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COMMENTARY

Advances in Immunopharmacology of Asthma

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ABSTRACT. Asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness and recurrent reversible airway obstruction. As there appears to be a preponderance of T-helper 2 (Th2) cells over Th1 cells in asthma, more attention has been focused on the role of Th2-derived cytokines such as interleukin (IL)-4 and IL-5 and their corresponding signaling pathways in the pathophysiology of the disease. These complex pathways may involve the activation of signal transducers and activators of transcription (STATs) and nuclear factor-κB (NF-κB). On the other hand, immunoglobulin (Ig) E-mediated mechanisms and the protein tyrosine kinase signaling cascade are important in triggering the release of mediators from inflammatory cells. In spite of all of these, host regulatory mechanisms exist to limit the inflammation. An increase in the 3′,5′-cyclic adenosine monophosphate (cAMP) level generally suppresses the activities of immune and inflammatory cells, and the level of cAMP is closely regulated by a family of phosphodiesterases (PDEs). Heparin, a glycosaminoglycan released exclusively from mast cells, also is believed to possess anti-inflammatory actions. Many new therapeutic agents have been developed either to attenuate the pro-inflammatory processes in asthma or to augment the host anti-inflammatory mechanisms. In this article, we discuss the immunopharmacology of several of these agents, which include heparin and inhibitors of PDEs, tyrosine kinases, and NF-κB, as well as antibodies and soluble receptors directed against IgE, IL-4, and IL-5. BIOCHEM PHARMACOL **59**;11:1323–1355, 2000.

KEY WORDS. phosphodiesterases; NF-κB; protein tyrosine kinase; IL-4; IL-5; heparin; immunoglobulin E

Asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness and recurrent reversible airway obstruction. The prevalence and mortality rate of asthma have been rising for the past decade despite our increased understanding of the pathogenesis of this airway disease. In industrialized countries, it has been observed that reduced microbial exposure and better childhood immunization predispose individuals to develop allergic asthma by driving the immune system to Th2†-dominant immunity [1]. Others have speculated that regular use of inhaled β_2 agonists is linked to the deterioration of asthma control by blocking the "protective" role of lung mast cells, down-regulating β_2-adrenoceptors, and increasing antigen load in the airways [2]. While these hypotheses remain to be confirmed, cumulative findings support the notion that Th2 cells, B cells, mast cells, and eosinophils contribute to the chronic inflammation of the airways [3] (Fig. 1).

Th2 cells produce a cytokine profile that includes predominantly IL-4 and IL-5. IL-4 is required for commitment of naïve T cells to the Th2 phenotype, isotype switching in B cells towards IgE synthesis, and up-regulation of VCAM-1 on endothelial cells to facilitate eosinophil infiltration into the lungs [3, 4]. IL-5 is critical for the growth and terminal differentiation of eosinophils and recruitment of eosinophils into the airways [4, 5]. On the other hand, IgE plays a significant role in both acute and late asthmatic responses by mediating the cross-linking of high-affinity Fc receptors (FcεRI) on mast cells, resulting in the release of a vast array of pro-inflammatory mediators including histamine, leukotrienes, and cytokines [6]. Apart from these mediators, heparin is also released upon mast cell degranulation. It possesses broad-spectrum anti-inflammatory activities thought to be important in modulating the asthmatic responses [7].

More recently, the critical role of various signal transduction pathways in mediating inflammatory cell responses has been confirmed. In general, an increase in the level of the intracellular second messenger cAMP is usually associated with the suppression of immune and inflammatory cells. The level of cAMP is tightly regulated by a family of PDEs [8]. On the other hand, it has been shown that the protein tyrosine kinase signaling cascade plays a critical role

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† *Abbreviations*: cAMP, 3′,5′-cyclic adenosine monophosphate; cGMP, 3′,5′-cyclic guanosine monophosphate; HMW, high-molecular weight; ICAM, intercellular cell adhesion molecule; Ig, immunoglobulin; IKK, IκB kinase; IL, interleukin; LMW, low-molecular weight; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MMW, medium-molecular weight; NAF, non-anticoagulation fraction; NF-κB, nuclear factor-κB; PDE, phosphodiesterase; PI3K, phosphatidylinositol 3′-kinase; PKA, cAMP-dependent protein kinase; RANTES, regulated upon activation normal T cell expressed and secreted; STATs, signal transducers and activators of transcription; Th, T-helper; TNF, tumour necrosis factor; ULMW, ultralow-molecular weight; and VCAM, vascular cell adhesion molecule.

