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COMMENTARY

Molecular Consequences of Human Mast Cell Activation Following Immunoglobulin E–High-Affinity Immunoglobulin E Receptor (IgE–FcεRI) Interaction

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ABSTRACT. The cross-linking by immunoglobulin E of its high-affinity receptor, FcεRI, on mast cells initiates a complex series of biochemical events leading to degranulation and the synthesis and secretion of eicosanoids and cytokines through the action of transcription factors, such as nuclear factor-κB. The initial activation involves the phosphorylation of FcεRI β- and γ-subunits through the actions of the tyrosine kinases lyn and syk. For the purposes of description, the subsequent events may be grouped in three cascades characterized by the key proteins involved. First, the phospholipase C–inositol phosphate cascade activates protein kinase C and is largely responsible for calcium mobilization and influx. Second, activation of Ras and Raf via mitogen-activated protein kinase causes the production of arachidonic acid metabolites. Third, the generation of sphingosine and sphingosine-1-phosphate occurs through activation of sphingomyelinase. While the early signaling events tend to be specific for the cited cascades, there is an increasing overlap of activated proteins with the downstream propagation of the signal. It is the balanced interaction between these proteins that culminates in degranulation, synthesis, and release of eicosanoids and cytokines. BIOCHEM PHARMACOL **58**;12:1841–1850, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. mast cell; FcεRI; IgE; signal transduction; MAP kinase; inositol phosphate; sphingosine

A focal point of interest in allergy research is mast cell activation and degranulation. Although mast cells can be triggered by a number of mechanisms, efforts have been concentrated on IgE†-mediated mast cell activation and its physiological consequences. This activation is elicited through the cross-linking of allergen-specific IgE bound to the high-affinity receptor for IgE, FcεRI, on the mast cell membrane, which results in the degranulation of the mast cell and the release of mediators that further aggravate the ongoing allergic process (reviewed in Ref. 1). Cytokines are pivotal in establishing the characteristic inflammatory processes occurring in asthma, and a number of cells, including T cells, mast cells, and eosinophils contribute with different quantities and kinetics of production to the cytokine pool. The observation that mast cells produce a panel of multifunctional cytokines upon activation, together with their strategic location and elevated numbers in asthma [2], support the hypothesis that they are implicated not only in the genesis of acute bronchoconstriction, but also in the late-phase and chronic components of bronchial inflammation and airway hyperresponsiveness.

Mast cells arise from $CD34^+$ pluripotent bone marrow precursors *in vivo* and circulate through the blood as undifferentiated $CD34^+$, $FcεRI^-$, and $c\text{-}kit^+$ cells. Migration into tissue is followed by their differentiation, under the influence of SCF and other locally produced cytokines, and accompanied by the acquisition of FcεRI expression. *In vitro*, FcεRI expression on differentiating mast cell precursors is inducible in the presence of SCF, IL-3, IL-4, and IL-6 [3, 4]. On human FcεRI-positive mast cells, IgE or IL-4 exposure can induce a further increase of functional receptor expression through increased FcεRIα *de novo* synthesis [5]. This amplification mechanism is likely to contribute to the perpetuation of allergic inflammation, where IgE and IL-4 levels are abundant, a notion supported by recent findings on human tissue mast cells [6, 7].

FcεRI EXPRESSION

FcεRI belongs to the family of multi-subunit immune response receptors that do not have intrinsic kinase activity, but associate reversibly with PTK [8, 9]. In its "classical"

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† *Abbreviations*: IgE, immunoglobulin E; FcεRI, high-affinity IgE receptor; PLC, phospholipase C; PKC, protein kinase C; MAP kinase, mitogen-activated protein kinase; SCF, stem cell factor; IL, interleukin; PTK, protein tyrosine kinase; RBL-cells, rat basophilic leukemia cell line; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; NF-AT, Nuclear Factor of Activated T cells; Btk, Bruton's tyrosine kinase; PH domain, pleckstrin homology domain; FAK, focal adhesion kinase; FAP, FAK associated protein; PAF, platelet-activating factor; Pyk2, proline-rich tyrosine kinase 2; Erk, extracellular signal regulated kinase; Jnk, c-jun NH_2 terminal kinase; PLA_2, phospholipase A_2; TNF, tumor necrosis factor; CAP, ceramide-activated protein; and RHD, Rel-homology domain.

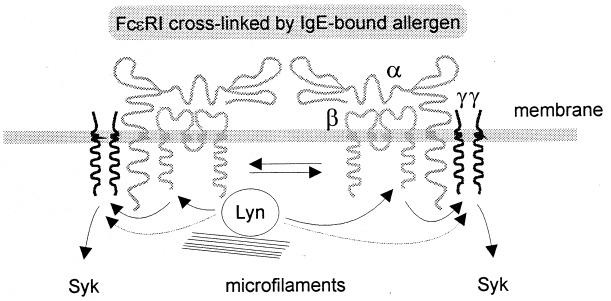

FIG. 1. The initial FcεRI signal. FcεRI cross-linking by IgE-bound antigen induces the recruitment of lyn, starting the signaling cascade and involving receptor trans-phosphorylation, the phosphorylation of the β- and γ-subunits, and the activation of syk.

form, FcεRI is expressed on the mast cell as a heterotetramer comprising α-, β-, and two γ-chains (Fig. 1). The binding site for IgE lies within the α-chain, which therefore confers ligand recognition and constitutes the major extracellular component of FcεRI. The α-chain carries seven N-linked glycosylation sites, which are essential for the proper folding of the α-subunit in the endoplasmic reticulum [10]. FcεRI surface expression is achieved after intracellular receptor assembly through γ-chain-mediated masking of a retention signal on the α-chain [11].

The β- and γ-subunits are responsible for the downstream propagation of the signal, through the phosphorylation of their ITAMs [12, 13]. The γ-dimer is essential for receptor surface expression and signal transduction, but is not a unique feature of FcεRI, since it is also a subunit of other Fc receptor complexes [14–16]. The FcεRI β-chain has been ascribed an amplifying role within the signaling pathway [17] and, in the human system, is not required for FcεRI expression. This characteristic allows for the FcεRI heterogeneity on human cells; i.e. the expression of the complete ("classical") $\alpha\beta\gamma_2$ complex on mast cells and basophils and $\alpha\gamma_2$ on monocytes and dendritic cells [18].

FcεRI Signal Transduction

In the following we attempt to give a concise summary of the signaling pathways activated through FcεRI cross-linking and their possible interactions. We will discuss the pathways separately (Figs. 1–4) and then bring them together in a summary (Fig. 5). This review is based essentially on findings in the rat basophilic leukemia (RBL) cell line, which is the predominant model system for studies on FcεRI signal transduction. It remains to be shown how well the signaling cascade elicited by FcεRI cross-linking on human mast cells matches the RBL model, particularly since results on cultured human mast cells indicate differences [19].

The Initial FcεRI Signal

FcεRI cross-linking is mediated through the interaction of multivalent antigen (allergen) with receptor-bound antigen-specific IgE, which leads to the recruitment of non-receptor tyrosine kinases (lyn, syk) to the receptor complex (Fig. 1). A number of models have been put forward to provide the theoretical background for the kinetics of this initial phosphorylation, which bears some unexpected findings. This concerns, in particular, the observations that (i) FcεRI phosphorylation reaches a plateau level well before the maximum aggregation of receptors, (ii) receptor aggregates formed late in the reaction do not signal as efficiently as those formed earlier on [8, 20], and (iii) there is a functional difference between stable and readily dissociable FcεRI cross-links [21]. The mechanisms proposed illustrate the complex interaction of FcεRI with its cellular environment and have been extrapolated from experimental evidence on RBL cells using ligands of well-defined structure and valency. First, the total receptor phosphorylation should be viewed as a dynamic balance between constant phosphorylation and dephosphorylation, modulated by phosphatases [22]; second, the receptor associates with detergent-resistant membrane domains and thus is influenced by membrane lipids and the cytoskeleton [21, 23, 24]; and third, the initiation kinase lyn has a dominant role on the FcεRI signal [25].

