

EDITORIAL

Clinical and Experimental Allergy

Novel approaches to the inhibition of mast cells in allergic disease

This editorial discusses the findings of the paper in this issue by Lee et al. [59], pp . . .

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IgE-mediated allergic disease is a global health burden and major drain on healthcare resources [1, 2]. Allergic disease can present in many ways depending on the tissues affected, from acute life-threatening episodes of anaphylaxis due to food allergy, the strictly seasonal symptoms due to grass pollen-induced summer rhinoconjunctivitis (hayfever), or the chronic on-going inflammation evident in bronchial asthma. Current treatment approaches for these common diseases are often of poor efficacy or have unacceptable side-effects, and there is therefore an unmet need for novel inhibitors of allergic disease. Mast cells play a central role in the pathophysiology of allergy through their ability to release a plethora of pro-inflammatory autacoid mediators, proteases and cytokines in response to both high-affinity IgE receptor (FcεRI)-dependent and -independent activation [3]. Within diseased tissues, mast cells relocate to key tissue structures such as the airway smooth muscle [4] and mucous glands [5] in asthma, facilitating the specific targeting of their mediators and adhesive cell-cell signals to these dysfunctional airway elements. Targeting mast cell activation therefore has the potential to attenuate allergic disease.

It is beyond the scope of this article to describe in detail the signalling pathways activated in mast cells following IgE-dependent activation, but there are several recent state-of-the-art reviews by others [6, 7]. It is important to note that most of the signalling events identified following FcεRI engagement have been defined in rodent models, with relatively little known about events in human mast cells. Where human cells have been investigated, some important differences in signalling components have been observed [8]. In brief, FcεRI is a

tetrameric structure that comprises an α-chain (FcεRIα) that binds IgE, a β-chain signal-amplifying subunit (FcεRIβ), and two γ-subunits that exist as a signalling homodimer (FcεRIγ) (Fig. 1). Signalling *in vivo* is initiated when multivalent allergen binds to allergen-specific IgE bound to the FcεRIα chain. This promotes FcεRI aggregation and can be mimicked *in vitro* by the use of anti-IgE antibodies. Receptor aggregation initiates association of the receptor with lipid rafts containing Lyn kinase, a membrane-anchored member of the Src family of protein tyrosine kinases [9]. Lyn phosphorylates tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAMs) of the β and γ-chains before binding strongly to the phosphorylated FcεRIβ ITAM. The phosphorylated ITAMs in the γ-chains promote the docking of spleen tyrosine kinase (Syk). Lyn and Syk are themselves phosphorylated by recruited Lyn and Syk kinases, promoting the Syk activation loop which results in a fully activated Syk with adjacent tyrosine phosphorylation of LAT (linker of activation for T cells) [10, 11]. This promotes a cascade of events leading to the activation of PLCγ and subsequent generation of inositol trisphosphate (IP₃) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate (PIP₂). The generation of IP₃ induces Ca²⁺ mobilization from intracellular stores, which initiates the influx of extracellular Ca²⁺ via store-operated Ca²⁺ channels (Fig. 1) [12]. DAG activates PKC which is required for degranulation to proceed and which contributes to gene transcription [13, 14]. In addition to the activation of the PLCγ pathway, there is also evidence for the involvement of PI-3 kinase-dependent [15], sphingosine kinase-dependent [16] and Ras-dependent signalling pathways [17, 18], which contribute to Ca²⁺ mobilization, arachidonic acid metabolism and gene transcription.

In terms of IgE-dependent mediator release in both rodents and humans, influx of extracellular Ca²⁺ is a critical requirement for the release of both pre-formed granule-derived mediators and newly generated autacoids

