



Review

Macromolecular protein signaling complexes and mast cell responses: a view of the organization of IgE-dependent mast cell signaling

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Abstract

The generation of signals following engagement of cell surface receptors is an ordered process that requires tight regulation as spurious signals could result in unwanted, and possibly deleterious, cellular responses. Like other cell surface receptors, stimulation of a mast cell via the high affinity IgE receptor (FcεRI) causes multiple biochemical events that ultimately result in cell activation and effector responses. Recently, our knowledge of how these events are ordered has increased. We now have identified some of the molecules involved, how they are organized into macromolecular complexes by FcεRI stimulation, and the role of some of the constituents of these macromolecular signaling complexes in mast cell effector responses. In brief, we review the knowledge on macromolecular signaling complexes used by FcεRI in mast cell activation and provide our view on the regulation of signal generation and its effect on mast cell activation.

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1. FcεRI structure, aggregation, and signaling competence

The subunit composition of the mast cell FcεRI has been determined by biochemical and genetic means (Perez-Montfort et al., 1983; Rivera et al., 1988; Blank et al., 1989). There is agreement that in rodents FcεRI comprises the IgE binding α chain, a single four transmembrane β subunit that functions to amplify signaling responses (Lin et al., 1996; Dombrowicz et al., 1998), and two disulfide linked γ subunits that are critically important to the signal competence of this receptor. Both the β and γ subunits contain an immunoreceptor tyrosine-based activation motif (ITAM) that imparts signaling competence to this receptor when novel protein binding sites are created by the phosphorylation of canonical tyrosine residues within these motifs. In humans, two isoforms of FcεRI exist. They differ in

the presence or absence of an associated β chain. Nevertheless, as in the rodent, the human β chain seemingly serves to amplify the signaling capacity of the human receptor. The structure and function of FcεRI will not be further discussed as it has recently been reviewed (Kinet, 1999). We will, however, briefly describe the early events associated with FcεRI phosphorylation as these events are important in the assembly and organization of macromolecular protein complexes involved in cell signaling.

Initiation of signaling requires aggregation of IgE-occupied FcεRI by antigen (Ag) recognized by the IgE antibody (IgE). This induces FcεRI ITAM phosphorylation by a mechanism that is not yet completely understood but clearly is dependent on the Src family tyrosine kinase, Lyn. Because of the brevity of this review we will not detail the proposed mechanisms for this event, however, the current views are discussed in the presentations of Drs. Draber, and Metzger in this issue. Regardless of the mechanism of phosphorylation, all views are consistent with the initial event of phosphorylation being mediated by Lyn. This is followed by additional recruitment of Lyn as a result of Lyn's phosphotyrosine recognizing SH2 domain with the phosphorylated FcεRI ITAM. Additionally, the phosphorylation of the FcεRI ITAMs causes the recruitment and activation of another tyrosine kinase, Syk, that functions as a "signal amplifying

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kinase” because it targets multiple proteins and causes their activation (for more details on Syk, see Dr. Siraganian’s presentation in this issue). For the most part, these initial steps have been well defined. It is known that Syk activity is required for the phosphorylation of molecules like the calcium regulating phospholipase PLC γ , the guanine nucleotide exchange factor (GEF) Vav1, and the adapter molecules SH2 domain-containing leukocyte protein of 76 kDa (SLP-76) and the linker for activation of T cells (LAT). However, it was not until recently that we have begun to understand how these molecules are organized to facilitate their function in cellular signaling. We now recognize that the cells solution to this problem was the evolution of adapter molecules to bring together signaling molecules and/or compartmentalize them (by use of scaffolding adapters) in a manner that concentrates their activity at a site where functional responses must be induced and governed. This assembly and regionalization of macromolecular signaling complexes is likely to be governed by the extracellular stimulus and the signaling protein expression profile in a given cell.

