extracorporeal membrane oxygenation and extracorporeal CO₂ removal. Systemically administered GCs are commonly administered to patients with ARDS, but their use is controversial: mixed results were obtained in clinical trials prior to the current period in which lung-protective ventilation is a key approach (Matthay et al., 2019). As indicated by its high mortality, effective pharmacological therapies do not currently exist for ARDS.

Figure 4 summarizes the pathobiology of ARDS resulting from viral infections (e.g., influenza, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome, SARS-CoV-2) of alveolar epithelial cells that induce lung injury. Numerous cell types in the lung contribute to pathophysiology and may be associated with secondary (typically bacterial) infection. The summary in Fig. 4 is based on
descriptions of ARDS pathobiology in SARS virus infections (Sriram and Insel, 2020a; Sriram et al., 2020; Horie et al., 2020), models for ALI/ARDS (e.g., Herrero et al., 2018), thromboinflammation in ARDS (e.g., Frantzeskaki et al., 2017), ARDS in influenza (Herold et al., 2015), and thromboinflammation, epithelial injury and complement activation in COVID-19 ARDS (Sriram and Insel, 2020a, 2020b; Sriram and Insel, 2021). In brief, alveolar inflammation induced by infection can produce inflammation of nearby endothelial cells and fibroblasts, increasing cytokine release from these cell types. Immune infiltration (primarily of innate immune cells, especially early in the infection) increases; immune cell activation and adhesion to epithelia further promote inflammation. In severe infections with virus-induced inflammation (perhaps along with underlying inflammatory conditions) hyperinflammation can ensue as a consequence of proinflammatory events that prevent viral clearance by innate and adaptive immune responses and, thereby, promote injury. Multiple mechanisms (complement signaling, tissue factor release, ADP release, platelet activation, and coagulopathy) are induced; platelet activation further contributes to inflammation via secreted factors and interactions with endothelial cells and neutrophils. In addition, epithelial disruption leads to reduced surfactant production and susceptibility to secondary bacterial pneumonia. Thus, a series of feedback loops is established, whereby multiple cell types interact to promote injury, inflammation, and coagulopathy, resulting in progressive alveolar damage and edema, ultimately leading to ARDS.

This progressive pathobiology is counteracted by protective immune mechanisms, in particular adaptive responses that follow seroconversion and can lead to pathogen clearance. Clinical features and patient outcomes thus result from the competing actions between progressive inflammation and injury (which can be enhanced by comorbidities associated with age and other factors) and protective responses (Sriram and Insel, 2020a; Sriram et al., 2020; Sriram and Insel, 2021). In this framework, drugs with anti-inflammatory effects and that target multiple cell types are predicted to be beneficial, especially with early administration prior to induction of hyperinflammation. These mechanisms provide a rationale for re-examining β2-AR agonists in the treatment of ALI/ARDS— in particular, inhaled LABAs administered early in viral respiratory infections.

IV. Evidence Regarding the Use of Long-Acting β2-Adrenergic Agonists in Acute Lung Injury/Acute Respiratory Distress Syndrome

Are β2-AR agonists (including LABAs) useful in treating ALI/ARDS? Evidence indicating a protective effect of β2-AR agonists in enhancing alveolar fluid
clearance (AFC), thereby reducing pulmonary edema (reviewed below and in Morty et al., 2007; Mutlu and Factor, 2008) led to the hypothesis that β2-AR agonists would be beneficial and protect from lung injury. Data showing a protective effect on the pulmonary endothelium (Morty et al., 2007; Mutlu and Factor, 2008) strengthened the rationale for testing β2-AR agonists in patients with ALI/ARDS. This hypothesis was also supported by evidence that prophylactic use of β2-AR agonists provided protection from high altitude pulmonary edema (HAPE) (Sartori et al., 2002). A prospective study in pediatric patients found that administration of inhaled β2-AR agonists on the first day of onset of ALI reduced mortality compared with those not treated (Flori et al., 2005). Retrospective analysis of data from mechanically ventilated patients also indicated a lower severity of ALI and longer survival in patients receiving a β2-AR agonist (Manocha et al., 2006). Other data in patients support a protective role for β2-AR agonists in ALI (Roca et al., 2008): the investigators assessed exhaled markers of nitrosative and oxidative stress and found that inhaled salbutamol reduced inflammation in ventilated patients. Systemic administration of salbutamol also improved AFC in a small cohort of patients with COPD, who had greater improvements than did healthy control subjects (Di Marco et al., 2012). Initial data from the first β-Agonist Lung Injury (BALTI) trial indicated improved lung function (i.e., reduced fluid accumulation) of patients with ALI/ARDS in an intensive care unit (ICU) systemically administered β2-AR agonists but lacked statistical power to detect improvement in mortality (Perkins et al., 2006).

However, subsequent larger randomized controlled trials (RCTs) undermined the case for using β2-AR agonists in ARDS. The BALTI-2 trial sought to expand on BALT-1 trial results by evaluating 28-day mortality and clinical assessments in patients with ARDS admitted to the ICU and administered salbutamol by infusion (Gao Smith et al., 2012; Gates et al., 2013). This trial found no improvement in mortality with salbutamol infusion and, in addition, noted AEs (especially cardiovascular AEs), thus indicating a net harm associated with the treatment. Similarly, the ALTA trial (National Heart, Lung, and Blood Institute et al., 2011) evaluated the effects of aerosolized salbutamol in patients with ALI receiving mechanical ventilation. No statistically significant differences were noted between treatment and placebo control groups, including ventilator-free days and 60-day mortality, although there was little evidence of AEs.

These trials led to reevaluation of the potential utility of β2-AR agonists in the treatment of ALI/ARDS. Subsequent analyses sought to reconcile encouraging preclinical and early clinical data with the negative results (Levitt and Matthay, 2012; Bassford et al., 2012). Explanations for the different results include possible off-target actions of β2-AR agonists, adverse cardiovascular effects, and concerns about whether pulmonary edema was reversible in the context of severe injury (Bassford et al., 2012). In the BALTI-1 and BALTI-2 trials, salbutamol was administered intravenously, a route that would be predicted to lead to greater cardiovascular AEs than would inhalational therapy. Another key issue involved the timing of β2-AR agonist administration: early administration of β2-AR agonists was suggested as more likely to produce benefits (Bassford et al., 2012). This idea reflects a paradigm shift in ARDS research, with greater emphasis on early treatment, including prophylactic approaches in at-risk patients (Ortiz-Diaz et al., 2013; Artigas et al., 2017; Griffiths et al., 2019).

Other studies necessitate re-examination of inhaled LABA and ICS treatment of ALI/ARDS. A retrospective study at the onset of ARDS among hospitalized, at-risk patients found that prehospital use of β2-AR agonists (but not ICS) was associated with reduced incidence of ARDS (Mangi et al., 2015). Recent retrospective data of patients with ARDS administered inhaled bronchodilators (most of which were a SABA/LAMA combination) found that increased cumulative doses of bronchodilators were associated with lower mortality, although the contributions of SABA versus LAMA were not analyzed (A.M. Scott et al., preprint, DOI: https://doi.org/10.21203/rs.3.rs-143660). A prior retrospective analysis by those authors indicated that patients with moderate or severe ARDS administered salbutamol had a dose-dependent improvement in survival (Scott and Ouellette, 2019). By their retrospective design, these studies were unable to control for certain confounders, such as patients with known reactive airway disease likely having been more frequently receiving β-agonist treatment.

In contrast with these supportive data, a 2019 Cochrane review of pharmacological agents for treating ARDS in adults found no statistically significant benefit of β-agonists, or any other agent evaluated, in reducing mortality (Lewis et al., 2019). The authors also noted a (non-statistically significant) indication of increased harm. This Cochrane review only included data from the BALTI-2, BALTI-1, and ALTA trials, which contrast with the approach that we propose: early administration prior to ICU admission.

A prospective study (Festic et al., 2016; Festic et al., 2017) compared the use of a LABA [formoterol/ICS (budesonide)] with placebo in patients presenting to emergency departments with hypoxemia and risk factors for ARDS (high prediction scores for lung injury). The key difference, compared with previous studies (e.g., the BALTI trials), was drug administration prior to the onset of ARDS/ALI, i.e., prophylaxis to mitigate lung injury. The authors found that
compared with placebo, LABA/ICS treatment reduced the incidence of ARDS, need for mechanical ventilation, length of intensive care, and duration of hospitalization. A recent study (Fouad et al., 2020) reported similar results: inhaled salbutamol as monotherapy or combined with ICS decreased the development of ARDS in hospitalized patients at risk for developing ARDS. Prioritizing early treatment, the same authors are currently conducting the ARREST Pneumonia trial that is testing whether early treatment of LABA/ICS in pneumonia can prevent exacerbation and respiratory failure (Levitt et al., 2021). The effect size LABAs in ARDS trials may also be reduced by concurrent administration of inotropes and vasopressors that have β-agonism (i.e., dobutamine, epinephrine, norepinephrine). Of note, an RCT with patients with COPD found that increased doses of LABA/ICS administered early in the onset of upper respiratory tract infection reduced the occurrence of severe exacerbations, thus highlighting the potential protective benefit of those drugs in that setting (Stolz et al., 2018).

Observational, retrospective studies have been conducted in patients with COPD, evaluating the effects of different inhaled therapies (various combinations of β-AR agonists, ICS, and LAMAs) and yielded ambiguous findings regarding the association between types of therapy and COVID-19 mortality (Bloom et al., 2021; Schultze et al., 2020). LABA use in the COVID-19 pandemic has received relatively little attention. Two RCTs have been initiated to investigate the use of inhalational LABA/ICS in COVID-19 and are recruiting patients as of March 2021 (NCT04331054 and NCT04331470).

The findings above thus imply the benefit of early treatment, perhaps with LABA/ICS, prior to severe lung injury. In subsequent sections, we review findings in pulmonary cell types that support early treatment with inhaled LABAs and ICS. The rationale for this approach is based on the following ideas:

1. Pulmonary pathology from infections with hyperinflammatory phenotypes results from interactions of multiple cell types and an interplay between cell death, inflammation, and coagulopathy that can lead to ARDS.
2. Feedback loops that drive disease progression are opposed by protective innate and adaptive immune responses, which contribute to tissue repair and clearance of pathogens. However, the protective responses are impeded by excessive inflammation.
3. β2-AR agonists have beneficial effects on multiple cell types (epithelial, immune, endothelial cells and fibroblasts) involved in alveolar injury by certain infections, effects that may be additive or synergize with actions of GCs.
4. Injurious mechanisms "compete" with protective effects of β2-AR agonists and GCs. Initial/moderate injury is potentially reversible, but severe injury may result in irreversible pathology and clinical progression, thus implying the need for early treatment before the onset of severe disease/injury.
5. Early treatment with inhaled LABAs (perhaps combined with ICS) prior to the onset of ARDS may counteract the pathologic feedback loops. By decreasing inflammation and cell death, those drugs help promote tissue repair and an effective immune response before severe pulmonary pathology occurs and progression to ARDS ensues.
6. This prophylactic strategy—early treatment with inhaled LABAs (and perhaps ICS)—for ALI/ARDS in pulmonary infections/damage in which progression of pathology involves an interplay between inflammation, injury, and coagulation thus represents a novel, potentially beneficial therapeutic approach.

V. Effects of β-Adrenergic Receptor Agonists on Pulmonary Cell Types in Alveolar Injury and Acute Respiratory Distress Syndrome

β-AR agonists preferentially activate β2-AR in cells in the lung (Figs. 1–3), and β2-ARs are expressed on multiple pulmonary and immune cell types that mediate diverse physiologic responses. These cell types include alveolar epithelial cells, pulmonary fibroblasts (FBs), endothelial cells (ECs), inflammatory cells associated with the innate immune response (e.g., dendritic cells, neutrophils, and macrophages), and adaptive immune cells (T cells and B cells).

A. Alveolar Epithelial Cells

AECs, in particular type 2 pneumocytes, and other types of pulmonary epithelial cells have high expression of β2-ARs (Mutlu and Factor, 2008). β2-AR expression increases toward the distal portion of the pulmonary airway network, with especially high expression in alveoli (Carstairs et al., 1985; Mak et al., 1996; Mutlu and Factor, 2008). Evidence for actions of β2-ARs includes studies conducted in cell-based models ex vivo, animal models, and human patients, (including those with COPD, asthma, and other conditions). Below we summarize the overall effects of β2-AR activation. We also include results for other types of respiratory epithelial cells [e.g., nasal and bronchial epithelial cells (BECs)] and various pulmonary epithelial cell lines as indicative of likely effects in AECs, although caveats exist with such ex vivo models (Hasan et al., 2018; Hiemstra et al., 2018).