Generally, the more we learn about FcεRI signaling, the more complex the mechanism appears. The principle of the initial FcεRI signal is well characterized. It is mediated by the aggregation of receptors and the kinase lyn, resulting in the phosphorylation of the FcεRI β-chain ITAM, the FcεRI γ-chain ITAMs, and the recruitment of syk kinase to the γ-chain ITAMs (reviewed in Refs. 9, 26, and 27). The association of syk with the γ-chain causes activation and a conformational change [28], and, based on experimental evidence, this is the last step common to all FcεRI downstream signals. The requirement of syk for FcεRI protein tyrosine phosphorylation and degranulation has been demonstrated through combinatorial expression of FcεRI, lyn, and syk in fibroblasts [29] and syk reconstitution in a syk-negative RBL cell variant [30]. Cbl, the product of the c-*cbl* proto-oncogene, activated via FcεRI, has been characterised as a negative regulator of syk phosphorylation [31].

Lyn is constitutively associated with FcεRI, albeit weakly and at low levels, and is a limiting factor in the elicitation of the downstream signal. Upon receptor aggregation, lyn redistributes from the low-affinity binding sites on non-phosphorylated receptors to the high-affinity ITAM binding sites on the phosphorylated receptor units, shuttles between receptor-aggregates, and amplifies the signal through trans-phosphorylation [8, 20].

Nevertheless, according to a recent report, one of the FcεRI-induced signaling cascades, the activation of the phosphatases SHP-1, SHP-2, and SHIP (see Ras pathway Fig. 3), appears to be independent of syk activation [32, 33]. They associate with the phosphorylated FcεRI β-ITAM and contribute to the dynamic balance of FcεRI phosphorylation by dephosphorylating the β- and γ-ITAMs (SHP-1 and SHP-2) and inositol polyphosphate (SHIP) and estab-

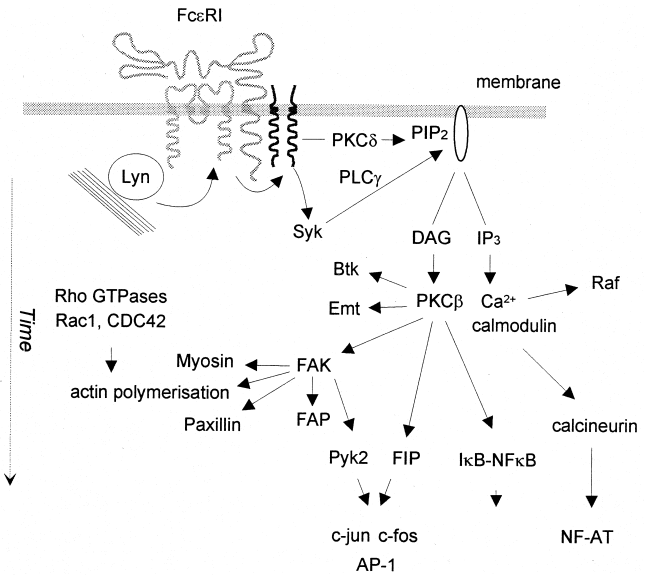

FIG. 2. The PLC–inositol phosphate cascade following lyn and syk activation and the hydrolysis of inositol phosphate. The crucial steps of this cascade are the activation of PKCβ and the mobilisation of intracellular calcium, both of which consequently activate their downstream effectors. Abbreviations: PKC, protein kinase C; PLC, phospholipase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; Btk, Bruton's tyrosine kinase; FAK, focal adhesion kinase; FIP, fos-interacting protein; NF-AT, Nuclear Factor of Activated T cells; and Ca^{2+}, intracellular calcium concentration.

lish the link to the ras pathway through their association with Grb2.

The Activation of Kinase Cascades

The FcεRI-mediated signal resembles a "snowball" effect, where increasing numbers of signaling molecules are activated during the downstream propagation of the signal. Although the cascades will be described one by one in the following (Figs. 2-4), it is important to note that they share a number of signaling components and thus form a closely interacting network, summarized in Fig. 5.

THE PLC–INOSITOL PHOSPHATE CASCADE. The first described and, therefore, best characterised signaling cascade involves syk-mediated phosphorylation of PLCγ (Fig. 2) (reviewed in Refs. 26 and 27). PLCγ, in turn, catalyzes the hydrolysis of PIP_2 to IP_3 and DAG. Inositol trisphosphate mediates the initial release of calcium from intracellular stores, followed by the influx of extracellular calcium, which binds to its receptor calmodulin, while DAG activates PKC. Both events are essential for mast cell degranulation and membrane ruffling [26, 34]. The calcium flux activates the calcium/calmodulin-dependent serine-threonine phosphatase calcineurin [35], which binds to the NF-AT family of transcription factors and causes their translocation to the nucleus (reviewed in Ref. 36; see Secretion and *De Novo* Synthesis). The PKC family of serine/threonine protein kinases consists of at least 12 isoforms; not all of them are expressed in mast cells, and it appears that they play different roles in receptor-mediated signaling and secretion [37]. PKC-δ, a calcium-independent isozyme, directly associates with the FcεRI γ-chain and promotes the hydrolysis of inositol phosphate [38], whereas the calcium-dependent isozyme PKC-β provides a link to the nuclear proteins c-Fos and c-Jun [39, 40] via fos-interacting protein [39] (see Ras Pathway). PKC regulates a number of downstream proteins; amongst them are the Tec-family kinases Btk and Emt. These kinases are activated enzymatically upon FcεRI cross-linking and regulated by PKC, to which they are associated via PH domains [41]. So far, reports on whether they are positively [42] or negatively [41] regulated by PKC are conflicting. Although their role in FcεRI-mediated signaling is not yet clearly established, two recent reports indicate that they are crucially important [43, 44]. PKC also promotes actin polymerization [45] and the activation of myosin [26], probably via FAK, ($pp125^{FAK}$); the latter in addition requires increased calcium levels for activation. FAK is also responsible for the phosphorylation of FAP, which may play a role in cell secretion [26]; paxillin, which forms a link between receptor-mediated signals and the cytoskeleton [26, 46]; and proline-rich tyrosine kinase 2 (Pyk2, RAFTK), a member of the FAK family, which phosphorylates potassium channels and acts as an upstream regulator of c-*jun* [47, 48]. The FcεRI-promoted actin polymerization and rearrangement of actin filaments are regulated by Rho GTPases, a family of ras-related small GTPases, in particular Rac1 and CDC42 (reviewed in Ref. 49). CDC42 controls cell adhesion and actin plaque assembly; Rac1 binds to tubulin [50], requires phosphatidylinositol-3-kinase for activation, and regulates the formation of membrane ruffles. GDP/GTP exchange on Rac1 also can be mediated by Vav [51] and is followed downstream by the activation of the transcription factor NF-AT [52].

