REVIEW

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Intracellular signaling pathways in IgE-dependent mast cell activation

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Abstract

Mast cells (MCs) are both central effectors and signaling cells in allergic reactions. Their key role in the immunopathology of asthma and other allergic diseases has been well documented. Molecular events leading to MC activation have not been yet fully established, however. Recent studies emphasize the key role of the protein tyrosine kinases Lyn and Fyn in MC signal transduction. The finding that Lyn kinase negatively regulates MC degranulation and that Fyn kinase enhances this effector response is of great importance. This creates new possibilities for therapeutic intervention in asthma and other allergic diseases. This review summarizes current knowledge on MC intracellular signaling and discusses the most recent strategies for the treatment of allergic diseases based on MC signaling pathway inhibition.

Key words: mast cell activation, intracellular signaling, FcεRI, Fyn kinase, Lyn kinase, phospholipase-C, MAPK.

Abbreviations: MC – mast cell, BMMCs – bone marrow-derived MC, Cbp – Csk-binding protein, CRH – corticotropin-releasing hormone, Csk – carboxy-terminal Src kinase, DAG – diacylglycerol, ERK – extracellular signal-regulated kinase, immunoglobulin E – IgE, FcεRI – high-affinity receptor for IgE, FcγRI – high-affinity receptor for IgG, Grb2 – growth factor receptor-bound 2, Gab2 – Grb2-associated binder protein 2, IP3 – inositol-1,4,5-trisphosphate, ITAMs – immunoreceptor tyrosine-based activation motifs, LAT – linker for T cell activation, MAPK – mitogen-activated protein kinase, MEK – mitogen-activated protein kinase, NF-κB – nuclear factor kappa B, PAR – protease-activated receptor, PI3K – phosphatidylinositol 3-OH kinase, PDK1 – PI3K-dependent protein kinase-1, PIP2 – phosphatidylinositol-4,5-bisphosphate, PIP3 – phosphatidylinositol-3,4,5-trisphosphate, PKC – protein kinase C, PLA2 – phospholipase A2, PLCγ1/2 – phospholipase Cγ1/2, PTK – protein-tyrosine kinase, SLP-76 – SH2-containing leukocyte-specific protein of 76 kDa, Syk – spleen tyrosine kinase, Vav1 – hematopoietic-specific guanine nucleotide exchange factor, VEGF – vascular endothelial growth factor.

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INTRODUCTION

Mast cells (MCs) play a central effector role in the development of allergic inflammatory reactions. In response to immunoglobulin E (IgE)-dependent or IgE-independent activation they produce and release a wide variety of preformed mediators (including histamine, neutral proteases, and proteoglycans), *de novo* synthesized lipids (the products of arachidonic acid, i.e. leukotrienes, prostaglandins, and thromboxanes) and many proinflammatory chemokinetic agents and immunomodulatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-3, IL-4, IL-6, IL-9, and IL-13 [42, 53]. The variety of chemical mediators leads to increased vascular permeability, tissue edema, bronchoconstriction, massive leukocyte recruitment, and inflammatory reaction in mucosa. IgE-dependent mechanisms, which result from allergen exposure, are crucial elements in the pathophysiology of asthma [29, 53]. Clinically, these processes are also important in anaphylaxis, angioedema, urticaria, and acute exacerbations of allergic rhinoconjunctivitis.

An important role of MCs in inducing chronic inflammation has also been confirmed. The accumulation and activation of MCs have been observed in several chronic inflammatory disorders, such as rheumatoid arthritis [9, 45], Crohn's disease [9], sarcoidosis, and multiple sclerosis [74]. Data obtained from histological studies have shown that MCs were noted to be involved in fibrotic reactions of inflammatory processes in scleroderma [1, 26], idiopathic pulmonary fibrosis [10], and glomerulonephritis [9]. Cardiac MCs have been suggested to be active and important in the pathogenesis of coronary spasm [16, 43], congestive heart failure devel-

opment [3, 25, 68, 91], as well as in the process of atherosclerosis and myocardial infarction [16, 43].

Several studies documented the important role of MCs in protective immunity against bacteria and helminthes [50, 92] and HIV infection [83]. It has also been demonstrated that MCs initiate adaptive immune response by their modulatory effect on dendritic cell trafficking [51]. In contrast to allergy, many of these latter MC-associated functions are initiated via IgE-independent activation pathways.