B. Increased Ion Transport and Fluid Clearance in Alveolar Epithelial Cells

The control of lung fluid homeostasis is a critical function of AECs (Mutlu and Factor, 2008; Morty et al., 2007; Huppert and Matthy, 2017). Dysregulation of AFC is an important contributor to pathology
in pulmonary infection and ARDS (Mutlu and Factor, 2008; Huppert and Matthay, 2017; Peteranderl et al., 2017; Berthiaume and Matthay 2007). AFC, i.e., removal of excess fluid to prevent flooding of the alveolar air space, is achieved by AECs via osmotic gradients generated by opening ion channels that regulate Na⁺ and Cl⁻ flux across the plasma membrane; this active transport occurs in both type 1 and type 2 pneumocytes (Matthay et al., 2005; Folkesson and Matthay, 2006; Mutlu and Factor, 2008; Bartoszewski et al., 2017). Na⁺ transport is primarily regulated in alveoli by the epithelial sodium channel (ENaC), CNG ion channels on the apical side, and Na⁺/K⁺-ATPase on the basolateral side (Mutlu and Factor, 2008; Huppert and Matthay, 2017; Peteranderl et al., 2017; Bartoszewski et al., 2017). Net transport of Na⁺ thus occurs from the alveolar space into the interstitium and generates an osmotic gradient that drives fluid clearance from the alveoli (Azzam and Sznajder, 2015). In addition, the cystic fibrosis transmembrane conductance regulator (CFTR) regulates Cl⁻ transport, primarily on the basolateral membrane of AECs. Activation of these various channels by signaling mechanisms, such as cAMP-PKA, is key for the regulation of AFC (Huppert and Matthay, 2017; Mutlu and Factor, 2008; Morty et al., 2007).

Initial evidence that β-ARs promote AFC involved physiologic β-AR agonists (e.g., epinephrine) in various mammalian systems, including human lung tissue; selective β2-AR agonists were subsequently shown to increase AFC (Morty et al., 2007; Mutlu and Factor, 2008). These increases resulted from enhanced Na⁺ transport and CFTR-mediated Cl⁻ transport, both enhanced by β2-AR agonism. Early data (prior to 2007) demonstrating these effects has been reviewed (Groszhaus et al., 2004; Mutlu and Factor, 2008; Morty et al., 2007). In addition, β2-AR agonists can stimulate AFC in lung injury, including in models of ALI (Vivona et al., 2001; Mutlu et al., 2004), ventilator-associated injury (Saldias et al., 2000) and pneumonia (Su et al., 2006). In addition to use of β2-AR agonists, overexpression of β2-ARs increases AFC in vivo in rats and mice, along with increased Na⁺ transport by endogenous catecholamines (McGraw et al., 2001; Dumasius et al., 2001; Factor et al., 2002); in contrast, β2-AR knockout mice have decreased AFC in lung injury models (Mutlu et al., 2004). Overexpression of β2-ARs in human AECs has confirmed these findings (Factor et al., 2002).

The importance of ENaC-driven Na⁺ currents in mediating effects of β2-ARs was also demonstrated by knockdown of ENaC, which reduces β2-AR–mediated increases in AFC (Li and Folkesson, 2006). Similar findings were obtained in mice with a loss-of-function mutation in the β-ENaC gene (Randrianarison et al., 2008). β-AR stimulation via norepinephrine increases abundance of Na,K-ATPase protein and, thus, is an additional mechanism by which β-ARs may regulate alveolar ion transport and AFC (Azzam et al., 2004). Subsequent work showed similar effects in rat lungs exposed to acute hypoxia: isoproterenol treatment restored AFC after hypoxia-induced lung injury while also increasing Na,K-ATPase expression on the AEC basolateral membrane (Litvan et al., 2006). This mechanism was also demonstrated by use of a selective β2-AR agonist in rat fetal distal lung epithelia (Rahman et al., 2010). Studies with primary human type II AECs confirmed a role for CFTR channels in mediating cAMP-driven alveolar fluid transport: CFTR inhibition blocked the AFC-promoting effects of the cAMP agonist forskolin (Fang et al., 2006). Subsequent data revealed a role for CNG channels in β2-AR–promoted AFC in rat lungs (Pedersen et al., 2012).

Ex vivo data, primarily in rat AECs, has provided pharmacological insight into the relationship between β2-AR activation, Na⁺ and Cl⁻ transport, and fluid transport by AECs (Morty et al., 2007; Mutlu and Factor, 2008). Moreover, inhaled β2-AR agonists can provide therapeutic levels of agonists in the distal air spaces in patients with pulmonary edema, supporting the hypothesis that inhaled β2-AR agonists may exert effects on the distal airways and alveoli (Atabai et al., 2002). Such data support the idea that β2-AR agonists can reduce pulmonary edema by increasing AFC in human subjects.

Since these early studies (Mutlu and Factor, 2008; Morty et al., 2007), subsequent data provide further evidence for the importance of β2-AR–promoted AFC and its role in disease. For example, β2-AR stimulation was shown to mitigate alveolar edema in a heart failure model in rats, with greater sensitivity to β2-AR–induced AFC in diseased animals than controls, perhaps from alveolar hyperplasia in response to heart failure (Maron et al., 2009), similar to previous data showing reduced data in sheep and rats (Frank et al., 2000; McAuley et al., 2004). Intratracheal administration of a β2-AR agonist to rats infected with Pseudomonas aeruginosa increased AFC and lowered the lung wet-to-dry ratio (Robriquet et al., 2011). Reduction in AFC induced by influenza A infection in mice was also decreased by early (48 hours postinfection) treatment (Wolk et al., 2008). Exposure of rats to hypercapnia for 1 week reduced AFC and induced endocytosis of Na,K-ATPase, effects blunted by prior treatment with a β2-AR agonist or a cAMP analog (Vadász et al., 2008). β2-AR agonist treatment after lung transplantation after 20 hours of ischemia in rats improved lung function by increasing AFC (Hamacher et al., 2009). Enhanced AFC and protective effects of β2-AR agonist inhalation in ischemia-reperfusion injury has also been shown in a canine
model (Kondo et al., 2015). Exposure of rat type II AECs ex vivo to hypoxia reduced Na\(^{+}\) and Cl\(^{-}\) currents, which were restored by \(\beta\)-2-AR agonist treatment (Loeh et al., 2010). Alteration of AFC by recombinant human FAS ligand (a proapoptotic factor, associated with alveolar injury) in mice was largely reversed by intratracheal administration of isoproterenol (Herrero et al., 2013). Inhaled LABA treatment also helped mitigate AFC in a mouse model of lung injury from chlorine exposure (Song et al., 2011). Hence, \(\beta\)-2-AR agonists and other cAMP-raising agents have been proposed as possible treatments for chlorine-induced lung injury (Hoyle, 2010). Cyclic AMP-mediated enhancement of AFC via ENaC channels has also been demonstrated by activation of adenosine 2B receptors (Wang et al., 2020).

\(\beta\)-2-AR-mediated increase in AFC has also been shown in humans. A key study that highlighted the potential utility of \(\beta\)-2-ARs in lung injury was a double-blind RCT that tested an inhaled \(\beta\)-2-AR agonist as a prophylactic for HAPE (Sartori et al., 2002). The results indicated a decreased risk of developing HAPE, which the investigators attributed to AFC-promoting effects of \(\beta\)-2-AR agonists. As noted above, data in the BALTI-1 trial that tested intravenous \(\beta\)-2-AR agonists reduced fluid accumulation in the lungs of patients with ARDS (Perkins et al., 2006), but the subsequent BALTI-2 trial did not support the use of systemically administered \(\beta\)-2-AR agonists in this setting (Gao Smith et al., 2012; Gates et al., 2013). \(\beta\)-AR activation by epinephrine was shown to stimulate AFC in perfused human lungs ex vivo, an effect prevented by inhibition of CFTR or Na,K-ATPase activity (Sakuma et al., 2006). AFC in response to \(\beta\)-AR stimulation has also been studied in human lungs rejected for transplantation: \(\beta\)-2-AR activation improved AFC ~2-fold above basal levels, even after prolonged ischemia (Frank et al., 2007). In vivo studies in exercising healthy human subjects, nonselective \(\beta\)-AR blockade reduced alveolar membrane diffusing capacity, indicating decreased AFC, which was attributed to \(\beta\)-2-ARs, as \(\beta\)-1-AR-selective blockade did not reduce AFC (Paolillo et al., 2013). Conversely, treatment with inhaled \(\beta\)-2-AR agonists in healthy subjects increased diffusing capacity and improved indicators of lung function, highlighting the ability of \(\beta\)-2-ARs to enhance AFC (Taylor et al., 2016). The authors subsequently showed that inhaled \(\beta\)-2-AR agonist could reduce elevated lung fluid accumulation in patients with reduced ejection fraction heart failure (Taylor et al., 2017). A clinical trial with intratracheal salbutamol plus surfactant in infants with ARDS decreased nasal continuous positive airway pressure and duration of hospitalization, responses attributed to improved AFC (Dehdashtian et al., 2016). Intravenous \(\beta\)-2-AR agonist infusion also can increase fluid clearance in patients with COPD and improve lung function independent of bronchodilation (Di Marco et al., 2012).

Thus, numerous studies demonstrate that \(\beta\)-2-ARs can improve AFC in healthy and diseased human subjects.

Other studies in experimental animals illustrate the effects of bacterial and viral infection and other injury models (e.g., exposure to cigarette smoke) in decreasing AFC by reducing the expression of ion channels that mediate \(\beta\)-2-AR–stimulated increases in AFC. In various models of pulmonary injury/disease, increasing alveolar inflammation and injury can reduce \(\beta\)-2-AR–stimulated AFC, thus showing “(patho-)physiological” functional antagonism of \(\beta\)-2-AR action. Examples are discussed below. Importantly, decreased activity of these ion channels, which reduces the ability of \(\beta\)-2-AR agonists to increase AFC, can be mitigated by corticosteroids, implying a potential synergy between ICS and LABAs in maintaining AFC.

Influenza A infection of rat type II AEC monolayers inhibits ENaC function via a mechanism mediated by Src and protein kinase C and also reduces ENaC-driven AFC in influenza-infected rats (Chen et al., 2004). The decrease in ENaC activity by influenza (in particular, the M2 influenza protein) in human pulmonary epithelial cell lines may result from reactive oxygen species (ROS) (Lazrak et al., 2009). Those authors observed that influenza infection also decreased CFTR activity (Londino et al., 2013, 2015). Influenza infection in mice can decrease epithelial expression and function of ENaC and CFTR; this decrease in CFTR activity is prolonged after infection; and decreased ENaC, CFTR, and Na,K-ATPase activities also occur in virally infected human AECs (Brand et al., 2018). Treatment of human BECs to restore CFTR function can ameliorate influenza infection-induced dysfunction of alveolar fluid transport. Similar results were observed in human and mouse AECs and in vivo in mouse lungs: influenza A infection decreased Na,K-ATPase expression and function, which may depend on epithelial interferon signaling (Peteranderl et al., 2016). Possible mechanisms that drive influenza-mediated dysregulation of alveolar fluid transport and its contributions to pulmonary pathology have been reviewed (Londino et al., 2017) as have stimuli that decrease ENaC function/expression (Matalon et al., 2015).

Other data indicate a decrease in AEC Na\(^{+}\) transport in bacterial pneumonia, e.g., downregulation of ENaC mRNA expression in mouse lungs infected with \(P.\) aeruginosa (Dagenais et al., 2005). Secreted products from \(P.\) aeruginosa [e.g., lipopolysaccharide (LPS) and flagellin] can decrease Na\(^{+}\) transport, likely via down-regulation of ENaC, in pulmonary epithelial cells, including BECs and type II AECs from rodents (Evans et al., 1998; Kunzelmann et al., 2006; Boncoeur et al., 2010). LPS appears to regulate ENaC transcription via the extracellular signal-regulated protein kinase 1/2.
(ERK1/2) and p38 mitogen-activated protein kinase (MAPK) pathways (Migneault et al., 2013) in rat AECs and via ERK1/2 in human AECs (Baines et al., 2010). Besides effects on ENaC, LPS can downregulate CFTR ex vivo and in vivo in rat type II AECs (Yang et al., 2013). *Staphylococcus epidermidis* and *Staphylococcus aureus* reduce CFTR and ENaC expression in human BECs (Hussain et al., 2013). Other findings indicate reduced ENaC expression, AFC, and stimulation of AFC by β2-ARs in ischemia-reperfusion injury in rats (Richard et al., 2019). Respiratory syncytial virus infection produces similar effects on Na+ and Cl− transport in mice and in human and mouse pulmonary epithelial cells (Chen et al., 2009).

Multiple cytokines associated with inflammatory and injury responses can decrease epithelial AFC-promoting ion channel expression. Decreased ENaC expression/function by tumor necrosis factor α (TNFα) occurs in type II rat AECs, thus reducing Na+ currents (Dagenais et al., 2004). Transforming growth factor (TGF) β can inhibit β2-AR-induced Cl− transport and reduce CFTR expression and activity in human and rat type II AECs in a phosphoinositide 3-kinase (PI3K)–dependent manner (Roux et al., 2010). IL-8, via PI3K, decreases CFTR activity in those cells (Roux et al., 2013). This effect of IL-8/CINC-1 (the rat IL-8 analog) inhibited AFC in a rat ALI model. Moreover, TGFβ1 and IL-8 act synergistically to reduce Cl− transport in human and rat AECs and reduce their response to β2-AR stimulation (Wagener et al., 2015). In addition, TNFα can reduce ENaC expression and activity in rats and in cultured type II AECs (Yamagata et al., 2009). Besides its effects on Cl− transport, TGFβ can decrease ENaC surface expression (by increasing internalization) in rabbits, mice, and human AECs from patients with ARDS (Peters et al., 2014). Unwalla et al. (2015) provided further evidence for TGFβ-mediated decrease in CFTR: β2-AR stimulation increased CFTR function in human BECs, which was reduced by TGFβ. Cigarette smoke extract produced similar inhibitory effects on CFTR. Additional data implicating secreted factors in the decrease in ENaC, CFTR, and Na,K-ATPase activity have been reviewed (Hamacher et al., 2018). Of particular relevance are TGFβ (associated with pulmonary fibrosis and injury response), IL-8 (an inflammatory cytokine implicated in ALI), and LPS. Studies in mice also show protective effects of β2-AR agonists against lung injury induced by LPS, due to anti-inflammatory responses of various cell types, including AECs, as well as fibroblasts and resident immune cells, thereby reducing alveolar injury (Bosmann et al., 2012).