2. Assembly of macromolecular signaling complexes: LAT an essential molecule for Fc ϵ RI-generated signals

LAT is an adapter protein first discovered in T cells (Zhang et al., 1998a) that contains multiple tyrosyl residues that can be modified by phosphorylation to create novel protein binding sites (analogous to ITAM phosphorylation). LAT is found in the plasma membrane where it is compartmentalized to cholesterol and sphingolipid-enriched regions commonly referred to as lipid rafts due to a post-translational dual acylation at its NH₂-terminus (Zhang et al., 1998b). Mast cells also express LAT (Zhang et al., 1998a) and in these cells it functions to assemble a complex of signaling proteins that include Grb2, Gads, SLP-76, Vav1, PLC γ 1, and PLC γ 2 (Arudchandran et al., 2000). Assembly of this complex requires the aggregation of Fc ϵ RI by IgE/Ag interactions and occurs in the lipid raft domains as biochemical isolation of lipid rafts by sucrose density centrifugation showed that LAT and Vav1 were co-localized in these domains and co-immunoprecipitated (Arudchandran et al.,

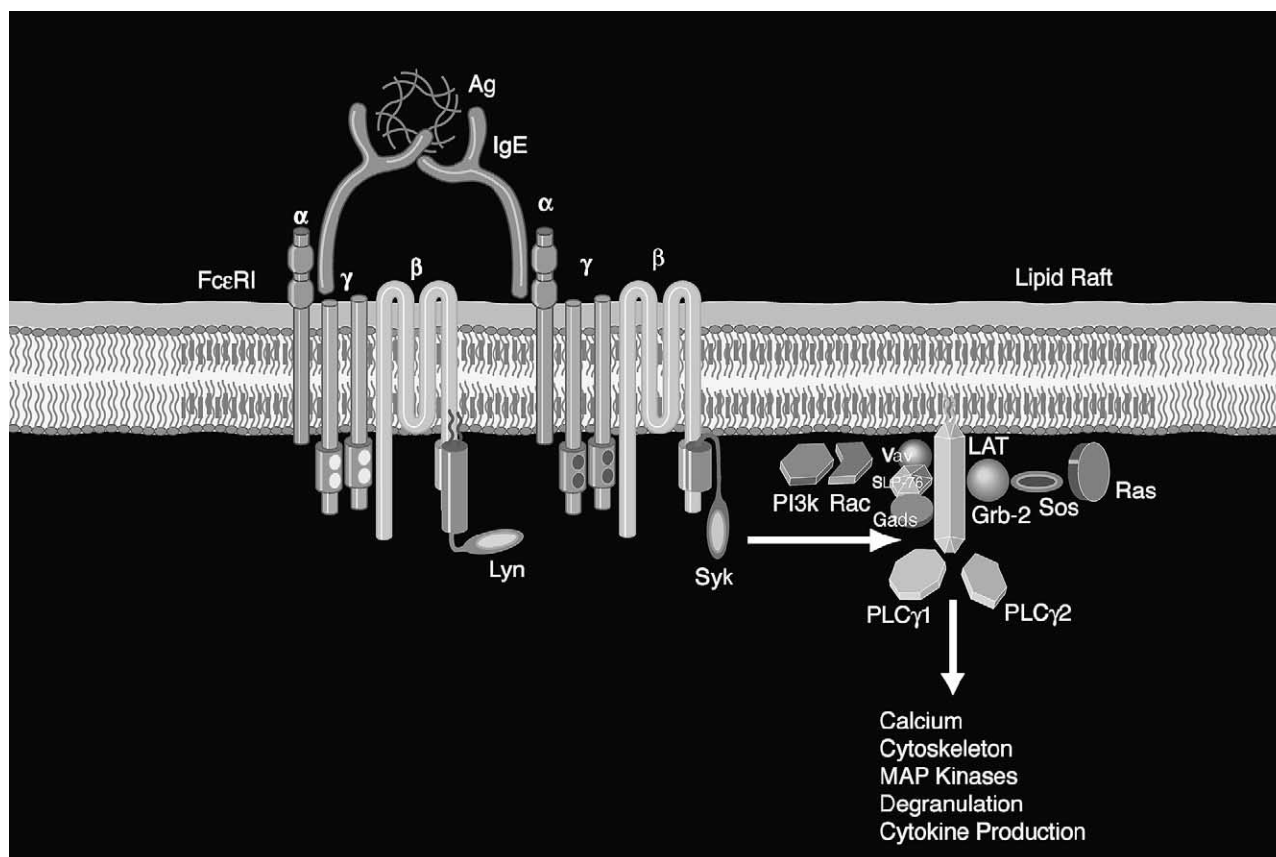


Fig. 1. Assembly of the LAT scaffolded macromolecular signaling complex occurs in lipid rafts in response to Fc ϵ RI aggregation. Antigen-induced aggregation of the multisubunit Fc ϵ RI causes its phosphorylation (activation), by receptor associated Lyn kinase, in the immunoreceptor tyrosine-based activation motif (ITAM, barrel shape segment shown with dots designating the canonical tyrosines that are phosphorylated). Aggregation of Fc ϵ RI also causes inclusion of this receptor in lipid rafts. The phosphorylated receptor binds Syk kinase causing its activation. This activity is critical for phosphorylation of LAT. Phosphorylated LAT binds multiple proteins primarily via phosphotyrosine–SH2 domain interactions. The assembled macromolecular signaling complex is important to activation of the small GTPases Ras and Rac as well as the phospholipases PLC γ 1 and PLC γ 2. Activation of these proteins play a significant role in calcium responses, cytoskeletal rearrangement, and MAP kinase activation. In turn, these activities modulate the ability of mast cells to degranulate and produce cytokines.

2000). Immunoprecipitation of the small amounts of LAT detected outside of the lipid raft domains did not provide evidence for complex formation outside of lipid rafts as Vav1 and PLC γ 1 were not detected as co-immunoprecipitates (unpublished data). Therefore, our results showed that the LAT scaffolded signaling complex assembles in the lipid raft domains as a consequence of Fc ϵ RI stimulation (Fig. 1).