THE RAS PATHWAY. As illustrated in Fig. 3, a number of signaling elements interact with the G-protein complex. Three negative regulators of FcεRI utilize this pathway: the above-mentioned syk-independent tyrosine phosphatases SHP-1 and SHP-2 [32] and the inositol-5-phosphatase SHIP [33, 53, 54]. SHP-2 reportedly associates with the adhesion molecule CD31 (PECAM-1) [55], which is tyrosine phosphorylated upon FcεRI cross-linking and is required for the transendothelial migration of leukocytes [56]. SHIP, on the other hand, has been identified as a "gatekeeper" for mast cell degranulation in SHIP knockout mice, where even monomeric IgE induced mast cell degranulation [57].

A molecule that requires prior activation of syk is the hematopoietic-specific guanine nucleotide exchange factor Vav, which controls actin polymerization via Rho GTPases [54, 58]. Vav forms a multimeric signaling complex with Shc, Grb2, and Sos [59, 60] and SLP-76 [58, 61]. This interaction mediates the downstream activation of MAP-

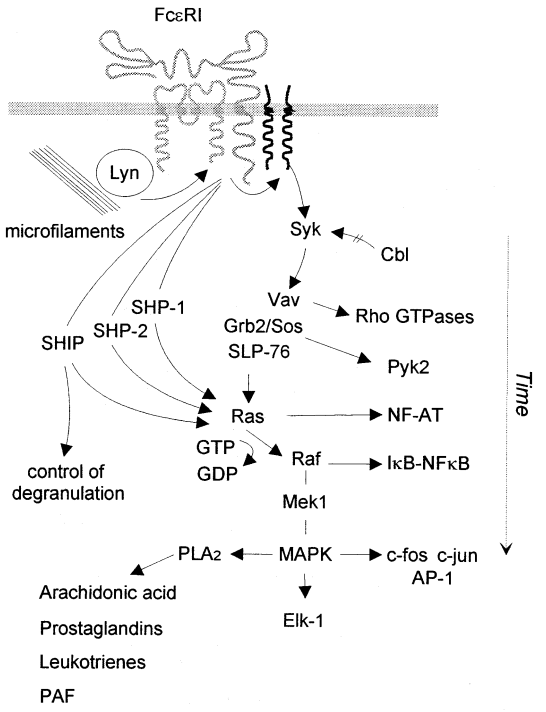

FIG. 3. The Ras pathway. A number of signaling elements can mediate the activation of Ras and Raf, which activate MAP kinase further downstream, and result, ultimately, in the release of arachidonic acid and its metabolites. Abbreviations: MEK1, MAP kinase kinase; MAP kinase, mitogen-activated protein kinase.

kinase via Ras and Raf, in the presence of GDP/GTP exchange [27, 59, 62]. Both Ras [63] and Raf [64] translocate to the cellular membrane upon activation, and for Ras the association with the membrane involves isoprenylation, proteolysis, and methylation (reviewed in Refs. 63 and 65). The above-mentioned Pyk2 [47, 48] can interact with Grb2 and thus establish a link between the G-protein- and the PLC/inositol phosphate pathways.

Erk, Jnk, and p38 MAP kinases are downstream components of this cascade [66] leading to nuclear import of a range of transcription factors including NFκB, NF-AT, ATF2, Elk-1 Jun, and SRF, and to activation of nuclear proteins, e.g. c-Fos and c-Jun [62]. It is proof of the close interaction between the FcεRI signaling pathways that Erk and Jnk activation occurs even in the presence of lovastatin, an inhibitor of isoprenoid metabolism, which prevents signaling via Ras [65, 67].

In addition, MAP-kinase activation regulates PLA_2 [68], which promotes the release of platelet-activating factor and the liberation of arachidonic acid, leukotrienes, and prostaglandins [69, 70].

THE SPHINGOSINE CASCADE. The connection of FcεRI to the ceramide/sphingosine pathway has been recognized only recently, and therefore the consequences are as yet poorly understood (Fig. 4) [71]. Earlier, this cascade was associated with growth factor signaling and cellular differentiation (reviewed in Refs. 72 and 73). It is initiated through the hydrolysis of sphingomyelin to ceramide plus fatty acid and sphingosine by sphingomyelinase. Sphingosine inhibits PKC [72] and PLCγ1 [74], and its second messenger sphingosine-1-phosphate mediates calcium release through its action on DAG [71, 75]. TNF and IL-1 utilize the sphingosine pathway, inducing increased ceramide levels, which activate CAP-phosphatase and the serine/threonine CAP-kinase. The latter interacts with Raf and thus forms a link to the MAP-kinase pathway and to NFκB activation [76].

Other Molecules Associated with FcεRI Signaling

The tyrosine phosphatases CD45 [77], HePTP [78], and the newly characterized MpIIPTP [79] have been implicated in FcεRI signaling, and so have the surface receptors CD81 [80] and CD28 [81] on murine mast cells. Further, the above-mentioned surface adhesion protein CD31 (PECAM-1) [56] is phosphorylated upon FcεRI aggregation, and can associate with SHP-2 [55].

The family of ITIM-bearing receptors are generally regarded as negative regulators of receptor-signaling via ITAM-motifs [82], and recently the inhibition of FcεRI signaling by co-cross-linking with FcγRIIb has been demonstrated on RBL cells [83].

Another group of regulatory structures is the membrane sphingo- and glycolipids. They have been attributed a role in cellular signaling in general [84] and in FcεRI signal transduction in particular. A number of studies demonstrate that an antibody against the ganglioside GD_{1b} on RBL cells (AA4) can act as a negative regulator of FcεRI activation [85]. The same ganglioside also has been identified as part of the detergent-resistant caveolae-like struc-

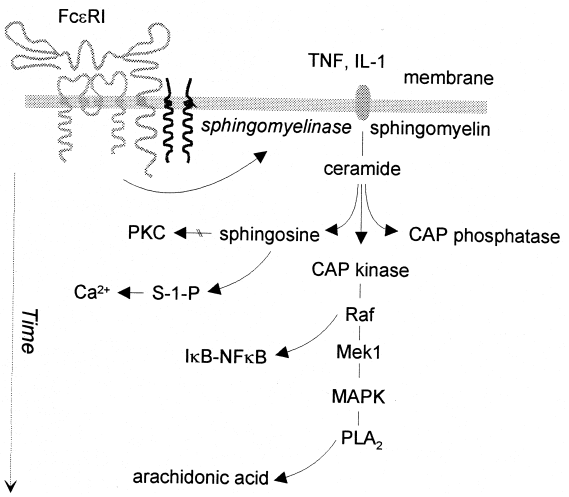

FIG. 4. The sphingosine cascade. Sphingomyelinase catalyses the metabolism of sphingomyelin into ceramide and sphingosine; the latter inhibits PKC, and further downstream sphingosine-1-phosphate mediates increased calcium flux. Ceramide, in addition, activates MAP kinase via intermediate steps. Abbreviations: CAP phosphatase, ceramide-activated protein phosphatase; CAP kinase, ceramide-activated protein kinase; PLA_2, phospholipase A_2; and S-1-P, sphingosine-1-phosphate.

tures, which contain the tyrosine kinase lyn [23] and thus may play a role in the recruitment of this kinase to the FcεRI complex. Another glycolipid with regulatory influence on FcεRI signaling is the C-type lectin MAFA (mast cell function-associated antigen), which upon clustering inhibits the FcεRI-induced RBL cell secretion [86]. Thus far, the human homologues for GD_{1b} or MAFA have not been identified.

