Targeting Insulin-Like Growth Factor-I and Insulin-Like Growth Factor–Binding Protein-3 Signaling Pathways

A Novel Therapeutic Approach for Asthma

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Abstract

Insulin-like growth factor (IGF)-I has been recognized to play critical roles in the pathogenesis of asthma, whereas IGF-binding protein (IGFBP)-3 blocks crucial physiologic manifestations of asthma. IGF-I enhances subepithelial fibrosis, airway inflammation, airway hyperresponsiveness, and airway smooth muscle hyperplasia by interacting with various inflammatory mediators and complex signaling pathways, such as intercellular adhesion molecule-1, and the hypoxia-inducible factor/vascular endothelial growth factor axis. On the other hand, IGFBP-3 decreases airway inflammation and airway hyperresponsiveness through IGFBP-3 receptor–mediated activation of caspases, which subsequently inhibits NF-κB signaling pathway. It also inhibits the IGF-I/hypoxia-inducible factor/vascular endothelial growth factor axis via IGF-I–dependent and/or IGF-I–independent mechanisms. This Translational Review summarizes the role of IGF-I and IGFBP-3 in the context of allergic airway disease, and discusses the therapeutic potential of various strategies targeting the IGF-I and IGFBP-3 signaling pathways for the management of asthma.

Keywords: insulin-like growth factor-I; insulin-like growth factor–binding protein-3; asthma

Current controllers for treating asthma are highly effective in approximately 90–95% of patients, but between roughly 5 and 10% of patients remain poorly controlled and account for approximately 50% of the health care costs of asthma (1). These patients poorly controlled on current pharmacologic agents are characterized by: (*1*) requirement of intensive treatment to control the disease; and (*2*) persistent symptoms, exacerbations, and airflow obstruction, which are collectively known as severe or refractory asthma (2). Furthermore, current treatment is far from a cure, as symptoms reappear when treatment is discontinued, and because it has little effect on inhibiting airway remodeling. Thus, new therapeutic approaches are necessary.

A focus of drug development for asthma has been to improve currently available drugs and to find novel compounds, often targeting T helper (Th) 2–driven airway inflammation (3). Although several agents targeting Th2-driven airway inflammation have been developed, only omalizumab has been marketed as a specific, targeted biological agent. Moreover, the efficacy and indication of omalizumab may be limited to IgE-related severe asthma. Therefore, there is a need for development of other agents modulating heterogeneous asthmatic features.

Fortunately, many new therapeutic approaches for the management of asthma have been under investigation. Among them, insulin-like growth factor I (IGF-I) has been reported as one of the key molecules in the pathogenesis of asthma. In fact, IGF-I has been reported to play important roles, especially in subepithelial fibrosis, airway inflammation, airway hyperresponsiveness (AHR), and airway smooth hyperplasia (Figure 1). Thus, regulation of the IGF-I signaling pathway might have therapeutic potential (4–6). On the other hand, recent studies have also shown that IGF-binding protein (IGFBP)-3 plays a critical role in inflammatory responses through

(Received in original form September 16, 2013; accepted in final form November 5, 2013)

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This work was supported by Korea Healthcare Technology R&D Project, Ministry for Health and Welfare, Republic of Korea grants A121931 (Y.C.L.) and A111992 (S.R.K.), and by the funds of the Biomedical Research Institute, Chonbuk National University Hospital.

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Am J Respir Cell Mol Biol Vol 50, Iss 4, pp 667–677, Apr 2014

Originally Published in Press as DOI: 10.1165/rcmb.2013-0397TR on November 12, 2013
Internet address: www.atsjournals.org