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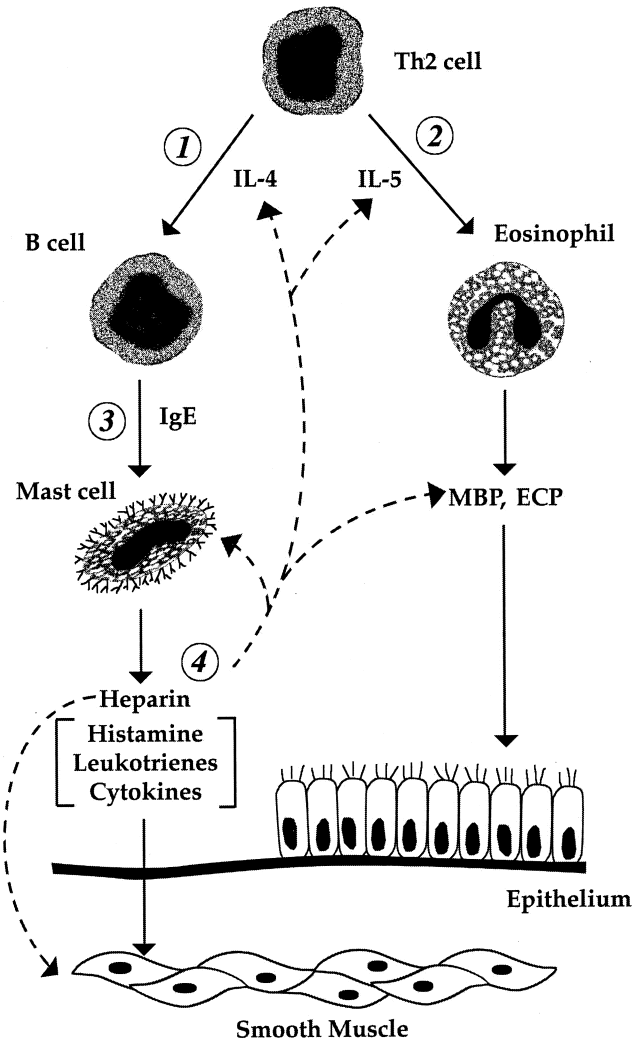

FIG. 1. Interactions among inflammatory cells in the pathogenesis of asthmatic airways. Key: (1) IL-4 inhibitors, (2) IL-5 inhibitors, (3) anti-IgE antibodies, and (4) heparin. Broken arrows denote the inhibitory effect of heparin on the various targets shown. Abbreviations: MBP, major basic protein; and ECP, eosinophil cationic protein.

in the activation of antigen receptors [9] and cytokine receptors [10], and the transcription regulator NF-κB is responsible for the expression of a variety of pro-inflammatory cytokines in the airways [11] (Fig. 2).

With the improved understanding of the pathophysiology of asthma at the cellular and molecular levels, many specific inflammatory processes or molecules have been identified as novel therapeutic targets for potential pharmacologic intervention. This review focuses on the immunopharmacology of some of these new therapeutic approaches to the treatment of asthma (Table 1).

INHIBITORS OF IL-4 AND IL-5

IL-4 is required for the commitment of naïve T cells to the Th2 phenotype [4]. It also promotes isotype switching of B cells towards IgE synthesis by initiating ϵ-germline transcription, and is synergistic with IgE in up-regulating mast cell FcϵRI expression and mediator release [12], suggesting that IL-4 may play a critical role in mediating IgE-dependent allergic reactions. IL-5, on the other hand, promotes the maturation of eosinophils from bone marrow precursors, prolongs their survival by inhibition of apoptosis, activates mature eosinophils, facilitates eosinophil recruitment to tissues via a synergistic effect with chemoattractants such as eotaxin, and promotes eosinophil adhesion to vascular endothelium [13].

In asthmatic subjects, inhalation of recombinant IL-4 resulted in airway hyperresponsiveness [14], whereas instillation of IL-5 into the airways produced eosinophilia in bronchial biopsies and bronchoalveolar lavage fluid [5]. It also has been reported that in both atopic and non-atopic asthma, there is an increase in expression of IL-4 and IL-5 mRNA and protein [15]. In addition, there is also an increase in expression of IL-5 receptors in asthmatic airways [16], and patients suffering from asthma exacerbations have a higher serum concentration of IL-5 than in periods of remission [17].

The recognition of the importance of IL-4 and IL-5 in asthma has led to the development of inhibitors of IL-4 and IL-5 as potential agents for the treatment of this airway disease. However, there are inherent difficulties in developing small molecule antagonists of the IL-4 and IL-5 receptors, as both cytokines exert their effects at high local concentrations during cell–cell interactions, and it is unlikely that a low molecular weight antagonist would be able to span the multichain binding domains of the IL-4 or IL-5 receptor to completely block the actions of these endogenous cytokines. Instead, substantial research effort has been expended on the development of inhibitory mutant or variant forms of these cytokines. Indeed, IL-4.Y124D, a macromolecular IL-4 mutant resulting from the replacement of Tyr 124 by aspartic acid, has been found to bind with high affinity to the IL-4 receptor and inhibit IL-4-dependent T-cell proliferation [18] as well as IL-4/IL-13-induced IgE synthesis [19]. IL-4δ2, a naturally occurring splice variant of IL-4, also blocked IL-4-induced T cell proliferation [20]. Similarly, mutation of IL-5 at Glu 13 (E13) [21] or charge reversal at position 12 (E12K) [22] resulted in mutant proteins that had antagonistic effects on IL-5-induced TF1 cell proliferation. Mutant IL-5 (E12K) also inhibited IL-5-induced eosinophil adhesion in a concentration-dependent manner without significantly blocking the effects of IL-3, granulocyte macrophage-colony stimulating factor, or TNF-α, indicating that IL-5 (E12K) is a specific IL-5 antagonist [22].