Nitric oxide, released by activated accessory cells, directly inhibits the IgE-mediated secretory function of mast cells [87] and, thus, could control local mast cell activation.

Mast cell adhesion to fibronectin, on the other hand, augments the FcεRI-mediated signal [88], possibly through the activation of PKC via integrin receptors [89] and/or the involvement of FcγR in mediating the adhesion step [90]. Similarly, IgE-induced mediator release from mast cells is enhanced in the presence of IL-4, which also supports the development of human mast cells [91] and the expression of the cytokines IL-3, IL-4, IL-8, and GM-CSF [92].

Secretion and De Novo *Synthesis*

Gene regulation at the transcriptional level is mediated largely by the interaction of transcription factors with specific promoter elements. It is interesting to note that the various signaling cascades with their numerous activated components terminate in the activation of relatively few transcription factors. In their activated state, transcription factors translocate to the nucleus and interact with specific binding sites on the gene promoters, an event that promotes transcription followed by translation and the synthesis of precursor proteins. Furthermore, transcription factors do not act as single units but in groups, with the final outcome depending on the pattern of factors activated.

NFκB and Rel proteins constitute a family of transcription factors involved in the regulation of several cytokines (IL-1, IL-6, TNFα) and adhesion molecules. Rel proteins, which carry the RHD, form the NFκB dimer. RHD is responsible for DNA binding, dimerization, and interaction with IκB family members. Due to its homology in the RHD, NF-AT is sometimes referred to as a member of the Rel family. NF-AT was described initially as critical for IL-2 production in T cells, and later in transcriptional activation of IL-4, GM-CSF, and TNFα (reviewed in Refs. 36 and 93). NFκB, in its inactive cytoplasmic form, is bound to IκB. Upon cell activation, the complex dissociates and activated NFκB translocates to the nucleus to interact with DNA (reviewed in Ref. 94).

The first transcription factors reported to be activated in FcεRI stimulated murine mast cells were an AP3-like transcription factor regulating the chemokine gene MARC and a purported NF-AT family member. Both of these factors have been shown subsequently to be members of the NF-AT family regulating IL-5. They are dependent on Ras, but independent of AP-1 as a cofactor [95, 96]. The binding sequence of AP3 (TGTGGA/TA/TA/T) has been shown to be similar to that of the common binding sequences of NFκB, and NF-AT. It comprises a cytoplasmic component encoded by an extensive gene family including NF-ATp, NF-ATc, NF-AT-3, and NF-AT-4/x, the last being composed of AP-1 family members (Fos/Jun), which translocate to the nuclei of stimulated cells and complex with an inducible nuclear component.

The activation of PKC family members, particularly PKCβ and PKCε, leads to the modulation of gene expression through interaction with the transcription factor AP-1 (Figs. 2 and 5). The PKC family, therefore, not only is involved in early signal transduction (see the PLC–inositol phosphate cascade), but plays a dual role, participating also in late FcεRI signaling events. PKCα and PKCε are negative regulators of PLCγ1 activation [97], and PKCβ and PKCδ isozymes are linked to secretion [98]. The two major components of the AP-1 complex are the proto-oncogene products c-Jun and c-Fos, which form a stable heterodimer and regulate transcription through the AP-1 binding site [99].

The MAP kinase family mediates diverse effects via the activation of the transcription factors Elk-1, an immediate early gene regulator, and NF-AT (Figs. 3 and 5). Transcriptional activation of Elk-1 is dependent on its COOH-terminal phosphorylation by members of the MAP kinase family, Erk-1/2, the jun kinase JNK, or the p38 kinase. Despite the potential for Elk-1 to be targeted by multiple MAP kinases, the regulation of Elk-1 requires the activity of the Erk activating kinase MEK.

Ras function is required for FcεRI induction of NF-AT, but alone is not sufficient. The cascade apparently involves the GTPase Rac-1, rather than Raf-1 and MEK [51]. The GTPases Ras, Rac-1, and CDC42, as well as the calcium/calcineurin-signaling pathway, play a crucial role in mast cell activation and cytokine production via NF-AT [51, 100, 101].

MAP kinase, as mentioned above, mediates the activation of PLA_2, located in the cytosol and in mast cell secretory granules [70]. Phospholipid hydrolysis by PLA_2 yields lyso-PAF, which upon acylation produces PAF and lysophospholipid, a potent membrane detergent capable of causing cell lysis, and in addition causes the release of arachidonic acid [69, 70], which is metabolised by cyclooxygenases and lipoxygenases into eicosanoids. In mast cells, the primary cyclooxygenase product is prostaglandin D_2 and the primary lipoxygenase product is leukotriene C_4 [102].

Finally, the activation of the signaling cascades described results in cytokine production, mast cell degranulation, and the release of pre-formed and newly synthesised inflammatory mediators. The mechanisms operative in these final steps remain to be characterised. They clearly are preceded by the activation of the above-described signaling cascades and include a transient rise of free calcium and cyclic AMP in the cytosol, which enable microtubular assembly and the fusion of secretory granules with the plasma membrane. Phosphatidyl transfer proteins, in addition to their involvement in the described signals (PIP_2, IP_3, DAG), appear to

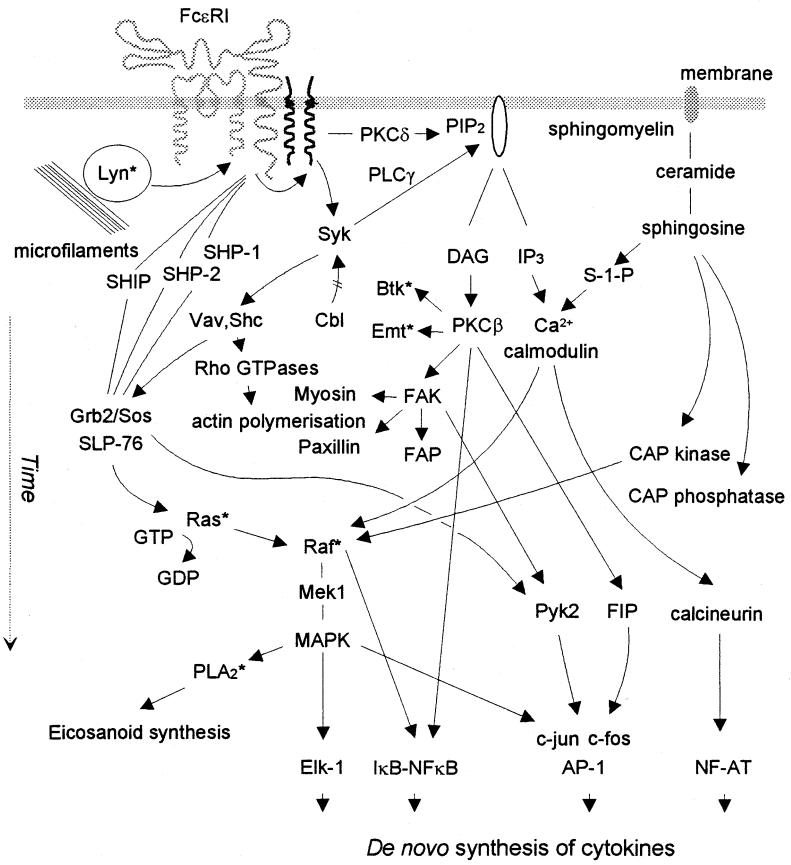

FIG. 5. The FcεRI signaling cascade. This is a compilation of the previous figures, with additional arrows indicating cross-talk between the pathways. Degranulation is not mentioned, since it requires cytoskeletal activation in combination with other signaling events. The asterisk (*) indicates proteins that are membrane-associated upon activation.

be involved in vesicular trafficking and cytoskeletal organisation, in particular 3-inositol lipids generated by phosphoinositide-3 kinases [103]. It is, however, clear that stimuli causing degranulation are not necessarily equipotent in releasing cytokines into the extracellular milieu: prostaglandins and lipopolysaccharide can induce IL-6 release in the absence of histamine liberation from rat peritoneal mast cells [104], and similarly, IFNα/β/γ inhibit TNFα secretion with no influence on histamine [105]. TNFα was shown to be generated *de novo* upon cell stimulation rather than being produced constitutively or stored in secretory granules [106].