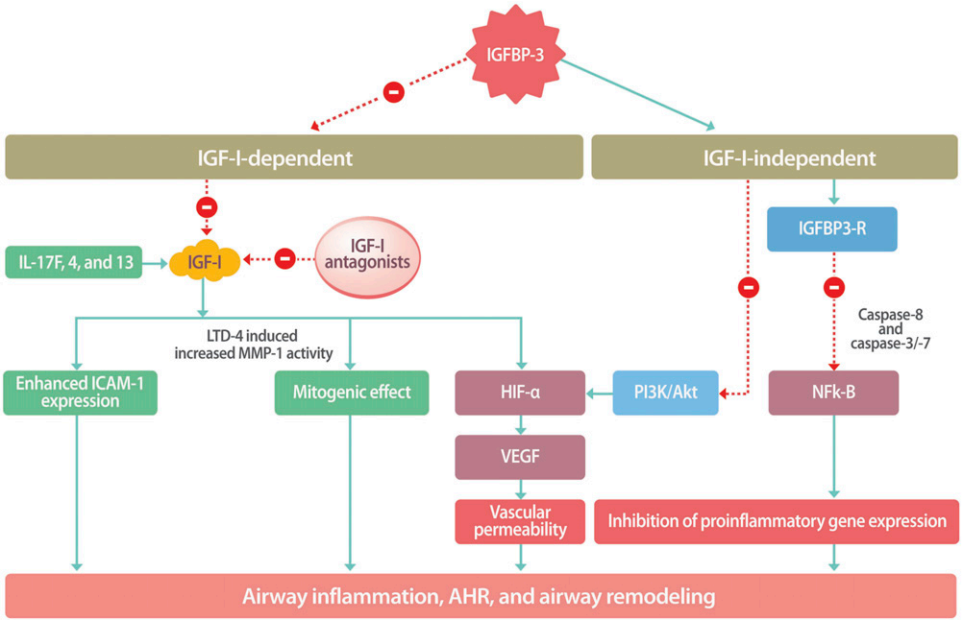

Figure 1. Roles of insulin-like growth factor (IGF)-I and IGF-binding protein (IGFBP)-3 in the pathogenesis of asthma. HIF, hypoxia-inducible factor; ICAM, intercellular adhesion molecule; PI3K, phosphoinositol-3 kinase; VEGF, vascular endothelial growth factor.

IGF-I–dependent and/or IGF-I–independent mechanisms (7–9).

In this *Review*, we discuss the roles of IGF-I and IGFBP-3 in airway inflammation, AHR, and airway remodeling of asthma, and scrutinize the therapeutic potential of targeting IGF-I and IGFBP-3 for bronchial asthma.

The IGF System

The IGF system has significant effects on cell growth and differentiation. The IGF system includes growth hormone (GH), IGF-I/IGF-II peptides, type I and II IGF receptors (IGF-IR and IGF-IIR), a family of IGFBPs (IGFBPs 1–6), and IGFBP proteases (10). Recently, an IGFBP-3–mediated novel cell death receptor (namely, IGFBP-3R) has been identified as a new member of the IGF system (11).

IGF-I and IGF-II Regulation

GH is the major inducer of IGF synthesis in the liver. GH is a polypeptide hormone that is synthesized and secreted by somatotrophs in the anterior pituitary. The stimulators of GH secretion are GH-releasing hormone, which is released from the hypothalamus (12), and ghrelin, which is released from the stomach (13). The inhibitors of GH secretion are IGF-I itself (14) and somatostatin (15). GH binding to the GH receptor in the liver stimulates IGF-I synthesis and release from the liver (14). The released IGF-I is then transported to the target organ through the circulation, and acts as an endocrine factor (14). IGF-I and IGF-II are small peptide hormones of roughly 7 kD molecular weight, and are composed of four domains: B, C, A, and D (sequentially from the N to the C terminus). The B and A domains of IGF-I and IGF-II have approximately 50% homology to the B and A chain of insulin (16). The C domain of IGF-I is shown to be required for high-affinity binding to IGF-IR (17). IGF-I and IGF-II contain eight and six amino acids in the D domain, respectively, and the amino acids form an extension of the carboxyl terminus (18).

There are two major mechanisms of IGF-I regulation (19). First, IGF-I is synthesized and secreted by the liver, acting as an extension of the GH axis in an endocrine manner. Second, IGF-I can be produced locally by many types of peripheral cells under basal conditions, as well as in response to inflammation. In this case, IGF-I acts as an autocrine or a paracrine factor like many cytokines and growth factors.

IGF-IR and IGF-IIR

IGF-IR is a transmembrane heterotetramer glycoprotein consisting of an α and a β subunit (20). The extracellular α subunit contains an IGF-binding domain, and the β subunit contains a tyrosine kinase domain (20). Thus, IGF-IR belongs to a family of transmembrane tyrosine kinase receptors, which also include the insulin receptor (IR) and IR-related receptor (21). There is roughly 60% homology between IGF-IR and IR (22). IGF-IR binds IGF-I, IGF-II, and insulin. However, the affinity of IGF-II and insulin for IGF-IR is much weaker than that of IGF-I (22).

Ligand binding to the α subunit of IGF-IR triggers conformational changes, leading to autoactivation of tyrosine kinase activity of the β subunit, followed by autophosphorylation in the kinase domains of the receptor (23). This process induces phosphorylation of the binding sites for docking proteins, such as IR substrates (IRSs) 1–4 and Src homology and collagen protein (24, 25). The phosphorylation of IRS-1 and Src homology and collagen protein leads to activation of adaptor protein Grb-2, which forms a complex with the Ras-activating protein son of sevenless. This complex leads to activation of p21 Ras (26), which then activates mitogen-activated protein kinase (27). The mitogen-activated protein kinase pathway is involved in cell growth.

IRS-1 can also activate phosphoinositol-3 kinase (PI3K), which generates

phosphatidyl inositol 1,4,5-trisphosphate by phosphorylating phosphatidyl inositol 4,5-bisphosphate (28). Binding of phosphatidyl inositol 1,4,5-trisphosphate to serine/threonine kinase Akt, which is also known as protein tyrosine kinase-B, leads to activation of mammalian target of rapamycin, p70/S6 kinase, and glycogen synthase kinase-3β (29). Thus, PI3K is involved in the protein synthesis, glucose transport, cell motility, and inhibition of apoptosis activated by IGF–IRS signaling.

