Signalling through the high-affinity IgE receptor FcεRI

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The FcεRI complex forms a high-affinity cell-surface receptor for the Fc region of antigen-specific immunoglobulin E (IgE) molecules. FcεRI is multimeric and is a member of a family of related antigen/Fc receptors which have conserved structural features and similar roles in initiating intracellular signalling cascades. In humans, FcεRI controls the activation of mast cells and basophils, and participates in IgE-mediated antigen presentation. Multivalent antigens bind and crosslink IgE molecules held at the cell surface by FcεRI. Receptor aggregation induces multiple signalling pathways that control diverse effector responses. These include the secretion of allergic mediators and induction of cytokine gene transcription, resulting in secretion of molecules such as interleukin-4, interleukin-6, tumour-necrosis factor-α and granulocyte-macrophage colony-stimulating factor. FcεRI is therefore central to the induction and maintenance of an allergic response and may confer physiological protection in parasitic infections.

Three distinct protein species compose the FcεRI complex[1]. The α-chain binds the Fc portion of *IgE** with high affinity, the β-chain has four transmembrane domains between amino- and carboxy-terminal cytoplasmic tails, and a homodimer of two disulphide-linked γ-chains completes the tetrameric structure. In humans, the tetrameric structure is not obligatory—an alternate form is present, comprising an $\alpha\gamma_2$ trimer. On the basis of several similarities in structural features and signalling targets, FcεRI has been placed in an *antigen* receptor superfamily with the B- and T-cell antigen receptors (BCR, TCR) and several immunoglobulin-γ (IgG) Fc receptor isotypes (Fig. 1)[1–3]. First, the α-chain contains two immunoglobulin-type domains, and similar domains are found in the ligand-binding subunits of other superfamily members. Second, the β- and γ-subunits of FcεRI contain conserved immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic tails[3,4]. These motifs are phosphoacceptors, through which the receptor subunits interact with signalling proteins. Finally, there is considerable homology between the signalling pathways initiated by FcεRI and other antigen receptors.

FcεRI binding of IgE

Signalling through the FcεRI is initiated by crosslinking of multivalent antigen bound to IgE; it is a prerequisite that IgE monomers have previously bound to the receptor through their Fc regions. Studies of the interaction between IgE and FcεRI have shown that the stoichiometry of the interaction is 1:1 (ref. 5) and that the interaction is high affinity (K_d = 10^{-9}–10^{-10} M). In addition, mutational analyses indicate that the third Ig constant domain (Cε3) of IgE may form the contact point between antibody and ligand[6,7], and that the α-subunit is highly *N*-glycosylated but that glycosylation is not necessary for IgE binding[8,9]. After much effort, the crystal structure of the FcεRIα chain has now been determined, providing some insight into the nature of the IgE interaction with its Fc receptor[10].

Crystallization reveals that the two Ig domains (D1 and D2) of FcεRIα are positioned at an acute angle to one another, forming a convex surface at the top of the molecule and a marked cleft enclosed by the two globular immunoglobulin domains (Fig. 2a). The relative orientation of these two domains differs between the various structures where tandem immunoglobulin domains are present and is determined by the composition of the interface region. Amino acids in the cleft are directed towards the membrane and will not interact with IgE. Residues forming the top surface of the protein have previously been implicated in binding to the IgE Fc portion. Here, four grouped tryptophan residues (contributed by D1, D2 and the interface region) form a hydrophobic patch that is a putative contact point for the binding of IgE Fc.

The proposed relationship between IgE and FcεRIα molecules is shown schematically in Fig. 2b. The IgE Fc region is homodimeric. Although two possible surfaces for interaction between FcεRIα and IgE Cε3 exist, the interaction stoichiometry is 1:1 (ref. 5). There are several possible reasons why two FcεRIα molecules are never bound to the two antibody immunoglobulin domains. First, the binding of one FcεRIα to IgE may partly occlude the other immunoglobulin domain and prevent, sterically, the interaction of a second receptor. Second, a conformational change may follow FcεRIα and IgE interaction, such that the Fc structure alters to prevent binding to another receptor.

IgE crosslinking by multivalent antigen

The IgE/FcεRIα interaction is followed by the antigenic crosslinking of receptors in the plane of the plasma membrane. FcεRIα chains do not aggregate in the absence of antigen, possibly as a result of the extensive glycosylation of the front and back of the molecule. This glycosylation does not extend to the top surface, where the IgE interaction is proposed to take place. *In vitro*, deglycosylated FcεRIα aggregates spontaneously without antigen. The glycosylation of potential α-chain interaction surfaces prevents premature aggregation and permits interaction with the synthetic machinery[9], whereas binding of multivalent antigen overcomes the intrinsic resistance of α-chains to interaction.

FcεRIα is not a conventional signal transducing molecule. It has a short (17 amino acids) cytoplasmic tail that has never been found to interact with a signalling target. In fact, deletion of the entire FcεRIα cytoplasmic domain does not compromise FcεRI signalling[11]. However, information concerning productive ligation of α-chains is passed to the β- and γ-chains (the signalling subunits) of the receptor, and an aggregation-induced change in FcεRIα must be sensed by the β- and γ-subunits. The crystal structure of FcεRIα now provides an enticing possibility that residues in the cleft formed by the opposition of D1 and D2 could interact with the extracellular portion of the β- or γ-subunit and transmit information regarding aggregation.