Figure 4 summarizes the balance between AFC, alveolar injury/inflammation, and edema. β2-AR activation enhances AFC [via increased expression and activity of Na+ and Cl− channels (ENaC, CFTR) and Na,K-ATPase], which helps reduce edema, thereby facilitating reduction in inflammation and enhancing recovery from lung injury. As discussed below, β2-AR agonists also reduce AEC apoptosis/inflammation and epithelial-to-mesenchymal transition (EMT). Increased edema and alveolar inflammation, induced by pathologic stimuli and inflammatory factors, can undermine this protective effect of β2-ARs by decreasing the expression and function of ion channels that promote AFC and that β2-ARs stimulate. The decrease in AFC can be mitigated by GCs, suggesting that β2-ARs/LABAs and GCs/ICS may synergize in protecting against pulmonary edema. We hypothesize that the balance between the pathologic decrease in AFC and protective stimulation of AFC can be approached therapeutically by early treatment with LABAs/ICS, thereby preventing/decreasing hyperinflammation and pulmonary edema.

C. Enhancement of Surfactant Production/Secretion

Surfactant is produced by type II AECs and plays a key role in protecting the lungs, in particular the alveolar epithelium, from infection (Griese, 1999; Frerking et al., 2001; Han and Mallampalli, 2015). Surfactant production is inhibited in numerous pathologic settings associated with alveolar injury, including viral and bacterial infections; depletion of surfactant contributes to the development of secondary pneumonia (Frerking et al., 2001; Wright et al., 2001; Glasser and Mallampalli, 2012; Mirastschijski et al., 2020). Administration of exogenous surfactant is an approach used to treat newborn respiratory distress syndrome (Chakraborty and Kotecha, 2013) and has been suggested as possible therapy for other types of ARDS, including for COVID-19 (Mirastschijski et al., 2020; Schousboe et al., 2020).

Cyclic AMP increases surfactant production by AECs by actions that include increased expression and cellular transport of Surfactant protein A, the major surfactant protein (Kazi et al., 2010; Benhabib et al., 2015). β2-ARs and adenosine 2B (A2B) receptors, via Gs-cAMP-PKA signaling, can enhance AEC surfactant production; the AC activator forskolin and cAMP analogs have similar effects (Wright and Dobbs, 1991). β2-AR agonists improved surfactant production in a rat model of sepsis (Wichert et al., 1988). Ex vivo studies of stem cell differentiation to type II AECs have shown that increased cAMP can induce expression and production of surfactant protein (Gonzales et al., 2002; Schmeckebier et al., 2013). cAMP-dependent surfactant mRNA expression and production can also occur by inhibition of PDE4 (which degrades cAMP) in type II human AECs (Hohne et al., 2012). β2-AR agonists/cAMP thus promote surfactant production and likely exert a beneficial effect in the context of viral infections. However, clear elucidation of this phenomenon in vivo—either in...
animal models or humans—is lacking and thus in need of further study.

**D. Reduced (and Improved Recovery from) Epithelial Injury; Impact on Epithelial-to Mesenchymal Transition**

Depending on the cell type, cAMP signaling can be either pro- or antiapoptotic (Insel et al., 2012a). Accumulating evidence suggests that β2-AR agonists and cAMP promote AEC resistance to apoptosis. In addition, β2-AR activation improves epithelial recovery from injury and reduces EMT and the susceptibility of AECs to damage from pathogens, while also potentially reducing viral/bacterial load. Below, we discuss examples of these protective effects.

Early data on the protective effects of β2-AR agonists on lung epithelia focused on airway and nasal ciliated epithelia (Salathe, 2002). Such observations emphasized that β2-AR agonists can blunt the reduction in ciliary beat frequency of epithelial cells exposed to bacterial products (Salathe, 2002). β2-AR agonists and cAMP improve mucociliary function of airway epithelial cells and thereby clear the lung of bacteria and other foreign matter; production of a mucus fluid layer and the actions of cilia propel mucus out of the lung (Bennett, 2002; Pappová et al., 2016).

Besides effects on ciliary beat frequency, bacterial products and infection induce damage/death of BECs; β2-AR agonists reduce these injurious effects while also inhibiting bacterial-epithelial adhesion (Dowling et al., 1997; Dowling et al., 1999; Coraux et al., 2004; Salathe, 2002). β2-AR activation of BECs and/or AECs also reduces inflammatory response, including intercellular adhesion molecule 1 (ICAM-1) and/or vascular cell adhesion protein 1 [markers of epithelial inflammation (Oddera et al., 1998; Salathe, 2002; Sabatini et al., 2003; Yamaya et al., 2011)] expression and production of IL-6, IL-8, and granulocyte macrophage colony-stimulating factor (GM-CSF) (Korn et al., 2001; Salathe, 2002; Sabatini et al., 2003; Strandberg et al., 2007) and stimulates wound healing of BECs (Salathe, 2002). Possible beneficial effects of β2-AR agonists on cilia have also been noted in-vivo in COPD patients (Piatti et al., 2005).

EMT, a feature of epithelial injury, is a profibrotic transformation of epithelial cells that can result in progressive fibrosis and inflammation, including in the lung in asthma, COPD, and pulmonary fibrosis, although the contribution of EMT in vivo is debated (Kage and Borok, 2012; Bartis et al., 2014; Salton et al., 2019). Considerable evidence implicates TGFβ as the profibrotic agonist that promotes EMT in pulmonary epithelial cells (reviewed in Willis and Borok, 2007; Chapman, 2011; Hill et al., 2019). Cyclic AMP inhibits EMT in many epithelial cell types via PKA and EPAC, which blunt pro-EMT effects of TGFβ (reviewed in Insel et al., 2012b; Zuo et al., 2019). This effect has been shown in human BECs, using a co-culture model of BECs with eosinophils, which stimulate EMT (Kainuma et al., 2017). LABA treatment of BECs reduced EMT, a response blocked by a β2-AR antagonist; treatment with forskolin also decreased EMT. Treating BECs from healthy subjects, smokers, or patients with COPD with a cAMP analog inhibited EMT; cAMP or PDE4 inhibition can block cigarette smoke extract–promoted EMT (Milara et al., 2012, 2014, 2015). Inhibition or knockdown of PDE4 decreased EMT in human AECs, blunting effects of TGFβ by inhibition of Rho kinase signaling (Kolosiornek et al., 2009). A2B receptor agonists also can decrease EMT and reduce TGFβ response in human AECs (Giacomelli et al., 2018). Such studies document the EMT-blunting effects of cAMP in AECs and BECs, implying that β2-AR agonists reduce EMT by cAMP signaling. As discussed below, in vivo data indicate that cAMP reduces pulmonary fibrosis, perhaps by combined antifibrotic actions of cAMP in AECs/BECs and fibroblasts.

Besides inhibiting EMT, β2-AR activation and cAMP can also decrease AEC apoptosis and/or stimulate alveolar repair. Cyclic AMP has antiapoptotic effects in numerous epithelial cell types, including renal, gastrointestinal, and pulmonary epithelial cells (Insel et al., 2012a; Barlow et al., 2008). Studies with murine AECs (Barlow et al., 2008) demonstrated that PKA–cAMP response element–binding protein (CREB) signaling decreases proapoptotic effects of H2O2, a response enhanced by CREB knockdown, implying a protective mechanism via Gs-cAMP-PKA-CREB in AECs, which may be activated by Gs-coupled GPCRs, such as β2-ARs. β2-AR agonists increase wound repair and enhance proliferation of human AECs and distal airway epithelial cells, effects that may contribute to improved endpoints in patients with ARDS treated with inhaled LABAs (Perkins et al., 2003). Moreover, treatment of AECs with bronchoalveolar lavage fluid from patients with ARDS treated with a β2-AR agonist stimulated wound healing of cultured AEC monolayers to a greater degree than bronchoalveolar lavage fluid from placebo-treated patients (Perkins et al., 2008). Wound healing effects of β2-ARs in AECs have also been demonstrated ex-vivo, in connection with an upregulation of matrix metalloproteinase-9 (MMP-9), which promotes AEC wound healing; upregulation of MMP-9 was also noted in BAL fluid from patients with ARDS (O’Kane et al., 2009).

β2-AR activation can stimulate growth of human AECs, likely via a PKA/CREB-dependent mechanism (Hu et al., 2016). PKA-dependent proliferation has also been shown in rat AECs: PKA inhibition reduces, whereas forskolin and cAMP analogs increase, AEC proliferation (Samuelsen et al., 2007). Studies in cells cultured from lung epithelial tumors support the role of β2-ARs in stimulating growth/proliferation/survival and have prompted interest in the use of β2-blockers in

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at ASPET Journals on September 15, 2023 pharmrev.aspetjournals.org Downloaded from
certain lung cancers (Al-Wadei et al., 2012; Nilsson et al., 2020). The growth-stimulating effect of β2-ARs may occur by transactivation of epidermal growth factor receptor, a promoter of growth in AECs and other epithelial cells (Berthiaume, 2003). In addition to β2-ARs, A2B receptor activation can reduce human AEC apoptosis after exposure to H2O2 (Xu et al., 2018). Similar effects were noted in human AECs treated with PGE2 acting via cAMP and PKA, likely via EP2 and EP4, Gs-coupled GPCRs (Leone et al., 2007). PGE2 also exerts protective effects on AECs, by blunting EMT (Crosby and Waters, 2010), responses analogous to the effects of β2-ARs. In addition, treatment of mice with PDE4 inhibitors can promote epithelial integrity, mitigating damage in an AEC-specific model of pulmonary fibrosis (Sisson et al., 2018). Thus, β2-AR signaling, likely via cAMP-PKA-CREB, has protective effects on AECs by reducing apoptosis, enhancing proliferation and wound repair, and stimulating restoration of the epithelium. These actions likely contribute to in vivo observations of antifibrotic effects of cAMP and improved recovery in models of lung injury, as discussed below.

E. Reduced Susceptibility to Epithelial Damage from Infectious Agents

Complementary early data showed a protective effect of β2-AR activation on epithelial damage involving AECs by bacterial products (Salathe, 2002). Supernatants from *P. aeruginosa* can reduce epithelial integrity in human AECs by decreasing expression of tight-junction proteins (Coraux et al., 2004), effects abolished by preincubation with an LABA. Human AECs infected with *S. aureus* have increased expression of inflammatory markers, such as IL-6, IL-8, and TNFα, mediated by nuclear factor κB (NF-κB) and activator protein 1 signaling; incubation of cells with a LABA/ICS combination blocks these responses (Fragaki et al., 2006). Similar results were obtained with human BECs treated with β2-AR agonists and corticosteroids (Edwards et al., 2006): mRNA expression of a range of inflammatory chemokines was reduced after exposure to RV16 rhinovirus. Beneficial effects of β2-AR agonists and corticosteroids were also noted in studies of RV16 rhinovirus infection in human BECs, with reduced production of the pro-remodeling factors fibroblast growth factor 2 and vascular endothelial growth factor (Volonaki et al., 2006). Studies with rhinovirus-infected primary human BECs support these results: β2-AR agonists plus corticosteroids reduced expression/release of proinflammatory factors without affecting viral replication; combination of the two drugs was synergistic (Bochkov et al., 2013). In vivo studies that tested the combined effects of β2-AR agonist and corticosteroid in a respiratory syncytial virus (RSV) model in mice also revealed a reduction in epithelial disruption and inflammation (Singam et al., 2006).

Alveolar Edema + Inflammation

Inflammation contributes to wider tissue injury, exerts effects on ECs, FBs, epithelial cells and immune cells

↑Na⁺ & Cl⁻ transport, driven by ENaC, CFTR, Na⁺/K⁺-ATPase

Decreases fluid buildup

Alveolar Fluid Clearance (AFC)

Enhance activity of AFC-promoting ion channels via transcriptional and functional regulation

β2-AR agonists

Downregulation of ion channel activity, expression and AFC

Downregulation blunted by corticosteroids

Corticosteroids

↑TGFβ, TNFα, IL8, etc. reduce ion channel expression

Primary & secondary infection worsen edema/inflammation

Viral & Bacterial infection

β2AR agonists and corticosteroids both reduce alveolar inflammation and susceptibility to infection

Fig. 5. The balance between AFC and alveolar edema plus inflammation. β2-AR agonists increase AFC and blunt edema by stimulating ion channel function directly and indirectly. Increases in edema and alveolar inflammation can reduce these protective effects by decreasing ion channel expression/activity, thereby worsening edema. Corticosteroids prevent this decrease in ion channels and may synergize with β2-AR agonists. β2-AR agonists and corticosteroids also reduce alveolar inflammation, cell death, and susceptibility to infection, thus protecting against edema and other consequences of alveolar injury.
In studies with human BECs, β2-AR agonists reduced *Mycoplasma pneumoniae* titers in infected cells while increasing expression of genes associated with antimicrobial defense (Gross et al., 2010). These protective effects of β2-AR agonists were reduced by IL-13 treatment and were less in cells from patients with COPD, results consistent with the paradigm presented in Fig. 5 regarding a balance between β2-AR-promoted protective effects and severity of pathology. Protective responses of β2-AR agonists and corticosteroids were also shown in human airway glandular cells infected with *S. aureus*: treatment restored mucus secretion, increased ion transport, and decreased inflammatory cytokine secretion (Zahm et al., 2010).