IL-4 receptor (IL-4R) signaling is well known as an important participant in Th2 cellular responses in various inflammatory disorders, including bronchial asthma. Interestingly, the sequence of amino acids important for IRS binding to IL-4R was determined by truncation mutational analysis (between aa 437 and 557). Within this interval, there is a homologous sequence that binds IRS proteins in the insulin and IGF-IR, known as the insulin and IL-4R motif, and IRS-1 is also tyrosine phosphorylated in response to IL-4 stimulation, which is critical for association of IRS proteins with the insulin and IL-4R motif of the IL-4R (30–32). This content suggests that IGF-I can participate in the pathogenesis of bronchial asthma through the interaction with IL-4 signaling (Figure 2).

IGF-IIR, which is identical to the cation-independent mannose-6-phosphate receptor, binds IGF-II with 500-fold–higher affinity than IGF-I (33). Unlike IGF-IR, IGF-IIR does not bind insulin. IGF-II binding to IGF-IIR results in internalization and degradation of extracellular IGF-II, leading to the suppression of IGF-II effects (33).

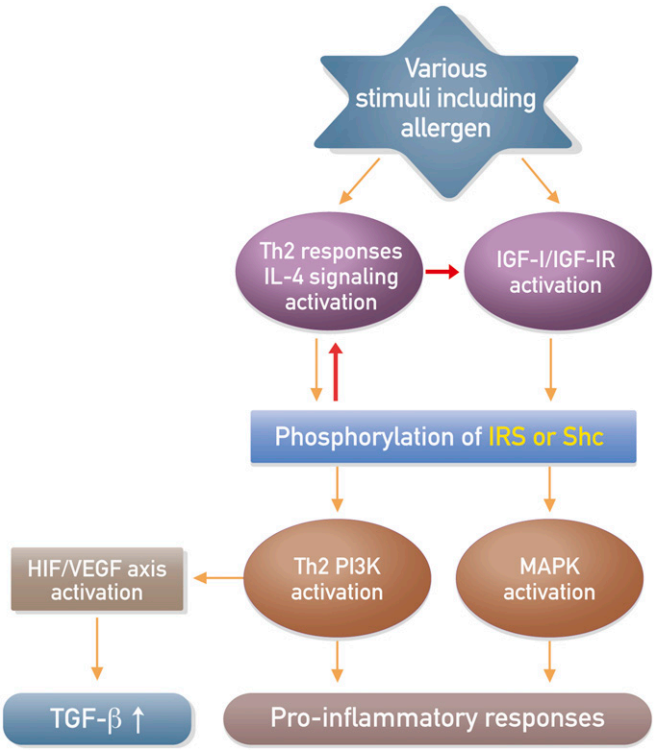

Figure 2. Schematic diagram for IGF-I and its related signaling pathways in bronchial asthma. IRS, insulin receptor substrate; Shc, Src homology and collagen protein; TGF, transforming growth factor; Th2, T helper type 2.

IGFBPs

The IGFBPs are composed of six high-affinity binding proteins (IGFBP-1 through IGFBP-6) (10). The IGFBPs transport IGFs in the bloodstream, regulate IGF action and bioavailability, and exhibit unique biological actions, including cell growth inhibition or promotion and induction of apoptosis. Among IGFBPs, IGFBP-3 is the most abundant form, with the highest affinity for IGF-I in the circulatory system, and which binds 75–90% of circulating IGF-I in a large ternary complex consisting of IGFBP-3, acid-labile subunit (ALS), and IGF-I. ALS prevents the ternary complex from crossing the capillary barrier to the extravascular compartment by stabilizing the structure (34). IGFBP-5 can also form a ternary complex with IGF-I and ALS (35). Other IGFBPs can carry IGF-I as binary forms. As a result, less than 1% of IGF-I circulates as a free form in the bloodstream (36).

If ALS dissociates from the ternary complex, the binary IGF-I/IGFBP complex crosses the capillary barrier. At local tissues, the IGFBPs are cleaved by IGFBP proteases, thereby releasing free IGFs from the binary complex and, thus, increasing free IGFs. Several IGFBP proteases, such as serine proteases, cathepsins, and matrix metalloproteinases (MMPs), have been discovered (37).

Biological functions of IGFBPs are divided into two aspects: those with IGF-dependent action, and those with IGF-independent action. First, IGFBPs function indirectly through modulation of IGFs (IGF-dependent action of IGFBPs). IGFBPs enhance the action of IGFs by forming a slow-releasing pool of IGFs (37). Conversely, IGFBPs can inhibit the actions of IGFs, because IGFs bind with higher affinity to IGFBPs than the IGF-IR, thereby reducing IGF bioavailability (38). Second, IGFBPs can also exert their own intrinsic biological roles, independent of IGFs (IGF-independent action of IGFBPs) (10, 39), including anti- (8, 9) or proinflammatory (7), anti- (40, 41) or proangiogenic (40, 42, 43), or profibrotic responses (7, 44). Among them, IGFBP-3 is a well documented inhibitor of cell growth and/or promoter of apoptosis. In addition, a very recent study has demonstrated that IGFBP-3 seems to regulate vascular endothelial growth factor (VEGF) production implicated in cell growth as well as vascular leakage via suppression of hypoxia-inducible factor (HIF)-1α/HIF-2α activity in ovalbumin (OVA)-induced allergic airway disease, resulting in dramatic improvement of asthmatic features (9). In this *Review*, therefore, we focus on IGFBP-3, and it is discussed in the next section.