Early FcεRI signalling events

Antigenic crosslinking of the FcεRI initiates a chain of phosphate transfer events within the receptor microenvironment. The system of information transfer relies on the ligation-dependent recruit-

* Terms in italic are defined in the glossary on p. B39.

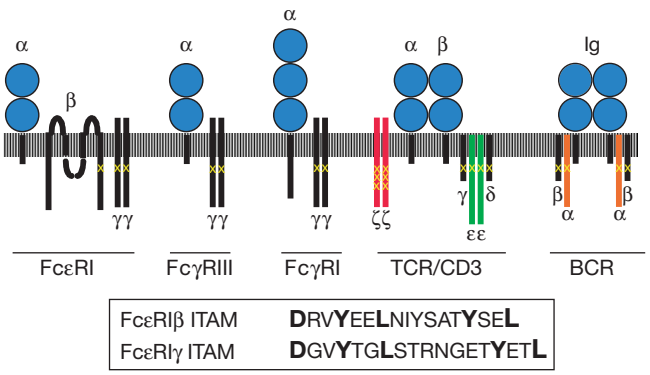

Figure 1 ITAM-containing immunoreceptors. The tetrameric FcεRI is shown in the context of other members of the immunoreceptor superfamily. Blue circles represent immunoglobulin domains. ITAM motifs are shown as yellow crosses. TCR, T-cell antigen receptor; BCR, B-cell antigen receptor; Ig, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif. Inset: sequences of the ITAM motifs contained within the β- and γ-chains of FcεRI. Canonical residues are shown in bold type.

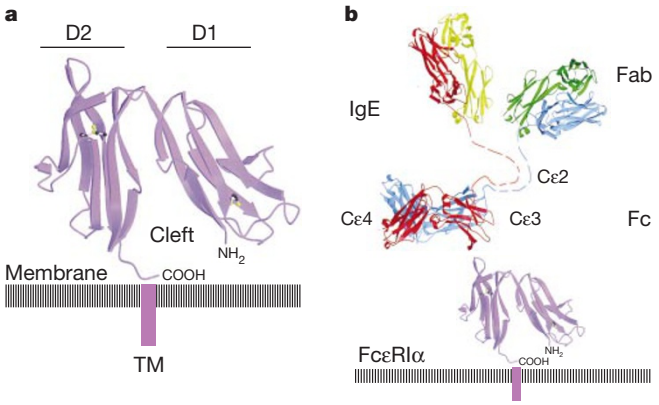

Figure 2 Structural relationship of FcεRIα and its ligand, IgE. **a**, Schematic structure of the FcεRIα chain. The opposing immunoglobulin domains, D1 and D2, form a marked cleft lined by residues that face the plasma membrane. TM, transmembrane domain. **b**, Interaction of IgE and FcεRIα. Contact residues have been localized to the top of D2 in FcεRIα, and the Cε2:Cε3 junction in the Fc region of IgE. Fab, antibody binding fragment; Fc, constant region.

ment of assembled signalling cassettes into the receptor context, providing an organizational parallel with the early events that occur after ligation of other antigen receptors. Phosphate transfer assists in this recruitment, and in the subsequent activation of recruited signalling proteins.

The β- and γ-chains of the FcεRI complex contain ITAMs, which are a conserved feature of antigen and some Fc receptors[1–4,12]. The ITAM consensus sequence is D/E-XX-YXXL-X_{7-11}-YXXL-L/I, where the tyrosine residues are phosphoacceptor sites for the action of receptor-associated protein tyrosine kinases (PTKs)[4]. Phospho-ITAMs provide a docking site for cytoplasmic proteins that contain the Src-homology-2 (SH2) domain and hence link receptor and signal transduction cascades, as the SH2 domain has high affinity for phosphorylated tyrosine residues[13]. In the context of FcεRI, the β- and γ-chain ITAMs have slightly different structures (Fig. 1). The β-chain ITAM is notable owing to its differences from the consensus ITAM sequence; the presence of a third tyrosine between the canonical tyrosine residues; and the short length of its spacer region. The β- and γ-chain ITAMs also have distinct functions. There are two species of FcεRI-associated PTK—the *src* family kinase Lyn and p72Syk kinase. The former is found associated with FcεRIβ, whereas the latter is able to bind both FcεRIβ and FcεRIγ but has higher affinity for interaction with FcεRIγ.