To understand the importance of the LAT-assembled signaling complex to Fc ϵ RI signaling and mast cell function we utilized mice where the LAT gene was genetically disrupted. The importance of LAT in mast cell responses was first demonstrated by *in vivo* experiments in which the LAT-deficient mice were compared to their wild type littermates for susceptibility to a passive systemic anaphylaxis challenge. Mice were systemically sensitized with allergen-specific IgE and subsequently challenged (after 24 h) with systemic administration of the allergen. Mast cell and basophil degranulation was measured quantitatively by measuring the histamine levels in the circulating blood. Unlike wild type mice, which showed increased levels of circulating histamine, LAT-deficient mice showed no significant rise in the levels of circulating histamine (Saitoh et al., 2000). This demonstrated a critical role for LAT in mast cell responses mediated by IgE/Ag.

We further explored this finding in BMMC from LAT-deficient mice. *In vitro* experiments demonstrated a defect in mast cell degranulation as it was lowered by 60–80% of that observed in wild type cells. Biochemical studies revealed a significant defect in the ability of LAT-deficient mast cells to mobilize calcium (Saitoh et al., 2000). Mechanistically, the defect appeared to be in an impaired ability to activate PLC γ 1 and PLC γ 2 as their tyrosine phosphorylation was significantly inhibited. Thus, the production of the second messenger inositol 1,4,5-trisphosphate (IP $_3$) which regulates calcium stores was impaired. Additionally, MAP kinase activation was also significantly impaired as ERK and JNK phosphorylation was almost completely absent in LAT-deficient mast cells. As this kinase family is important for gene expression, we also analyzed the production of cytokines in LAT-deficient mast cells following Fc ϵ RI stimulation. A broad defect was observed as IL-2, IL-3, IL-4, IL-6, IL-10, TNF- α , and GM-CSF mRNA and protein (for those tested) was greatly reduced. However, it should be noted that some chemokines, like MCP-1, appeared to be unaffected (Saitoh et al., 2000). Collectively, these studies demonstrated a central and essential role for LAT in the regulation of mast cell responses following Fc ϵ RI stimulation (Fig. 1).