IGFBP-3 and IGFBP-3R

IGFBP-3

Structure of IGFBP-3. The gene of IGFBP-3 is located on chromosome 7 (45). Mature

deglycosylated human IGFBP-3 consists of 264 amino acids. IGFBP-3 contains three distinct domains, in which additional critical subdomains or functional motifs exist and contribute to various actions, such as interacting with IGFs, ALS, IGFBP-3R, and nuclear localization. IGFBP-3 possesses distinctive characteristics compared with other IGFBPs. For example, IGFBP-3 has heparin binding motifs, nuclear localization sequences, and serine residues that can be phosphorylated (10).

The N terminus of mature IGFBP-3 peptide contains 87 amino acids after the signal peptide. A total of 18 cysteines exist in IGFBP-3, 12 of which are located in this domain. IGF binding sites are known to be in this domain (46, 47), and a subdomain that mediates IGF-I–independent inhibition of mitogenesis has been suggested to be located in this region (48, 49).

The midregion of IGFBP-3 has 95 amino acids, is highly variable within IGFBPs, and shares less than 15% similarity with other IGFBPs. Post-translational modifications have been demonstrated to occur in this region. Because post-translational modifications affect cell interaction, IGF-binding affinity and susceptibility to proteases, such modification, might influence IGFBPs targeting to tissues differentially (50). The midregion of IGFBP-3 is responsible for binding to a novel cell death receptor, IGFBP-3R (11).

The C-terminal domain of IGFBP-3 contains six cysteines, and three disulfide bonds exist within this domain. It contains IGF-binding residues (51–53), and may form an IGF-binding pocket together with the N-terminal domain (10). IGFBP-3 can also bind fibrinogen, fibrin, and plasminogen via this region (54, 55). This domain contains a functionally important 18-residue basic motif with heparin-binding activity, and can bind heparin, other glycosaminoglycans, and proteoglycans (56, 57). The basic region, Lys228–Arg232, is essential for interaction with ALS (58), and additional basic residues are present that interact with the cell surface and matrix, the nuclear transporter importin-β (59), and other proteins. Moreover, this region contains a short metal-binding domain (60) and caveolin-scaffolding domain consensus sequence (10).

Regulation of IGFBP-3. GH stimulates the production of IGFBP-3 as well as IGF-I, which is one of the inducers of IGFBP-3 (61, 62). It has been suggested that the liver is the major source of circulating IGFBP-3, and that GH is the primary inducer of hepatic IGFBP-3 expression (63, 64). However, a recent study has revealed that increased circulating IGFBP-3 by GH administration is due to increased formation of the ternary complex, not via hepatic IGFBP-3 synthesis (65). The levels of circulating IGFBP-3 and IGF-I are affected by many other factors, such as age, hormones, nutrition, and combined diseases. Both circulating IGFBP-3 and IGF-I levels decline with advancing age (66). Circulating IGFBP-3 level is low in patients with GH deficiency (67), and is increased in patients with GH excess (68). Several chronic diseases and malnutrition are associated with low IGF-I levels and relatively unchanged IGFBP-3 levels (37). Insulin also up-regulates IGFBP-3 levels (61).

IGFBP-3 is also produced by peripheral tissues (37), and can be induced by a variety of molecules, such as GH (69), IL-1 (70), TNF-α (70, 71), transforming growth factor (TGF)-β_1 (72–74), glucocorticosteroids (75), retinoic acid (73), vitamin D (76), antiestrogens (77), and antiandrogens (78). Tumor suppressor genes, including p53 (79) and phosphatase and tensin homolog (80), have also been shown to up-regulate IGFBP-3 at the transcriptional level.

Down-regulation of IGFBP-3 can be achieved by various factors during the process of translation. Aberrant DNA methylation and histone acetylation have been demonstrated to be associated with the silencing of IGFBP-3 transcriptional expression in many cancers (81–86). Some transcription factors, including CDX2 (*Drosophila* caudal-related homeobox transcription factor) (87) and EWS/FLI1 (Ewing's sarcoma fusion protein) (88, 89) also suppress IGFBP-3 transcription through binding to the IGFBP-3 gene promoter. In addition, after the secretion of IGFBP-3, IGFBP-3 proteases cleave IGFBP-3, thereby inhibiting both IGF-I–dependent and –independent action of IGFBP-3.