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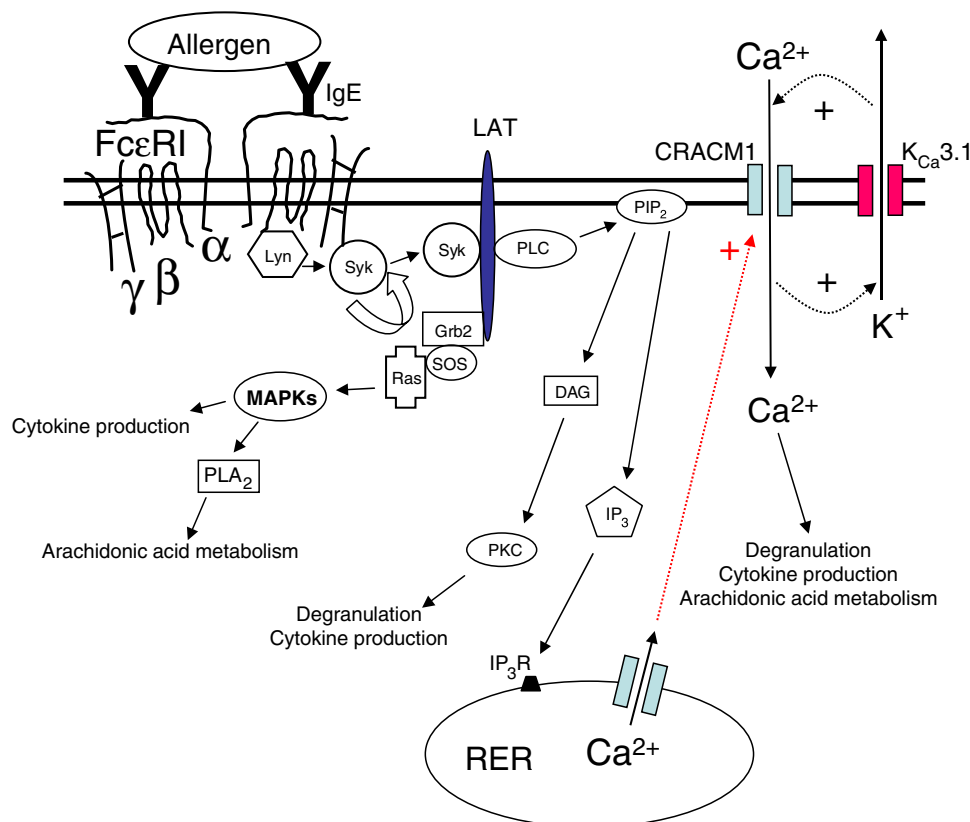


Fig. 1. Simplified schematic demonstrating the proximal FcεRI-dependent signalling pathway and its link to the PLCγ and Ras-MAP kinase signalling modules (see text for details). Abbreviations: DAG, diacylglycerol; Grb2, growth factor receptor-bound protein 2; IP₃, inositol-1,4,5-trisphosphate; LAT, linker for activation of T cells; MAPK, mitogen-activated protein kinases; PIP₂, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLA₂, phospholipase A₂; PLC, phospholipase C; RER, rough endoplasmic reticulum; SOS, son-of-sevenless homologue.

(leukotriene C₄ and prostaglandin D₂ from membrane arachidonic acid) and many cytokines [19, 20]. The process of Ca²⁺ entry through the plasma membrane as a result of the depletion of Ca²⁺ from internal stores is termed capacitive Ca²⁺ entry. The Ca²⁺ current passing through the plasma membrane is known as the Ca²⁺ release activated Ca²⁺ current (I_{CRAC}) [12], and is believed to play a central role in many physiologic processes such as gene transcription, proliferation and cytokine release [21]. I_{CRAC} has been well characterized electrophysiologically in several cells including rodent mast cells [22], but the channel carrying it has only recently been identified as CRACM1 (also known as Orai1) [23–26]. In mouse mast cells at least, CRACM1 appears to make a major contribution to degranulation, and LTC₄ and TNF-α release in response to IgE-dependent activation [27]. Human mast cells also express several members of the transient receptor potential family of ion channels that might also contribute to Ca²⁺ influx following immunologic activation [28, 29]. K⁺ channels are also of interest because they modulate Ca²⁺ influx and hence mediator release due to their profound effects on the cell membrane potential [30]. The K⁺ channels present in human mast

cells differ from those in rodents, highlighting an example of species-dependent heterogeneity. For example, although human mast cells express K_{ir} family mRNA [28, 31], they do not express functional K_{ir} currents [8, 30, 32–36], in contrast to rodent mucosal mast cells [37, 38]. Following IgE-dependent activation, human mast cells rapidly open the intermediate conductance Ca²⁺-activated K⁺ channel K_{Ca}3.1 [8, 34, 36, 39], which has not been described to date in rodent mast cells. The K_{Ca}3.1 channel indirectly enhances Ca²⁺ influx and histamine release, but is not critical for secretion, and can thus be considered as increasing the gain of an immunologic stimulus [30]. This channel is closed by compounds that inhibit mast cell secretion such as β₂-adrenoceptor agonists [34] and adenosine [36], providing a mechanism for the coupling of receptor activation and impaired secretion. Interestingly, this effect appears to act through a cAMP-independent mechanism [34].