3. The role of Vav1 in the LAT-organized signaling complex

As mentioned above, the GEF Vav1 associates with LAT in the lipid raft domains upon Fc ϵ RI stimulation. To understand its role in this complex, we performed *in vivo* and *in vitro* studies on Vav1 genetically disrupted mice and

BMMC derived from these mice, respectively. We searched for similarities in the functional phenotype of Vav1- and LAT-deficiencies, as this would indicate an overlap in their functional roles. *In vivo* studies revealed that the absence of Vav1 caused a reduction in mast cell degranulation, as measured by circulating histamine concentrations. However, this was not as dramatic as that observed for LAT-deficiency with the response being inhibited by, approximately 30% when compared to wild type mice (Manetz et al., 2001). Additionally, analysis of cytokine production revealed a more selective defect in Vav1-deficient mast cells, where primarily IL-2 and IFN γ mRNA production was severely impaired.

Immunoprecipitation experiments demonstrated that LAT was normally phosphorylated in Vav1-deficient cells and that the adapter SLP-76 and the phospholipases PLC γ 1 and PLC γ 2 could complex with LAT in the absence of Vav1. However, analogous to the LAT-deficiency, Vav1-deficient mast cells showed a defect in the phosphorylation of PLC γ 1 and PLC γ 2 (Manetz et al., 2001). This led to reduced IP $_3$ production and a defective calcium response. The magnitude of the defect (50% decrease) was identical to that observed for LAT-deficient mast cells. The calcium response and PLC γ 1 and PLC γ 2 phosphorylation could be restored by reconstituting the Vav1-deficient mast cells with wild type Vav1 (Manetz et al., 2001). However, reconstitution with a GEF-deficient Vav1 mutant (Dbl domain mutant) did not restore the calcium response demonstrating the importance of Rac activation and possibly cytoskeletal rearrangement for this event. Therefore, the overlap of Vav1-deficiency with LAT-deficiency is in the regulation of PLC γ phosphorylation and subsequent calcium responses, suggesting that Vav1 is the critical component in the LAT-organized signaling complex which regulates these activities. Nevertheless, it should be noted that in the absence of either LAT or Vav1, some responses were unaltered thus indicating that other LAT-independent pathways might exist.

4. A LAT-independent signaling complex that is negatively regulated by Lyn

The discovery of the Grb2 associated binder (Gab) family of proteins, which are distantly related to the insulin receptor substrate (IRS) family of adapter proteins, has identified a new family of adapter proteins that function downstream of cytokine, growth factor, and antigen receptors (Gu et al., 1998; Gadina et al., 2000; Nishida et al., 1999). One family member, Gab2 (Gu et al., 1998; Zhao et al., 1999), is expressed in mast cells and can be found to associate with phosphatidylinositol-3-OH kinase (PI3K) and the phosphatase SHP-2 (Gu et al., 1998, 2001). Analysis of its functional role in response to Fc ϵ RI was studied in Gab2-deficient mice and BMMC thereof. This study demonstrated that Gab2 contributes to mast cell degranulation and cytokine production by its central role in the regulation of PI3K in mast cells (Gu et al., 2001).

We explored the possibility that Gab2 could scaffold a signaling complex that might be independent of LAT. LAT-deficient mast cells were stimulated by Ag aggregation of FcεRI and Gab2 phosphorylation was measured in comparison to wild type cells. In the absence of LAT, the phosphorylation of Gab2 was normal (Parravicini et al., submitted for publication), providing evidence for the independence of this event from LAT function. Additionally, no significant difference was found in the FcεRI-mediated association of PI3K with Gab2 from LAT-deficient versus wild type cells. We further explored the relationship of these two molecules by co-immunoprecipitation experiments and failed to detect the presence of LAT in Gab2 immunoprecipitates or vice versa. Additionally, it was recently shown that LAT and Gab2 did not co-localize and