Action of IGFBP-3. **IGF-I–dependent action of IGFBP-3.** Interestingly, IGFBP-3 can enhance as well as inhibit IGF-I action. As discussed previously here, IGFBP-3 has a high affinity for IGF-I, and binds most of the circulating IGF-I (> 70%). Moreover, the binding affinity of IGFBP-3 for IGF-I is greater than that of IGF-IR, so that IGFBP-3 can sequester the active hormone, thereby reducing IGF-I/IGF-IR signaling (38). In addition, another proposed mechanism for the dual effects of IGFBP-3 on IGF-I action is that IGFBP-3 might function as a reservoir of IGF-I, presenting and slowly releasing IGF-I to interact with its receptor, while protecting the receptor from down-regulation (90). Thus, a low level of IGFBP-3 enhances IGF-I action, whereas a high level of IGFBP-3 reduces IGF-I action, decreasing free IGF-I level (37).

IGF-I–independent action of IGFBP-3. IGFBP-3 has its own biological actions independent of IGF-I, which are known as IGF-I–independent actions of IGFBP-3 (10, 39). Although IGFBP-3 has been known to inhibit cell growth and/or promote apoptosis, it can promote cell growth in various cell types (91, 92). In addition, IGFBP-3 has other functional roles, such as a proangiogenic effect on endothelial precursor cells (42), induction of a fibrotic phenotype in fibroblasts *in vitro* (43, 93), inhibition of human preadipocyte differentiation and differentiated adipocyte function (94), and anti-inflammatory actions *in vivo* and *in vitro* (8, 9, 95).

However, the underlying mechanisms mediating these biological actions of IGFBP-3 are largely unknown. To date, IGF-I–independent actions of IGFBP-3 have been demonstrated to be mediated through cell surface receptors, inhibition of NF-κB, and interaction with retinoid X receptor-α (10).

IGFBP-3R

Recently, a new cell death receptor, IGFBP-3R, has been cloned, and mediates cell death when activated by IGFBP-3. IGFBP-3R, which is a single-span membrane protein, binds to IGFBP-3 specifically, but not to other IGFBPs (11).

IGFBP-3R has two unique characteristics: (*1*) a leucine zipper sequence, which is involved in dimerization/olimerization of membrane proteins, and is located in the putative transmembrane domain; and (*2*) IGFBP-3R can interact with the initiator of the apoptosis cascade, caspase-8, in the absence of a DD sequence that interacts with caspase-8 in other death receptors. Caspase-8 has been known to interact with the cytoplasmic tail of IGFBP-3R, because a C-terminal truncated IGFBP-3 mutant cannot interact with caspase-8. These

findings suggest that IGFBP-3R and caspase-8 exist as one complex in the resting state, and that IGFBP-3 binding to IGFBP-3R may facilitate dimerization/oligomerization of IGFBP-3R, resulting in activation of caspase-8, followed by activation of executioner caspases (caspase-3, -6, and -7) and NF-κB inhibition (8, 11, 96).

It has been suggested that the IGFBP-3/IGFBP-3R axis can exert different biological functions depending on cell types (8). For example, although the IGFBP-3/IGFBP-3R axis induces growth inhibition and apoptosis in breast and prostate cancer cells, this axis does not induce apoptosis, and actually reduces airway inflammation in bronchial epithelial cells.

Role of IGF-I and IGFBP-3 in the Pathogenesis of Asthma

Role of IGF-I in the Pathogenesis of Asthma

IGF-I is likely to play a crucial role in asthma, especially in subepithelial fibrosis, airway inflammation, AHR, and airway smooth hyperplasia (Figure 1).

A study employing endobronchial biopsies from patients with asthma has shown that IGF-I mRNA level is significantly elevated and is correlated with subepithelial fibrosis (4). These observations have suggested that IGF-I may act as a growth factor involved in airway inflammation and remodeling. Supporting this hypothesis, treatment of OVA-challenged mice with an IGF-I neutralizing antibody (Ab) inhibited the elevation of airway resistance, airway inflammation, and an increase in airway wall thickening, indicating that inhibition of IGF-I signaling may be a promising therapeutic strategy for asthma (6). Furthermore, administration of the IGF-I–neutralizing Ab decreased expression of intercellular adhesion molecule-1 in a dose-dependent manner without changing the level of IL-4, -5, and -13. This suggests that anti-inflammatory effects from neutralization of IGF-I may be due to suppression of intercellular adhesion molecule-1 expression, but not alteration of the expression of Th2 cytokines.

Recently, a noteworthy study has demonstrated a novel mechanism by which IGF-I exerts its pathogenic effect in asthma using a murine model (9). This study has demonstrated that IGF-I induces airway inflammation and AHR via enhanced HIF-α activity and VEGF expression. VEGF plays a role as a proinflammatory mediator, as well as a vascular permeability factor (9). Moreover, VEGF is shown to be associated with subepithelial fibrosis by regulation of TGF-β_1 expression through the PI3K/AKT signaling pathway (97). Taken together, these findings suggest the intriguing hypothesis that IGF-I may induce subepithelial fibrosis via the IGF-I/HIF/VEGF/TGF-β_1 axis, and that inhibition of this axis may reduce subepithelial fibrosis (Figure 2).