Other studies with human AECs have demonstrated that β2-AR agonists alone and synergistically with corticosteroids reduce viral titers and inflammatory readouts in cells infected with RV14 rhinovirus (Yamaya et al., 2011, 2013, 2014). Multiple mechanisms appear to mediate these effects, including decreased expression of viral receptors, increased acidification of endosomes (which reduces viral action) and decreased activity of pathways, such as NF-κB, that promote inflammation. Similar findings have been obtained with human BECs infected with the coronavirus HCoV-229E (Yamaya et al., 2020).

Those findings are supported by studies with RSV-A2–infected human BECs treated with forskolin or cAMP analogs, which protected the epithelial barrier by decreasing cytoskeletal and morphologic changes induced by viral infection and prevented disassembly of intercellular junctions (Rezaee et al., 2017). Viral titer and mRNA replication were also reduced. BECs from patients with asthma indicated an antiviral role for cAMP in RV16 rhinovirus infection with reductions in cell death, viral replication, and inflammatory marker expression (Roth et al., 2017). Moreover, LABA-promoted increases in cAMP sensitized human BEC response to corticosteroids, lowering the concentration dependence of GC response element–mediated transcriptional effects (Kaur et al., 2008).

In summary, substantial data from studies with human AECs indicate that β2-AR agonists reduce barrier dysfunction and epithelial cell death, apoptosis, and microbial titer and can restore ion transport. Such effects in AECs and other pulmonary epithelial cells imply the potential utility of β2-AR/cAMP in blunting alveolar injury and potential progression to ARDS.

**F. Complementarity with Glucocorticoid Signaling in Epithelial Cells**

β2-AR agonists have synergistic effects with GCs in a range of settings, including reduction of inflammation in asthma and COPD (reviewed in Barnes, 2002; Johnson, 2004; Tamm et al., 2012; Newton and Giembycz, 2016). These synergies include anti-inflammatory effects by both drug classes on airway smooth muscle and immune cells. The mechanisms include increased gene expression of β2-ARs by GCs, enhanced nuclear translocation of GC receptors due to β2-AR/cAMP signaling, and GC signaling effects that reduce β2-AR desensitization. Both GC and β2-AR/cAMP signaling inhibit pathways such as NF-κB and MAPK and aspects of calcium signaling, likely contributing to the complementarity of GC and β2-AR signaling.

GCs and β2-ARs/cAMP have other complementary effects in pulmonary epithelia. Examples include 1) increase by β2-AR agonists of anti-inflammatory transcriptional effects by GCs in BECs (Kaur et al., 2008); 2) reduction by β2-AR agonists and GCs of RSV replication and endocytosis (Yamaya et al., 2011, 2013, 2014) 3) suppression of rhinovirus-induced proinflammatory factors in BECs (Volonaki et al., 2006; Bochkov et al., 2013); 4) prevention by GCs of β2-AR agonist-induced release of proinflammatory factors in BECs, yielding a net anti-inflammatory effect (Edwards et al., 2007; Holden et al., 2010); 5) enhancement of regulator of G-protein signaling 2 function by GC and β2-AR/cAMP signaling (regulator of G-protein signaling 2 terminates Gq/G11 signaling) (Newton and Giembycz, 2016); 6) mutual enhancement by GC and β2-ARs of transcriptomic effects in human bronchial biopsies (van den Berge et al., 2014), BECs, and AECs (e.g., Rider et al., 2011; Joshi et al., 2015; Mostafa et al., 2019); 7) enhancement by PDE inhibitors of GC-mediated regulation of expression of proinflammatory factors (BinMahfouz et al., 2015; Milara et al., 2015); and 8) GC-stimulated expression of AFC-promoting ion channels, most notably ENaC, whose expression and function are also enhanced by β2-AR agonists (Lazrak et al., 2000; Nakamura et al., 2002; Itani et al., 2002; McTavish et al., 2009). Serum and glucocorticoid-regulated kinase 1 promotes ENaC and CFTR function; GCs enhance serum and glucocorticoid-regulated kinase 1 expression (Lang and Shumilina, 2013). Corticosteroid treatment can enhance AFC in animal models (e.g., Folkesson et al., 2000) and prevent ENaC downregulation by TNFα in human AECs ex vivo (Dagenais et al., 2006).

GCs thus exert multiple effects promoting AFC, likely in a manner additive to or synergistic with β2-AR agonists. Combined with the other protective/anti-inflammatory synergistic effects discussed above, ICS and LABAs administered together likely decrease pulmonary inflammation and injury while promoting AFC, thereby protecting against pulmonary edema in a complementary fashion (Fig. 5).

**VI. Fibroblasts**

Cyclic AMP has antifibrotic actions that include reduced profibrotic gene/protein expression and...
decreases in FB proliferation, migration/invasion, and secretion of proinflammatory factors. Such effects occur in FBs from multiple tissues, including lung, heart, and skin (Insel et al., 2012b; Lu et al., 2013; Zuo et al., 2019; Delaunay et al., 2019). Substantial data indicate that β2-AR agonists, via cAMP, promote an antifibrotic phenotype, including in studies of lung injuries that induce pulmonary fibrosis.

β2-AR agonists exert antifibrotic effects (decrease FB proliferation and expression of adhesion marker proteins associated with a profibrotic/remodeling phenotype) in human lung FBs in vitro (Silvestri et al., 2001). In that study, corticosteroids also reduced expression of adhesion markers. Treatment of human lung FBs with a β-AR agonist (isoproterenol), other agents that increase cAMP (PGE2, forskolin, PDE inhibition), or overexpression of AC6 decreased FB proliferation, collagen synthesis, and expression of collagen genes (Liu et al., 2004; Liu et al., 2010). β2-AR agonist and corticosteroid treatment reduce TGF-β-stimulated differentiation of human lung FBs to myofibroblasts (myoFBs) and TNFα-mediated IL-6 secretion (Baouz et al., 2005). LABA treatment of human lung FBs also blocks profibrotic and proinflammatory effects of TGF-β (Goulet et al., 2007). Combining SABAs/LABAs with corticosteroids was antifibrotic and anti proliferative in human bronchial FBs (Descalzi et al., 2008). Reduction in human lung FB proliferation is PKA-dependent (Kohyama et al., 2009).

β2-ARs are highly expressed in human lung FBs and FB cell lines, and LABAs reduce proliferation, collagen synthesis, and expression of α-smooth muscle actin (a fibrotic marker) (Lamyel et al., 2011). Roflumilast (a PDE4 inhibitor) potentiates LABA-promoted antifibrotic and anti-inflammatory effects in human lung FBs (Tannheimer et al., 2012b). Antifibrotic effects of combined β2-AR agonist/corticosteroid treatment also occur in fibrocytes: the drugs blunted the transformation of peripheral blood mononuclear cell–derived fibrocytes (isolated from patients with severe asthma) into myoFBs (Lo et al., 2017). A broad range of studies thus demonstrate antifibrotic effects of β2-AR stimulation, including in tandem with corticosteroids.

Other studies document the antifibrotic role of cAMP in lung FBs. Studies prior to 2012 (Insel et al., 2012b) identified antifibrotic effects by cAMP that include decreased proliferation, reduced FB-myofibroblast transformation, increased death of FBs/myofBFs, and reduced FB motility and expression/synthesis/release of extracellular matrix (ECM) components, including various collagens. Similar findings occur in FBs from other tissues, including heart, kidney, and skin. These effects appear to be mediated by EPAC (in particular, EPAC1) and PKA, with fibrotic transformation able to blunt such antifibrotic signaling.

The antifibrotic effects of PGE2, via EP2 and EP4 prostanoid receptors that raise cAMP, has been extensively studied (Bozyk and Moore, 2011) and reveal similar responses in lung FBs to those induced by β2-AR agonists and cAMP-elevating agents. PGE2 inhibits α-smooth muscle actin expression in human lung FBs by reducing nuclear accumulation of myocardin-related transcription factor-A and serum-response factor complexes and expression of serum-response factor mRNA, thereby inhibiting p38 MAP kinase activation and TGFβ response (Penke et al., 2014). PGE2 can exert antifibrotic effects (via EP2 and EP4) in human lung FBs from healthy donors and patients with idiopathic pulmonary fibrosis (IPF) by inhibiting Ca2+ signaling and decreasing TGFβ-promoted Ca2+ oscillations and activation of downstream kinases (Mukherjee et al., 2019). PGE2 can also reverse TGFβ-stimulated profibrotic changes in gene expression, suggesting actions in myoFBs (Wetlaufer et al., 2016). Lung epithelial cells produce PGE2; co-culture of epithelial cells and FBs reduces TGFβ-stimulated profibrotic effects in a PGE2-dependent manner (Epa et al., 2015).

Additional work on prostanoid receptors has shown that activation of prostacyclin receptors (PTGIR, a Gs-coupled GPCR) has antifibrotic effects in lung FBs by increasing cAMP, which can inhibit YAP/TAZ-mediated profibrotic transcriptional signaling and differentiation into myoFBs (Zmajkovicova et al., 2019). Inhibition of YAP/TAZ profibrotic signaling has also been observed for Gs-coupled dopamine D1 receptors, suggesting that YAP/TAZ inhibition may be a mechanism for antifibrotic effects of cAMP (Haak et al., 2019, 2020). Treprostinil (an agonist for EP2, EP4, and prostacyclin receptors) can inhibit platelet-derived growth factor-B and TGFβ-mediated profibrotic effects (e.g., expression of ECM proteins) and reduce lung FB proliferation (Lambers et al., 2018). Prostaglandin D2 and agonists for the prostanoid DP1 receptor (a Gs-coupled GPCR) have similar cAMP-dependent antifibrotic effects (e.g., decreasing TGFβ-driven transformation to myoFBs) (Ayabe et al., 2013). Thus, multiple Gs-coupled GPCRs exert cAMP-mediated antifibrotic effects in human lung FBs ex vivo.

In vivo studies using β2-AR agonists and other cAMP-elevating agents support these ex vivo data. Early evidence of the importance of β2-ARs in vivo in lung fibrosis was shown in models of lung injury in mice (induced by bleomycin, hyperoxia, or butylated hydroxytoluene): prior treatment of mice with the β-AR antagonist propranolol increased lung collagen (Lindenschmidt and Witschi, 1985). Similar results were obtained with rats treated with propranolol for 1–3 weeks (Smith and Smith, 1988). Treatment with propranolol increased collagen and interstitial fibrosis.
in the lung and produced alveolar remodeling. Subsequent studies indicated that propranolol treatment increases lactate dehydrogenase release in vivo and in fibroblasts cultured ex vivo (Smith and Smith, 1989) and induces fibrotic effects that involved ECs (Sommers Smith and Smith, 2002). LABA treatment has antifibrotic effects in lung FBs from normal subjects and patients with IPF (Herrmann et al., 2017). The latter study also tested inhalational LABA treatment of bleomycin-induced lung fibrosis in mice, using both a “preventive” treatment regimen (administration of LABA at the start of bleomycin treatment) or a “therapeutic” regimen (7 days after bleomycin exposure, i.e., once injury and fibrosis occurred). The authors found that LABA treatment in both regimens reduced weight loss, immune cell infiltration, and inflammatory and fibrotic marker expression in bronchoalveolar lavage fluid. Early LABA treatment also reduced collagen deposition and enhanced forced vital capacity. The investigators also tested LABA treatment in TGF-β1-overexpressing mice that spontaneously develop fibrosis; early, but not late, treatment with LABA had strong antifibrotic effects.

Treatment with inhaled LABA/ICS (formoterol/beclomethasone) has been tested in a 4-week pilot study of patients with IPF (Wright et al., 2017). LABA/ICS-treated patients had reductions in several readouts of coagulopathy and platelet activation, reduced sputum eosinophil counts, and improved 1-second forced expiratory volume. These pilot study data provide evidence in support of efforts to test LABA/ICS in settings that can cause lung injury associated with fibrosis and underscore the potential complementarity/synergy between LABAs and corticosteroid.

In vivo studies with other cAMP-raising agents (including other Gs-coupled GPCR agonists) support the findings with β2-AR agonists. Examples are as follows: dibutyryl cAMP was tested prophylactically in a bleomycin pulmonary fibrosis model in hamsters, with treatment commencing 48 hours prior to bleomycin exposure (O’Neill et al., 1992). Treatment with dibutyryl cAMP reduced the bleomycin-induced increase in inflammation and the rate of fibrotic lesion formation and growth. A more recent study that tested dcAMP in a silicosis model of pulmonary fibrosis in rats (Liu et al., 2017) showed reductions in Gxi expression and the extent of fibrotic scar formation along with enhanced abundance of PKA and phospho-CREB protein; ex vivo data indicated a cAMP-PKA-CREB mediated effect, with reductions in phosphorylated SMAD2/3 binding to CREB binding protein. In vivo inhibition of fibrosis by cAMP has been demonstrated in FB-specific AC6-overexpressing mice with bleomycin-mediated lung injury (Liu et al., 2010). AC6 overexpression also reduced immune cell infiltration in vivo, with ex vivo data showing enhanced response to β2-AR agonist stimulation; larger increases in cAMP produced stronger antifibrotic effects.