Gab2 is not present in lipid rafts (Wilson et al., 2001, and Dr. Wilson's presentation in this issue). This is consistent with our finding that all of Gab2 and the vast majority of PI3K is in the high density fractions of sucrose gradients rather than the low density sucrose fractions where lipid rafts localize (Parravicini et al., submitted for publication). Additionally, when the activity of PI3K in LAT-deficient mast cells was indirectly assessed by the phosphorylation of PKB/AKT, a kinase substrate of phosphatidylinositol 3-OH kinase-dependent kinase (PDK), it was found to be intact. Thus, because Gab2 was previously shown to be a major regulator of PI3K activity in mast cells (Gu et al., 2001) and we found PI3K activity to be intact in LAT-deficient mast cells, there appears to be no functional interdependence or physical association of LAT

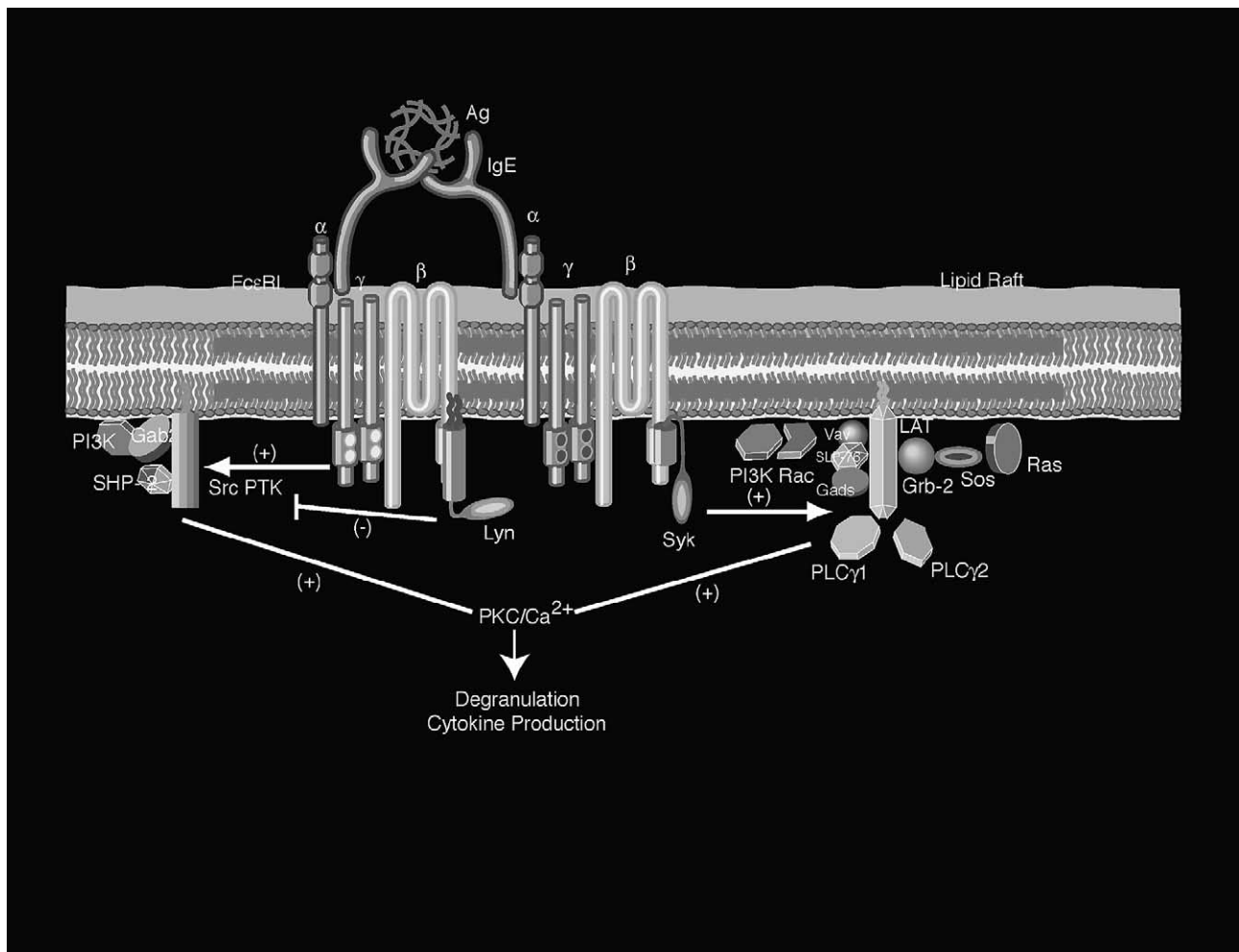


Fig. 2. FcεRI signaling diverges following its aggregation by utilizing more than one macromolecular signaling complex. Aggregation of FcεRI induces the phosphorylation of the adapters LAT and Gab2. These phosphorylated adapters scaffold distinct complexes comprised of proteins that are recruited by SH2 domain recognition of specific phosphotyrosine motifs found in LAT and Gab2. Whereas the LAT complex is formed in lipid rafts, the Gab2 complex does not reside in these domains. The LAT-organized complex requires the activity of Lyn and Syk as phosphorylation of LAT is dependent on these kinases. The Gab2 complex requires the activity of a yet undefined Src family protein tyrosine kinase (Src PTK). Lyn and Syk are not involved in the phosphorylation of Gab2. However, Lyn negatively regulates Gab2 phosphorylation as Lyn's absence causes the constitutive phosphorylation of Gab2. Signals generated by the LAT- and Gab2-organized complexes synergize in the modulation of PKC and calcium. These signals are critical for mast cell degranulation and contribute to the induction of cytokines.

and Gab2 proximal to Gab2 phosphorylation and PI3K activation.

These findings showed that Gab2 functioned upstream or in parallel to the LAT complex. We addressed the possibility that it might function upstream by asking whether Lyn kinase might be needed for Gab2 phosphorylation as it is for LAT phosphorylation (Parravicini et al., submitted for publication). In Lyn-deficient mast cells, Gab2 was found to be phosphorylated constitutively although FcεRI stimulation did enhance the phosphorylation (Parravicini et al., submitted for publication). However, treatment of