SCF promotes development, survival, degranulation, and histamine release from both pulmonary and bone marrow-derived mast cells [107, 108] and, similar to FcεRI activation, induces increased mRNA for the AP-1 factors c-Fos, c-Jun, and Jun-B in mouse bone marrow-derived mast cells [109]. In particular, airway-derived mast cells proliferate in response to the single stimulus of SCF, IL-3, IL-4, or IL-10 [110]. SCF also promotes the generation of the CC-chemokine MCP-1 in human lung mast cells, an effect also seen with anti-IgE, and could, therefore, enable the mast cell to attract MCP-1-responsive cells to sites of inflammation [111]. Purified human lung mast cells constitutively express mRNA for IL-5, IL-6, IL-8, and TNFα, and stimulation with anti-IgE induces IL-4, GM-CSF, and increased levels of IL-5 and TNFα [1, 112, 113]. In asthmatic patients, the bronchial mast cells have been documented to be a source of IL-4, IL-5, IL-6, and TNFα [114].

CONCLUSION

Finally, the signaling pathways discussed above are brought together in Fig. 5, providing a summary of ways in which the cascades may interact. However, since the parts of this mosaic are pieced together from results obtained with various experimental models, the relative contributions and importance of the individual components in human mast cells remain to be determined.

References

1. Church MK and Levi-Schaffer F, The human mast cell. *J Allergy Clin Immunol* **99:** 155–160, 1997.
2. Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH and Holgate ST, Mucosal inflammation in asthma. *Am Rev Respir Dis* **142:** 434–457, 1990.
3. Metcalfe DD, Mekori JA and Rottem M, Mast cell ontogeny and apoptosis. *Exp Dermatol* **4:** 227–230, 1995.
4. Toru H, Ra C, Nonoyama S, Suzuki K, Yata J and Nakahata T, Induction of the high-affinity IgE receptor (FcεRI) on human mast cells by IL-4. *Int Immunol* **8:** 1367–1373, 1996.
5. Xia H-Z, Du Z, Craig S, Klisch G, Noben-Trauth N, Kochan JP, Huff TH, Irani A-MA and Schwartz LB, Effect of recombinant human IL-4 on tryptase, chymase, and Fcε receptor type I expression in recombinant human stem cell

factor-dependent fetal liver-derived human mast cells. *J Immunol* **159:** 2911–2921, 1997.
6. Pawankar R, Okuda M, Yssel H, Okumura K and Ra CS, Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* **99:** 1492–1499, 1997.
7. Rajakulasingam K, Durham SR, O'Brien F, Humbert M, Barata LT, Reece L, Kay AB and Grant JA, Enhanced expression of high-affinity IgE receptor (FcεRI) α chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. *J Allergy Clin Immunol* **100:** 78–86, 1997.
8. Wofsy C, Torigoe C, Kent UM, Metzger H and Goldstein B, Exploiting the difference between intrinsic and extrinsic kinases: Implications for regulation of signaling by immunoreceptors. *J Immunol* **159:** 5984–5992, 1997.
9. Jouvin M-H and Kinet J-P, Expression and function of the high-affinity receptor for IgE in asthma. In: *Asthma and Allergic Diseases: Physiology, Immunopharmacology and Treatment* (Eds. Marone G, Austen KF, Holgate ST, Kay AB and Lichtenstein LM), pp. 59–74. Academic Press, San Diego, 1998.
10. Letourneur O, Sechi S, Willette-Brown J, Robertson MW and Kinet J-P, Glycosylation of human truncated FcεRI α chain is necessary for efficient folding in the endoplasmic reticulum. *J Biol Chem* **270:** 8249–8256, 1995.
11. Letourneur F, Hennecke S, Démollière C and Cosson P, Steric masking of a dilysine endoplasmic reticulum retention motif during assembly of the human high affinity receptor for immunoglobulin E. *J Cell Biol* **129:** 971–978, 1995.
12. Cambier JC, Daëron M, Fridman W, Gergely J, Kinet JP, Klausner R, Lynch R, Malissen B, Pecht I, Reinherz E, Ravetch J, Reth M, Samelson L, Sandor M, Schreiber A, Seed B, Terhorst C, van de Winkel J and Weiss A, New nomenclature for the Reth motif (or ARH1/TAM/ARAM/YXXL). *Immunol Today* **16:** 110, 1995.
13. Jouvin M-H, Numerof RP and Kinet J-P, Signal transduction through the conserved motifs of the high affinity IgE receptor FcεRI. *Semin Immunol* **7:** 29–35, 1995.
14. Ernst LK, Duchemin A-M and Anderson CL, Association of the high-affinity receptor for IgG (FcγRI) with the γ subunit of the IgE receptor. *Proc Natl Acad Sci USA* **90:** 6023–6027, 1993.
15. Scholl PR and Geha RS, Physical association between the high-affinity IgG receptor (FcγRI) and the γ subunit of the high-affinity IgE receptor (FcεRIγ). *Proc Natl Acad Sci USA* **90:** 8847–8850, 1993.
16. Pfefferkorn LC and Yeaman GR, Association of IgA-Fc receptors (FcαR) with FcεRIγ_2 subunits in U937 cells: Aggregation induces the tyrosine phosphorylation of γ_2FcγRI. *J Immunol* **153:** 3228–3236, 1994.
17. Lin S, Cicala C, Scharenberg AM and Kinet J-P, The FcεRIβ subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. *Cell* **85:** 985–995, 1996.
18. Bieber T, FcεRI on antigen-presenting cells. *Curr Opin Immunol* **8:** 773–777, 1996.
19. Suzuki H, Takei M, Yanagida M, Nakahata T, Kawakami T and Fukamachi H, Early and late events in FcεRI signal transduction in human cultured mast cells. *J Immunol* **159:** 5881–5888, 1997.
20. Torigoe C, Goldstein B, Wofsy C and Metzger H, Shuttling of initiating kinase between discrete aggregates of the high affinity receptor for IgE regulates the cellular response. *Proc Natl Acad Sci USA* **94:** 1372–1377, 1997.
21. Pierini L, Harris NT, Holowka D and Baird B, Evidence supporting a role for microfilaments in regulating the coupling between poorly dissociable IgE-FcεRI aggregates and downstream signaling pathways. *Biochemistry* **36:** 7447–7456, 1997.
22. Paolini R, Jouvin M-H and Kinet J-P, Phosphorylation and dephosphorylation of the high-affinity receptor for immunoglobulin E immediately after receptor engagement and disengagement. *Nature* **353:** 855–858, 1991.
23. Holowka D and Baird B, Antigen-mediated IgE receptor aggregation and signaling: A window on cell surface structure and dynamics. *Annu Rev Biophys Biomol Struct* **25:** 79–112, 1996.
24. Sheets ED, Holowka D and Baird B, Membrane organization in immunoglobulin E receptor signaling. *Curr Opin Chem Biol* **3:** 95–99, 1999.
25. Ortega E, Lara A, Lee I, Santana C, Martinez AM, Pfeiffer JR, Lee RJ, Wilson BS and Oliver JM, Lyn dissociation from phosphorylated FcεRI subunits: A new regulatory step in the FcεRI signaling cascade revealed by studies of FcεRI dimer signaling activity. *J Immunol* **162:** 176–185, 1999.
26. Hamawy MM, Mergenhagen SE and Siraganian RP, Protein tyrosine phosphorylation as a mechanism of signalling in mast cells and basophils. *Cell Signal* 7: 535–544, 1995.
27. Beaven MA and Baumgartner RA, Downstream signals initiated in mast cells by FcεRI and other receptors. *Curr Opin Immunol* **8:** 766–772, 1996.
28. Shiue L, Zoller MJ and Brugge JS, Syk is activated by phosphotyrosine-containing peptides representing the tyrosine-based activation motifs of the high affinity receptor for IgE. *J Biol Chem* **270:** 10498–10502, 1995.
29. Scharenberg AM, Lin S, Cuenod B, Yamamura H and Kinet J-P, Reconstitution of interactions between tyrosine kinases and the high affinity IgE receptor which are controlled by receptor clustering. *EMBO J* **14:** 3385–3394, 1995.
30. Zhang J, Berenstein EH, Evans RL and Siraganian RP, Transfection of Syk protein tyrosine kinase reconstitutes high affinity IgE receptor-mediated degranulation in a Syk-negative variant of rat basophilic leukemia RBL-2H3 cells. *J Exp Med* **184:** 71–79, 1996.
31. Ota Y and Samelson LE, The product of the proto-oncogene c-*cbl*: A negative regulator of the Syk tyrosine kinase. *Science* **276:** 418–420, 1997.
32. Kimura T, Zhang J, Sagawa K, Sakaguchi K, Appella E and Siraganian RP, Syk-independent tyrosine phosphorylation and association of the protein tyrosine phosphatases SHP-1 and SHP-2 with the high affinity IgE receptor. *J Immunol* **159:** 4426–4434, 1997.
33. Kimura T, Sakamoto H, Appella E and Siraganian RP, The negative signaling molecule SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP) binds to the tyrosine-phosphorylated β subunit of the high affinity IgE receptor. *J Biol Chem* **272:** 13991–13996, 1997.
34. Hirasawa N, Sato Y, Yomogida S, Mue S and Ohuchi K, Role of phosphatidylinositol 3-kinase in degranulation induced by IgE-dependent and -independent mechanisms in rat basophilic RBL-2H3 (ml) cells. *Cell Signal* **9:** 305–310, 1997.
35. Klee CB, Ren H and Wang X, Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. *J Biol Chem* **273:** 13367–13370, 1998.
36. Rao A, Luo C and Hogan PG, Transcription factors of the NFAT family: Regulation and function. *Annu Rev Immunol* **15:** 707–747, 1997.
37. Chang E-Y, Szallasi Z, Acs P, Raizada V, Wolfe PC, Fewtrell C, Blumberg PM and Rivera J, Functional effects of overexpression of protein kinase C-α, -β, -δ, -ε, and -ζ in the mast cell line RBL-2H3. *J Immunol* **159:** 2624–2632, 1997.
38. Haleem-Smith H, Chang EY, Szallasi Z, Blumberg PM and Rivera J, Tyrosine phosphorylation of protein kinase C-δ in response to the activation of the high-affinity receptor for