It is important to note that mast cells have the potential to be activated through numerous FcεRI-independent mechanisms which may play important roles in inflamed tissues. Examples include proteases including the mast cell protease tryptase [40], cytokines such as SCF, TNF-α,

and IFN- γ [41–44], adenosine [45], Toll-like receptor ligands [46, 47], neuropeptides (particularly, skin mast cells) [48], complement [49] and hyperosmolality [50]. The pattern of mediators released by mast cells varies depending on the stimulus indicating the versatility of the mast cell in being able to respond to a number of diverse tissue insults with an appropriate pattern of mediator secretion and gene expression aimed at initiating an appropriate programme of inflammation and repair [51]. The signalling pathways involved in these Fc ϵ RI-independent mechanisms of activation are poorly understood or unknown.

From the above outline, it is evident that there are numerous points at which mast cell activation or its effects could be targeted. Starting proximally, these include allergen avoidance or immunotherapy, anti-IgE therapy to inhibit both allergen-dependent and monomeric IgE-dependent signalling [52, 53], inhibition of the various signalling components highlighted earlier in the text including the final effector ion channels required for normal stimulus secretion coupling, or antagonism of released mediators. A further approach might be to prevent the relocation of mast cells to key tissue structures such as the airway smooth muscle in asthma, and the CXCL10/CXCR3 axis and K $_{Ca}$ 3.1 ion channel appear to be promising targets for this [35, 54]. Allergen avoidance is difficult to achieve, desensitizing immunotherapy is effective but complicated and occasionally hazardous, while anti-IgE therapy is partially effective but prohibitively expensive in many healthcare systems. Some drugs are described as mast cell 'stabilizers' because they can inhibit IgE-dependent mast cell activation *in vitro*, but *in vivo* they are of poor efficacy. For example, disodium cromoglycate is only a weak inhibitor of IgE-dependent HLMC secretion with maximal inhibition of histamine release *in vitro* of 10–20% when used in the high micromolar range and it also exhibits rapid tachyphylaxis [55]. β_2 -Adrenoceptor agonists such as salbutamol are more potent inhibitors of HLMC mediator release *in vitro* [55], but again there is rapid tachyphylaxis, so that with chronic administration, the clinical evidence is that they do not attenuate mast cell secretion in the asthmatic airway and may even enhance it [56–58]. Specific inhibitors of mast cell-derived mediators such as antihistamines and leukotriene receptor antagonists are partially efficacious in some diseases such as rhinoconjunctivitis but not in all patients. Identifying effective inhibitors of mast cell mediator release therefore remains a major therapeutic goal.

In this issue of the journal Lee et al. investigated the anti-allergic properties of several plant extracts [59]. They demonstrated that the leaf extract of *Camellia japonica* (LECJ) is able to markedly attenuate the IgE-dependent degranulation of rodent bone marrow-derived mast cells, rat basophilic leukaemia (RBL) cells, and human cord

blood-derived mast cells, as well as markedly inhibit IL-4 and TNF- α mRNA expression and protein secretion from RBL cells. LECJ did not inhibit thapsigargin-induced degranulation, indicating that the active components were inhibiting the proximal Fc ϵ RI-dependent signalling pathway. This was confirmed with the demonstration that LECJ inhibited Syk and LAT tyrosine phosphorylation inhibited Lyn activity in an *in vitro* phosphorylation assay and inhibited phosphorylation of several MAP kinases. LECJ was also active *in vivo* in the mouse model of passive cutaneous anaphylaxis, where it attenuated the extravasation of Evan's blue dye. HPLC identified eugenol and quercetin-3- β -D-glucoside as two components of LECJ, which were able to attenuate mast cell degranulation in the micromolar range. These results are very similar to the effects of the herb *Polygoni cuspidati* radix reported previously by this research group [60]. Although the active components were distinct, the *P. cuspidati* radix extract was very similar in potency to LECJ on a microgram basis.