wild type or Lyn-deficient cells with the Src-selective inhibitor PP2 caused a dramatic inhibition of Gab2 phosphorylation whereas the Syk-selective inhibitor piceatannol had no effect. Additionally, Syk was only weakly active in the Lyn-deficient mast cells. Thus, all evidence suggested a possible role for another Src kinase family member in FcεRI-mediated Gab2 phosphorylation. Studies identifying this kinase are too preliminary to discuss in this review. Regardless, these findings argue that FcεRI utilizes an alternate pathway in the regulation of PI3K that is independent of LAT and is negatively regulated by Lyn kinase. Additionally, we found that PKC activity is enhanced in the absence of Lyn suggesting that the enhanced Gab2/PI3K activity in these cells is important in PKC activation. Therefore, proximal to FcεRI stimulation, the Gab2 complex assembly diverges from the assembly of the LAT complex (Fig. 2). We conclude that FcεRI engages these complexes independent of each other by a mechanism not yet defined and that these two complexes synergize in the regulation of calcium (primarily through LAT) and PKC (primarily through Gab2).

5. Regulation of macromolecular complex assembly and effect on mast cell responses

Mast cells express a given profile of signaling proteins but yet the elicited response is dependent on the stimulus. This suggests that the generated signals (proteins utilized and/or macromolecular complexes formed) differ depending on the stimulus. Indeed, this has been demonstrated to be the case, as G protein coupled receptors form different signaling complexes from those of receptor tyrosine kinases or cytokine receptors, although many components may be shared in common (Miller and Lefkowitz, 2001). However, differences in signal generation can also be much more subtly regulated because in many cases a given stimulus (ligand, which refers to Ag/IgE interactions in the case of FcεRI) can elicit different cell responses depending on its concentration and/or affinity (Chang et al., 1997; Torigoe et al., 1998). We will not dwell on the effects of affinity of an Ag as this is briefly covered in Dr. Metzger's presentation in this issue. Instead, we briefly focus on the relationship between receptor stimulation, assembly of macromolecular complexes and signal generation. Due

to the brevity of this review we cannot detail all studies, however, we will point out several highlights of our own studies that provide what we think is a reasonable hypothesis of how selective responses may be elicited by the same stimulus.