The majority of the molecular mechanisms responsible for MC degranulation have already been established. Even though our knowledge concerning the intracellular signaling pathways has markedly increased in recent years, further research is needed to elucidate the intracellular network. Understanding the molecular signaling during MC activation also has therapeutic implications. It holds the promise for the creation of novel and more selective drugs efficient not only in the treatment of allergic diseases, but also useful in other MC-related pathological disorders.

LYN KINASE IN THE IgE-DEPENDENT PATHWAY OF MASTOCYTE ACTIVATION

MCs are most frequently activated via the interaction of an allergen with the specific IgE bound to the high-affinity receptor for IgE (FcεRI), expressed on the MC surface [82]. The structure of FcεRI is well established [54, 55, 71]. The receptor belongs to the family of multi-subunit immune-response receptors and is expressed on the MC as a heterotetramer of an α, a β, and two γ chains. The α chain constitutes the extracellular component. Within this chain is located the binding site for IgE, responsible for ligand recognition. The β and γ subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs). Phosphorylation of ITAMs plays a crucial role in the amplification and downstream propagation of the intracellular signaling [38, 64]. The Src family non-receptor protein-tyrosine kinases (PTKs) are also involved in the phosphorylation process. Lyn has been described as the first kinase activated after FcεRI aggregation, and the Lyn-dependent molecular pathway has long been known as the only pathway for MC activation [94]. The detailed molecular mechanisms of the initial engagement of the Lyn kinase and FcεRI are, however, not yet fully understood. Recent studies have shown that the Lyn kinase is anchored in the plasma membrane and moves toward regions of FcεRI aggregation after stimulation of this receptor [41]. Kovarova et al. [41] revealed that the redistribution of Lyn kinase depends on fatty acylation in the N-terminal end of the molecule. Lack of acylation results in impaired anchoring of Lyn into the plasma membrane and early disruption of the early signaling pathway at the stage of FcεRI phosphorylation. If the redistribution of Lyn proceeds correctly, it binds to the β subunit of FcεRI and initiates the phosphorylation of the β- and γ-ITAMs [21, 41]. Phosphorylated γ-ITAMs then serve as binding sites for the Syk kinase that is also activated by Lyn [21, 77]. Syk kinase by itself is considered a downstream cellular signal amplifier. Several adapter proteins have been identified in a molecular pathway following Syk activation. Among theses, the linker for T cell activation (LAT) plays a crucial role in MC activation [73]. After Syk-phosphorylation, this membrane-located protein forms a molecular complex that includes other adapter proteins, such as SH2-containing leukocyte-specific protein of 76 kDa (SLP-76), growth factor receptor-bound (Grb)2, hematopoietic-specific guanine nucleotide exange factor (Vav1), and phospholipase Cγ1/2 (PLCγ1/2). Assembling of the LAT complex requires phosphorylation of at least four tyrosyl residues that are critical for PLCγ1/2 binding [98]. The lack of particular components of this complex has serious consequences for the MC response. Saitoh et al. [73] reported that a LAT deficiency in MCs results in the loss of calcium signal transduction and, in consequence, blocks MC degranulation due to inhibition of PLCγ1/2 activation. The SLP-76 null MCs are also defective in calcium signal transduction and subsequent degranulation [69]. In turn, phosphorylated PLCγ1/2 of the complex hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) to inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG), initiating the phospholipase-C-inositol-phosphate cascade, which was first described as a Syk-mediated pathway of signal transduction [7, 24]. In the next step, IP3 causes initial release of calcium ions from intracellular magazines, while DAG activates protein kinase C (PKC), responsible for promoting the activation of various molecular pathway transcription factors, which results in proinflammatory cytokines and growth factor synthesis [7]. The above interactions play a crucial role in MC activation, as it has been well established that MC degranulation critically depends on intracellular calcium concentration and the activation of PKC [65].

LYN-SYK-DEPENDENT PATHWAY OF MITOGEN-ACTIVATED PROTEIN KINASE IN SIGNAL TRANSDUCTION FROM THE SURFACE TO THE NUCLEUS

During the propagation of the Lyn-Syk-dependent pathway, the molecular events leading to the production of arachidonic metabolites are also activated. The hematopoietic-specific guanine nucleotide exchange factor Vav and the adapter protein Grb2, other components of the LAT-molecular membrane signaling complex, play a crucial role in the initiation of the mitogen-activated protein kinase (MAPK) pathway, resulting in the activation and regulation of cytosolic phospholipase A2 (PLA2) [30]. Recent advances in MAPK signaling research have been compiled by Armstrong [4]. The MAPKs are a group of protein (serine/threonine) kinases. They mediate signal transduction from the cell surface to the nucleus. MAPK activation largely requires Ras-dependent