Substantial in vivo data support a role for prostanoid signaling in mitigating lung fibrosis via Gs-cAMP–driven mechanisms. Using a synthetic PGE2 analog in a bleomycin mouse model, Failla et al. (2009) treated the mice at the onset or 72 hours after bleomycin administration and observed a decrease in inflammation, lung injury, and fibrosis. By contrast, bleomycin-treated cyclooxygenase-2–deficient mice exposed to bleomycin had more severe inflammation and fibrosis, likely because of reduced PGE2 generation (Hodges et al., 2004; Lovgren et al., 2006). Protective effects of PGE2 are likely time-sensitive: Dackor et al. (2011) showed such effects if PGE2 or the PGE2 analog, iloprost, were administered to mice 7 days prior to, but not 14 days after, bleomycin treatment. Studies using iloprost (Zhu et al., 2010; Aytemur et al., 2012) or the corticosteroid methylprednisone (Aytemur et al., 2012) also showed that early administration blunted inflammation and fibrosis in bleomycin-treated mice. Orotracheal administration of mice with treprostinil prior to bleomycin exposure had protective antifibrotic and anti-inflammatory effects and reduced vascular remodeling and may protect from pulmonary hypertension induced by bleomycin injury (Nikitopoulou et al., 2019).

Novel delivery methods for PGE2 in fibrosis models further highlights this protective effect of prostanoids (and likely other cAMP-raising agents) in decreasing fibrosis in vivo. An example is inhalation of liposome-containing PGE2 after bleomycin exposure (Ivanova et al., 2013), which delivers drug to the lung and reduces fibrosis. Inhaled PGE2-containing nanoparticles (used with small interfering RNAs against certain profibrotic genes) can also protect from bleomycin-induced fibrosis in mice (Garbuzenko et al., 2017). Thus, multiple inhalational approaches may be ways to increase cAMP and treat lung fibrosis.

Other studies underscore the protective effects of prostanoid signaling against fibrosis in vivo. Examples include the following: 1) upregulation of PGE2 signaling can protect plasminogen activator inhibitor 1–deficient mice from lung fibrosis (Bauman et al., 2010); 2) synergistic protection from lung fibrosis by hepatocyte growth factor and PGE2s (Yoon et al., 2013); 3) inhibition of angiotensin II signaling by losartan enhances PGE2 production and associated antifibrotic effects (Molina-Molina et al., 2006); 4) mice deficient in microsomal prostaglandin E2 synthase-1 are susceptible to more severe bleomycin-induced fibrosis (Wei et al., 2014); 5) intravenous injection of retrovirally transfected FBs with prostaglandin synthase into bleomycin-exposed mice reduces lung injury, edema, and fibrosis (Ando et al., 2003); 6) greater susceptibility to bleomycin-induced fibrosis in
SLCO2A1 (prostaglandin transporter)–deficient mice (Nakanishi et al., 2015); and 7) fibroblasts from patients with IPF are less susceptible to apoptosis, but PGE2 induces apoptosis in profibrotic cells and protects AECs from FAS-ligand–induced apoptosis, implying both an antifibrotic and epithelial-protective effect of PGE2 and cAMP (Maher et al., 2010).

Other Gs-coupled GPCR agonists and PDE inhibitors have antifibrotic actions in vivo. A peptide targeting relaxin receptors (RXFP1/LGR7) administered at the time of or 7 days after bleomycin treatment had protective effects; ex vivo studies in human cells showed that these effects are cAMP-mediated, consistent with antifibrotic responses to relaxin in other cell types (Pini et al., 2010). Relaxin also reduced fibrosis in bleomycin-exposed mice 14 days after exposure; ex vivo experiments in FBs derived from patients with IPF and in murine FBs showed that relaxin reduces myoFB contractility and RhoA signaling (Huang et al., 2011). Treatment of bleomycin-exposed mice with a DRD1 agonist 10 days after exposure reduced FB contractility, enhanced cAMP, and inhibited YAP/TAZ signaling (Haak et al., 2019). Inhibition of PDE4 immediately after bleomycin exposure can exert antifibrotic and anti-inflammatory effects in rats (Pan et al., 2009) and mice (Udalov et al., 2010). Treatment of bleomycin-exposed rats 10 days after exposure with a PDE4 inhibitor also alleviated fibrosis and vascular remodeling in vivo (Cortijo et al., 2009).

Thus, numerous studies with human and rodent lung FBs ex vivo and studies in vivo demonstrate that cAMP and Gs-coupled GPCRs, including β2-ARs and certain prostanoid receptors, reduce the profibrotic phenotype of lung FBs and blunt inflammation, vascular remodeling, and lung injury. The ex vivo data imply that raising cAMP concentrations reduces fibrotic signaling in FB/myoFB. In vivo findings likely result from actions of cAMP-elevating agents on multiple cell types besides fibroblasts, including AECs, endothelial cells, and immune/inflammatory cells. Antifibrotic effects appear to optimally occur if cAMP is increased near the onset of profibrotic stimulation, emphasizing the need for early intervention with β2-AR agonists and other agents and consistent with data above for alveolar epithelial injury and edema.

VII. Endothelial Cells

Numerous studies document a protective effect of cAMP on the pulmonary endothelium by enhancing the integrity of the endothelial barrier. Increase in cAMP reduces endothelial permeability, thereby decreasing the severity of edema and immune cell infiltration (Sayner, 2011; Birukova et al., 2013; Birukov and Karki, 2018; Millar et al., 2016; Liu et al., 2015; Claesson-Welsh et al., 2021). These barrier-protective effects of cAMP appear to be mediated by both PKA and EPAC and oppose the effects of RhoA, which enhances endothelial permeability. The barrier-protective effects of cAMP on ECs are of particular relevance in the context of pneumonia that leads to ALI/ARDS in which endothelial integrity is compromised (Gonzales et al., 2015; Millar et al., 2016; Matthay et al., 2019). EPAC has been shown to have protective effects on the endothelium, reducing vascular permeability and disruption and modulating endothelial inflammation in a range of settings (Roberts and Dart, 2014).

In ECs of other tissues, cAMP increases (including by β2-ARs) may promote angiogenesis and protect from apoptosis (Dormond and Ruegg, 2003; Namkoong et al., 2005; O’Leary et al., 2015; Sorriento et al., 2011). However, other findings suggest that cAMP may have opposite, concentration-dependent actions, perhaps mediated by p38 and AKT, respectively (Sorriento et al., 2011). Angiogenesis, a critical process during recovery after lung injury, including in ARDS, restores function (e.g., gas exchange) in the recovering lung (Voelkel et al., 2007), underscoring a potential benefit of β2-AR agonists during this postinjury phase.

The endothelial barrier-protective effects of cAMP are complicated by its role in subcellular compartments. Certain ACs increase cAMP concentration near the plasma membrane and produce barrier-protective effects (Sayner, 2011). By contrast, cytosolic pools of cAMP, perhaps derived from soluble AC, can have opposite actions in ECs. β2-ARs are functional in these cells, but previous reviews (e.g., Sayner, 2011; Millar et al., 2016; Birukov and Karki, 2018; Claesson-Welsh et al., 2021) that discuss effects of cAMP on the pulmonary endothelium have limited discussion of β2-ARs and such effects. β2-AR agonists regulate microvascular endothelial permeability, which is complementary to β2-AR agonist actions on AECs in regulating edema. Below we discuss examples of endothelial barrier-protective effects of β2-AR agonists.

β2-AR agonists can reduce pulmonary vascular permeability in humans (Basran et al., 1986): ten patients with ARDS were systemically administered a β2-AR agonist; radiolabeled transferrin was used to test for plasma protein extravasation and accumulation (PPA). β2-AR agonist treatment reduced PPA in patients who survived; nonresponders succumbed to ARDS. Thus, in a subset of patients, β2-AR agonists reduce vascular permeability. Administration of β2-AR agonists to sheep reduced thrombin-induced increases in protein flux (Minnear et al., 1985). In studies with bovine pulmonary arterial ECs, β2-AR agonists reduced endothelial permeability, including if enhanced by z-thrombin or thrombin peptides; response to a β2-AR agonist was abolished by a β2-AR antagonist (Minnear et al., 1993). An inhaled LABA
inhibited histamine-induced increases in pulmonary PPA and reduced LPS-induced neutrophil accumulation in guinea pig lungs, indicating that inhaled LABAs can regulate microvascular endothelial permeability in vivo (Whelan et al., 1993). Systemic administration of a LABA also reduced endothelial gaps in rat tracheal ECs (Baluk and McDonald, 1994).

Data from isolated, perfused rat lungs showed a similar endothelial barrier protective effect, as assessed by high pulmonary venous pressures to measure increased capillary filtration rates (Parker and Ivey, 1997). β2-AR agonist infused in the pulmonary vein reduced capillary filtration rates and protected against fluid accumulation. Intravenous β2-AR agonist also reduced microvascular permeability/permeability (evaluated by estimating plasma protein extravasation) induced by antigen (Ikezono et al., 2005). Studies in rats, using a model of high-volume ventilation, yielded similar results: intraperitoneal β2-AR agonist treatment reduced microvascular permeability and helped reduce lung fluid accumulation (deProst et al., 2008). β2-AR agonists thus can decrease edema not only by effects on AFC (discussed above) but also by regulation of endothelial permeability. However, continuous systemic administration of β2-AR antagonists in vivo can induce EC death (Sommers Smith and Smith, 2002).

β2-ARs may have an endothelial-protective role in infection. Influenza A infection of ex vivo human pulmonary microvascular ECs increased permeability of endothelial monolayers, with similar increases in microvascular permeability noted in vivo in mice (Armstrong et al., 2012). Treatment with a LABA (ex vivo or in vivo) largely abrogated these influenza-induced changes in permeability and protected infected mice from pulmonary edema. As noted above, a complex relationship exists between the ability of β2-ARs to promote AFC and the decrease in this β2-AR-mediated effect by edema-associated pathology. Similarly, in the pulmonary endothelium, LPS treatment of cultured rat ECs reduces β2-AR expression at the cell surface (by promoting internalization) and increases endothelial permeability (Yang et al., 2015). Conversely, pretreatment of ECs with a β2-AR agonist prevents LPS-mediated increases in endothelial permeability.

Additional protective effects of β2-AR in pulmonary ECs may result from increased nitric oxide synthase (NOS) activity and nitric oxide (NO) production (Horvath and Wanner, 2006). Inhaled NO has been tested as a therapeutic approach in ALI/ARDS (Gebistorf et al., 2016). Increased NO production and vasodilation in response to β2-AR agonists was shown in pulmonary arteries of normoxic mice and mice exposed to chronic hypoxia (mimicking a pulmonary hypertensive phenotype) (Leblais et al., 2008). Similar effects were noted in pulmonary arteries of mice, with β2-AR stimulation increasing endothelial NO production and cGMP levels and reducing PDE5 expression (Davel et al., 2015). Certain effects of β2-AR signaling in ECs may occur by signaling from Src-kinase in murine pulmonary arterial caveolae, leading to PI3K/Akt phosphorylation and NOS activation (Banquet et al., 2011). Evidence in pulmonary microvascular ECs is sparse, but β2-AR–mediated NO production has been shown in microvascular ECs from other tissues (Conti et al., 2013), suggesting that this effect also occurs in microvascular ECs in the vicinity of alveoli.

A contributing factor to angiogenesis promoted by β2-ARs is their stimulation of endothelial progenitor cells (EPCs), as precursors for angiogenesis and re-endothelialization. β2-AR agonists stimulate proliferation and migration and increase vascular endothelial growth factor release in EPCs isolated from mice and rats (Galasso et al., 2013). Injection of EPCs from wild-type, but not β2-AR knockout, mice exposed to ischemic injury increased angiogenic response. Ke et al. (2016) observed a similar proangiogenic effect with overexpression of β2-ARs in human-derived EPCs studied in vitro, with increases in proliferation, migration, adhesion, and endothelial nitric oxide synthase activity. Further experiments in mice using a carotid artery injury model showed that β2-AR–overexpressing EPCs induce greater re-endothelialization. The latter findings were replicated and extended in studies that revealed enhancement by β2-ARs in shear stress–induced increases in AKT phosphorylation and endothelial nitric oxide synthase activity (Hu et al., 2020), implicating β2-ARs in promotion of EPC-mediated endothelial restoration.

Both ex vivo and in vivo studies thus document endothelial barrier-protective effects of cAMP and β2-ARs. The findings include regulation of endothelial permeability that contributes to protection from edema along with other protective effects on the endothelium and alveoli. β2-ARs/cAMP also likely promote NO production, angiogenesis, and re-endothelialization, thus helping to restore function after inflammation while possibly also inhibiting endothelial apoptosis.