Several studies have contributed to a model for the events that occur immediately after FcεRI aggregation[1,14]. The salient features of this model are as follows. First, the β- and γ-chains act cooperatively. Both *in vitro* and genetic reconstitution studies illustrate this point. Reconstitution of FcεRI-deficient *mast-cell* lines showed that mutation of the two canonical tyrosines in the β-chain ITAM abolished activation-dependent phosphorylation of both the β- and the γ-chain ITAMs[15], which indicates that the phosphorylation of the former has bearing on the status of the latter. Moreover, whereas $Lyn^{-/-}$ mast cells exhibit no β- or γ-chain phosphorylation[16], $Syk^{-/-}$ cells have intact β- and γ-chain phosphorylation but still lack downstream signalling events[17,18]. Second, Lyn binds to FcεRIβ under resting conditions. An obvious candidate for the mediation of this interaction is the Lyn SH2 domain, as FcεRIβ is slightly tyrosine-phosphorylated under resting conditions. However, we have shown that the Lyn SH2 domain is not required for FcεRI phosphorylation (S. Lin and J.P.K., unpublished data), and others have demonstrated that the 'unique' (SH4-containing) domain of Lyn interacts with FcεRIβ[19]. In addition, Lyn is likely to rest passively in the receptor context owing to its myristoylation, which constitutively targets the enzyme to the membrane. Third, active Lyn phosphorylates the β- and γ-chain ITAMs. Upon receptor aggregation, Lyn is activated and its catalytic activity becomes directed towards the β- and γ-chain ITAMs. Syk is then recruited to the receptor γ-chain through one of the tandem SH2 domains in the kinase[18]. An important feature is that Lyn may transphosphorylate ITAM in other receptor complexes[20]. Fourth, Syk binding to FcεRIγ leads to Lyn-dependent tyrosine phosphorylation and activation of the kinase. This step enables the productive interaction of active Syk with its many targets[21].

One step that remains unclear in the initiation of the early events in FcεRI signalling is how FcεRIα aggregation causes transition of Lyn from an inactive to an active state. Moreover, analyses of the CD45 phosphatase and Src kinases indicate that there may be subtle subdivisions in the activation status of Lyn. In its inactive/closed state, Lyn is phosphorylated on a C-terminal regulatory tyrosine by the Src kinase Csk[22]. This phosphotyrosine provides a docking site for the Lyn SH2 domain, which binds and occludes the activation domain of Lyn[23]. Moreover, by analogy with studies on Hck, there is also an intramolecular interaction of the Lyn SH3 domain that must be displaced before the kinase is fully activated. The first of these intramolecular interactions is thought to be released by dephosphorylation of the inhibitory tyrosine residue by CD45. The displacement of the intramolecular SH3 interaction is not well understood, but it seems likely that some aggregation-induced change in a receptor subunit or associated molecule provides a higher affinity SH3 ligand that successfully competes for the Lyn SH3 domain. The SH3 domain is directed towards proline-rich regions[13], and such a ligand has not yet been identified in the receptor context. Lyn then moves into an inactive/open conformation, priming the kinase for activation[24]. Autophosphorylation of open Lyn is then required for full activity of the kinase.

The mechanism by which α-chain aggregation is sensed by the signalling subunits and subsequently alters the interplay between CD45 and Lyn, Lyn and Csk, and Lyn with other possible molecules (such as SHP phosphatases) is unknown (Fig. 3a). CD45 does not, however, continue to dephosphorylate either the activatory tyrosine on Lyn or other phosphotyrosines in the receptor context. It therefore seems likely that, after aggregation, CD45 is actively excluded from this context, perhaps as a result of the partition of receptor aggregates into lipid microdomains[24,25].

Initiation of intracellular signalling cascades

Activation of Lyn and Syk drives intracellular signal transduction cascades that regulate cellular functions. Some basic principles that underlie the complex network of events occurring immediately proximal to the receptor can be identified. First, signalling molecules are recruited to the receptor context; second, a post-translational modification (for example, tyrosine phosphorylation) activates the catalytic activity of the signalling protein; and third, pluripotent adapter proteins have inducible or constitutively associated effector proteins that direct their activity towards a particular intracellular target.

One of the primary targets of antigen-receptor signalling is phospholipase Cγ1 (PLCγ1), which catalyses the breakdown of membrane phospholipids to generate two second messengers—inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and diacylglycerol (DAG). These signalling molecules are responsible for calcium release from intracellular stores and activation of various protein kinase C (PKC) isoforms, respectively. PLCγ1 must be recruited to the membrane (the location of its substrate pool) and tyrosine-phosphorylated for activation. In T cells, this recruitment is accomplished by the linker for activation of T cells (LAT) adapter protein[26], a membrane anchored substrate for the Syk analogue ZAP-70 (ref. 27). Mast cells have recently been shown to contain LAT[28], and it is likely that T and mast cells share this LAT-dependent mechanism for PLCγ1 membrane targeting. Once within the membrane, PLCγ1 is exposed to activating tyrosine phosphorylation. Two types of kinase contribute to this process—Syk and either the tyrosine kinase Itk or Bruton's tyrosine kinase (Btk), both of which are members of the Tec family of non-receptor tyrosine kinases. As described above, Syk activation derives from membrane targeting followed by Lyn-dependent phosphorylation, leading to full activation. Similarly, Btk is first membrane-targeted by binding of a phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$) moiety to its pleckstrin-homology (PH) domain[29,30]. It then becomes an accessible substrate for Lyn and is able to autophosphorylate, giving full activation[31]. Btk then phosphorylates and activates PLCγ1, a process requiring the adapter Slp-76.