Soluble receptors and monoclonal antibodies also have been developed as specific inhibitors of IL-4 and IL-5. In a mouse model, soluble IL-4 receptor (sIL-4R) inhibited IgE synthesis and prevented development of airway inflammation [23], whereas soluble IL-5 receptor α chain (sIL-5Rα) suppressed antigen-induced eosinophilia [24] and inhibited inflammatory mediator release [25]. A genetically engineered sIL-4R has been developed, and results of phase II

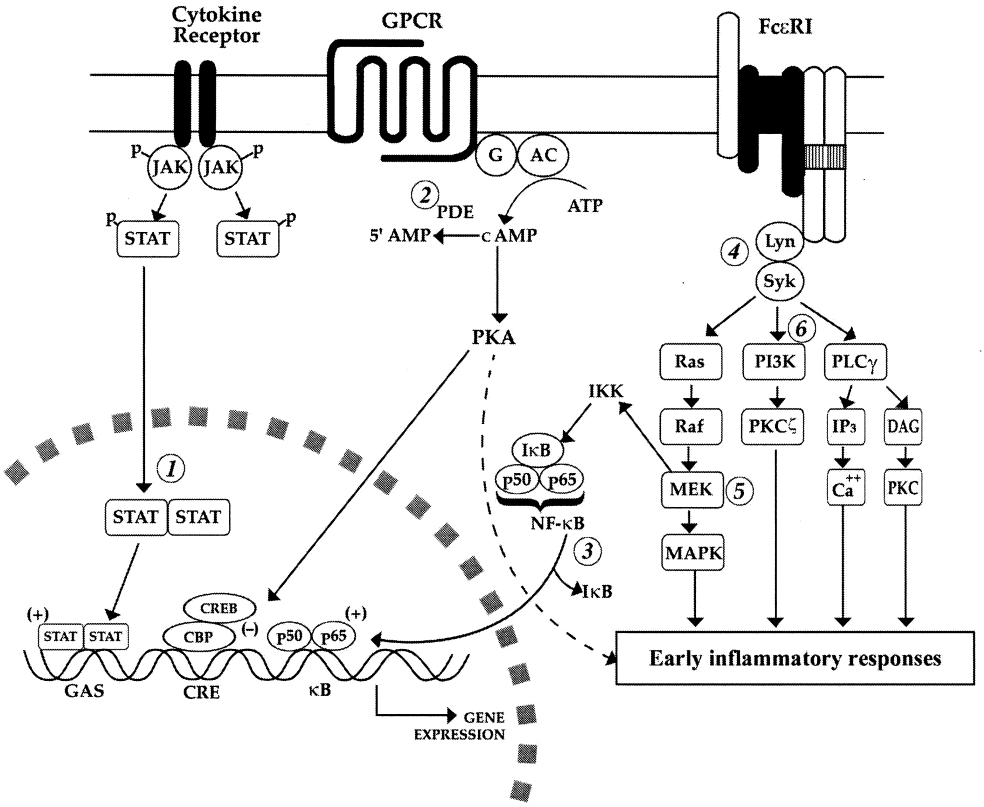

FIG. 2. Signal transduction pathways that have been implicated in the acute and chronic inflammatory responses in asthma. Key: (1) STAT inhibitors, (2) PDE inhibitors, (3) NF-κB inhibitors, (4) tyrosine kinase inhibitors, (5) MAPK kinase inhibitors, and (6) PI3K inhibitors. Abbreviations: 5′-AMP, 5′-adenosine monophosphate; AC, adenylate cyclase; cAMP, 3′,5′-cyclic adenosine monophosphate; CBP, CREB-binding protein; CRE, cAMP response element; CREB, CRE binding protein; DAG, diacylglycerol; G, G-protein; GAS, γ-interferon activation site; GPCR, G-protein-coupled receptor; IKK, IκB kinase; IP_3, inositol 1,4,5-triphosphate; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PDE, phosphodiesterase; PI3K, phosphatidylinositol 3′-kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; and STAT, signal transducer and activator of transcription. The symbol (+) denotes activation. A broken arrow or the symbol (−) denotes inhibition.

clinical trials have shown that nebulized recombinant human IL-4 receptor (rhIL-4R) possesses good anti-inflammatory activity [26]. Antibodies against IL-4 and IL-5 also have been proven to be a promising option. In animal models, treatment with anti-IL-4 mAb [27, 28] or anti-IL-4R antibody [29] blocked IgE production, prevented development of airway hyperreactivity, and attenuated airway eosinophilia. Likewise, treatment with anti-IL-5 mAbs inhibited pulmonary eosinophilia and, in some cases, bronchial hyperresponsiveness as well [27, 28, 30]. The administration of murine antibodies to humans, however, almost always elicits an immune response resulting in the production of neutralizing antibodies against the administered antibodies. In an attempt to circumvent the problem of antigenicity, a humanized, non-immunogenic antibody to IL-5 (SCH 55700) has been developed using complementarity determining region grafting techniques. Animal studies have shown that SCH 55700 reduced antigen-induced pulmonary eosinophilia in monkeys by 75% and suppressed the eosinophil response for up to 6 months after administration of the antibody [31]. The efficacy and safety of SCH 55700 remain to be confirmed in clinical trials.