VIII. Immune Cells

β2-ARs are expressed on numerous immune cell types, including dendritic cells, macrophages, monocytes, neutrophils, and others (Theron et al., 2013; Padro and Sanders, 2014; Arumugham et al., 2017). β2-ARs are the predominant AR expressed on B cells and T cells and play a modulatory role on the adaptive immune response (Padro and Sanders, 2014). The general paradigm for the actions of β2-AR activation and cAMP in these cell types is suppression of inflammation. The localized delivery of β2-AR agonists (i.e., inhaled LABAs) may thus help mitigate increases in pulmonary hyperinflammation that can
occur in pulmonary infections, such as certain viral and bacterial infections, thereby decreasing disease severity. Other approaches to increase cAMP, e.g., PDE inhibition, can also reduce inflammation in the lungs, for example, infections by rhinovirus (Edwards et al., 2016) or influenza (Sharma et al., 2013). We discuss the evidence for immune-modulating effects of β2-AR activation and cAMP in the following text and accompanying tables. Figure 6 summarizes actions of β2-ARs/cAMP signaling in adaptive and innate immune cells. These observations are relevant to pulmonary inflammation and, more generally, as effects of β2-AR/cAMP signaling.

A. Innate Immune Cells

1. Macrophages/Monocytes. The consensus conclusion (Table 2) is that β2-ARs, via cAMP, suppress inflammatory responses/phenotypes in macrophages and monocytes. These responses occur by inhibition of the production of proinflammatory factors (TNFα, IL-6, IL-1β, and others) and increased production/secrection of anti-inflammatory factors, most notably IL-10. In addition, Toll-like receptor signaling can be inhibited by β2-AR activation/cAMP increase. β2-AR/cAMP signaling can promote a phenotypic switch from M1 (proinflammatory) to M2 (anti-inflammatory) macrophages and enhance expression of a proresolving phenotype in circulating monocytes. β2-AR/cAMP signaling can also reduce the killing of bacteria by macrophages and may promote the clearance of apoptotic cells by proresolving macrophages and monocytes. β2-AR/cAMP signaling can enhance, whereas blockade of such signaling can worsen, inflammatory responses to stimuli (e.g., LPS). β2-AR agonists regulate inflammation in both acute (e.g., response to infection) and chronic (e.g., diabetes, obesity) settings. Anti-inflammatory effects of β2-AR/cAMP signaling appear to be mediated by both PKA and EPAC. cAMP may also have effects that reduce oxidative stress by promoting NO production in macrophages (Serezani et al., 2008). Limited evidence suggests that β2-AR activation, especially in settings of high catecholamine concentrations, can have proinflammatory effects.

Hence, β2-AR activation promotes macrophage/monocyte-driven mechanisms of tissue repair and recovery and suppresses proinflammatory mechanisms. The immunosuppressive effects of β2-AR activation or other approaches that increase cAMP signaling may represent a “double-edged sword.” In addition, such treatments can enhance cytokine responses that induce a T helper (Th) type 2 versus Th1 phenotype in T cells, an action that can alter pathobiology and clinical manifestations.

2. Dendritic Cells. As with monocytes/macrophages, a consensus (Table 3) indicates that β2-ARs/cAMP exert anti-inflammatory effects on dendritic cells (DCs). These effects include a reduction in proinflammatory cytokine release and increased release of anti-inflammatory IL-10. β2-ARs/cAMP promote DC-mediated differentiation of T helper cell lineages away from a Th1 phenotype and toward Th2 and Th17 phenotypes. However, definitive conclusions regarding such effects in human DCs, including as...
TABLE 2

Review articles (listed chronologically) that summarize effects of β2-AR/cAMP signaling on macrophages and monocytes.

<table>
<thead>
<tr>
<th>Source</th>
<th>Stimulus</th>
<th>Response/phenotype</th>
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<tbody>
<tr>
<td>Shirshev, 2011</td>
<td>cAMP/EPAC activation</td>
<td>EPAC1 increases with differentiation of monocytes to macrophages and contributes to anti-inflammatory effects of cAMP in macrophages (but less so in monocytes) while also promoting prosurvival/proliferation signaling via AKT.</td>
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<td>cAMP via EPAC1 reduces macrophage phagocytosis and production of IL-1β and ROS.</td>
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<td>Kolmus et al., 2015</td>
<td>β2-ARs</td>
<td>Reduce inflammation, ROS production, LPS-induced MIP-1α, ICAM-1, IL-12, TNFα, IFN-γ, IL-18, IL-12 in macrophages and monocytes.</td>
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<td></td>
<td>Blockade of β2-ARs can reduce circulating M2 monocytes. Macrophages may produce catecholamines, creating an autocrine-paracrine signaling loop especially in stress, injury, inflammation. Can reduce macrophage infiltration and bacterial phagocytosis.</td>
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<td>In certain settings, especially with high concentrations of catecholamines, may be proinflammatory.</td>
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<tr>
<td>Theron et al., 2013</td>
<td>β2-AR activation, cAMP/PKA-mediated effects</td>
<td>β2-ARs exert numerous anti-inflammatory effects, e.g., reduce signaling/release of PGE2, IL-1β, IL-6 and other proinflammatory interleukins, ERK1/2, GM-CSF, increase release of IL-10.</td>
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<tr>
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<td>cAMP/PKA enhance glucocorticoid receptor-activated gene transcription, which promotes expression of anti-inflammatory genes.</td>
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<td>cAMP/PKA-mediated effects reduce transcriptional activity of AP-1 and NF-κB, which promote expression of various proinflammatory genes.</td>
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<td>EPAC1 reduces bacterial killing and production of ROS (H₂O₂).</td>
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<td>EPAC1, via PI3K/Akt signaling, can stimulate macrophage proliferation.</td>
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<td>Mice deficient in AC7 have decreased cAMP in the immune system, resulting in increased proinflammatory LPS response in macrophages.</td>
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<td>Subpopulations of macrophages may be more susceptible to β2-AR–induced M1/M2 switching. In addition to secreted factors listed above, β2-ARs reduce IL-27 (which promotes Th1 T-cell lineages) and increase anti-inflammatory IL-13. Flumilast, a PDE4 inhibitor, reduces release of inflammatory factors from macrophages by inhibiting NF-κB, p38, and JNK pathways. Crisaborole, a PDE4 inhibitor, reduces inflammatory cytokine release (e.g., TNFα, IL-1β, IL-6) in macrophages and monocytes.</td>
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<td>Release by macrophages of inflammatory cytokines (e.g., TNFα, IL-6, IL-1β, CCL2-4), lung–resident macrophages may resist this response. Reduce inflammatory phenotype of monocytes by reducing the expression of ICAM-1.</td>
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<td>Similar anti-inflammatory effects on monocytes. May reduce macrophage–driven inflammation in various disease settings. PKA via CREB-mediated effects typically increases IL-10 and reduces TNFα in macrophages/monocytes, yielding anti-inflammatory responses. LABAs block LPS-driven lethality in mice, likely via IL-10 enhancing effects in macrophages.</td>
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<td></td>
<td>Such effects can be bidirectional in monocytes: in some studies, EPAC1 may have pro-inflammatory effects. β2-AR activation blunts LPS-induced activation of macrophages and inflammatory secretion (TNFα, IL-6, CCL2-4).</td>
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<tr>
<td></td>
<td></td>
<td>Promotion of phenotype switching from M1 to M2 macrophages reduces inflammation, including with in vivo exposure to LPS.</td>
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<tr>
<td></td>
<td></td>
<td>In some settings/cell lines, β2-AR activation may have proinflammatory effects, perhaps via β-arrestin-2.</td>
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(continued)
related to Th cell differentiation, especially at therapeutically relevant LABA concentrations, is not yet possible. Most findings support the idea of an overall reduction in T-cell priming and antigen presentation by DCs, with the broadly suppressive effects of cAMP on DCs used by regulatory T (T reg) cells to control DC function. Whether the effects of LABAs on DCs are beneficial during acute inflammation accompany- ing pulmonary infection is likely influenced by the rel- ative role of hyperinflammation in driving pathobiology and of humoral and cell-based immune responses in resolving infection.

3. Neutrophils. β2-AR activation and cAMP signal- ing induce anti-inflammatory actions in neutrophils (Table 4). Such actions include inhibition of chemotaxis/inheritation of neutrophils, release of ROS, neutrophil extracellular trap (NET) formation, adhesion of neutrophils to endothelial and epithelial cells at sites of inflammation/injury, and regulation of pro- and anti- inflammatory factors. PDE4 inhibition decreases neutro- philic inflammation by increasing cAMP actions via PKA and EPAC. Inhibition of Ca2+ signaling, which promotes neutrophil activation, appears to be one mechanism of the anti-inflammatory effects of cAMP. EPAC1 may also play a role via mechanisms independent of Ca2+ signaling. NET formation, ROS production, and neutrophil infiltration/actions may be major contributors to pulmonary hyperinflammation in infec- tions that include influenza (Narasaraju et al., 2011) and SARS-CoV-2/COVID-19 (Barnes et al., 2020). Reduced neutrophil activation, NETosis, and ROS production are associated with lower neutrophil apoptosis (Brostjan and Oehler, 2020); hence antiapoptotic responses are observed with β2-AR activation/cAMP signaling. Clearance of apoptotic neutrophils by macro- phages is enhanced by cAMP. Some evidence suggests that neutrophils and macrophages can produce cAMP/PKA signaling, via IL-10 and IL-4, increases M1 to M2 switching and ERK1/2-mediated recruitment of anti-inflammatory macrophages. cAMP signaling promotes a proresolving phenotype during inflammation, including in vivo models (exposure to LPS). Similar anti-inflammatory effects noted for EPAC signaling, including reduced IFN-β, CCL3/4, and GSK3β inhibition. cAMP/PKA enhances efferocytosis of apoptotic cells by pro-resolving macrophages, thereby decreasing inflammation. β2-AR activation has an anti-inflammatory effect in macrophages (including via PAMPs) via a broad-based suppression of TLR signaling. Mice deficient in β2-ARs in macrophages have reduced IL-10, elevated TNFα, and more susceptibility to LPS-induced injury. M2 macrophages reportedly can synthesize catecholamines, which can regulate inflammation in an autocrine and paracrine manner. Numerous studies (reviewed in Barnes et al., 2015) report catecholamine synthesis by macrophages, including with exposure to LPS. Other studies are unable to replicate such results, in particular in macrophages involved in adipose thermogenesis.

4. Natural Killer Cells. Data for β2-AR function in natural killer (NK) cells are complex and somewhat contradictory, thus requiring further study. Initial work indicated that β2-AR activation is likely inhibitory, reducing NK cell cytotoxicity, adhesion, and migration (Marino and Cosentino, 2013), effects that may contribute to immune suppression/evasion in tumors (Scanzano and Cosentino, 2015). Similar effects were also noted with PGE2-mediated increases in cAMP (Kalinski, 2012). However, other findings indicate an enhancement of migration and killing with β2-AR activation in rodents, including in certain tumor models (Scanzano and Cosentino, 2015; Qiao et al., 2018). Recent data from viral infection models in rodents show contrasting effects of β2-AR signaling on NK cell function (Sharma and Farrar, 2020). β2-ARs in other types of innate lymphoid cells, in particular group 2 innate lymphoid cells, may also exert anti- inflammatory and antiproliferative effects and have been shown to mitigate pulmonary and intestinal inflammation in mice (Moriyama et al., 2018).

5. Other Granulocytes: Mast Cells, Basophils, and Eosinophils. In addition to the sources noted above (Table 5), an earlier review (Weston and Peachell, 1998) discussed work that assessed effects of cAMP in mast cells and basophils, showing inhibition by β2-AR activation/cAMP in both cell types. The overall find- ings are that β2-AR activation likely induces anti-
TABLE 3