Btk controls PLCγ1 tyrosine phosphorylation, and Btk recruitment/activation requires $PtdIns(3,4,5)P_3$ (ref. 32). In turn, this raises the question of how FcεRI directs $PtdIns(3,4,5)P_3$ synthesis. $PtdIns(3,4,5)P_3$ derives from phosphorylation of pre-existing phosphatidylinositol-4,5-bisphosphate, a reaction catalysed by phosphatidylinositol-3-OH kinase (PI(3)K)[33]. PI(3)K comprises a regulatory subunit of relative molecular mass 85,000 (M_r 85K), which is tyrosine phosphorylated by receptor-associated PTKs, and a catalytic subunit (M_r 110K), which must be recruited to the membrane in order to locate its substrate pool. One model is therefore that receptor-associated PTKs phosphorylate p85, which causes activation and contributes to recruitment of p110. We have recently shown that Syk is required for PI(3)K activation in mast cells (L. Beitz and J.P.K., unpublished data), potentially placing Syk upstream of p85 tyrosine phosphorylation, or indirectly affecting p110 recruitment, or both.

Membrane proximal adapter proteins are important in early FcεRI signalling. Adapter proteins are common in antigen-receptor signalling cascades, are composed of modular protein–protein interaction domains and lack intrinsic enzymatic activity. Adapter proteins are excellent examples of the modular design of signalling proteins, which have evolved conserved structural domains that allow for diversification of function within a single polypeptide[13].

The Grb-2 adapter comprises two SH3 (proline-directed) domains and one SH2 (phosphotyrosine-directed) domain. Grb-2 links upstream phosphotyrosine residues (substrates for receptor-associated PTKs) and effector molecules that form complexes with its SH3 domain. Grb-2 does not adapt directly to FcεRI ITAMs. Rather, indirect association is mediated either by the Shc adapter or by the LAT phosphoprotein. In mast cells, two prominent Grb-2 effector molecules are Sos and Slp-76 (ref. 34).

Grb-2 contributes to the recruitment and presumably the orientation of Sos towards its substrate, the Ras GTPase. Sos may also be membrane-targeted by its PH domain/phospholipid interaction[35], but it is unclear whether this assists, precedes or follows the Grb-2 interaction. Sos is a guanine nucleotide exchange factor for Ras, a membrane-resident GTPase. Sos promotes GTP loading and hence activation of Ras and its multiple downstream effector pathways, including activation of other GTPases (in fibroblasts, there is crosstalk at the level of Sos between Ras and Rac GTPase activation[36]), PI(3)K and the Raf/MEK/ERK cascade[37].

Slp-76 protein is a critical effector in antigen signalling and can apparently act at several different positions in the signalling network. In T and mast cells it has been shown that one function of Slp-76 is to recruit Vav (itself a substrate for receptor-associated PTKs)[38]. Vav is a guanine nucleotide exchange factor for the Rac GTPase[39], and thus through Slp-76 a further set of GTPase effector pathways are initiated (cytoskeletal rearrangements, p21-activated kinase (PAK) activity and Rho activation). However, in T cells there is also clear genetic evidence that a deficiency in Slp-76 uncouples receptor-associated PTKs from PLCγ1 activation, placing Slp-76 upstream of both $Ins(1,4,5)P_3$ generation and Ras activation in the same cell type[27]. This pathway probably operates also in mast cells.

However, new data obtained from T cells may complicate the model for mast cells. Although Grb-2 can mediate the interaction between LAT and Slp-76, it now seems that this is also accomplished by GADS, a novel haematopoietic adapter protein[40]. GADS also binds Shc, and it is unclear whether Grb-2 and GADS complexes coexist in the mast cell and what their relative roles are in downstream signalling pathways. Where multiple routes to a downstream target exist, it is likely that subtle choices are made by receptors and that these may not be visible in current assay systems.

GTPase/kinase networks

As described above, FcεRI aggregation results in activation of guanine nucleotide exchange factors that promote the GTP loading, and hence increased activity, of the small GTPases Ras, Rac and Rho. This class of proteins receive and react to information on many cellular processes. In the context of the mast cell, the involvement of GTPases in transcriptional activation and secretion of allergic mediators has been described.

It is well established that each GTPase has available a wide range of effector molecules[37], each of which in turn regulate complex networks of signalling pathways such as mitogen-activated protein (MAP) kinase cascades. The terminal targets of these pathways include transcription factors. For example, in the mast cell, the Ras GTPase initiates a classical Ras/Raf-1/MEK/ERK signalling cascade that targets the Elk-1 transcription factor, which is involved in immediate early gene regulation. Rac-1, on the other hand, uses an unknown effector pathway to regulate the subcellular location of the *cytokine* gene transcription factor, NFAT[41,42]. Both Ras and Rac-1 are involved in regulation of the AP-1 class of transcription factors, which independently regulate some sites and act in concert with NFAT.

Some GTPase pathways do not have nuclear targets, as two studies using mast cells have shown. First, GTPγS, which activates all classes of GTPase, can stimulate secretion of allergic mediators in mast cells. Because this is an exocytotic process, some groups have addressed the question of whether the Rab class of GTPase, which is involved in vesicle trafficking, may be important in regulation. Indeed, dominant inhibitory forms of Rab3d do block antigen-driven exocytosis[43]. Second, profound morphological change is associated with FcεRI aggregation on mast cells, including the formation of membrane ruffles and filopodia. The role of Ras, Rac and Rho GTPases in regulating such changes is well established in fibroblasts[44] and similar mechanisms exist in the mast cell.