IGF-I is known to be associated with airway smooth muscle (ASM) hyperplasia and enhanced contraction. *In vitro* studies with rabbit ASM cells have demonstrated that IGF-I promotes ASM proliferation (98–100). The mitogenic effect of IGF-I is enhanced by leukotriene D_4 (98). Moreover, an *in vitro* study with human ASM cells has demonstrated that this enhanced ASM proliferation by leukotriene D_4 is mediated by MMP-1, which is one of the IGFBP proteases, thus enhancing IGF-I activity (101). In addition to these effects, IGF-I induces Rho-kinase–dependent sustained contraction of human ASM (100). A subsequent study, which used airway tissues from patients with asthma, has shown that MMP-1 levels and activity are enhanced, and that IGFBPs exist as cleaved forms in the airway tissues (5).

Although regulation of IGF-I expression in allergic airway diseases is not well defined, IL-17F, a putative mediator of severe asthma, has been shown to induce IGF-I gene expression in bronchial epithelial cells (102). Costimulation with other Th2 cytokines (IL-4 and IL-13) enhances IGF-I production, suggesting an important relationship among the IGF-I signaling pathway, Th2, and Th17 cells in asthma (102). In fact, IL-17 has been reported as one of the key players in eosinophilic as well as neutrophilic airway inflammation using animal models of asthma induced by toluene diisocyanate or OVA (103, 104). In these airway disorders, the blockade of IL-17 with neutralizing Ab significantly suppressed the airway inflammation, including Th2 responses and AHR (103, 104). Moreover, IL-17 expression is regulated by peroxisome proliferator–activated receptor γ and PI3K signaling in antigen-induced airway inflammation (103, 104). Considering that these transcriptional factors are also associated with the IGF-I system, targeting IGF-I can be a good way to regulate IL-17, one of the main cytokines in the pathogenesis of bronchial asthma.

Role of IGFBP-3 in the Pathogenesis of Asthma

Fragments of IGFBP-3 have been identified in tissues and bronchoalveolar lavage fluid from patients with asthma, and an association between IGFBP-3 and asthma has been suggested (7). Moreover, a growing body of evidence has indicated that IGFBP-3 plays a therapeutic role, dampening allergic airway inflammation (8, 9) (Figure 3). As discussed previously here, IGFBP-3 has its own biological activities, known as IGF-I–independent actions, such as suppression of NF-κB signaling pathway via IGFBP-3R and antitumor action via interaction with retinoid X receptor-α.

As for bronchial asthma, a study with wild-type and IGFBP-3 transgenic mice has demonstrated that IGFBP-3 inhibits airway inflammation and AHR via activation of IGFBP-3R signaling and cross-talk with NF-κB (8). In addition, the study has shown that IGFBP-3 is suppressed in OVA-challenged mice, and that restoration of IGFBP-3 by administration of recombinant human IGFBP-3 (rhIGFBP-3) or transfer of the IGFBP-3 gene normalizes crucial manifestations of asthma, such as antigen-induced inflammation, proinflammatory cytokine production in lung tissues and bronchoalveolar lavage fluid, and AHR. These unique effects of IGFBP-3 are likely to be IGF-I independent, because a non–IGF-binding IGFBP-3 mutant (IGFBP-3^{GGG}) shows similar results. Regarding the mechanism of IGFBP-3 action, IGFBP-3 not only inhibits phosphorylation of IκBα, but also degrades IκBα and p65–NF-κB through activation of caspases, in particular caspase-8 and caspase-3/-7. This caspase-dependent action of IGFBP-3 appears to be mediated through IGFBP-3R, because knockdown of endogenous IGFBP-3R completely negates the biological effect of IGFBP-3. During this process, it seems that binding of IGFBP-3 to IGFBP-3R extracellularly activates the IGFBP-3R intracellular signaling into caspase, which results in reduction of total NF-κB protein levels as well as phospholylated ones in airway

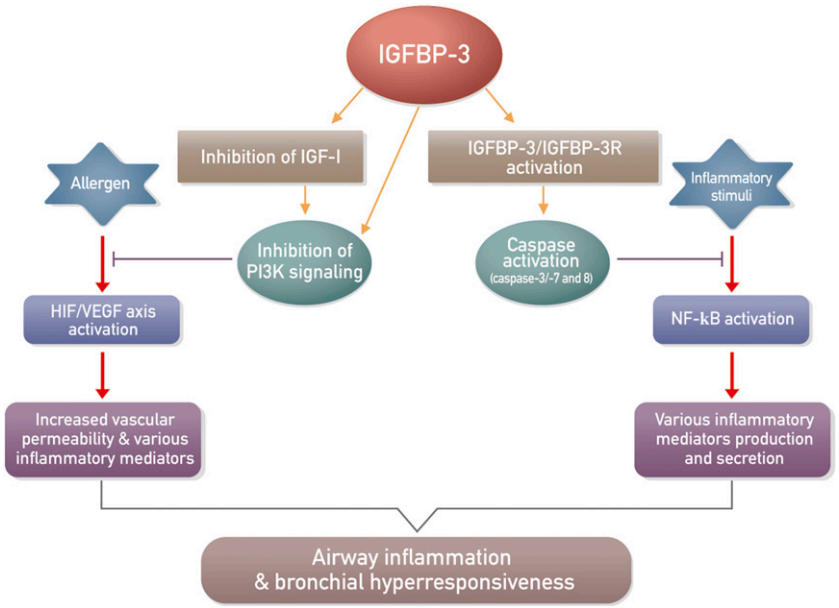

Figure 3. Roles of IGFBP-3 and its related signaling pathways in bronchial asthma.