<table>
<thead>
<tr>
<th>Source</th>
<th>Stimulus</th>
<th>Phenoype</th>
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<tbody>
<tr>
<td>Marino and Cosentino,</td>
<td>/2-AR agonists/cAMP</td>
<td>Reduce the induction of Th1 and increase the induction of Th2 and Th17</td>
</tr>
<tr>
<td>2013; Scanzano and</td>
<td></td>
<td>differentiation by DCs, promoted by increased IL-33 released from DCs,</td>
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<tr>
<td>Cosentino, 2015; Agać</td>
<td></td>
<td>although proinflammatory effects of IL-33 on DCs themselves are blunted</td>
</tr>
<tr>
<td>et al., 2018</td>
<td></td>
<td>by /2-AR agonists (Helbig et al., 2020).</td>
</tr>
<tr>
<td>Theron et al., 2013</td>
<td>/2-AR agonists/cAMP</td>
<td>cAMP/PKA/CREB increase IL-10 in DCs, exerting anti-inflammatory effects.</td>
</tr>
<tr>
<td>Kolmus et al., 2015</td>
<td>/2-AR–mediated inhibition of NF-κB</td>
<td>Decrease LPS-induced activation of DCs (by blunting NF-κB signaling);</td>
</tr>
<tr>
<td></td>
<td>signaling</td>
<td>reduce release of IL-1/β, TNFα, IL-6, and IL-12 but increase IL-10</td>
</tr>
<tr>
<td>Padro and Sanders, 2014</td>
<td>Nonselective adrenergic and /2-AR–selective agonists</td>
<td>/2-AR activation promotes DC chemotaxis and motility but overall reduces DC antigen processing and presentation, reduces production of IL-1, IL-6, IL-12, and TNFα, and inhibits TLR, NF-κB, and MAPK-driven inflammation.</td>
</tr>
<tr>
<td>Raker et al., 2016</td>
<td>cAMP/PDE inhibitors</td>
<td>Promote differentiation of T helper cells to both Th2 and Th17 lineages</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(reducing the Th1 population).</td>
</tr>
<tr>
<td>Rueda et al., 2016</td>
<td>cAMP signaling in DCs, induced by T reg cells</td>
<td>T reg cells communicate with DCs via transfer of cAMP itself plus release of adenosine, which elevates cAMP in DCs via adenosine receptors.</td>
</tr>
<tr>
<td>Arumugham et al., 2017</td>
<td>cAMP, PKA, and EPAC</td>
<td>Increase in cAMP in DCs decreases their surface expression of co-stimulatory molecules and increases expression of inhibitory molecules.</td>
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<td>Increased cAMP enhances DC migration to T reg cells, separating DCs from other T-cell populations by reducing DC–T-cell interactions.</td>
</tr>
<tr>
<td>Wu et al., 2018</td>
<td>/2-AR agonists</td>
<td>Suppress of DC activity by cAMP is primarily mediated by EPAC and blunted by PDE4.</td>
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<tr>
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<td></td>
<td>Increased cAMP/PDE4 inhibition reduces polarization to Th1, promoting differentiation to the Th17 lineage.</td>
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<tr>
<td></td>
<td></td>
<td>cAMP has different effects at different stages of DC maturation (e.g., PGE2 stimulates DC maturation in immature DCs but inhibits mature DCs).</td>
</tr>
<tr>
<td>Chinn and Insel, 2020</td>
<td>cAMP, PKA, and EPAC</td>
<td>T reg cells produce adenosine, which increases DC migration via EPAC1 signaling, facilitating DC–T reg cell interactions.</td>
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<tr>
<td></td>
<td></td>
<td>Reduce differentiation of monocytes to DCs.</td>
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<td></td>
<td>Reduced expression of CD86 and MHC-II, decreasing release of proinflammatory factors in interacting T cells.</td>
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<td></td>
<td>Enhance migration of DCs to lymphoid tissue but decrease antigen presentation to CD 8+ (cytotoxic) T cells.</td>
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<td></td>
<td>In presence of LPS, /2-AR agonists reduce IL-12/IL-23 ratio, promoting differentiation of T helper (CD 4+) cells, in particular to the Th17 lineage.</td>
</tr>
<tr>
<td>Tavares et al., 2020</td>
<td>cAMP, PKA, and EPAC</td>
<td>Lack of cAMP (from deficiency of Gαs in mice) in DCs is associated with increase in allergic inflammation and pulmonary pathology that resembles asthma, suggesting that increase in cAMP (via PKA) in DCs is a potential means to treat allergic inflammation.</td>
</tr>
<tr>
<td>Sharma and Farrar, 2020</td>
<td>/2-ARs</td>
<td>Anti-inflammatory effects attributed to EPAC signaling, including reduced IFN-β, IL-10, and IL-6, and TNFα.</td>
</tr>
</tbody>
</table>

CCL, C-C motif chemokine ligand; GSK3β, Glycogen Synthase Kinase 3 Beta; IFN-β, interferon-β; MHC-II, major histocompatibility complex II; TLR, Toll-like receptor.

inflammatory effects in granulocytes, reducing production of proinflammatory factors, adhesion to cell surfaces and ECM, infiltration into inflammatory sites, and degranulation.

B. Adaptive Immune Cells

1. T Cells. Most studies of T cells (Table 6) focus on effects of cAMP and its signaling with fewer studies of β2-ARs. Increases in cAMP suppress T-cell inflammatory action and have complex effects on T-cell differentiation and subsequent immune response. In addition to β2-ARs, Gs-coupled GPCRs in T cells include adenosine (A2A, A2B) and PGE2 (EP2/EP4) receptors. T cells can release ligands that activate these receptors, thereby providing autocrine/paracrine communication that may modulate T-cell activation. Most evidence indicates that increases in cAMP promote differentiation of T reg cells and their subsequent suppressive effects on other T-cell populations and DCs. Increases in cAMP also promote the differentiation of CD4+ T cells toward...
have been emphasized as protective in ALI/ARDS and can contribute to further inflammation. T reg cells with increased tissue injury (Hufford et al., 2015) and whose production is reduced by cAMP (e.g., TNF)

In addition, proinflammatory factors released by T cells can further contribute to the disease by interfering with an effective adaptive immune response, protecting T cells from apoptosis and promoting T-cell retention in tissues. Moreover, hyperinflammation may protect T cells from apoptosis and promote T-cell retention in tissues. The net effect of cAMP on T-cell–mediated immune response to infection is difficult to define, perhaps because of T-cell subpopulations. Although cAMP has immunosuppressive actions, increases in cAMP may protect T cells from apoptosis and promote T-cell retention in tissues. Moreover, hyperinflammation interferes with an effective adaptive immune response, a feature of SARS-1 and COVID-19 disease (Chpanannavar and Perlman, 2017; Sriram and Insel, 2020a). In addition, proinflammatory factors released by T cells and whose production is reduced by cAMP (e.g., TNF, macrophage inflammatory protein-1x) are associated with increased tissue injury (Hufford et al., 2015) and can contribute to further inflammation. T reg cells have been emphasized as protective in ALI/ARDS and subsequent cytokine storm (Lin et al., 2018). Conversely, severe COVID-19 (in which hyperinflammation and subsequent ALI/ARDS and cytokine storm can occur) results in depletion of forkhead box P3 (FOXP3)–expressing T reg cell populations and hyperactivation of other CD4+ lineages, including Th1 cells (Zhou et al., 2020; Yang et al., 2020; Kalfaoglu et al., 2020; Stephen-Victor et al., 2020). Transfer of T reg cell populations to subjects with inflammatory diseases is a topic of active study (Romano et al., 2019; Stephen-Victor et al., 2020), including the administration of T reg cells to treat ARDS in patients with COVID-19 (Gladstone et al., 2020).

2. B Cells. Relative to other immune cell types, the literature is sparse regarding effects of β2-AR/cAMP signaling in B cells (Table 7). However, certain reviews (e.g., Sanders, 2012) provide insight into such effects. Increases in cAMP levels tend to suppress most immune cell types, but signaling via PKA can increase antigen-induced antibody production. Moreover,
increased B-cell retention in lymph nodes appears to enhance response to antigens and antibody production. In tandem with the Th2-promoting effects of β2-AR/cAMP on DCs and naïve CD4+ T cells discussed above, the net effect of β2-AR/cAMP appears to be enhancement of humoral immune response via complementary actions of the three cell types. B cells produce IL-10 in response to β2-AR/cAMP stimulation, consistent with the response in innate immune cells (and possibly T cells), pointing to a modulation of inflammation that accompanies enhancement in humoral response. Inflammation can increase B-cell populations in pulmonary lymphoid tissue (Moyron-Quiroz et al., 2007). It is unclear whether therapeutic doses of inhaled LABAs enhance antibody production by B cells in vivo in humans—including B cells in lymphoid tissue in lungs.

**IX. Do Inhaled Long-Acting β-Adrenergic Agonists Reduce Pulmonary Inflammation in Patients?**

Preclinical studies of the effects of β2-AR/cAMP in immune cells reviewed above indicate that β2-AR agonists exert multiple anti-inflammatory effects on both innate and adaptive immune cell types. Consistent with those observations, numerous studies have examined the impact of inhaled LABAs on inflammatory markers in patients (primarily those with COPD or asthma). Anti-inflammatory effects have been observed, as discussed in examples below.

Bronchial biopsies and sputum from patients with COPD treated with LABA/ICS had a decrease in the number of neutrophils, eosinophils (Bathoorn et al., 2009), CD45+ cells (leukocytes), CD4+ T cells, and TNFα- and interferon-γ-expressing cells, implying multiple anti-inflammatory effects in vivo (Barnes et al., 2006). Moreover, bronchial biopsies from patients with COPD indicated that LABA/ICS, but not ICS treatment alone, reduced the numbers of infiltrating macrophages and CD8+ T cells (Bourbeau et al., 2007). Inhaled LABAs/ICS can reduce inflammatory cell number in sputum while increasing the proportion of circulating FOXP3+ T reg cells among CD4+ T cells (Yang et al., 2011). Use of inhaled LABA by patients with COPD has been associated with reduced ROS production in peripheral blood neutrophils (Santus et al., 2012). Similar effects were noted in circulating lymphocytes: administration of inhaled LABA/ICS acutely reduced inflammatory (LPS) response of lymphocytes isolated from patients with asthma (Rudiger et al., 2013), consistent with ex vivo preclinical data that showed that LABAs blunt LPS-mediated inflammation. These results agree with other data that show inhalation of LPS increased proinflammatory factors (IL-6, IL-8, TNFα, etc.) in bronchiolar lavage fluid, along with neutrophil influx, protein leakage, and macrophage activation, effects suppressed in subjects pretreated with an inhaled LABA (Maris et al., 2005). Administration of LABA/ICS in patients with COPD exacerbation was associated with reduced levels of proinflammatory IL-8 and increased anti-inflammatory IL-10 in sputum (Feng et al., 2013), findings akin to preclinical data showing that LABAs increase IL-10.

**TABLE 5**

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<thead>
<tr>
<th>Source</th>
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<th>Phenotype</th>
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<tbody>
<tr>
<td>Theron et al., 2013</td>
<td>β2-ARs</td>
<td>β2-ARs on MCs reduce histamine release, TNFα, GM-CSF, MIP-1, and other pro-inflammatory factors. Histamine, IL-4, and IL-13 release/production are reduced in basophils. β2-AR activation reduces histamine release from MCs, including in the lung and in circulation, promoting MC stabilization (elaborated in Kay and Peachell, 2005). Response of MCs to antigens, including proliferation, adhesion, and release of inflammatory factors, is also reduced. Similarly, β2-AR activation reduces production of inflammatory factors, adhesion, and recruitment of eosinophils. cAMP/PKA signaling reduces RhoA-mediated adhesion and migration of granulocytes, blunting recruitment and contribution to inflammation. PDE4 inhibitors exert similar inhibitory effects on granulocyte-driven inflammation and degranulation. Elevation of cAMP/PDE inhibition increased apoptosis and clearing/effecytosis of granulocytes by macrophages, reducing inflammation.</td>
</tr>
<tr>
<td>Scanzano and Cosentino, 2015</td>
<td>β2-ARs</td>
<td></td>
</tr>
<tr>
<td>Tavares et al., 2020</td>
<td>cAMP</td>
<td></td>
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MC, mast cell; MIP-1, macrophage inflammatory protein-1.
**TABLE 6**

Review articles (listed chronologically) that summarize effects of β2-AR/cAMP signaling on T cells.

<table>
<thead>
<tr>
<th>Source</th>
<th>Stimulus</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>Mosenden and Taskén, 2011</td>
<td>cAMP</td>
<td>Increased cAMP can increase Th2-related (e.g., IL-4, IL-10) while reducing Th1-related (e.g., IL-12, TNFα) cytokines in naive CD4+ T cells. cAMP enhances expression of GPR83, a novel GPCR whose upregulation drives differentiation of FOXP3-expressing T reg cells. Under basal conditions, T eff cells contain little cAMP; T reg cells (which are rich in cAMP) provide cAMP stimulation to suppress T eff cell function. Elevation of cAMP via Gs-GPCR agonists or PDE inhibition reduces T cell–induced inflammation via PKA-Csk signaling.</td>
</tr>
<tr>
<td>Bodor et al., 2012</td>
<td>cAMP</td>
<td>FOXP3 regulates cAMP concentrations in T reg cells by reducing expression of miR-142-3p, which suppresses AC activity. T reg cells suppress functions of other CD4+ T cells via cAMP-mediated activation of ICER, which inhibits NFAT transcriptional activity, IL-2 expression, and activation of T eff cells. Activation of ICER by cAMP induces CD4+ Th1 cells to adopt a more T reg-like phenotype, suppressing Th1 functions.</td>
</tr>
<tr>
<td>Wah et al., 2018</td>
<td>cAMP</td>
<td>Regulation of cAMP via PKA and EPAC sustains increases in cAMP reduce T-cell activation, chemotaxis, and cytokine secretion.</td>
</tr>
<tr>
<td>Rauen et al., 2013</td>
<td>cAMP actions, mediated by CREM/ICER</td>
<td>Effects from CREM/ICER regulation of transcription are suppressive of T-cell activation, expression of IL-2, Foxp3, MIP-1β, AP-1, etc.</td>
</tr>
<tr>
<td>Rueda et al., 2016</td>
<td>cAMP, PKA, and EPAC</td>
<td>T reg cells suppress activation, proliferation, and cytokine production of other T-cell populations by direct transfer of cAMP via gap junctions, plus generation of adenosine, driven by CD39 and CD73, subsequently activating Gs-coupled adenosine receptors on T cells.</td>
</tr>
<tr>
<td>Padro and Sanders, 2014</td>
<td>Nonselective adrenergic and β2-AR–selective agonists</td>
<td>Th2/Th17 lineages, and contributes to immune suppression in tumors.</td>
</tr>
<tr>
<td>Li et al., 2018</td>
<td>Regulation of cAMP signaling by PDEs</td>
<td>PDE4 inhibitors/cAMP have anti-inflammatory effects on T helper cell subtypes, reducing secretion of major cytokines associated with each lineage, by inhibiting TCR signaling via increased cAMP concentration.</td>
</tr>
<tr>
<td>Wu et al., 2018</td>
<td>β2-ARs</td>
<td>β2-AR expression in CD8+ T cells is greater than in CD4+ T cells and results in greater suppression of IFN-γ in CD8+ than in CD4+ T cells. Anti-inflammatory/suppressive effects of β2-ARs are primarily via suppression of secreted factors, e.g., GM-CSF, IFN-γ, and IL-3.</td>
</tr>
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(continued)
TABLE 6—Continued

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<tr>
<th>Source</th>
<th>Stimulus</th>
<th>Phenotype</th>
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</thead>
<tbody>
<tr>
<td>Sharma and Farrar, 2020</td>
<td>β2-ARs</td>
<td>CD8+ T eff cells and memory T cells and CD4+ Th1 cells express β2-AR; Th2 cells have lower expression. β2-AR expression increases with inflammation, including by proinflammatory cytokines (e.g., IL-2 and IL-12). β2-AR agonists reduce release of IFN-γ and TNF-α from Th1 cells and from the CD8+ population that has less cytolytic activity. β2-AR activation increases T reg cell differentiation in CD4+ T cells, thereby regulating T-cell–mediated inflammation.</td>
</tr>
</tbody>
</table>

AKAP, A-kinase anchoring protein; AP-1, activator protein 1; APC, antigen-presenting cell; COX-2, cyclooxygenase-2; CREM, cAMP responsive element modulator; Csk, C-Terminal Src Kinase; GPR83, G Protein-Coupled Receptor 83; ICER, Inducible cAMP early repressor; IFN, interferon; LCK, LCK Proto-Oncogene; MIP-1α, macrophage inflammatory protein-1α; NFAT, Nuclear Factor of Activated T cell; RAP1, Ras-related protein 1; T eff, T effector; TCR, T cell receptor.