Elevation of intracellular free calcium

FcεRI aggregation causes a biphasic modulation in intracellular free calcium ($[Ca_i^{2+}]$). First, Ins(1,4,5)P_3 generated by PLCγ1 binds and opens the Ins(1,4,5)P_3 receptor calcium channels on the surface of intracellular calcium stores (primarily the endoplasmic reticulum (ER)), which are then depleted of calcium. The cell is now presented with a problem: the finite amount of stored calcium is soon exhausted and cannot deliver sustained calcium signals. Moreover, stored calcium needs to be replenished. FcεRI-effector functions rely on sustained elevation in $[Ca_i^{2+}]$. For example, transcriptional activation of cytokine genes in mast cells, such as the gene for interleukin (IL)-4, requires sustained increases in cytoplasmic calcium[41,45]. Here, calcium signals are required for the dephosphorylation and nuclear import of the transcription factor NFAT, as NFAT is dephosphorylated by the calcium-dependent phosphatase calcineurin. If calcium is removed, rephosphorylation by a nuclear resident kinase occurs and NFAT is exported to the cytosol where it no longer contacts its target genes[46].

It is also apparent that sustained calcium signals act in synergy with those provided by cytoplasmic kinases to induce secretion in mast cells. Generalized activation of PKC and, indirectly, other

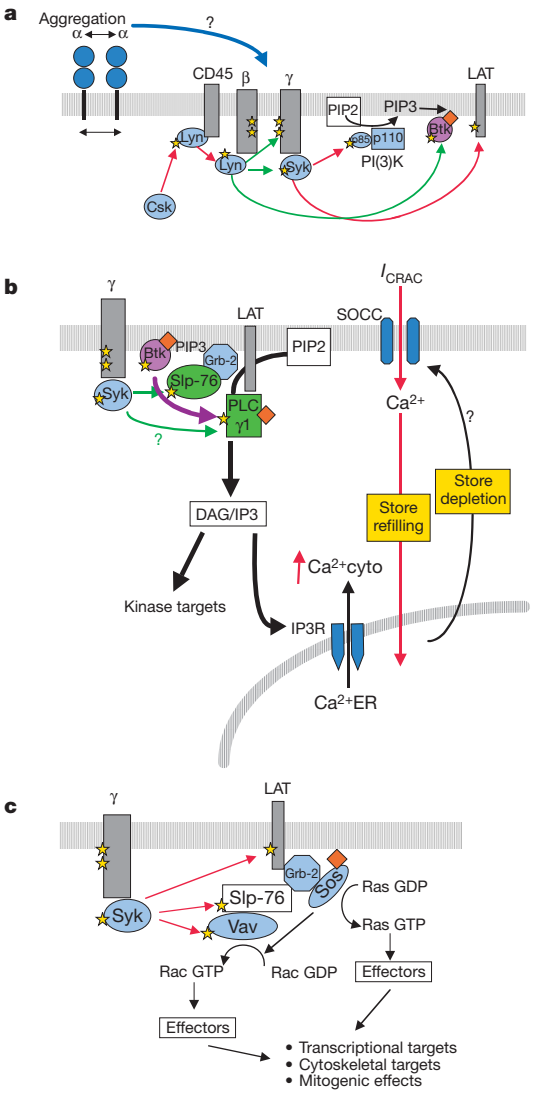

Figure 3 FcεRI activation induces complex networks of signalling events. **a**, Early events in FcεRI signalling. FcεRIα chains are aggregated by crosslinking of bound IgE by multivalent antigen. Information concerning aggregation is passed, by an unknown mechanism, to the β- and γ-chain signalling subunits. Lyn activation status is defined by the opposing actions of the CD45 phosphatase and the Csk kinase. Activated Lyn phosphorylates the β- and γ-chain ITAMs, the latter is then able to recruit Syk, which is in turn activated by Lyn. Targets of Lyn and Syk include the activation of PI(3)K to produce PtdIns(3,4,5)P_3 (PIP3), phosphorylation of the BTK and phosphorylation of the membrane-localized adapter protein LAT. **b**, FcεRI control of $[Ca_i^{2+}]$. BTK, which is localized at the membrane by binding to PIP3, contributes to activation of PLCγ1, which in turn has been brought to the membrane by the phosphorylated LAT adapter. PLCγ1 acts on membrane inositol phospholipids to generate Ins(1,4,5)P_3 (IP3) and DAG. The intracellular targets of the latter include PKC isoforms, whereas IP3 binds to the IP3 receptors (IP3R) on the surface of the ER calcium stores, leading to store depletion and elevation of cytoplasmic calcium levels. Depletion of ER calcium stores is coupled to opening of plasma membrane channels for calcium influx; the calcium release activated current (I_{CRAC}) is mediated by these store-operated calcium channels (SOCC), and is responsible for sustained elevations in cytosolic calcium and refilling of ER stores. **c**, FcεRI-regulated adapter molecules contribute to activation of GTPase/kinase cascades. Syk targets include the LAT adapter molecule, which anchors Grb-2 and Slp-76 adapters. These in turn contribute to the recruitment/localization of guanine nucleotide exchange factors for Ras family GTPases. GTP loading of GTPases initiates a wide range of effector responses. These include the induction of serial kinase cascades such as the Ras/Raf-1/MEK/ERK cascade, where the terminal targets are transcription factors, and the rearrangement of the actin cytoskeleton to produce changes in cell morphology. Diamonds represent PH domains/phospholipid interaction; stars represent phosphorylations.

