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Crosslinking-mediated activation of the FcεRI: Does it need antigen for success?

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Abstract: Mast cells (MCs), hematopoietic cells of the myeloid lineage, are well-known for their pro-inflammatory nature contributing to the development of various allergic and autoimmune diseases. One of the characteristic receptors on MCs, the high-affinity receptor for IgE (FcεRI), is activated in its IgE-bound state via binding and crosslinking by polyvalent antigen. This results in its phosphorylation by the SRC family kinase LYN, initiating differential signaling pathways, eventually triggering immunological effector functions, such as degranulation and cytokine production. Few publications have reported on FcεRI-dependent but antigen-independent MC activation by antibody-mediated crosslinking of membrane molecules (e.g., transmembrane proteins and glycosphingolipids) that are both localized in membrane rafts and in close vicinity to the FcεRI. In this Viewpoint we will briefly introduce FcεRI-mediated MC stimulation, cite examples of FcεRI-proximal molecules, the crosslinking of which can cause FcεRI-dependent MC activation, and discuss the potential of certain viruses as well as auto-antibodies to act as indirect FcεRI-crosslinking agents. In latter cases, antigen-independent FcεRI-mediated pro-inflammatory MC activation could contribute to the development of detrimental cytokine storms.

Introduction

Mast cells (MCs) are innate immune cells and belong to the hematopoietic lineage. They are strategically located at the borders between self and environment of eukaryotic organisms, particularly in skin, lung and gastrointestinal tract (Metz and Maurer, 2007; Huber et al., 2019). Though being evolutionary older than the adaptive immune system, MCs are endowed with immunoglobulin-binding Fc receptors, among which the high-affinity receptor for IgE (FcεRI) is one of the most characteristic MC receptors. The FcεRI belongs to the family of multichain immune recognition receptors (MIRRs). It comprises an IgE-binding α-chain, a signal-modulating tetraspanin β-chain, and a disulfide-bridged signal-generating γ-chain homodimer. Both β- and γ-chains contain so-called ITAMs (immunoreceptor tyrosine-based activation motifs), which are phosphorylated by the tyrosine kinase LYN upon receptor stimulation (Fig. 1A). Concerning these initial tyrosine phosphorylation events that spark the downstream IgE-mediated signaling and effector processes, three models are discussed, which most likely are not mutually exclusive: i) the transactivation model,

ii) the lipid raft model, and iii) the kinase-phosphatase interplay model (Bugajev et al., 2010). In principle, FcεRI stimulation can be provoked via crosslinking of the IgE-bound FcεRI by polymeric antigens (Fig. 1B), eventually triggering several intricately interconnected signaling pathways (e.g., phosphatidylinositol-3-kinase (PI3K) pathway, phospholipase C-γ (PLC-γ) pathway, and mitogen-activated protein kinase (MAPK) pathways). Antigen-triggered MCs immediately release preformed mediators (e.g., proteases, histamine, and proteoglycans) stored in secretory lysosomes in a process called degranulation, and de novo synthesize arachidonic acid metabolites (e.g., leukotrienes and prostaglandins) as well as various cytokines and chemokines such as IL-6 and TNF. Regarding this direct crosslinking-mediated mechanism of FcεRI activation triggered by polyvalent antigens, it is tempting to speculate that indirect crosslinking of single FcεRI complexes provoked by the aggregation of raft-localized transmembrane proteins proximal to the FcεRI might result in MC activation, as well.

A First Proof of Principle

Compared to other MIRRs like the B- and T-cell receptors, the number of established co-receptors for the FcεRI is considerably smaller. Thus, several studies were undertaken,