All of our studies to date suggest that productive signals can be generated under conditions where a mast cell does not degranulate. We and others have previously demonstrated that Ag concentrations or other stimuli that did not elicit degranulation from mast cells are fully capable of eliciting the induction of cytokine mRNA accumulation and cytokine release (Leal-Berumen et al., 1994; Gupta et al., 1996; Chang et al., 1997). Thus, it is obvious that the appropriate signaling complexes must be formed to generate the required signals and these complexes do not generate signals that culminate in degranulation. Our current findings provide some clues to the mechanisms involved. First, preliminary evidence suggests the possibility that Gab2 phosphorylation may be more sensitive to a stimulus than LAT phosphorylation. Second, mast cells stimulated by Ag under conditions where IgE occupancy of receptors is low respond by showing a selective profile of cytokine production (Gonzalez-Espinosa et al., in preparation). Third, selective activation of signaling proteins under the conditions of low receptor occupancy and/or low Ag concentrations was observed in our studies (Gonzalez-Espinosa et al., in preparation). This included the detection of one signaling complex in the absence of the detection of others (Parravicini et al., submitted for publication; Gonzalez-Espinosa et al., in preparation). Thus, we propose that the formation of macromolecular signaling complexes and the signals generated are differentially sensitive to the strength of the stimulus. This may also affect what proteins are assembled in the signaling complex, although current evidence for such an effect in FcεRI signaling is still lacking.

6. Concluding remarks

As is evidenced in the proceedings summarized in this issue, our knowledge of the molecular events following FcεRI activation of mast cells and basophils has significantly advanced. We now know that signaling is organized by protein–protein and protein–lipid interactions. This serves to compartmentalize the activity of the signaling proteins generating productive signals. FcεRI-mediated signals utilize at least two macromolecular signaling complexes scaffolded by the adapters LAT and Gab2. There is accumulating evidence that the formation of these signaling complexes and thus the signals generated are regulated by the strength of receptor engagement (aggregation). Thus, varying the concentration of the antigen or the level of receptor occupancy by IgE can elicit differing responses (selective) because it causes the generation of selective signals. This may provide new insights on why individuals with a similar allergy may show differing symptomatic responses.