signalling pathways using phorbol esters induces sub-maximal secretion. Combination with sustained calcium signals from ionophore treatment causes a synergistic increase in secretion, although ionophore alone is not a strong secretory stimulus. If external calcium is removed (that is, if influx ceases), secretion is halted abruptly. In the presence of a calcium chelator such as EGTA, the FcεRI itself cannot induce significant secretion. The precise targets for these calcium/PKC signals within the secretory machinery in mast cells are not known.

The second phase of calcium signalling induced by FcεRI satisfies the requirement for sustained calcium signals. Store depletion causes the influx of calcium from the surrounding environment (Fig. 3b). It is not yet clear how store depletion is coupled to this influx, but it is thought that a class of 'store-operated' calcium channels (SOCC)[47] mediates influx. These channels are defined by their opening in response to any store-depletion stimulus, and they are thought to be distinct from known channel families such as the Trp channels or voltage-operated calcium channels. In mast cells, where voltage-operated or ligand-gated calcium channels are not expressed, the sole entry mechanism for calcium is thought to be through SOCCs. More specifically, the calcium release activated current, I_{CRAC}, has been defined, in electrophysiological terms, as the primary mechanism for calcium entry and store refilling in mast cells[48,49]. I_{CRAC} is a highly calcium selective cation current but the channel proteins underlying the I_{CRAC} current and their regulatory mechanisms are unknown. This remains a major question in immunological research, as most lymphoid and mast cells are reliant on these channels for the sustained calcium influx needed to drive cellular activation.

Turning the receptor off

The potent mast-cell effector function initiated by FcεRI does not last indefinitely. Activation persists only as long as the receptor is engaged and phosphorylated[50]. A unique feature of FcεRI among antigen receptors is that its engagement with multivalent antigen can be reversed rapidly by the addition of monovalent hapten[50]. Signalling is terminated completely by receptor disengagement from multivalent antigen, indicating that rapid response mechanisms are in place to stop the induction of signalling pathways in the absence of continued stimulation. Although it is unlikely that receptor disengagement by competing monovalent antigen would occur *in vivo*, it is worthwhile examining the underlying mechanisms for receptor deactivation.

The off-rate of multivalent antigen is likely to be slow and cannot be relied upon as a method of signal termination, in contrast with other antigen receptors where the on- and off-rates of short peptide ligands are relatively fast. In addition, rapid receptor internalization that follows crosslinking may not be an effective signal terminator, as competent signalling complexes may still be present. Phosphorylation of phosphoacceptors in both receptor subunits and intracellular targets initiates and maintains signalling. Therefore, as a receptor-level termination mechanism, dephosphorylation by specific phosphatases seems likely.

The β-chain ITAM binds, *in vitro,* to the protein tyrosine phosphatases SHP1 and SHP2, and the inositol phospholipid-directed phosphatase SHIP also associates with FcεRI[51,52]. However, the SHP2 interaction has been regarded as irrelevant owing to its low affinity as assayed by surface plasmon resonance[53]. Our recent data suggest that SHP1 is most important in termination of FcεRI signals (D. Lin and J.P.K., unpublished data). By analogy with the BCR, SHIP is likely to be important in terminating signalling by lipid second messengers. Open questions still concern the preference or specificity of the phosphatases deactivating FcεRI, and the nature of their own activation/deactivation cycles.

Tuning the receptor

Studies using chimaeric receptors have shown that the FcεRIγ/Syk complex is competent to drive cell activation in the absence of a β-chain ITAM, so prompting the question 'What is the role of FcεRIβ?'. We have observed that, *in vitro* and *in vivo*, FcεRIβ acts as a signal amplifier[54,55]: FcεRIβ does not have autonomous cell-activation properties but does produce a five- to sevenfold amplification in the intensity of signals from FcεRIγ (as measured by Syk activation and calcium mobilization). The mechanism of amplification involves enhanced Lyn recruitment and increased Lyn phosphorylation of FcεRIγ. *In vivo*, we have shown exhaustively that FcεRIβ is an amplifier of responses using humanized FcεRIα transgenic mice expressing either $\alpha\gamma_2$ or $\alpha\beta\gamma_2$ complexes at the mast-cell surface. The amplifying effect of FcεRIβ is observed *in vivo* at the level of receptor phosphorylation, calcium mobilization, mediator secretion and IL-6 generation. Even *in vivo anaphylaxis* was enhanced threefold in $\alpha\beta\gamma_2$ complexes compared with $\alpha\gamma_2$ complexes.