This study suggests that LECJ and its components have the potential to attenuate diseases, where IgE-dependent mast cell activation plays a dominant role. However, Lyn and Syk are used widely for signalling by many receptors in many cell types, so mast cell-independent effects are therefore also likely to occur. However, in inflamed tissues where many cell types are active, this may be an advantage, and so LECJ and its components might prove to be more active and effective than anti-IgE therapy, for example, which is much more mast cell-specific. While Lyn is undoubtedly an important initiator of Fc ϵ RI-dependent signalling, knockdown/knockout experiments in mice have sometimes revealed enhanced Fc ϵ RI-dependent mast cell activation (reviewed in Refs. [5] and [6]). This is probably because Lyn can also deliver inhibitory signals which are related to the intensity of the stimulus. As LECJ appears to act primarily on the inhibition of Lyn, its effects on Fc ϵ RI-dependent mast cell activation *in vivo* may therefore not necessarily be inhibitory. A Syk inhibitor R112 demonstrated modest short-term efficacy in patients with seasonal allergic rhinitis when administered intranasally [61], but with regular dosing for 1 week this was ineffective [62], and R112 did not attenuate the nasal response to allergen challenge [63]. It will be interesting to see how LECJ compares with this. A further area that will require investigation is the ability of LECJ to inhibit IgE-independent mast cell activation. The importance of these IgE-independent pathways is probably underestimated in 'allergic' disease, and the signalling pathways are poorly understood.

In summary, LECJ represents a novel inhibitor of mast cell degranulation and cytokine secretion *in vitro*, which acts on the proximal Fc ϵ RI-dependent signalling pathway. It will be of great interest to see whether this will translate into a useful therapy for the treatment of allergic disease.

References

- 1 Asher MI, Montefort S, Bjorksten B *et al.* Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet* 2006; **368**:733–43.
- 2 Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004; **59**:469–78.
- 3 Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006; **117**:1277–84.
- 4 Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; **346**:1699–705.
- 5 Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; **57**:677–82.
- 6 Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006; **6**:218–30.
- 7 Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 2006; **117**:1214–25.
- 8 Duffy SM, Lawley WJ, Conley EC, Bradding P. Resting and activation-dependent ion channels in human mast cells. *J Immunol* 2001; **167**:4261–70.
- 9 Kovarova M, Tolar P, Arudchandran R, Draberova L, Rivera J, Draber P. Structure–function analysis of Lyn kinase association with lipid rafts and initiation of early signaling events after Fcepsilon receptor I aggregation. *Mol Cell Biol* 2001; **21**:8318–28.
- 10 Saitoh S, Arudchandran R, Manetz TS *et al.* LAT is essential for Fc(epsilon)RI-mediated mast cell activation. *Immunity* 2000; **12**:525–35.
- 11 Saitoh S, Odom S, Gomez G *et al.* The four distal tyrosines are required for LAT-dependent signaling in FcepsilonRI-mediated mast cell activation. *J Exp Med* 2003; **198**:831–43.
- 12 Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 1992; **355**:353–6.
- 13 Ozawa K, Szallasi Z, Kazanietz MG *et al.* Ca²⁺-dependent and Ca²⁺-independent isoforms of protein kinase C mediate exocytosis in antigen-stimulated rat basophilic RBL-2H3 cells. Reconstitution of secretory responses with Ca²⁺ and purified isoforms in washed permeabilized cells. *J Biol Chem* 1993; **268**:1749–56.