References

- Arudchandran, R., Brown, M.J., Peirce, M.J., Song, J.S., Zhang, J., Siraganian, R.P., Blank, U., Rivera, J., 2000. The Src homology 2 domain of Vav is required for its compartmentation to the plasma membrane and activation of c-jun NH(2)-terminal kinase 1. *J. Exp. Med.* 191, 47–60.
- Blank, U., Ra, C., Miller, L., Metzger, H., Kinet, J.-P., 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature* 337, 187–189.
- Chang, E.-Y., Szallasi, Z., Acs, P., Raizada, V., Wolfe, P.C., Fewtrell, C., Blumberg, P.M., Rivera, J., 1997. Functional effects of overexpression of protein kinase C- α , - β , - δ , - ϵ , and - η in the mast cell line RBL-2H3. *J. Immunol.* 159, 2624–2632.
- Dombrowicz, D., Lin, S.Q., Flamand, V., Brini, A.T., Koller, B.H., Kinet, J.-P., 1998. Allergy-associated FcR β is a molecular amplifier of IgE- and IgG-mediated in vivo responses. *Immunity* 8, 517–529.
- Gadina, M., Sudarshan, C., Visconti, R., Zhou, Y.J., Gu, H., Neel, B.G., O'Shea, J.J., 2000. The docking molecule Gab2 is induced by lymphocyte activation and is involved in signaling by interleukin-2 and interleukin-15 but not other common γ chain-using cytokines. *J. Biol. Chem.* 275, 26959–26966.
- Gu, H., Pratt, J.C., Burakoff, S.J., Neel, B.G., 1998. Cloning of p97/Gab2, the major SHP2-binding protein in hematopoietic cells, reveals a novel pathway for cytokine-induced gene activation. *Mol. Cell* 2, 729–740.
- Gu, H., Saito, K., Klamann, L.D., Shen, J., Fleming, T., Wang, Y., Pratt, J.C., Lin, G., Lim, B., Kinet, J.-P., Neel, B.G., 2001. Essential role for Gab2 in the allergic response. *Nature* 412, 186–190.
- Gupta, A.A., Leal-Berumen, I., Croitoru, K., Marshall, J.S., 1996. Rat peritoneal mast cells produce IFN- γ following IL-12 treatment but not in response to IgE-mediated activation. *J. Immunol.* 157, 2123–2128.
- Kinet, J.-P., 1999. The high-affinity IgE receptor (Fc ϵ RI): from physiology to pathology. *Annu. Rev. Immunol.* 17, 931–972.
- Leal-Berumen, I., Conlon, P., Marshall, J.S., 1994. 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.
- Lin, S., Cicala, C., Scharenberg, A.M., Kinet, J.-P., 1996. The Fc ϵ RI β subunit functions as an amplifier of Fc ϵ RI γ -mediated cell activation signals. *Cell* 85, 985–995.
- Manetz, T.S., Gonzalez-Espinosa, G., Arudchandran, R., Xirasagar, S., Tybulewicz, V., Rivera, J., 2001. Vav1 regulates phospholipase C γ activation and calcium responses in mast cells. *Mol. Cell. Biol.* 21, 3763–3774.
- Miller, W.E., Lefkowitz, R.J., 2001. Expanding roles for β -arrestins as scaffolds and adapters in GPCR signaling and trafficking. *Curr. Opin. Cell Biol.* 13, 139–145.
- Nishida, K., Yoshida, Y., Itoh, M., Fukada, T., Ohtani, T., Shirogane, T., Atsumi, T., Takahashi-Tezuka, M., Ishihara, K., Hibi, M., Hirano, T., 1999. Gab-family adapter proteins act downstream of cytokine and growth factor receptors and T- and B-cell antigen receptors. *Blood* 93, 1809–1816.
- Perez-Montfort, R., Kinet, J.-P., Metzger, H., 1983. A previously unrecognized subunit of the receptor for immunoglobulin E. *Biochemistry* 22, 5722–5728.
- Rivera, J., Kinet, J.-P., Kim, J., Pucillo, C., Metzger, H., 1988. Studies with a monoclonal antibody to the β subunit of the receptor with high affinity for immunoglobulin E. *Mol. Immunol.* 25, 647–661.
- Saitoh, S., Arudchandran, R., Manetz, T.S., Zhang, W., Sommers, C.L., Love, P.E., Rivera, J., Samelson, L.E., 2000. LAT is essential for Fc ϵ RI-mediated mast cell activation. *Immunity* 12, 525–535.
- Torigoe, C., Inman, J.K., Metzger, H., 1998. An unusual mechanism for ligand antagonism. *Science* 281, 568–572.
- Wilson, B.S., Pfeiffer, J.R., Surviladze, Z., Gaudet, E.A., Oliver, J.M., 2001. High resolution mapping of mast cell membranes reveals primary and secondary domains of Fc ϵ RI and LAT. *J. Cell Biol.* 154, 645–658.
- Zhang, W., Sloan-Lancaster, J., Kitchen, J., Tribble, R.P., Samelson, L.E., 1998a. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* 92, 83–92.
- Zhang, W., Tribble, R.P., Samelson, L.E., 1998b. LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. *Immunity* 9, 239–246.
- Zhao, C., Yu, D.-H., Shen, R., Feng, G.-S., 1999. Gab2 a new pleckstrin homology domain-containing adapter protein, acts to uncouple signaling from ERK kinase to Elk-1. *J. Biol. Chem.* 274, 19649–19654.