stimulation. Phosphorylation of Grb2 in the LAT-complex initiates the activation of Ras protein in the presence of GDP/GTP. Ras protein binds directly to a serine-threonine kinase, Raf, forming a transient membrane-anchoring signal. Active Raf kinase phosphorylates MEK. MEK is a dual-specific kinase that in turn phosphorylates tyrosine and threonine residues by extracellular signal-regulated kinases (ERKs). The major targets of activated ERKs are pp90 ribosomal S6 kinase and the cytoplasmic PLA2. Once PLA2 is activated, it translocates into the cell membrane, where it releases arachidonic acid, triggering leukotrienes and prostaglandins formation [78].

The above-described molecular pathways of MC activation are Lyn kinase dependent. The linear model, with the central positive role of Lyn kinase as the only Src family PTK required for MC degranulation, has been long accepted. Findings in Lyn-deficient bone marrow-derived MCs (BMMCs) have partially changed this view. Nishizumi and Yamamoto [58] revealed impaired tyrosine phosphorylation and calcium mobilization, but not degranulation, in Lyn-deficient MCs. Further studies have demonstrated that degranulation of Lyn-deficient MCs was still massive even though FcεRI phosphorylation was reduced [37, 41]. These results suggested the existence of an alternative pathway with Lyn kinase-independent activity, which would be fundamental for MC effector response.

FYN-DEPENDENT SIGNALING PATHWAY IN MC DEGRANULATION

Recent studies have shown that Fyn kinase activity is responsible for the intense degranulation of Lyn-deficient MCs [59]. The Fyn-dependent signaling pathway and its role in MC degranulation were first described by Parravacini et al. [67]. Fyn kinase was shown to be a membrane-localized Src family non-receptor PTK. It is activated similarly to Lyn kinase, i.e. by the interaction of allergen with the specific IgE bound to the FcεRI on the MC surface. Fyn activation induces rapid phosphorylation of Grb2-associated binder protein 2 (Gab2), followed by formation of a signaling complex organized around Gab2 which contains tyrosine phosphatase and phosphatidylinositol 3-OH kinase (PI3K). Gab 2 has multiple binding sites for the p85 subunit of PI3K and functions as a critical regulator of PI3K activity [23, 47, 96]. Activation of PI3K results in the production of phosphatidylinositol-3,4,5-trisphosphate (PIP3), which plays an important role in calcium influx. Fyn-deficiency leads to impaired PI3K activity and MC degranulation, although the phosphorylation of FcεRI, Syk, LAT, and ERK2 still takes place [67]. Next, PIP3 acts as an activator of PI3K-dependent kinase 1 (PDK1), which is an effector of Gab signaling [23]. Then the protein kinase Akt is activated by PDK1. Akt regulates the activity of many transcription factors, such as nuclear factor (NF)-κB, NF-AT, and AP-1, crucial for the expression of the IL-2 and TNF-α genes [39].

Recent studies on Lyn-deficient MCs revealed no impairment of Gab2 activation [67]. Enhanced Fyn activation and increased levels of PIP3 were observed instead [59]. Moreover, research data demonstrate enhanced levels of serum IgE, increased cell numbers, increased cell surface expression of the high-affinity IgE receptor, and eosinophilia in Lyn-deficient mice, proving Lyn's role as a major player especially in intra-MC signaling. The reason for Fyn's hyperactivity is yet to be established. It has been suggested that impairment of the phosphorylation of the membrane scaffold adapter, Csk-binding protein (Cbp), plays an important role in MC responsiveness in Lyn-null mice [59]. Activated Cbp rejects the cell membrane Src PTK negative-regulatory kinase Csk, which is responsible for inactivating of Fyn phosphorylation [35, 60]. Thus, loss of Cbp phosphorylation in Lyn-deficient MC is likely to increase the fraction of active Src PTKs. These data explain the high Fyn activity in the absence of Cbp phosphorylation [59].