- 14 Razin E, Szallasi Z, Kazanietz MG, Blumberg PM, Rivera J. Protein kinases C-beta and C-epsilon link the mast cell high-affinity receptor for IgE to the expression of c-fos and c-jun. *Proc Natl Acad Sci U S A* 1994; **91**:7722–6.
- 15 Ali K, Bilancio A, Thomas M *et al.* Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. *Nature* 2004; **431**:1007–11.
- 16 Olivera A, Urtz N, Mizugishi K *et al.* IgE-dependent activation of sphingosine kinases 1 and 2 and secretion of sphingosine 1-phosphate requires Fyn kinase and contributes to mast cell responses. *J Biol Chem* 2006; **281**:2515–25.
- 17 Turner H, Cantrell DA. Distinct Ras effector pathways are involved in Fc epsilon R1 regulation of the transcriptional activity of Elk-1 and NFAT in mast cells. *J Exp Med* 1997; **185**:43–53.
- 18 Jabril-Cuenod B, Zhang C, Scharenberg AM *et al.* 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* 1996; **271**:16268–72.
- 19 Church MK, Pao GJ, Holgate ST. Characterization of histamine secretion from mechanically dispersed human lung mast cells: effects of anti-IgE, calcium ionophore A23187, compound 48/80, and basic polypeptides. *J Immunol* 1982; **129**:2116–21.
- 20 Kim TD, Eddlestone GT, Mahmoud SF, Kuchty J, Fewtrell C. Correlating Ca²⁺ responses and secretion in individual RBL-2H3 mucosal mast cells. *J Biol Chem* 1997; **272**:31225–9.
- 21 Lewis RS. Calcium oscillations in T-cells: mechanisms and consequences for gene expression. *Biochem Soc Trans* 2003; **31**:925–9.
- 22 Fasolato C, Hoth M, Matthews G, Penner R. Ca²⁺ and Mn²⁺ influx through receptor-mediated activation of nonspecific cation channels in mast cells. *Proc Natl Acad Sci USA* 1993; **90**:3068–72.
- 23 Feske S, Gwack Y, Prakriya M *et al.* A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006; **441**:179–85.
- 24 Vig M, Peinelt C, Beck A *et al.* CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science* 2006; **312**:1220–3.
- 25 Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006; **443**:230–3.
- 26 Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 2006; **443**:226–9.
- 27 Vig M, DeHaven WI, Bird GS *et al.* Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol* 2008; **9**:89–96.
- 28 Bradding P, Okayama Y, Kambe N, Saito H. Ion channel gene expression in human lung, skin, and cord blood-derived mast cells. *J Leukoc Biol* 2003; **73**:614–20.
- 29 Wykes RCE, Lee M, Duffy SM, Yang W, Seward EP, Bradding P. Functional transient receptor potential melastatin 7 (TRPM7) channels are critical for human mast cell survival. *J Immunol* 2007; **179**:4045–52.
- 30 Duffy SM, Berger P, Cruse G, Yang W, Bolton SJ, Bradding P. The K⁺ channel IK_{Ca1} potentiates Ca²⁺ influx and degranulation in human lung mast cells. *J Allergy Clin Immunol* 2004; **114**:66–72.
- 31 Dewson GD, Conley EC, Bradding P. Multigene family isoform profiling from blood cell lineages. *BMC Genomics* 2002; **3**:22.
- 32 Duffy SM, Leyland ML, Conley EC, Bradding P. Voltage-dependent and calcium-activated ion channels in the human mast cell line HMC-1. *J Leukoc Biol* 2001; **70**:233–40.
- 33 Duffy SM, Lawley WJ, Kaur D, Yang W, Bradding P. Inhibition of human mast cell proliferation and survival by tamoxifen in association with ion channel modulation. *J Allergy Clin Immunol* 2003; **112**:970–7.
- 34 Duffy SM, Cruse G, Lawley WJ, Bradding P. Beta2-adrenoceptor regulation of the K⁺ channel IK_{Ca1} in human mast cells. *FASEB J* 2005; **19**:1006–8.
- 35 Cruse G, Duffy SM, Brightling CE, Bradding P. Functional K_{Ca3.1} K⁺ channels are required for human lung mast cell migration. *Thorax* 2006; **61**:880–5.
- 36 Duffy SM, Cruse G, Brightling CE, Bradding P. Adenosine closes the K⁺ channel K_{Ca3.1} in human lung mast cells and inhibits