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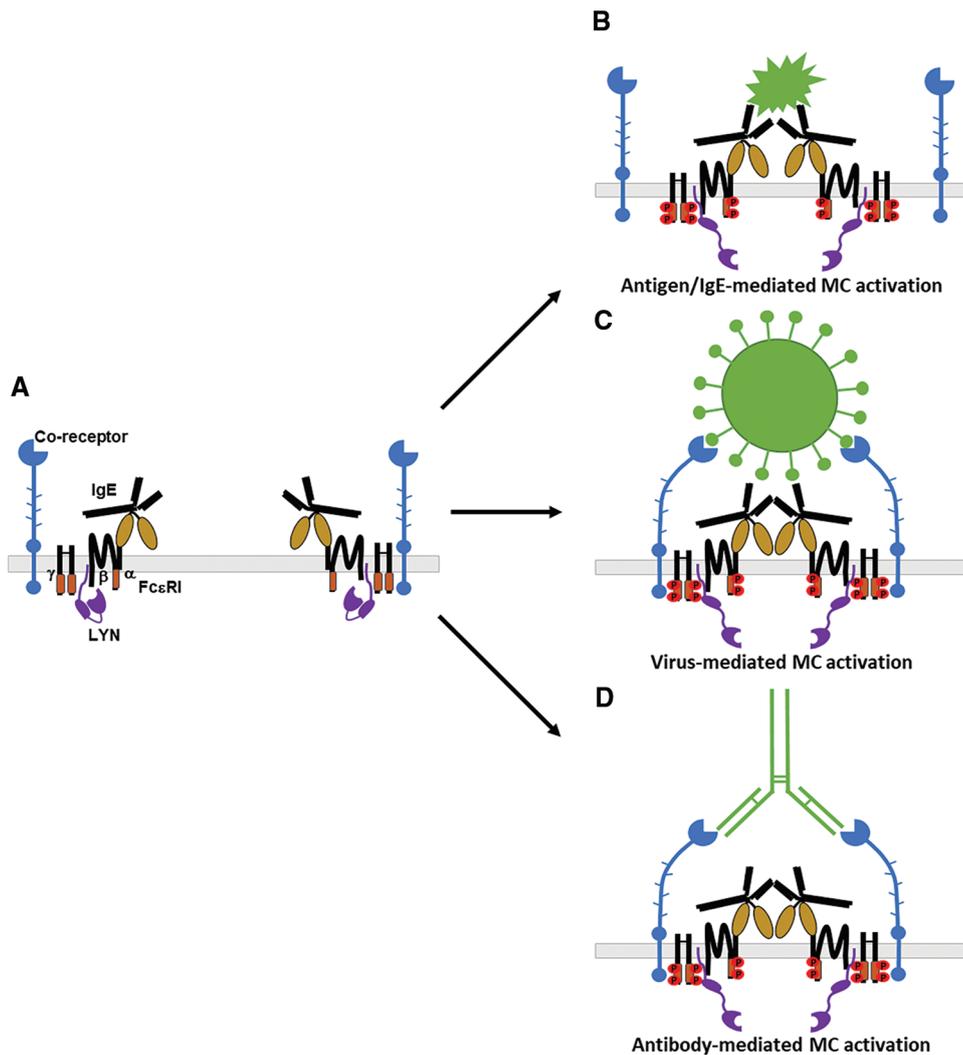
FIGURE 1. Well-known antigen-dependent and potential antigen-independent FcεRI-mediated mast cell activation. In the resting state, single FcεRI complexes consisting of the α, β, and γ subunits are located in the plasma membrane, pre-associated with the SRC family kinase LYN and (various) coreceptors (A). IgE-dependent binding to polyvalent antigens induces the canonical FcεRI activation followed by LYN-dependent phosphorylation of ITAMs in the β and γ subunits (B). Activation of the FcεRI could be achieved by crosslinking of associated co-receptors that bind polyvalent antigens, like viruses (C), or by co-receptor-targeting antibodies (D). Depending on the epitope, which the crosslinking antibody recognizes, or the type of targeted co-receptor, these non-canonical ways of FcεRI activation do or do not require IgE bound to the α chain.

in which antibodies against MC membrane structures were produced to be analyzed with respect to their MC activating or suppressing potential either when applied alone or when used for stimulation in combination with antigen. Schwarz et al. (2000) raised respective monoclonal antibodies by immunizing mice with membrane preparations of the rat MC line RBL-2H3 and tested for their potency to influence FcεRI-triggered MC activation. Intriguingly, one of these antibodies (belonging to the IgM class) was able to induce degranulation of IgE-preloaded MCs in an antigen-independent manner. However, this antibody did not bind to any FcεRI-interacting membrane protein but rather to four types of glycosphingolipids. This fits perfectly into the plethora of data describing the importance of glycosphingolipid/cholesterol-rich membrane domains (so-called rafts) for antigen-triggered activation of the FcεRI (Varshney et al., 2016). Thus, co-aggregation of IgE-loaded FcεRI by the glycosphingolipid-specific antibody sufficed to initiate signaling mechanisms necessary for induction of the secretion process. Importantly, these data indicated that productive crosslinking of IgE-loaded FcεRI does not only depend on IgE-specific antigen but can also be promoted by aggregation of FcεRI-interacting or proximal membrane molecules. This suggests that crosslinking of FcεRI-associated, raft-localized transmembrane proteins in

physiological or pathophysiological settings could be capable of fueling inflammatory processes by triggering MC degranulation, arachidonic acid metabolism and/or cytokine secretion. Such antigen-independent crosslinking might be accomplished by auto-antibodies against these FcεRI-associated membrane proteins or by other multivalent ligands binding to such proteins.