epithelial cells. In fact, previous studies have also demonstrated that IGFBP-3 inhibits TNF-α–induced NF-κB activity (105). IGFBP-3 significantly enhances TNF-related apoptosis–inducing ligand–induced cell death by inhibiting NF-κB activation in response to the induction of apoptosis by TNF-related apoptosis–inducing ligand in cancer cells (106). Considering that NF-κB plays an important role in the pathogenesis of bronchial asthma, it is noteworthy that IGFBP-3 treatment results in inhibition of the nuclear translocation of NF-κB in bronchial asthma.

In addition, a recent study has provided another mechanism of IGFBP-3 action in allergic airway inflammation, in which exogenous recombinant IGFBP-3 attenuates asthmatic features through the inhibition of VEGF and HIF expression (9). A study with OVA-challenged mice has revealed that administration of rhIGFBP-3 reduced levels of IGF-I, VEGF, Th2 cytokines, and activity of HIF-1α and HIF-2α in the lung (9). Administration of rhIGFBP-3 also decreased infiltration of inflammatory cells in the airway, production of Th2 cytokines in the lung, OVA-specific IgE production in serum, plasma exudation, and AHR. Using IGF-I–neutralizing Ab and PI3K inhibitors, LY294002 and wortmannin, we have also revealed that IGFBP-3 signaling involves the HIF-1α/HIF-2α–VEGF axis through IGF-I–dependent and/or IGF-I–independent mechanisms, thereby attenuating asthmatic features, including vascular permeability.

Based on these mechanisms of IGFBP-3 action in the pathogenesis of bronchial asthma, there can be speculation on the potential roles of IGFBP-3 in subepithelial fibrosis and mucus metaplasia. First, VEGF is known to induce subepithelial fibrosis in the lung (107) and to enhance the production of TGF-β1, which plays an important role in the pathogenesis of structural changes, including fibrosis, in a number of chronic lung diseases (108). Furthermore, VEGF has been reported to regulate TGF-β1 expression through the PI3K/Akt signaling pathway in a murine model of bronchial asthma (97). Therefore, the inhibitory effects of IGFBP-3 on VEGF expression/production may result in the prevention of airway subepithelial fibrosis.

Second, the IGF-I signaling pathway can cross-talk with the epidermal growth factor pathway (109) that is involved in the development of mucus metaplasia (110). The activation of HIF-1α and inhibition of forkhead box transcription factor 2, which are inducible by IGF-I, have been suggested to induce mucus metaplasia through activation of the muc5ac promoter (111–114). These observations suggest that IGFBP-3 may also play a role in the pathogenesis of mucus metaplasia by modulating IGF-I signaling.

Finally, IGFBP-3 may also have an antiproliferative effect on ASM cells in allergic airway diseases. A study with human bronchial and tracheal smooth muscle cells has shown that an IGFBP protease, MMP-1, degrades intact IGFBP-3 and promotes ASM hyperplasia (5).

IGFBP-3 and HIF/VEGF Signaling in the Respiratory System

As described previously here, IGFBP-3 as well as IGF-I appear to be closely associated with HIF/VEGF signaling in bronchial asthma. VEGF has been shown to stimulate endothelial cell mitogenesis, cell migration, vasodilatation, and vascular permeability. In addition, VEGF is a mediator of vascular and extravascular remodeling, and plays a crucial role in Th2-mediated inflammation (107). With many reports that an increase in VEGF level has been observed in tissues and biological samples from individuals with asthma (115–117), mounting evidence has demonstrated that VEGF is a pivotal player in the pathogenesis of various airway disorders (107, 118, 119). As for HIF-1α/HIF-2α, they have been well known as a transcriptional factor for VEGF in various pathologic conditions. Determination of HIF-1α and/or HIF-2α protein level in nuclear extracts has revealed that these protein levels are increased in several pulmonary inflammations, including allergen-induced asthma or exogenous oxidant–inhaled lung injury (118–122). On the basis of these observations, the control of HIF/VEGF signaling via the IGFBP-3 and IGF-I system seems to be promising for the development of therapeutics for inflammatory lung disorders.

Targeting IGF-I and IGFBP-3 for Treatment of Asthma

Because IGF-I and IGFBP-3 signaling pathways are implicated in the pathogenesis of asthma, targeting IGF-I and IGFBP-3 can be an attractive therapeutic strategy for asthma. There are two major potential strategies: (*1*) inhibition of IGF-I action; and (*2*) up-regulation of IGFBP-3.