- their migration via the adenosine A_{2A} receptor. *Eur J Immunol* 2007; **37**:1653–62.
- 37 McCloskey MA, Cahalan MD. G protein control of potassium channel activity in a mast cell line. *J Gen Physiol* 1990; **95**:205–27.
 - 38 McCloskey MA, Qian YX. Selective expression of potassium channels during mast cell differentiation. *J Biol Chem* 1994; **269**:14813–9.
 - 39 Kaur D, Berger P, Duffy SM, Brightling CE, Bradding P. Co-cultivation of mast cells and FcεRIα⁺ dendritic-like cells from human hip bone marrow. *Clin Exp Allergy* 2005; **35**:226–33.
 - 40 He S, Aslam A, Gaca MD *et al.* Inhibitors of tryptase as mast cell-stabilizing agents in the human airways: effects of tryptase and other agonists of proteinase-activated receptor 2 on histamine release. *J Pharmacol Exp Ther* 2004; **309**:119–26.
 - 41 Columbo M, Horowitz EM, Botana LM *et al.* The human recombinant c-kit receptor ligand, rhSCF, induces mediator release from human cutaneous mast cells and enhances IgE-dependent mediator release from both skin mast cells and peripheral blood basophils. *J Immunol* 1992; **149**:599–608.
 - 42 Yanagida M, Fukamachi H, Takei M *et al.* Interferon-gamma promotes the survival and FcεRI-mediated histamine release in cultured human mast cells. *Immunology* 1996; **89**:547–52.
 - 43 Sperr WR, Czerwenka K, Mundigler G *et al.* Specific activation of human mast cells by the ligand for c-kit: comparison between lung, uterus and heart mast cells. *Int Arch Allergy Immunol* 1993; **102**:170–5.
 - 44 Coward WR, Okayama Y, Sagara H, Wilson SJ, Holgate ST, Church MK. NF-κB and TNF-α: a positive autocrine loop in human lung mast cells? *J Immunol* 2002; **169**:5287–93.
 - 45 Forsythe P, McGarvey LP, Heaney LG, MacMahon J, Ennis M. Adenosine induces histamine release from human bronchoalveolar lavage mast cells. *Clin Sci* 1999; **96**:349–55.
 - 46 Varadaradjalou S, Feger F, Thieblemont N *et al.* Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* 2003; **33**:899–906.
 - 47 Kulka M, Alexopoulou L, Flavell RA, Metcalfe DD. Activation of mast cells by double-stranded RNA: evidence for activation through toll-like receptor 3. *J Allergy Clin Immunol* 2004; **114**:174–82.
 - 48 Benyon RC, Robinson C, Church MK. Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli. *Br J Pharmacol* 1989; **97**:898–904.
 - 49 Oskeritzian CA, Zhao W, Min HK *et al.* Surface CD88 functionally distinguishes the MCTC from the MCT type of human lung mast cell. *J Allergy Clin Immunol* 2005; **115**:1162–8.
 - 50 Eggleston PA, Kagey-Sobotka A, Schleimer RP, Lichtenstein LM. Interaction between hyperosmolar and IgE-mediated histamine release from basophils and mast cells. *Am Rev Respir Dis* 1984; **130**:86–91.
 - 51 Okumura S, Kashiwakura J, Tomita H *et al.* Identification of specific gene expression profiles in human mast cells mediated by toll-like receptor 4 and FcεRI. *Blood* 2003; **102**:2547–54.
 - 52 Cruse G, Kaur D, Yang W, Duffy SM, Brightling CE, Bradding P. Activation of human lung mast cells by monomeric immunoglobulin E. *Eur Respir J* 2005; **25**:858–63.
 - 53 Cruse G, Cockerill S, Bradding P. IgE alone promotes human lung mast cell survival through the autocrine production of IL-6. *BMC Immunol* 2008; **9**:2.
 - 54 Brightling CE, Ammit AJ, Kaur D *et al.* The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. *Am J Respir Crit Care Med* 2005; **171**:1103–8.
 - 55 Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987; **90**:421–9.
 - 56 Roberts JA, Bradding P, Britten KM *et al.* The long-acting beta₂-agonist salmeterol xinafoate: effects on airway inflammation in asthma. *Eur Respir J* 1999; **14**:275–82.
 - 57 Giannini D, Carletti A, Dente FL *et al.* Tolerance to the protective effect of salmeterol on allergen challenge. *Chest* 1996; **110**:1452–7.
 - 58 Swystun VA, Gordon JR, Davis EB, Zhang X, Cockcroft DW. Mast cell tryptase release and asthmatic responses to allergen increase with regular use of salbutamol. *J Allergy Clin Immunol* 2000; **106**:57–64.
 - 59 Lee J-H, Kim J-W, Mun S-H *et al.* Camellia japonica suppresses IgE-mediated allergic response by the inhibition of Syk kinase activation in mast cells. *Clin Exp Allergy* 2008;doi: 10.1111/j.1365-2222.2008.02936.x
 - 60 Lim BO, Lee JH, Ko NY *et al.* *Polygoni cuspidati* radix inhibits the activation of Syk kinase in mast cells for antiallergic activity. *Exp Biol Med* 2007; **232**:1425–31.
 - 61 Meltzer EO, Berkowitz RB, Grossbard EB. An intranasal Syk-kinase inhibitor (R112) improves the symptoms of seasonal allergic rhinitis in a park environment. *J Allergy Clin Immunol* 2005; **115**:791–6.
 - 62 Masuda ES, Schmitz J. Syk inhibitors as treatment for allergic rhinitis. *Pulm Pharmacol Ther* 2007.
 - 63 Guyer BJ, Shimamoto SR, Bradhurst AL, Grossbard EB, Dreskin SC, Nelson HS. Mast cell inhibitor R112 is well tolerated and affects prostaglandin D₂ but not other mediators, symptoms, or nasal volumes in a nasal challenge model of allergic rhinitis. *Allergy Asthma Proc* 2006; **27**:208–13.