We have recently explored whether enhancement of signalling by surface-resident receptors is the only mechanism by which FcεRIβ exerts its amplifying effect. In fact, there seems to be a second role for FcεRIβ in that it associates with $\alpha\gamma_2$ complexes early in biosynthesis and promotes the transport of intracellular FcεRIα chains from ER to Golgi, the latter being where the α-chain is terminally glycosylated. Detergent studies revealed that the β-chain stabilizes the FcεRI complex, leading us to the final observation that the β-chain positively affects FcεRI surface expression; the presence of β-chains markedly increases the number of surface α-chains (fully assembled receptors) at the surface of transfected cells (E. Donnadieu and J.P.K., unpublished data).

The presence of an intrinsic molecular amplifier is so far unique to the FcεRI among antigen receptors. It is unclear whether certain subunits of other multimeric receptors can modify the intensity of signalling. However, in the case of FcεRI, small changes in β-chain function could profoundly affect signalling and the resulting effector functions controlled by FcεRI. It is therefore intriguing to note that genetic studies have revealed that a locus in the long arm (q) of chromosome 11 at bands 12–13 (11q12–13), which contains the FcεRIβ gene, is linked with the allergic disorder *atopy*[56,57].

Further analysis has revealed that several *polymorphisms* of FcεRIβ associate with allergic phenotypes in various populations. These include the substitutions I181L and V183L, usually observed in tandem, and E237G[58,59]. We investigated *in vitro* whether mutant FcεRIβ containing the former mutations, singly or doubly, or the latter single substitution, functioned differently than wild-type FcεRIβ. We assayed the signal-amplifier effect on calcium mobilization and the trafficking-based effect of the β-chain on FcεRI surface expression. None of the β-chain polymorphisms tested enhanced or diminished FcεRIβ function. However, it is appropriate to note that polymorphisms in FcεRIβ may, in fact, be in *linkage disequilibrium* with another genetic factor that is phenotypically associated with atopy.

There is one further level at which FcεRI regulates its own function. FcεRI levels at the surface of mast cells and *basophils* are modulated by the serum IgE concentration; receptor occupancy seems to increase receptor levels at the cell surface. This is an important mechanism as there is a concomitant increase in the severity of effector responses[60]. Ligand-mediated FcεRI upregulation is seen in rodent and human, and is not dependent on FcεRI structure ($\alpha\beta\gamma_2$ compared with $\alpha\gamma_2$). The upregulation is biphasic: a cyclohexamide-insensitive phase precedes a sustained phase that is highly dependent on protein synthesis. IgE-mediated amplification can be modelled as follows. First, IgE binding stabilizes receptor complexes at the surface and protects them from degradation, the result being surface FcεRI accumulation from the intracellular pool without the need for *de novo* synthesis. Later, when all intracellular FcεRI is at the surface, continued upregulation could be maintained only by the synthesis of new complexes from pre-existing transcripts.

The modular nature of FcεRI: implications for animal models

In the field of signal transduction, there has been a general progression from the study of *in vitro* interactions between signalling molecules in cell lines towards the assignment of relevance to such interactions *in vivo*, followed by study of their possible roles in pathology. Because *allergy* and atopy are diseases of the immune system with signal transduction at their core, it is tempting to extend these *in vivo* analyses to the study of mast-cell signalling pathways. However, there are major differences between FcεRI complexes in mouse and human systems that should be noted.

Rodent FcεRI has an obligatory tetrameric $\alpha\beta\gamma_2$ structure. In humans, both $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ complexes are observed. Rodent FcεRI complexes are confined to the surface of mast cells and basophils. In humans, however, it is now recognized that there is a far wider distribution of FcεRI. On the mast cell and basophil surface there is a mixture of $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ complexes, whereas *monocytes*, *Langerhans cells*, *eosinophils* and *dendritic cells* express surface $\alpha\gamma_2$ (ref. 1). Finally, in rodent but not human there is crosstalk between IgE- and IgG-mediated cellular activation. In the rodent, the IgE Fc region can bind to two classes of low-affinity FcγRII, that is, activatory and inhibitory isotypes[61]. Both of these are expressed on mast cells and so there is a route by which IgE or IgG *immune complexes* may regulate mast-cell function independently of FcεRI.

The first question to address is why the rodent FcεRI reaches the cell surface only as an assembled tetramer. Transfection experiments showed that rodent α-chain contains an ER retention signal that is masked by co-transfection of, and assembly with, $\beta\gamma_2$; this allows export to the cell surface. In humans, however, there is $\alpha\gamma_2$ at the cell surface with no β-chain, indicating that the γ-chain alone can balance the human ER retention signal. Why is this not sufficient in rodent? Our data indicate that an additional ER retention signal is placed in the rodent extracellular domain of the α-chain[62]. How this would be deactivated by FcεRIβ to allow surface expression is not clear, although it is now possible to infer from the crystal structure of the α-chain that the D1/D2 cleft could interact with the extracellular portion of FcεRIβ.