Inhibition of the IGF-I System

The inhibition of IGF action can be achieved at several different levels: suppression of

ligands with antibodies, induction of IGFBPs, and signaling blockade using IGF-IR inhibitors (123). Although *in vitro*, preclinical, and early clinical studies have suggested therapeutic potential for the inhibition of IGF-I action in particular cancers, these modalities do not benefit all patients uniformly (124). A neutralizing Ab for IGF-I, MEDI-573 (a dual IGF-I/IGF-II–neutralizing Ab), has been developed and evaluated as a possible anticancer drug for patients with advanced cancers (125). In addition, some small-molecule tyrosine kinase inhibitors and anti–IGF–IR monoclonal Abs have been evaluated in clinical trials for patients with cancers (124, 126). However, to date, there are no available data on the therapeutic effects of these pharmacologic agents and clinical trials for patients with bronchial asthma, although an IGF-I–neutralizing Ab has been reported to reduce airway resistance, airway inflammation, and airway wall thickening in a murine model of asthma (6). We eagerly await clinical trials to evaluate whether the recently developed pharmacologic agents that inhibit IGF-I action can improve features of asthma, including airway inflammation, AHR, subepithelial fibrosis, mucus metaplasia, and ASM hyperplasia. Some potential compounds are listed in Table 1.

Up-Regulation of IGFBP-3

As we discuss here, IGFBP-3 is a very promising target for management of bronchial asthma, although there is scant clinical information on the role of IGFBP-3 in bronchial asthma. In fact, studies with animal models have demonstrated that administration of rhIGFBP-3 inhibits crucial manifestations of asthma in mice (9). An IGFBP-3 mutant that does not bind IGF-I binds to IGFBP-3R and acts as an IGFBP-3R agonist, thus enhancing IGFBP-3R–mediated anti-inflammatory responses (8). To sum up, it is expected that reinforcement of IGFBP-3 action can be provided by treatment with rhIGFBP-3 or other IGFBP-3R agonists/activators for patients with asthma (127). Therefore, the discovery and development of such novel agents should have a high priority in the management of asthma, specifically severe or refractory asthma.

Conclusions and Perspectives

Despite enormous improvements in our understanding and insight into the causative mechanisms implicated in bronchial asthma, especially severe or refractory asthma, treatment of patients with asthma is still challenging. Recently, accumulating findings suggest that IGF-I and IGFBP-3 are prospective molecular therapeutic targets for various pulmonary disorders, including bronchial asthma. Despite success in mice, there are no published clinical trials that have evaluated the therapeutic effects of the pharmacologic agents targeting IGF-I and IGFBP-3 in humans. In addition, because the IGF-I system and IGFBP-3 play essential roles in the body, such as in glucose metabolism and growth, the side effects of the pharmacologic intervention targeting

Table 1: Targeting IGF-I for Treatment of Asthma

	Agent	Mechanism	Side effects	Development phase	Route	References
IGF-I neutralizing Abs	MEDI-573	Human monoclonal Ab, which inhibits both IGF-I and IGF-II, thus inhibits IGF-IR, IR-A, and IGF-IR/IR-A hybrid signaling	Anorexia, nausea, diarrhea, fatigue, and anemia	Phase II	IV	(125)
IGF-IR inhibitors						
IGF-IR specific tyrosine-kinase Inhibitors	BMS-754807	Tyrosine kinase inhibitors prevent autophosphorylation of the tyrosine kinase domain of cell surface receptors	To be determined	Phase I/II	PO	(128, 129)
	Insm-18 (NDGA)		Nausea, vomiting, and syncope due to dehydration	Phase I/II	PO	(126)
Monoclonal Ab against IGF-IR	MK-0646 (dalotuzumab)	Inhibits IGF-induced IGF-IR activation and induces receptor internalization and degradation	Fatigue, nausea, rash, diarrhea, neutropenia, thrombocytopenia, hyperglycemia, and diarrhea	Phase III	IV	(126)
	AMG 479 (ganitumumab)		Thrombocytopenia, neutropenia, hyperglycemia, transaminitis, fatigue, fever, and rash	Phase III	IV	(130, 131)
	AMG A12 (cixutumumab)		Hyperglycemia, anemia, thrombocytopenia, and fatigue	Phase III	IV	(132–135)

Definition of abbreviations: Ab, antibody; IGF, insulin-like growth factor; IGF-IR, IGF-I receptor; IR-A, insulin receptor isoform A; IV, intravenous; NSCLC, non–small cell lung cancer; PO, per oral.
Search strategy: ongoing or planned trials registered on ClinicalTrials.gov per March 2013.

IGF-I and IGFBP-3 must be considered. Therefore, it may be desirable to develop novel agents that manipulate IGF-I/IGFBP-3 actions for the treatment of bronchial asthma as an inhaled formulation allowing local action while minimizing systemic side effects.

In summary, IGF-I and IGFBP-3 are potentially exciting targets for the development of compounds to achieve better management of bronchial asthma, especially severe or refractory asthma in which steroids and other current agents are less effective. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgments: The authors thank Professor Mie-Jae Im (Chonbuk National University Medical School, Jeonju, South Korea) for critical readings of the manuscript.

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