Recent findings point to Fyn kinase as a positive regulator of not only MC degranulation, but also of arachidonic acid metabolites. Studies on Fyn-deficient MCs revealed markedly decreased secretion of the leukotrienes B4 and C3 in the absence of Fyn [22]. The failure of Fyn-deficient MCs to release arachidonic acid and to generate both LTB4 and LTC4 was dependent on impaired phosphorylation of MAP kinases, which are implicated in eicosanoid production. Moreover, the same data showed a selective negative regulatory role of Fyn activation in the production of certain cytokines. The secretion of IL-2, IL-3, IL-6, and TNF-α was significantly reduced in Fyn-deficient MCs. A key regulatory signal for these cytokines, downstream of Fyn, is the transcription NF-κB. Fyn has been shown to be a positive regulator of NF-κB activation. It may be concluded that Fyn is positively required for MC degranulation and cytokine production; Lyn, in turn, negatively regulates the Gab2 pathways and the production of mediators, but positively regulates the activation of Syk and phosphorylation of LAT, a complex that is required for normal MC calcium-driven reactions.

Finally, the signaling pathways discussed above are brought together in Fig. 1, providing a summary of ways in which the cascades may interact.

IgE-INDEPENDENT MC ACTIVATION

MC degranulation following FcεRI aggregation is generally believed to be the major mechanism of MC activation, leading to its immunological response. However, recent data have revealed that other receptors expressed on the MC surface can promote MC degranulation and cytokine production. This IgE-independent activation plays an important role in the pathogenesis of various chronic inflammatory disorders, such as rheumatoid arthritis, multiple sclerosis, psoriasis, atherosclerosis, and many others (as well as non-atopic asthma and rhinitis).

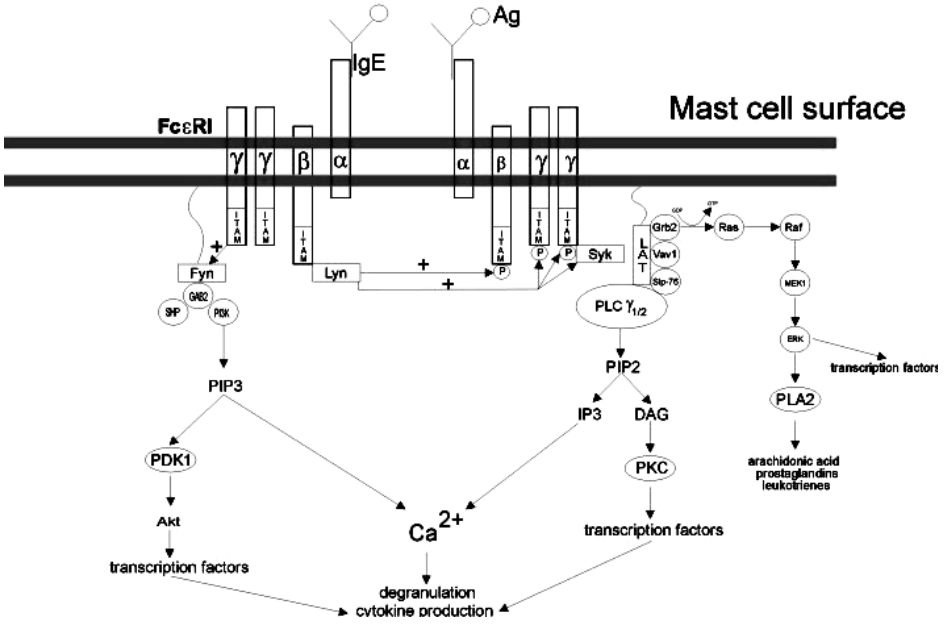

Fig. 1. FcεRI-mediated mast cell (MC) signal transduction. Interaction of multivalent antigen with FcεRI induces the activation of the non-receptor tyrosine kinases Lyn and Syk through the phosphorylation of FcεRI β and γ subunits. Activated Syk kinase together with the adapter proteins LAT, Grb2, Vav1, and SLP-76 initiates the phospholipase C-inositol-phosphate cascade. This Syk-dependent pathway involves the activation of PLCγ1/2 and hydrolysis of the PIP2 to IP3 and DAG. DAG activates PKC, responsible for the induction of transcription factors. IP3 initiates calcium influx and mobilization from intracellular stores. This results in MC degranulation and cytokine production. Activation of Ras and Raf via mitogen-activated protein kinase causes the production of arachidonic acid and its metabolites. The Fyn-dependent pathway leading to MC degranulation involves phosphorylation of the scaffolding adapter protein Gab2 and PI3K activation. Induction of PI3K results in the production of PIP3, which plays an important role in calcium influx. PIP3 serves as the first inductor of PI3K-dependent kinase 1, which participates in the activation of the protein kinase Akt. Akt regulates the activity of transcription factors.