ANTI-IgE ANTIBODIES

The role of IgE in mediating allergic reactions is well-established, and a causal relationship between serum IgE levels and the presence of asthma has been confirmed [32]. Two structurally distinct IgE receptors exist, namely the high-affinity FcεRI found on mast cells, basophils, and antigen-presenting cells, and the low-affinity FcεRII (CD23) found on a wide variety of cells including B cells, eosinophils, monocytes, and macrophages. The Cε3 domain of IgE is critical for its interaction with FcεRI. The binding of IgE to FcεRI on mast cells and basophils and the subsequent cross-linking of FcεRI by IgE/antigen complexes result in the release of histamine, leukotrienes, and cytokines (e.g. IL-4, IL-5, IL-6, and TNF-α). Among these cytokines, IL-4 promotes IgE production (see above), and IgE in turn up-regulates FcεRI [33, 34], suggesting the presence of an IgE-dependent mechanism for the amplification of allergic responses. In addition, IgE–antigen complexes also may interact with FcεRII on B cells to facilitate antigen presentation to T cells [35], induce lung eosinophilia, and increase cytokine production from Th2 cells [36].

TABLE 1. New therapeutic agents that may be used in the treatment of asthma

Mechanisms	Examples	Effects	Ref.
Anti-IL-4			
IL-4 mutant protein Anti-IL-4 mAb Soluble IL-4R Anti-IL-4R	IL-4.Y124D	Blocks IgE production Inhibits eosinophil infiltration	18, 19, 23, 26–29
Anti-IL-5			
IL-5 mutant protein Anti-IL-5 mAb Soluble IL-5R	E13, E12K SCH 55700	Inhibits eosinophil adhesion, infiltration, and mediator release	22, 24, 25, 27, 28, 30, 31
Anti-IgE			
Anti-IgE mAb	rhuMab-E25 CGP 51901	Inhibits lung eosinophil infiltration Reduces number of IgE-producing B cells Down-regulates FcεRI on basophils	36, 38
Proteoglycans	Heparin ODS-heparin NAF-heparin	Inhibits neutrophil/eosinophil infiltration Binds and inactivates IL-4, RANTES, MBP, L- and P-selectins Inhibits airway smooth muscle proliferation	7, 50, 51, 53, 58, 60, 61
PDE4 inhibition	Rolipram SB207499 CDP840 RP73401	Prevents eosinophil degranulation Inhibits generation of MBP and ECP Inhibits proliferation of Th2 clones Inhibits IL-4 release from T cells	73, 74, 76, 77, 79–81
Tyrosine kinase inhibition	Genistein Piceatannol ER-27319 PP1 Terreic acid Herbimycin A Leflunomide	Prevents antigen-induced activation of mast cells, T and B lymphocytes, and granulocytes Inhibits eotaxin-induced eosinophil chemotaxis	90, 91, 93–96, 98
MAPK kinase inhibition	PD098059	Attenuates antigen-induced airway smooth muscle contraction	92
NF-κB inhibition	Triflusal TPCK PAO Hymenialdisine Helenalin MG-132 IKK inhibitors	Inhibits neutrophil chemotaxis, VCAM-1 and prostaglandin endoperoxide synthase-2 mRNA expression, iNOS induction, IL-8 production and release	115, 116, 118, 122

Since IgE mediates a wide spectrum of effects in asthma, various strategies have been proposed to inhibit the actions of IgE. These include anti-IgE antibodies, anti-CD23 antibodies, soluble IgE-binding α-subunits of FcεRI, as well as IgE-derived peptides and oligonucleotides to prevent IgE from binding to FcεRI [37]. Of these, neutralization of IgE by antibodies has been hailed as the most promising approach. Most of the available anti-IgE antibodies, however, have a tendency to activate mast cells, and the administration of these antibodies very likely will precipitate allergic symptoms. This problem has been overcome by the development of newer anti-IgE antibodies that bind IgE at the same site normally recognized by IgE receptors (i.e. the Cε3 domain of IgE heavy chains), so that IgE that is already bound to its receptor can no longer interact with anti-IgE antibodies to cause mediator release. Studies in animal models revealed that these non-anaphylactogenic antibodies are able to reduce the number of IgE-forming cells and the serum IgE levels [38], lung eosinophil infiltration [36], and antigen-induced bronchoconstriction [39].

More recently, non-immunogenic, non-anaphylactogenic anti-human IgE antibodies have been developed from mouse antibodies using chimerization and humanization techniques. Two such antibodies, CGP 51901 (chimeric) and rhuMAb-E25 (humanized), are undergoing trials currently for the treatment of allergic diseases such as rhinitis and asthma. Treatment with CGP 51901 or rhuMAb-E25 in atopic subjects resulted in a significant reduction in free serum IgE levels [40–43] and substantial reversible down-regulation of FcεRI on human basophils [44, 45]. Preliminary results obtained from Phase III clinical trials on subjects with moderate to severe asthma revealed that 12-week therapy with rhuMAb-E25 resulted in symptom improvement and increases in peak expiratory flow rate

[46], and continued treatment for a further 8 weeks led to a decrease in asthma exacerbations with corresponding reduction in corticosteroid use and β-agonist rescue [47]. Apart from the occasional urticarial rash [42], rhuMAb-E25 appears to be well-tolerated by most patients.