immunoglobulin E modifies its substrate recognition. *Proc Natl Acad Sci USA* **92:** 9112–9116, 1995.
39. Lewin I, Jacob-Hirsch J, Zang ZC, Kupershtein V, Szallasi Z, Rivera J and Razin E, Aggregation of the FcεRI in mast cells induces the synthesis of Fos-interacting protein and increases its DNA binding-activity: The dependence on protein-kinase C-β. *J Biol Chem* **271:** 1514–1519, 1996.
40. Hutchinson LE and McCloskey MA, FcεRI-mediated induction of nuclear factor of activated T-cells. *J Biol Chem* **270:** 16333–16338, 1995.
41. Kawakami Y, Yao LB, Han W and Kawakami T, Tec family protein-tyrosine kinases and pleckstrin homology domains in mast cells. *Immunol Lett* **54:** 113–117, 1996.
42. Kawakami Y, Yao L, Tashiro M, Gibson S, Mills GB and Kawakami T, Activation and interaction with protein kinase C of a cytoplasmic tyrosine kinase, Itk/Tsk/Emt, on FcεRI cross-linking on mast cells. *J Immunol* **155:** 3556–3562, 1995.
43. Hata D, Kitaura J, Hartman SE, Kawakami Y, Yokota T and Kawakami T, Bruton's tyrosine kinase-mediated interleukin-2 gene activation in mast cells. Dependence on the c-Jun N-terminal kinase activation pathway. *J Biol Chem* **273:** 10979–10987, 1998.
44. Bolland S, Pearse RN, Kurosaki T and Ravetch JV, SHIP modulates immune receptor responses by regulating membrane association of Btk. *Immunity* **8:** 509–516, 1998.
45. Apgar JR, Polymerization of actin in RBL-2H3 cells can be triggered through either the IgE receptor or the adenosine receptor but different signaling pathways are used. *Mol Biol Cell* **5:** 313–322, 1994.
46. Hamawy MM, Swieter M, Mergenhagen SE and Siraganian RP, Reconstitution of high affinity IgE receptor-mediated secretion by transfecting protein tyrosine kinase $pp125^{FAK}$. *J Biol Chem* **272:** 30498–30503, 1997.
47. Torigoe C, Tadakuma T and Nakanishi M, Single-cell observation of calcium signals in T cells and antigen-presenting cells during antigen presentation. *Immunol Lett* **46:** 75–79, 1995.
48. Okazaki H, Zhang J, Hamawy MM and Siraganian RP, Activation of protein-tyrosine kinase Pyk2 is downstream of Syk in FcεRI signaling. *J Biol Chem* **272:** 32443–32447, 1997.
49. Hall A, Rho GTPases and the actin cytoskeleton. *Science* **279:** 509–514, 1998.
50. Best A, Ahmed S, Kozma R and Lim L, The Ras-related GTPase Rac1 binds tubulin. *J Biol Chem* **271:** 3756–3762, 1996.
51. Guillemot J-C, Montcourrier P, Vivier E, Davoust J and Chavrier P, Selective control of membrane ruffling and actin plaque assembly by the Rho GTPases Rac1 and CDC42 in FcεRI-activated rat basophilic leukemia (RBL-2H3) cells. *J Cell Sci* **110:** 2215–2225, 1997.
52. Turner H, Gomez M, McKenzie E, Kirchem A, Lennard A, and Cantrell DA, Rac-1 regulates nuclear factor of activated T cells (NFAT) C1 nuclear translocation in response to Fcε receptor type 1 stimulation of mast cells. *J Exp Med* **188:** 527–537, 1998.
53. Osborne MA, Zenner G, Lubinus M, Zhang XL, Songyang Z, Cantley LC, Majerus P, Burn P and Kochan JP, The inositol 5′-phosphatase SHIP binds to immunoreceptor signaling motifs and responds to high affinity IgE receptor aggregation. *J Biol Chem* **271:** 29271–29278, 1996.
54. Teramoto H, Salem P, Robbins KC, Bustelo XR and Gutkind JS, Tyrosine phosphorylation of the *vav* proto-oncogene product links FcεRI to the Rac1-JNK pathway. *J Biol Chem* **272:** 10751–10755, 1997.
55. Sagawa K, Kimura T, Swieter M and Siraganian RP, The protein-tyrosine phosphatase SHP-2 associates with tyrosine-phosphorylated adhesion molecule PECAM-1 (CD31). *J Biol Chem* **272:** 31086–31091, 1997.
56. Sagawa K, Swaim W, Zhang J, Unsworth E and Siraganian RP, Aggregation of the high affinity IgE receptor results in the tyrosine phosphorylation of the surface adhesion protein PECAM-1 (CD31). *J Biol Chem* **272:** 13412–13418, 1997.
57. Huber M, Helgason CD, Damen JE, Liu L, Humphries RK and Krystal G, The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. *Proc Natl Acad Sci USA* **95:** 11330–11335, 1998.
58. Fischer KD, Tedford K and Penninger JM, Vav links antigen-presentation signaling to the actin cytoskeleton. *Semin Immunol* **10:** 317–327, 1998.
59. Jabril-Cuenod B, Zhang C, Scharenberg AM, Paolini R, Numerof R, Beaven MA and Kinet J-P, Syk-dependent phosphorylation of Shc: A potential link between FcεRI and the Ras/mitogen-activated protein kinase signaling pathway through SOS and Grb2. *J Biol Chem* **271:** 16268–16272, 1996.
60. Song JS, Gomez J, Stancato LF and Rivera J, Association of a p95 Vav-containing signaling complex with the FcεRI γ-chain in the RBL-2H3 mast cell line. Evidence for a constitutive *in vivo* association of Vav with Grb2, Raf-1, and ERK2 in an active complex. *J Biol Chem* **271:** 26962–26970, 1996.
61. Hendricks-Taylor LR, Motto DG, Zhang J, Siraganian RP and Koretzky GA, SLP-76 is a substrate of the high affinity IgE receptor-stimulated protein tyrosine kinases in rat basophilic leukemia cells. *J Biol Chem* **272:** 1363–1367, 1997.
62. Graves JD, Campbell JS and Krebs EG, Protein serine/threonine kinases of the MAPK cascade. *Ann NY Acad Sci* **766:** 320–343, 1996.
63. Rommel C and Hafen E, Ras—a versatile cellular switch. *Curr Opin Genet Dev* **8:** 412–418, 1998.
64. Morrison DK and Cutler RE, The complexity of Raf-1 regulation. *Curr Opin Cell Biol* **9:** 174–179, 1997.
65. Zhang FL and Casey PJ, Protein prenylation: Molecular mechanisms and functional consequences. *Annu Rev Biochem* **65:** 241–269, 1996.
66. Burgering BMT and Bos JL, Regulation of Ras-mediated signaling: More than one way to skin a cat. *Trends Biochem Sci* **20:** 18–22, 1995.
67. Graham TE, Pfeiffer JR, Lee RJ, Kusewitt DF, Martinez AM, Foutz T, Wilson BS and Oliver JM, MEK and ERK activation in Ras-disabled RBL-2H3 mast cells and novel roles for geranylgeranylated and farnesylated proteins in FcεRI-mediated signaling. *J Immunol* **161:** 6733–6744, 1998.
68. Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, Tischfield JA and Kudo I, The functions of five distinct mammalian phospholipase A_2s in regulating arachidonic acid release. Type IIA and type V secretory phospholipase A_2s are functionally redundant and act in concert with cytosolic phospholipase A_2. *J Biol Chem* **273:** 14411–14423, 1998.
69. Hirasawa N, Santini F and Beaven MA, Activation of the mitogen-activated protein kinase/cytosolic phospholipase A_2 pathway in a rat mast cell line. Indications of different pathways for release of arachidonic acid and secretory granules. *J Immunol* **154:** 5391–5402, 1995.
70. Chock SP, Schmauder-Chock EA, Cordella-Miele E, Miele L and Mukherjee AB, The localization of phospholipase A_2 in the secretory granule. *Biochem J* **300:** 619–622, 1994.
71. Choi OH, Kim JH and Kinet J-P, Calcium mobilization via sphingosine kinase in signalling by the FcεRI antigen receptor. *Nature* **380:** 634–636, 1996.
72. Hannun YA and Bell RM, Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. *Science* **243:** 500–507, 1989.