Numerous clinical trials, especially with patients with COPD, have tested if LABA monotherapy or in combination with ICS or LAMAs increase the frequency of respiratory infections, perhaps as a consequence of chronic treatment being immunosuppression in the lungs. Based on concerns regarding fatal exacerbations in patients with asthma administered LABA monotherapy, LABAs are typically only administered in combination with other drugs (e.g., ICS) (Cloutier et al., 2020; Global Initiative for Asthma, 2020). Limited information is available regarding whether LABAs alone increase the risk of respiratory infections in patients with asthma. In the text below and Table 8, we discuss recent RCTs and meta-analyses that have focused on patients with COPD.

Therapeutic approaches in such RCTs include inhaled LABAs, ICS, and LAMAs as monotherapies, as well as combinations (including LABA/LABA/LAMA, triple therapy). Table 8 lists selected RCTs (in particular, large multicenter RCTs, notably the TORCH (Calverley et al., 2007) and SUMMIT trials (Vestbo et al., 2016]) and recent meta-analyses. The overall conclusion is that LABA treatment as monotherapy or combined with LAMAs does not increase the risk of respiratory infections. Moreover, increasing LABA dose does not correlate with an increase in risk of infections. By contrast, prolonged ICS therapy appears to increase the risk of pneumonia, both when used alone or in combination with LABAs, although the SUMMIT trial suggests no increase in pneumonia by ICS treatment (Vestbo et al., 2016). Meta-analyses in the Cochrane Database of Systematic Reviews have considered the risk of serious AEs in COPD and have compared LABAs with placebo (Kew et al., 2013) and LABA/LAMA to placebo (Maqsood et al., 2019). Neither study found an increase in serious AEs of LABAs versus placebo; pneumonia risk was not quantified separately, but the studies included this risk among the serious AEs. A meta-analysis of 20 RCTs reached a similar conclusion: no risk of infections with LABA monotherapy in COPD and minimal risk of serious AEs (Decramer et al., 2013). Although a theoretical concern exists regarding the immunosuppressive effects of inhaled LABAs, the findings in patients with COPD appear to allay these concerns.

Pathologic Pulmonary Immune Suppression in Patients?

As described above, β2-AR agonists suppress a range of immune cell types but in some cases promote immune response (e.g., adaptive responses), such as in B cells. An important concern regarding the potential utility of inhaled LABAs in treating/preventing ARDS is whether such treatment might worsen infections and/or increase the risk of secondary infections, thereby enhancing disease severity. This concern is especially relevant, given preclinical data discussed above, indicating that LABAs reduce bacterial phagocytosis by various immune cell types.
concerns. Perhaps such results derive from immune-enhancing effects of LABAs discussed above or from their prevention of excessive inflammation, which helps protect immune response while also promoting epithelial barrier integrity. Hence, available evidence implies that inhaled LABAs are unlikely to meaningfully compromise immune response to infection in the setting of acute infection–induced ALI/ARDS. However, this may not be the case if inhaled LABAs are combined with ICS in such settings, especially once seroconversion begins. The examples discussed above in which addition of ICS is associated with increased risk of pneumonia involve chronic exposure to ICS. Data for acute administration of ICS/LABA combinations in ARDS do not indicate increased risk of pneumonia (e.g., Mangi et al., 2015; Festic et al., 2017). Of note, emerging evidence from trials in patients with COVID-19 indicates the benefit of early ICS administration in preventing severe disease (Ramakrishnan et al., 2021; the STOIC trial), supporting the hypothesis that combinations of inhaled medications that suppress inflammation may protect patients from progressive pulmonary injury. Further support for this hypothesis is provided by interim results from the PRINCIPILE trial (Lee2021), which is testing the effects of adding ICS to the standard of care for patients with COVID-19 with risk factors for developing severe disease. ICS had a significant improvement in the time to recovery of these patients and also indicated the likelihood of reduced mortality and rates of admission to ICUs.

### XI. Platelets

Elevation of cAMP inhibits platelet activation, aggregation, adhesion, and proliferation. These responses from increased cAMP occur via PKA, inhibition of Ca2+, and RhoA signaling (Noé et al., 2010; Aburima et al., 2013; Fuentes and Palomo, 2014; Nagy and Smolenksi, 2018). Gs-coupled GPCRs thus inhibit platelet activation, whereas Gq/11-coupled GPCRs (which increase Ca2+) and G12/13-coupled GPCRs (which promote RhoA signaling), and Gi-coupled GPCRs (which lower cAMP) promote platelet activation and coagulation. Inhibitors of G- and Gq-coupled GPCRs [e.g., protease-activated (thrombin) receptors and P2Y12 purinergic (ADP) receptors] are widely prescribed as antiplatelet drugs (Sriram and Insel, 2021). PDE inhibition has also been studied as a means to reduce platelet activation; platelets highly express PDE3, which is cAMP-selective (Fuentes and Palomo, 2014). Raising platelet cAMP via
agonists for Gs-coupled A2A and A2B receptors has also been suggested (Wolska and Rozalski, 2019).

Older studies reported that $\beta_2$-AR activation inhibits platelet aggregation (Woulfe, 2005; Anfossi et al., 1996). Study of $\beta_2$-ARs in platelets has been sparse in recent years, perhaps because epinephrine promotes platelet activation via $\zeta$2a-ARs, a G-coupled GPCR that is the more prominently expressed AR in platelets (Motulsky and Insel, 1982; Woulfe, 2005; Anfossi et al., 1996).

$\beta_2$-ARs were identified on human platelet membranes using radioligand ([125I]-iodopindolol) binding, together with $\beta_1$-AR– and $\beta_2$-AR–selective antagonists (Wang and Brodde, 1985). Isoproterenol, likely via $\beta_2$-ARs, can increase cAMP and blunt aggregation of human and rat platelets (Yu and Latour, 1977; Perry and Scruutton, 1983; Winther et al., 1985; Andersson and Vinge, 1991). Isoprotrenol inhibits ADP-induced aggregation of platelets from healthy and diabetic rats (Yu and Latour, 1977; Austin et al., 1995) and thrombin-induced release of serotonin from rat platelets (Koutouzov et al., 1985). Subsequent work demonstrated that activation of $\beta_2$-ARs in human platelets enhances NOS activity (NO production); $\beta_2$-ARs inhibit thrombin-induced platelet adhesion to endothelial cells in a platelet-expressed, NOS-dependent manner; $\beta_2$-ARs also reduced thrombin-mediated platelet aggregation in a NOS-independent manner (Queen et al., 2000). Human subjects acutely given systemic doses of $\beta_2$-AR agonists had reduced platelet aggregation (Larsson et al., 1992) and platelet number (Fredén et al., 1978). Recent data indicate disruption of platelet indices in patients with COPD: reduced mean platelet width and volume, interpreted as markers of thromboinflammation, associated with increased platelet function at sites of inflammation (Hlapčić et al., 2020). LABA/ICS therapy appears to restore these platelet indices, although whether this is an action on platelets or an indirect anti-inflammatory effect requires further study.

The regulation of platelets by $\beta$-AR agonists is a potentially overlooked effect of inhaled LABAs, i.e., on platelets in the lung, especially in capillaries in the vicinity of alveoli, that merits further study. Patients with IPF show improvements in markers of coagulation and platelet activation after treatment with inhaled LABAs/ICS (Wright et al., 2017). Cross-talk between signaling pathways associated with inflammation may promote coagulation and pulmonary thromboinflammation in certain viral infections (Sriram and Insel, 2020b, Sriram and Insel, 2021). If LABAs exert anti-inflammatory effects in the lung, those agents may reduce platelet activation and help explain the findings in patients with IPF. Existing evidence thus provides a basis to suggest that LABAs may have inhibitory effects on platelet aggregation, but this idea requires additional study.

**XII. Summary and Conclusions**

Figure 7 summarizes the effect of $\beta_2$-AR activation/ cAMP on a range of cell types involved in ARDS pathobiology (Fig. 4). In this review, we identify
evidence (from experimental ex vivo and in vivo studies plus clinical data) in support of potential efficacy of β2-AR agonists, in particular LABAs, and perhaps other agents that increase cAMP, e.g., other Gs-coupled GPCR agonists (Sun and Ye, 2012; Wendell et al., 2020), and PDE [e.g., PDE4] inhibitors (Philips, 2020; Giorgi et al., 2020), in the setting of ALI/ARDS. Beneficial effects of β2-AR agonists/cAMP include protection of epithelia (including in AECs) and reductions in profibrotic response of lung FBs, in inflammatory responses in multiple cell types, activation of innate immune cells (with possible potentiation of adaptive immune responses), and EC injury (accompanied by increased endothelial repair). In addition, some evidence implies a reduction in platelet activation. Thus, thromboinflammation and accompanying tissue injury—characteristics of ARDS—are likely blunted by β2-AR agonists/cAMP-elevating agents. Key advantages of β2-AR agonists include the expression of β2-ARs on multiple cell types that participate in ARDS pathology and the widespread use of inhaled LABAs as approved therapies, thus offering the opportunity to repurpose such agonists.

β2-AR agonists (especially inhaled LABAs) can reduce pulmonary inflammation apparently without harmful immune suppression. Inhaled LABAs are widely used as monotherapies in treating COPD with few serious AEs, but safety concerns, especially regarding LABA monotherapy in asthma, led to the use of combination therapies with ICS and/or LAMAs. What are the implications with respect to LABAs in ARDS? The safety risks associated with LABA use in asthma are largely skewed toward fatal exacerbations in juvenile patients (McMahon et al., 2011; Cates et al., 2012; Kersten et al., 2017). By contrast, serious viral infections, e.g., influenza and SARS coronaviruses, that can result in ARDS occur more commonly in older subjects.

The evidence we have discussed suggests that inhaled LABA monotherapy and/or combination therapies should be tested in settings of infections associated with ARDS. Such an approach would be especially appropriate for adults, especially those over age 60, who have higher mortality with infections that can lead to ARDS. LABAs in combination with ICS and/or LAMAs might be assessed for their ability to prevent disease progression and improve the outcome of patients with ARDS.

Importantly, we interpret the available data on action of LABAs on various cell types, combined with current understanding of the pathobiology of ARDS, as providing a strong rationale to administer LABAs early in the course of the disease. Such an approach will likely maximize the probability that beneficial effects—such as restoration of AFC, prevention of epithelial and endothelial injury, inhibition of fibroproliferative effects, and suppression of hyperinflammation—can occur before injury reaches an irreversible stage. Early treatment with inhaled LABAs is thus a potential

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**Fig. 7.** Based on the framework for cell-cell interactions and cell-based effects that drive alveolar injury presented in Figure 4, the effects of β2-AR agonists (mediated by cAMP) on the same cell types that are involved in ARDS/ALI pathobiology.
prophyaxis against severe lung injury. Limited evidence in recent studies supports this hypothesis (Festic et al., 2017; Fouad et al., 2020) and contrasts with previous trials [e.g., the BALTI trials (Perkins et al., 2006; Gao Smith et al., 2012; Gates et al., 2013)] in which treatment was initiated in patients with ARDS who were already in intensive care. A prophylactic strategy that focuses on early treatment with LABAs may offer a potential to improve the currently poor outcomes and high mortality of patients with ARDS. This approach includes treatment of COVID-19, where disease progression to ARDS is characterized by the development of hyperinflammation and thromboinflammation (Sriram and Insel, 2021). Those features may be slowed/prevented by early treatment with LABAs, potentially in combination with ICS or LAMAs. Indeed, initial data from clinical trials suggests the utility of ICS treatment in patients with COVID-19, including use early in the disease (Ramakrishnan et al., 2021; Lee, 2021).

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References