PROTEOGLYCANS

Heparin is a member of the structurally complex, polyanionic glycosaminoglycan family, which also includes heparan sulphate, chondroitin sulphate, and hyaluronic acid. It is composed of highly sulphated repeating disaccharide units consisting of iduronic acid 1,4-linked to glucosamine, with an average molecular weight range of 12,000–15,000. Endogenous heparin is found exclusively in the granules of most mammalian mast cells and is released upon mast cell degranulation [7]. Because of its polyanionic nature, heparin has been shown to bind to a variety of proteins with positively charged amino acids through electrostatic forces. The most well-characterized of such interactions is the formation of the heparin–antithrombin III complex in the modulation of blood coagulation, whereby a pentasaccharide sequence in the heparin polysaccharide chain is required for specific binding to antithrombin III [48]. In addition to its anticoagulant activity, heparin has been shown to possess various anti-inflammatory properties, including inhibition of T lymphocyte function [49], neutrophil and eosinophil infiltration into the lungs [50], allergen-induced early and late asthmatic responses [51], exercise-induced asthma [52], and airway smooth muscle proliferation [53]. These anti-inflammatory effects of heparin most probably can be attributed to its physical binding to a variety of heparin-binding proteins such as TNF-α, IL-4, RANTES, secretory leukocyte protease inhibitor, neutrophil-derived elastase and cathepsin G, eosinophil-derived major basic protein, and L- and P-selectins [7, 54, 55]. Alternatively, heparin has been shown to specifically inhibit the inositol 1,4,5-triphosphate signal transduction pathway, which is important for a vast array of inflammatory cellular responses [56, 57].

Although heparin has therapeutic potential for the treatment of asthma, its effectiveness is limited by its inherent anticoagulant activity. A series of chemically modified heparins with little or no anticoagulant activity has been developed and examined for their anti-inflammatory effects. In a study using a selective 2-*O*- and 3-*O*-desulphated (ODS) heparin (10,500 Da) produced under extreme alkaline conditions (pH $\geq$ 13) with almost all anticoagulant activity being eliminated, the levels of anti-inflammatory effects obtained were comparable to those of unfractionated heparin [58]. The ODS heparin inhibited the protease activity of neutrophil-derived elastase and cathepsin G, inhibited airway smooth muscle proliferation, and attenuated vagally induced airway hyperreactivity in antigen-challenged guinea pigs. Similar inhibitory effects could be reproduced by polyanionic dextran sulphate with sulphation contents ranging from 12 to 17% [58]. These findings indicate that selective desulphation of unmodified heparin can eliminate anticoagulant activity, while the contents and patterns of sulphation determine the anti-inflammatory effect of heparin. In another series of studies looking into the molecular weight-dependent effects of the "non-anticoagulant fraction" of heparin (NAF-heparin) on allergic airway responses, the ULMW (2,400 Da) NAF-heparin consistently demonstrated more potent inhibitory effects on allergen-induced acute bronchoconstriction and airway hyperresponsiveness to carbachol in allergic sheep than the HMW (10,500 Da), MMW (6,500 Da), and LMW (4,270 Da) NAF-heparins [59, 60]. On the other hand, inhibition of mast cell degranulation requires a NAF-heparin of at least the LMW level, since ULMW NAF-heparin failed to block antigen-induced histamine release [59, 61], suggesting that the anti-inflammatory effects of heparin are molecular weight-dependent. Another study, however, showed that NAF-heparins of all different molecular weights failed to prevent antigen-induced histamine release from sheep lungs [60]. The inhibitory role of heparin in mast cell degranulation thus remains to be clarified.

Clinical studies using inhaled heparin to treat asthma are limited by its potential anticoagulant activity. Two studies in patients with asthma showed that inhaled heparin prevented allergen- and exercise-induced acute bronchoconstriction [62, 63]. However, another clinical study showed that inhaled heparin substantially blocked the allergen-induced late asthmatic response, but not the early asthmatic response [7]. This finding is consistent with the anti-inflammatory effects of heparin in various *in vivo* and *in vitro* models of inflammation. The beneficial effects of non-anticoagulant heparins in asthmatics await confirmation in clinical studies.

PDE TYPE 4 INHIBITORS

PDE is responsible for hydrolyzing intracellular second messenger 3′,5′-cyclic nucleotides, e.g. cAMP and cGMP, to nucleoside 5′-monophosphates, e.g. 5′-AMP and 5′-GMP, resulting in a decrease in the levels of cAMP or cGMP. There are at least seven families of isozymes identified in the PDE superfamily [8], and their expression exhibits a certain degree of tissue and organ specificity. Among these isozymes, the cAMP-specific PDE4 has been shown to be the predominant form of PDE expressed in immune and inflammatory cells [8]. In general, an increase in the cytoplasmic cAMP level suppresses the activities of immune and inflammatory cells. Cumulative evidence shows that cAMP inhibits mast cell degranulation, eosinophil chemotaxis, superoxide anion production in neutrophils, TNF-α release from monocytes, and airway smooth muscle proliferation [8, 64]. Many of these anti-inflammatory effects of cAMP are mediated by a family of PKAs and PKA-activated transcription regulators called cyclic AMP response element binding proteins (CREB), which can regulate gene expression [65].