73. Gómez-Muñoz A, Modulation of cell signalling by ceramides. *Biochim Biophys Acta* **1391:** 92–109, 1998.
74. Matecki A, and Pawelczyk T, Regulation of phospholipase C δ1 by sphingosine. *Biochim Biophys Acta* **1325:** 287–296, 1997.
75. Desai NN, Zhang H, Olivera A, Mattie ME and Spiegel S, Sphingosine-1-phosphate, a metabolite of sphingosine, increases phosphatidic acid levels by phospholipase D activation. *J Biol Chem* **267:** 23122–23128, 1992.
76. Kolesnick R and Golde DW, The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. *Cell* **77:** 325–328, 1994.
77. Adamczewski M, Numerof RP, Koretzky GA and Kinet J-P, Regulation by CD45 of the tyrosine phosphorylation of high affinity IgE receptor β- and γ-chains. *J Immunol* **154:** 3047–3055, 1995.
78. Swieter M, Berenstein EH, Swaim WD and Siraganian RP, Aggregation of IgE receptors in rat basophilic leukemia 2H3 cells induces tyrosine phosphorylation of the cytosolic protein-tyrosine phosphatase HePTP. *J Biol Chem* **270:** 21902–21906, 1995.
79. Hampe CS and Pecht I, Purification and preliminary characterization of an Fcε-receptor-activated protein-tyrosine phosphatase from mast cells. *Eur J Biochem* **251:** 964–970, 1998.
80. Fleming TJ, Donnadieu E, Song CH, Van Laethem F, Galli SJ and Kinet J-P, Negative regulation of FcεRI-mediated degranulation by CD81. *J Exp Med* **186:** 1307–1314, 1997.
81. Tashiro M, Kawakami Y, Abe R, Han W, Hata D, Sugie K, Yao L and Kawakami T, Increased secretion of TNF-α by costimulation of mast cells via CD28 and FcεRI. *J Immunol* **158:** 2382–2389, 1997.
82. Vivier E and Daëron M, Immunoreceptor tyrosine-based inhibition motifs. *Immunol Today* **18:** 286–291, 1997.
83. Malbec O, Fong DC, Turner M, Tybulewicz VLJ, Cambier JC, Fridman W and Daëron M, Fcε receptor I-associated *lyn*-dependent phosphorylation of Fcγ receptor IIB during negative regulation of mast cell activation. *J Immunol* **160:** 1647–1658, 1998.
84. Brown RE, Sphingolipid organization in biomembranes: What physical studies of model membranes reveal. *J Cell Sci* **111:** 1–9, 1998.
85. Swaim WD, Minoguchi K, Oliver C, Hamawy MM, Kihara H, Stephan V, Berenstein EH and Siraganian RP, The anti-ganglioside monoclonal antibody AA4 induces protein tyrosine phosphorylations, but not degranulation, in rat basophilic leukemia cells. *J Biol Chem* **269:** 19466–19473, 1994.
86. Bocek P Jr., Guthmann MD and Pecht I, Analysis of the genes encoding the mast cell function-associated antigen and its alternatively spliced transcripts. *J Immunol* **158**: 3235–3243, 1997.
87. Eastmond NC, Banks EMS and Coleman JW, Nitric oxide inhibits IgE-mediated degranulation of mast cells and is the principal intermediate in IFN-γ-induced suppression of exocytosis. *J Immunol* **159:** 1444–1450, 1997.
88. Hamawy MM, Mergenhagen SE and Siraganian RP, Cell adherence to fibronectin and the aggregation of the high affinity immunoglobulin E receptor synergistically regulate tyrosine phosphorylation of 105–115-kDa proteins. *J Biol Chem* **268:** 5227–5233, 1993.
89. Wolfe PC, Chang E-Y, Rivera J and Fewtrell C, Differential effects of the protein kinase C activator phorbol 12-myristate 13-acetate on calcium responses and secretion in adherent and suspended RBL-2H3 mucosal mast cells. *J Biol Chem* **271**: 6658–6665, 1996.
90. Dastych J, Hardison MC and Metcalfe DD, Aggregation of low affinity IgG receptors induces mast cell adherence to fibronectin: Requirement for the common FcR γ-chain. *J Immunol* **158:** 1803–1809, 1997.
91. Toru H, Eguchi M, Matsumoto R, Yanagida M, Yata J and Nakahata T, Interleukin-4 promotes the development of tryptase and chymase double-positive human mast cells accompanied by cell maturation. *Blood* **91:** 187–195, 1998.
92. Coleman JW, Buckley MG, Taylor AM, Banks EM, Williams CM, Holliday MR and Thompson J, Effects of interleukin-4 or stem cell factor on mast cell mediator release and cytokine gene expression. *Int Arch Allergy Immunol* **107:** 154–155, 1995.
93. Weiss DL, Hural J, Tara D, Timmerman LA, Henkel G and Brown MA, Nuclear factor of activated T cells is associated with a mast cell interleukin 4 transcription complex. *Mol Cell Biol* **16:** 228–235, 1996.
94. Ghosh S, May MJ and Kopp EB, NF-κB and Rel proteins, Evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* **16:** 225–260, 1998.
95. Prieschl EE, Pendl GG, Harrer NE and Baumruker T, $p21^{ras}$ links FcεRI to NF-AT family member in mast cells. The AP3-like factor in this cell type is an NF-AT family member. *J Immunol* **155:** 4963–4970, 1995.
96. Jarmin DI, Kulmburg PA, Huber NE, Baumann G, Prieschl-Strassmayr EE and Baumruker T, A transcription factor with AP3-like binding specificity mediates gene regulation after an allergic triggering with IgE and Ag in mouse mast cells. *J Immunol* **153:** 5720–5729, 1994.
97. Ozawa K, Yamada K, Kazanietz MG, Blumberg PM and Beaven MA, Different isozymes of protein kinase C mediate feedback inhibition of phospholipase C and stimulatory signals for exocytosis in rat RBL-2H3 cells. *J Biol Chem* **268:** 2280–2283, 1993.
98. Ozawa K, Szallasi Z, Kazanietz MG, Blumberg PM, Mischak H, Mushinski JF and Beaven MA, Ca^{2+}-dependent and Ca^{2+}-independent isozymes of protein kinase C mediate exocytosis in antigen-stimulated rat basophilic RBL-2H3 cells. Reconstitution of secretory responses with Ca^{2+} and purified isozymes in washed permeabilized cells. *J Biol Chem* **268:** 1749–1756, 1993.
99. Razin E, Szallasi Z, Kazanietz MG, Blumberg PM and Rivera J, Protein-kinases C-β and C-ε link the mast-cell high-affinity receptor for IgE to the expression of c-fos and c-jun. *Proc Natl Acad Sci USA* **91:** 7722–7726, 1994.
100. Turner H and Cantrell DA, Distinct Ras effector pathways are involved in FcεRI regulation of the transcriptional activity of Elk-1 and NFAT in mast cells. *J Exp Med* **185:** 43–53, 1997.
101. Woodrow M, Clipstone NA and Cantrell D, $p21^{ras}$ and Calcineurin synergize to regulate the nuclear factor of activated T cells. *J Exp Med* **178:** 1517–1522, 1993.
102. Church MK, Benyon RC, Rees PH, Lowman MA, Campbell AM, Robinson C and Holgate ST, Functional heterogeneity of human mast cells. In: *Mast Cell and Basophil Differentiation and Function in Health and Disease* (Eds. Galli SJ and Austen KF), pp. 161–170. Raven Press, New York, 1989.
103. Cockcroft S, Phosphatidylinositol transfer proteins: A requirement in signal transduction and vesicle traffic. *Bioessays* **20:** 423–432, 1998.
104. Leal-Berumen I, Conlon P and Marshall JS, IL-6 production by rat peritoneal mast cells is not necessarily preceded by histamine release and can be induced by bacterial lipopolysaccharide. *J Immunol* **152:** 5468–5476, 1994.
105. Bissonnette EY, Chin B and Befus AD, Interferons differentially regulate histamine and TNF-α in rat intestinal mucosal mast cells. *Immunology* **86:** 12–17, 1995.
106. Baumgartner RA, Yamada K, Deramo VA and Beaven MA, Secretion of TNF from a rat mast cell line is a brefeldin