Second, in view of the model presented above, where FcεRIβ and Lyn are required for Syk recruitment and activation, we have to ask how the $\alpha\gamma_2$ complex accomplishes competent signalling. The answer to this question may lie in the fact that recruitment to FcεRIβ is not the only mechanism by which Lyn is membrane-targeted. Lyn is myristoylated in its N-terminal 'unique' domain, allowing it to interact with the membrane. In $\alpha\gamma_2$ complexes, a population of Lyn would in fact be present, anchored passively in the receptor context by myristoylation. Here, Lyn will contribute to FcεRIγ/Syk activation, but less efficiently than where specific recruitment and orientation by FcεRIβ occurs.

We should also consider that there is a wider cell distribution of FcεRI in humans than rodents. In humans, FcεRI was detected on normal dendritic and Langerhans cells, as well as the eosinophils of hypereosinophilic patients and monocytes of atopics. In the latter cell types the $\alpha\gamma_2$ receptor has been shown to have a significant role in IgE-mediated antigen presentation[63,64]. The level of FcεRI expression on these cell types is very low, presumably due to the lack of FcεRIβ to assist in trafficking. Because $\alpha\gamma_2$ complexes are less competent at signalling than the $\alpha\beta\gamma_2$ on mast cells and basophils (they lack the β signal amplifier), the function of antigen presentation by the antigen-presenting cells (APCs) may be accomplished with only moderate induction of intracellular signalling pathways. Antigen presentation by $\alpha\gamma_2$ complexes on APCs does lead to some intracellular signalling[63], which is possibly involved in delivering co-stimulatory signals to T cells. Thus the antigen-presenting function of FcεRI provides a direct link between IgE-mediated responses and T cells, and this may be critical to the long-term maintenance of an IgE-driven immune response.

IgE-mediated antigen presentation occurs in humans but not rodents. Moreover, there are other differences between the two systems that lead us to question the validity of rodent models for human allergy. In rodent, multimeric IgE immune complexes can interact with activatory and inhibitory Fcγ receptors (FcγR) on the surface of mast (and other) cells. Obviously, IgG immune complexes can also interact with these FcγR. In humans, there is no crosstalk between IgE- and IgG-mediated responses, so IgE, monomeric or in immune complex, cannot interact with FcγR. This has a particular bearing on *in vivo* experiments, which challenge immunized rodents with high levels of antigen (often delivered intravenously). These antigen doses will form IgE or IgG immune complexes, which will be directed towards FcγR, and the activatory consequence of this will be an immune-complex-based disease state, or Arthus reaction. Small amounts of antigen could in theory drive a monomeric IgE/FcεRI reaction in mice, but in this delivery system it would tend to form immune complexes in the blood and be destroyed as such in the spleen before development of a true allergic reaction. Conversely, in humans, the tiny amounts of *allergen* that are delivered locally (to the skin and lungs) and that are needed to trigger IgE/FcεRI-mediated allergic reactions would never reach the levels needed to form immune complexes.

The observation that IgE-deficient mice can raise anaphylactic reactions should be viewed in the light of these species differences[65]. In fact, this observation is unsurprising, as IgG immune complexes formed when challenging these mice intravenously with high antigen doses will cause FcγR-mediated cellular activation. Moreover, in a recent mouse study, a mechanism was proposed where binding of IgE/antigen to the inhibitory low-affinity IgG receptor FcγRIIb modulates the anaphylactic response[66]. However, because IgE does not bind to FcγR in humans, the relevance of this observation to human allergy is limited.

This is not to argue that mouse models cannot be useful. It is important to be mindful of the fundamental differences between a mouse experiment and a human allergic response. First, IgE-mediated antigen presentation occurs in humans but not in rodents, and so it is unlikely that *in vivo* immunization of rodents (by injection or aerosol) will mimic the response seen in atopic humans. Second, the human allergic response results from monomeric IgE/FcεRI interactions and is not an immune complex disease. Most rodent experiments use amounts of antigen that will undoubtedly form IgG and IgE immune complexes, precisely because smaller amounts of antigen do not produce a biological response. Finally, IgE immune complexes bind to activating and inhibitory FcγR on rodent but not human mast cells. Thus there is a clear demarcation between IgE–FcεRI and IgG–FcγR networks in humans but not in rodents.

Interfering with FcεRI function

Modulation of FcεRI responses has long been considered as a therapeutic strategy in allergy. The field is now in a better position than ever to make rational decisions about drug design. There is a wealth of new information derived from the α-chain crystal structure that may be used to design peptide blockers of the IgE/FcεRIα chain interaction, or possibly of the proposed interaction between the D1/D2 cleft and the extracellular regions of the β- and γ-chains. Intracellular targets within signalling pathways are still not obvious. There are such significant parallels between the signalling cassettes used by FcεRI and other antigen receptors that specificity may be an insurmountable problem, although in humans the FcεRIβ/Lyn interaction occurs only in mast cells and basophils and may therefore offer a viable target. Finally, the recognition that the FcεRI expression levels correlate with serum IgE levels has led to an exciting new development. An anti-IgE immunotherapy is currently in phase-III human clinical trials, based on the concept that resulting decreased serum IgE will prevent levels of FcεRI on the surface of mast cells from accumulating, and therefore prevent a build-up of intensity in IgE-mediated cellular responses. □

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