As such, selective inhibition of PDE4 appears to be a

promising therapeutic approach to modulate airway inflammation in asthma. Indeed, rolipram, the prototype for the novel class of PDE4 inhibitor, has been shown to elicit substantial *in vitro* and *in vivo* anti-inflammatory actions [8]. However, rolipram causes severe nausea and vomiting in animals and human subjects, which is believed to be a result of activation of emetic reflexes in the central nervous system and the gastrointestinal tract via PDE4 inhibition [66–68]. In an effort to improve the side-effect profiles of PDE4 inhibitors, PDE4 was found to exist in two distinct and catalytically active conformational states. One of the conformers is a high-affinity rolipram-binding PDE4 (HPDE4), and the other is a low-affinity rolipram-binding PDE4 (LPDE4) [8, 69]. Inhibition of the LPDE4 is often associated with anti-inflammatory effects of rolipram [70, 71], whereas inhibition of the HPDE4 appears to be related to side-effects [67, 72]. As such, a panel of second-generation PDE4 inhibitors has been developed with increased affinity for LPDE4 and reduced affinity for HPDE4, which includes SB207499 (Ariflo) [73], CDP840 [74, 75], and RP73401 (Piclamilast) [76].

In animal models of asthma, CDP840 and SB207499 have been shown to inhibit antigen-induced bronchoconstriction by blocking the release of mediators, and pulmonary eosinophilia and eosinophil degranulation [73, 74, 77, 78]. In addition, RP73401 inhibited leukotriene B_4-induced generation of superoxide, major basic protein, and eosinophil cationic protein from guinea pig eosinophils [76]. On the other hand, SB207499 and RP73401 were found to inhibit TNF-α production from human monocytes [71, 79, 80], and SB207499 was shown to be more effective in inhibiting proliferative responses of Th2 clones than those of Th1 clones [81]. At present, both SB207499 and CDP840 are undergoing clinical trials for the treatment of asthma [8]. Initial clinical results showed that CDP840 significantly ablated the late asthmatic response, but not the early asthmatic response to allergen in asthmatic subjects, suggesting that the anti-asthma effect of CDP840 is mediated mainly by its broad anti-inflammatory actions on immune and inflammatory cells. CDP840 was well-tolerated in the study and did not generate any nausea or vomiting in treated subjects [75]. Although these early clinical results offer optimism for a novel approach to treat asthma, the efficacy and improved safety profiles of selective PDE4 inhibitors remain to be confirmed.

PROTEIN TYROSINE KINASE INHIBITORS

Protein tyrosine kinases can be subdivided into two classes: receptor tyrosine kinases and non-receptor tyrosine kinases. The family of receptor tyrosine kinases includes the insulin receptor and receptors for various growth factors such as epidermal growth factor and platelet-derived growth factor. The family of non-receptor tyrosine kinases can be divided into eleven subfamilies, which include Src, Syk, JAK, Btk, and Csk, among many others [82]. The tyrosine kinase signaling cascade mediates a diverse array of cellular functions including proliferation, differentiation, cell survival, and acute immune reactions in inflammatory cells in response to growth factors, cytokines, chemokines, and neurotransmitters [82–84]. Recently, the role of certain subfamilies of non-receptor tyrosine kinases in allergic diseases such as asthma is emerging, and inhibitors of tyrosine kinases have been examined in various models of allergic inflammation [82].

Cumulative evidence shows that cross-linking of antigen-receptors on mast cells, T and B lymphocytes, and granulocytes results in instant activation of certain non-receptor tyrosine kinases [9, 82]. For instance, in mast cells, engagement of the FcεRI produces immediate activation of Lyn (Src-related tyrosine kinase), Syk, and Btk [85, 86], resulting in tyrosine phosphorylation and activation of downstream signaling molecules such as phospholipase Cγ1 and MAPK [87, 88], and eventually leading to mast cell degranulation. It has been shown that non-selective tyrosine kinase inhibitors including genistein, tyrphostin 47, lavendustin A, and methyl-2,5-dihydroxycinnamate significantly blocked antigen-induced protein tyrosine kinase activation and histamine release from mast cells [89, 90]. In an *in vitro* guinea pig model of allergic asthma, genistein and tyrphostin 47, two tyrosine kinase inhibitors, and PD098059, a MAPK kinase inhibitor, were found to attenuate antigen-induced airway smooth muscle contraction and release of histamine and peptidoleukotrienes from lung fragments [91, 92]. More recently, studies showed that selective inhibition of Syk by piceatannol [93] or ER-27319 [94], of Lyn by PP1 [95], or of Btk by terreic acid [96] resulted in inhibition of phospholipase Cγ1 activity, inositol 1,4,5-triphosphate generation, histamine and β-hexosaminidase release, and TNF-α production.

In eosinophils, studies showed that chemoattractants including RANTES, C5a, and platelet-activating factor were able to induce tyrosine kinase-mediated activation of PI3K, a downstream molecule of the tyrosine kinase signaling cascade [97]. Eotaxin-induced eosinophil chemotaxis and leukotriene B_4-induced respiratory burst in eosinophils were found to be inhibited by tyrosine kinase inhibitors such as herbimycin A, lavendustin A, and erbastatin [98, 99]. In addition, eosinophil degranulation induced by IgG and respiratory burst in eosinophils induced by zymosan could be blocked by LY294002, a selective inhibitor of PI3K [97, 100].