It has been well documented that MCs express on their surface the high-affinity receptor for monomeric IgG (FcγRI) following interferon (IFN)-γ stimulation [62, 95]. The main source of IFN-γ are Th1 lymphocytes. The FcγRI comprises a single IgG-binding chain (FcγRIα) and an FcRγ homodimer, which is also expressed as part of the FcεRI [70]. It binds monomeric IgG as well as complexed IgG. The γ chains of this receptor contain ITAMs, which upon receptor crosslinking become phosphorylated. Sequential activation of Src and Syk family tyrosine kinases results in activation of a signaling cascade, similarly to that observed after FcεRI activation [88]. However, aggregation of the FcγRI induces a markedly higher degree of Syk phosphorylation [61]. Although both receptors similarly activate the MAPK- and PI3K-dependent pathways, the latter seems to be preferentially employed after FcγRI aggregation [63]. Consequently, markedly higher levels of proinflammatory cytokines, such as IL-1, IL-5, IL-6, IL-13, and especially TNF-α, are noted in response to FcγRI rather than FcεRI activation [61]. TNF-α has been shown to play a key role in inflammation associated with rheumatoid arthritis, Crohn's disease, and other autoimmune disorders. The above observations provide support to the concept that, in a Th1 environment, MCs could be involved in autoimmune disease induction.

Recent findings also indicate that MC may be implicated in chronic inflammatory diseases, especially those exacerbated by stress [76, 85]. Stress activates the hypothalamic-pituitary-adrenal axis through the release of corticotropin-releasing hormone (CRH). This results in the secretion of catecholamines and glucocorticoids and, consequently, suppression of the immune response [14]. On the other hand, acute stress reveals proinflammatory effects that appear to be mediated through activation of MCs [32, 87]. MCs are highly activated in areas which stain strongly positive for CRH. The number of MCs has been reported to be elevated in the joints of patients with rheumatoid arthritis and psoriasis, conditions known to be worsened by stress [28, 40]. Acute stress triggers MC degranulation and leads to increased CRH peptide content in rat skin [76]. Moreover, inflammatory arthritis, which does not occur in MC-deficient mice, is markedly attenuated in CRH-deficient mice [45]. Human MCs are reported to synthesize and secrete both CRH and urocortin in response to FcεRI crosslinking [86]. Recently, Cao et al. [12] investigated the expression of CRH receptors and CRH-induced MC activation in a human leukemic MC line. Their observations indicate that CRH-induced MC activation results in selective secretion of vascular endothelial growth factor (VEGF) without release of either preformed mediators or cytokines. It is well documented that VEGF is directly implicated in the pathogenesis of psoriasis, atopic dermatitis, and rheumatoid arthritis-conditions

worsened by stress [6, 57, 97]. Selective production of VEGF in MCs is mediated through the activation of the CRH receptor and adenylate cyclase with increased intracellular cAMP, activation protein kinase A, and MAPK signaling pathway [11]. CRH receptor antagonists could be used to inhibit MC activation and provide new therapeutic options for chronic inflammatory diseases exacerbated by stress.

Recent data have also shown that proinflammatory IL-1 selectively stimulates the secretion of IL-6 from human MCs [31]. In consequence, elevated levels of IL-6 in autoimmune disease and chronic inflammatory disorders are observed [66].

There is strong evidence that serine proteases (e.g. trypsin, chymotrypsin, thrombin) directly induce MC activation [20, 72]. Serine proteases have been known to be important regulators of many biological functions, such as hemostasis, fibrinolysis, tissue repair, and the digestion of dietary protein. They are also implicated in inflammatory responses through their interactions with the recently described protease-activated receptors (PARs) [79]. Generally, PARs play an important role in tissue remodeling, injury, and inflammation. Four types of PAR family receptors have been described [17]. PAR-1, PAR-3, and PAR-4 have been shown to be activated by thrombin, and PAR-2 is stimulated by trypsin, tryptase, and factor Xa [17, 49, 79]. Both PAR-1 and PAR-2 have been identified on the MC surface [80]. In previous studies it was shown that thrombin causes degranulation of rat and human BMMCs [90, 93]. Recently it was observed that *in vitro* stimulation of rat peritoneal MCs with thrombin induced a dose-dependent release of proinflammatory mediators. Similarly, PAR-2 agonists have also been shown to be important stimuli for MC activation in the gut [27]. Moreover, the antagonists of PAR-2 and PAR-4, such as curcumin, significantly inhibited MC degranulation in the gastrointestinal tract [5]. These findings support the hypothesis that MCs, through PAR-mediated degranulation, play an essential role in inflammatory bowel disease.