A-sensitive and a calcium/protein kinase C-regulated process. *J Immunol* **153:** 2609–2617, 1994.
107. Bischoff SC and Dahinden CA, c-*kit* Ligand: A unique potentiator of mediator release by human lung mast cells. *J Exp Med* **175:** 237–244, 1992.
108. Taylor AM, Galli SJ and Coleman JW, Stem-cell factor, the kit ligand, induces direct degranulation of rat peritoneal mast cells *in vitro* and *in vivo*: Dependence of the *in vitro* effect on period of culture and comparisons of stem-cell factor with other mast cell-activating agents. *Immunology* **86:** 427–433, 1995.
109. Tsai M, Tam SY and Galli SJ, Distinct patterns of early response gene expression and proliferation in mouse mast cells stimulated by stem cell factor, interleukin-3, or IgE and antigen. *Eur J Immunol* **23:** 867–872, 1993.
110. Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, Iikura Y, Awaji T, Tsujimoto G, Yanagida M, Uzumaki H, Takahashi G, Tsuji K and Nakahata T, Selective growth of human mast cells induced by steel factor, IL-6, and prostaglandin E_2 from cord blood mononuclear cells. *J Immunol* **157:** 343–350, 1998.
111. Baghestanian M, Hofbauer R, Kiener HP, Bankl HC, Wimazal F, Willheim M, Scheiner O, Füreder W, Müller MR, Bevec D, Lechner K and Valent P, The c-kit ligand stem cell factor and anti-IgE promote expression of monocyte chemoattractant protein-1 in human lung mast cells. *Blood* **90:** 4438–4449, 1997.
112. Okayama Y, Semper AE, Holgate ST and Church MK, Multiple cytokine mRNA expression in human mast cells stimulated via FcεRI. *Int Arch Allergy Immunol* **107:** 158–159, 1995.
113. Bressler RB, Lesko J, Jones ML, Wasserman M, Dickason RR, Huston MM, Cook SW and Huston DP, Production of IL-5 and granulocyte-macrophage colony-stimulating factor by naive human mast cells activated by high-affinity IgE receptor ligation. *J Allergy Clin Immunol* **99:** 508–514, 1997.
114. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH and Holgate ST, Interleukin-4, -5 and -6 and tumor necrosis factor-α in normal and asthmatic airways: Evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* **10:** 471–480, 1994.