On the other hand, JAK family tyrosine kinases have been shown to play a critical role in cytokine receptor-mediated signal transduction and cellular responses. Each JAK member (JAK1–3 and Tyk 2) is physically associated with the intracellular domains of a variety of cytokine receptors, and each cytokine receptor can activate multiple JAKs. The activated JAKs, in turn, phosphorylate and activate a family of transcription factors called STATs for the regulation of cytokine-induced gene transcription [10, 82]. At present, seven distinct STATs (STAT1–4, STAT5a and 5b, and STAT6) have been identified. Recent findings revealed that IL-4 receptor activation

stimulates the activities of JAK1 and JAK3, leading to specific tyrosine phosphorylation and activation of STAT6 [10]. In mice made STAT6-deficient by targeted gene disruption, severe defects in IL-4-dependent immune responses were observed, which include impairments in Th2 cell differentiation, B-cell proliferation, immunoglobulin class switching to IgE, antigen-induced airway hyperresponsiveness and mucus production, and expression of cell surface markers such as CD23 and major histocompatibility complex class II [101–103]. Whereas specific inhibition of JAK1 or JAK3 likely will affect the cellular responses to multiple pleiotropic cytokines, which can be undesirable, selective inhibition of STAT6 appears to be an attractive therapeutic approach for IL-4-dependent allergic diseases such as asthma. It has been reported that leflunomide, a tyrosine kinase inhibitor, diminished tyrosine phosphorylation of STAT6 and prevented STAT6 from binding to the STAT6 DNA binding site in the IgG1 promoter region, resulting in a reduction in IgG1 production [104].

Substantial evidence supports the notion that Lyn, Syk, and JAK-stimulated STAT6 play a critical role in the pathogenesis of asthma, and inhibition of these signaling molecules likely will produce beneficial effects. However, protein tyrosine kinase constitutes an extraordinarily large family of kinases with multiple subtypes in each subfamily; this poses enormous obstacles to the design of inhibitors with high enough specificity for distinct tyrosine kinase subtypes [105]. The effectiveness and safety profile of this group of inhibitors for the treatment of asthma remain to be determined.

NF-κB INHIBITORS

In addition to the STATs, NF-κB represents another family of transcription factors that may play a pivotal role in chronic inflammation in asthma. At present, five known mammalian NF-κB/Rel proteins have been identified, and the prototypic activated form of NF-κB is a heterodimer consisting of the p50 and p65 subunits. In the absence of cell stimulation, NF-κB is localized to the cytoplasm as an inactive complex with the endogenous inhibitory protein, IκB, the most abundant isoform being IκBα. Upon cellular stimulation, IκBα is activated by IκBα-specific kinase complex (IKK), resulting in ubiquitination and degradation of IκB by the proteasome. This is followed by the translocation of the free active NF-κB to the nucleus, where it binds specific κB elements and initiates transcription of genes that encode pro-inflammatory proteins such as cytokines (IL-1β, TNF-α), chemokines (IL-8, RANTES, eotaxin), and adhesion molecules (VCAM-1, ICAM-1) [11].

In asthmatic bronchial epithelial cells, exposure to the allergen Der p1 resulted in NF-κB activation and increased expression of granulocyte macrophage-colony stimulating factor and RANTES [106]. In addition, airway epithelial cells and macrophages obtained from asthmatic patients, but not from chronic obstructive pulmonary disease patients, exhibited increased expression of the p65 subunit as well as increased NF-κB DNA binding [107]. It has been reported that mice deficient in the p50 subunit of NF-κB were incapable of mounting eosinophilic airway inflammation due to a diminished capacity to produce IL-5 and eotaxin [108]. All these point towards a putative role of NF-κB in the pathogenesis of asthma. Selective inhibition of this transcription factor, therefore, would seem to be a promising strategy for the treatment of this airway disease.

Most approaches developed to date are targeted at either the signaling pathway leading to the activation of NF-κB, or the binding of NF-κB to DNA. Inhaled glucocorticoids are the mainstay of asthma management, and they inhibit the production of many inflammatory products, some of which are a consequence of NF-κB activation [109]. Glucocorticoids have been shown to directly inhibit NF-κB via a protein–protein interaction between glucocorticoid receptors and NF-κB, and they may also enhance the expression of IκB in some cell types [110]. The immunosuppressive agents cyclosporin A and FK506 also have been shown to inhibit the activation of NF-κB, possibly via inhibition of calcineurin by the cyclosporin A/cyclophilin A or FK506/FKBP12 complex [111], or by inhibition of proteolysis of IκB by the proteasome [112]. On the other hand, the immunosuppressive agent PG490 (triptolide) inhibits NF-κB action by blocking transcriptional activation of NF-κB at a step after specific DNA binding [113]. Substances such as the antioxidant pyrrolidine dithiocarbamate, gold-containing compounds, aspirin, and other salicylates also have been shown to inhibit NF-κB [114]. All these compounds, however, are non-selective inhibitors of NF-κB and likely will produce substantial adverse effects. Nonetheless, they may be used as lead compounds for the development of more specific and more potent NF-κB inhibitors. Indeed, it has been shown that 4-trifluoromethyl derivatives of salicylate, such as triflusal and its metabolite 2-hydroxy-4-trifluoromethylbenzoic acid, are more potent inhibitors of TNF-α-induced NF-κB activation than are aspirin and sodium salicylate [115].