According to the observations that MCs are often found in close association with blood vessels and nerves, the concept of MC-related induction of neurogenic inflammation has been raised [56]. Recent studies confirmed this hypothesis. MCs have been shown to be activated by substance P released from sensory neurons via the neurokinin-1 receptor expressed on their surface. The response of MCs to the neuropeptide substance P is dependent on MC subtype and cytokine microenvironment (mucosal MCs are described as unresponsive) [44]. In rat's brain and human skin, rapid MC degranulation follows substance P stimulation [15]. However, this activation pathway results in markedly impaired eicosanoid production in comparison with the IgE-dependent response. It was reported earlier that BMMCs treated with recombinant stem cell factor and IL-4 gained the functional ability for arachidonic acid metabolite production [34]. More recently, Karimi et al. [33] reported that MCs stimulated by IL-3 do not respond to substance P by degranulation, but selectively generate prostaglandin D2 and leukotriene C4. Taken together, there are several IgE-independent MC-stimulation pathways in which the cytokine microenvironment determines whether MC stimulation leads only to lipid mediator secretion or full activation and degranulation.

THE BLOCKADE OF MC ACTIVATION: A NEW APPROACH TO MC-RELATED DISEASES

The possibility of a pharmacological blockade of several critical signaling molecules (such as tyrosine protein kinases) associated with MC activation has become an attractive target for anti-inflammatory therapy in MC-related diseases. Beneficial effects of such inhibitors have been reported in *in vivo* and *in vitro* studies of animal models of allergic inflammation.

Genistein [8, 84] and tyrphostin [36, 46], one of the first nonselective tyrosine kinase inhibitors described, have been shown to inhibit antigen-induced protein tyrosine phosphorylation and the subsequent release of preformed mediators from MCs. Genistein inhibits PTK activity through competitive inhibition at the ATP-binding domain of the kinase, whereas tyrphostin acts by competitive inhibition at the substrate site of the kinase [2]. Recently it has also been reported that they can markedly attenuate antigen-induced acute bronchoconstriction and airway hyperresponsiveness to inhaled methacholine in a guinea pig model of asthma [89]. Similar observations of Syk-selective inhibition have been published [75, 89]. Piceatannol, a Syk-selective inhibitor, significantly diminished antigen-induced bronchial smooth muscle contraction and release of histamine and leukotrienes from lung fragments isolated from sensitized guinea pigs [75]. In addition, Stenton et al. [81] showed that aerosolized Syk-selective antisense oligonucleotides (ASOs) suppressed antigen-induced contraction of isolated tracheal smooth muscle from sensitized rat. Their results also confirmed the beneficial role of ASOs in suppressing antigen-induced bronchial eosinophil infiltration and TNF-α production in a rat model of asthma [13]. Recently, an intranasally available selective Syk inhibitor (R112) was synthesized and the first study on its efficacy and safety in volunteers with seasonal allergic rhinitis was performed [52]. R112 was shown to markedly reduce allergic rhinitis symptoms compared with placebo. As early as 45 min after dosing, R112 showed a significant improvement in symptoms over placebo, and the duration of this effect exceeded 4 h [52]. Adverse effects were indistinguishable between the groups and clinically insignificant. Nevertheless, these promising results remain to be confirmed in further studies.

Other blockers of MC activation also represent attractive targets for anti-inflammatory therapy in allergic diseases. PD 098059, a selective MAPK kinase

inhibitor, and UO126, a potent and selective MEK1/2 blocker, are known to inhibit FcεRI-mediated signal transduction and airway hyperresponsiveness in mouse and rat models of asthma [19, 89]. Using LY294002, a highly specific inhibitor of PI3K, a significant suppression of antigen-induced inflammatory cell influx and cytokine production has been shown [18].

In summary, the tyrosine kinase signaling pathway inhibitors represent an attractive target for anti-inflammatory therapy in MC-related diseases. Preliminary results obtained from animal models are very promising, but further research is needed to verify their potential long-term clinical usefulness and safety in human.

FINAL REMARKS

Over the last few years, enormous progress has been made in mast cell signaling. New alternative pathways complementary to one another and many adapter proteins have been identified. The finding that Lyn kinase negatively regulates MC degranulation and that Fyn kinase enhances this effector response is of great importance. This creates new possibilities of therapeutic intervention in asthma and other allergic diseases.

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