Other potent inhibitors of NF-κB activation include the serine protease inhibitor *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), and the tyrosine phosphatase inhibitor phenylarsine oxide (PAO) [116]. TPCK was shown to inhibit IκBα degradation in a concentration-dependent manner, whereas PAO completely abolished TNF-α-induced degradation of IκB and NF-κB activation. Several natural products also have been found to possess potent and specific NF-κB inhibitory activities. It was reported that gliotoxin, a fungal metabolite, prevented degradation of IκBα at nanomolar concentrations without affecting the activation of other transcription factors such as nuclear factor of activated T cells and CREB [117]. Likewise, hymenialdisine, a marine natural product, reduced DNA binding of NF-κB without affecting the activity of protein kinase C or the binding of activator protein-1, CCAAT/enhancer binding protein, and Sp1 to their DNA consensus motifs [118]. In addition, helenalin, a sesquiter-

pene lactone extracted from the flowerheads of *Arnica montana* and *A. chamissonis*, inhibited NF-κB activation by selectively alkylating the p65 subunit of NF-κB without affecting the activity of Oct-1, TBP, Sp1, and STAT5 [119, 120]. Other potential blockers of NF-κB activation include inhibitors of ubiquitin ligase [121], the proteasome [122], and IKK complexes [123].

CONCLUSION

This review has sought to provide the reader with current knowledge on the development of novel therapeutic agents targeted at several newly identified inflammatory processes or signaling molecules for the treatment of asthma. Some of these agents are already in their last phase of clinical trials, while the others are at the early stage of experimental investigation. The scope of this review is limited to those novel approaches that primarily modulate the unbalanced immunological responses in asthma. Therefore, the development of other novel pharmacological agents such as adenosine antagonists [124], tachykinin and kinin antagonists [125, 126], and K^+ channel openers [127] is not included.

Among the various strategies mentioned, anti-IgE mAb and PDE4 inhibitors are the two most promising approaches that have reached their final phase of clinical trials. Anti-IgE mAb likely will be the first therapeutic antibody to be used in asthma. The major concern about mAb therapy is the development of anti-idiotypic host antibodies, resulting in rapid clearance of the therapeutic mAb and prevention of repeated usage. However, the humanized rhuMAb-E25 seems to be well tolerated by test subjects, with hardly any incidence of it inducing anti-idiotypic responses. The second-generation PDE4 inhibitors have demonstrated potent anti-inflammatory effects against immune and inflammatory cells in asthmatics. The major adverse effects of nausea and vomiting associated with the use of rolipram, the first-generation PDE4 inhibitor, have been reduced substantially with the use of the second-generation PDE4 inhibitors (i.e. SB207499 and CDP840).

Although several clinical experiments reported beneficial effects using inhaled heparin in asthmatics, the usefulness of this agent is still limited by the concern of its inherent anticoagulant activity. Whereas heparin acts by physically binding to a variety of pro-inflammatory molecules, whether it may also non-selectively bind to and down-regulate some other endogenous anti-inflammatory molecules remains to be confirmed. It is essential to determine the optimal molecular weight range and the pattern and degree of sulphation of the so-called "NAF-heparin," and to compare the various aspects of anti-inflammatory activities of unfractionated heparin with those of NAF-heparin [128].

IL-4 traditionally has been regarded as a pro-inflammatory cytokine in asthma. As such, IL-4 soluble receptors and anti-IL-4 mAb have been developed as therapeutic approaches to neutralize the undesirable effects of this pleiotropic cytokine. Recent findings, however, indicated that IL-4 also possesses anti-inflammatory actions such as reduction in gene expression of RANTES [129], TNF-α and IL-1 [130], suppression of the biosynthesis of metalloproteinases [131], and inhibition of mitogen-induced proliferation of airway smooth muscle cells [132]. While IL-4 soluble receptors and anti-IL-4 mAb generally have demonstrated useful anti-inflammatory actions in animals and asthmatics, their long-term dampening effects on the anti-inflammatory actions of IL-4 cannot be ignored. In contrast, the expression of IL-5 receptor is restricted mainly to eosinophils and basophils. The use of anti-IL-5 mAb is, therefore, relatively specific for the attenuation of eosinophilic inflammation in asthma. On the other hand, it has been shown that binding of IL-4 and IL-5 to their cognate receptors activates multiple JAKs and STATs for cellular responses. Since STAT6 has been shown to be selectively stimulated by IL-4 receptor activation, it is an even more specific target for pharmacological intervention. Development of a STAT6 inhibitor has now become one of the major research efforts in the immunopharmacology of asthma.

On the other hand, Lyn and Syk are the two major non-receptor protein tyrosine kinases that are activated rapidly upon antigen receptor occupation, and are responsible for initiation of intracellular signal transduction upon antigen challenge. Inhibition of Lyn or Syk activity has been shown to interrupt the downstream signaling cascade and the antigenic responses. From the viewpoint of mechanism-based pharmacological research, Lyn and Syk are very attractive targets for drug development. However, the family of protein tyrosine kinases is so large, with multiple subfamilies, that it poses a huge, yet surmountable, hurdle for the design of drugs with high enough specificity for these two protein tyrosine kinase subtypes. NF-κB, a downstream transcription factor of the tyrosine kinase signaling cascade, is responsible for the production of a variety of pro-inflammatory cytokines and seems to be a promising therapeutic target for pharmacological manipulation. However, studies in knockout mice have demonstrated that NF-κB deficiency results in immune deficit or even lethal developmental abnormalities [133, 134]. Additional studies need to be carried out to determine the effectiveness and side-effect profiles of NF-κB inhibitors.

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