Viruses and Auto-Antibodies as Potential FcεRI-Dependent MC Activators

Particularly viruses, being evolutionary-perfected multivalent antigens, would have what it takes to activate MCs in an FcεRI-dependent but antigen-independent manner, potentially promoting the contribution of MCs to the development of pathological cytokine storms in the course of virus infections. In an endeavor to find novel FcεRI-interacting proteins, potentially exerting co-receptor activities, we identified CD13/aminopeptidase N as an FcεRI-interacting membrane protein and a negative regulator of MC activation (Zotz et al., 2016). CD13 was co-internalized upon antigen-mediated FcεRI triggering, verifying close proximity of these raft-associated proteins. Remarkably, a CD13-specific monoclonal antibody induced pro-inflammatory cytokine production and secretion in IgE-loaded murine bone marrow-derived MCs (BMMCs) in

a strictly FcεRI-dependent manner (Fig. 1D). Moreover, the anti-CD13 antibody synergistically enhanced antigen-triggered cytokine production (Zotz et al., 2016). This raised the question if CD13-mediated FcεRI crosslinking can be of any physiological or pathophysiological relevance. Though not proven yet, it appears interesting that in humans CD13 acts as receptor for coronavirus 229E that causes upper respiratory tract infections (Yeager et al., 1992). Hence, the occurrence of an infection with coronavirus 229E might be able to aggravate IgE-driven allergic diseases via interaction with CD13 and consequent indirect crosslinking of the FcεRI (Fig. 1C). Moreover, production of CD13-specific auto-antibodies was reported in bone marrow-transplanted, cytomegalovirus-positive patients (Rahbar et al., 2006). This suggests that auto-antibodies raised against membrane proteins, which have been caught in viral membranes during virus budding, in principle can have what it takes to bind and crosslink these membrane proteins. Given that such auto-antibodies concern FcεRI-proximal MC proteins, MC activation might be the consequence. Intriguingly, in a very recent study, Wang et al. (2021) analyzed a cohort of 194 SARS-CoV-2 infected COVID-19 patients for auto-antibodies against proteins of the so-called exoproteome, including cell surface proteins. Compared to healthy controls, COVID-19 patients exhibited drastic increases in auto-antibodies against immunomodulatory proteins. Given that such proteins are raft-localized in MCs, respective antibodies could drive inflammation via indirect FcεRI crosslinking and activation, and thus contribute to the generation of detrimental cytokine storms in severe cases of COVID-19.

Along these lines, CD26, a.k.a. dipeptidyl peptidase IV (DPP4), which was recently identified on human skin MCs (Gschwandtner et al., 2017), has been demonstrated to act as a functional receptor for the MERS coronavirus (hCoV-EMC) (Raj et al., 2013). Fitting to the localization of the FcεRI, CD26 in T lymphocytes is expressed in lipid rafts. Moreover, it has been shown that crosslinking of CD26 by anti-CD26 antibodies can cause co-aggregation of the transmembrane tyrosine phosphatase CD45 in lipid rafts, hence promoting activation of the tyrosine kinase LCK mandatory for T cell antigen receptor activation (Ishii et al., 2001). Though MCs express several SRC family kinases, LCK is not amongst them; however, one of the central functions of LCK in T cells, namely to phosphorylate the ITAMs in the CD3 chains of the T cell antigen receptor, is taken on by LYN in MC FcεRI signaling, which is also dephosphorylated by CD45 at its C-terminal inhibitory tyrosine residue, and thus activated to phosphorylate the ITAMs of the FcεRI β- and γ-chains (Bugajev et al., 2010). In line with a positive regulatory role of CD45, murine MCs deficient for CD45 show dramatic attenuation of antigen-triggered degranulation and cytokine production (Grochowy et al., 2009). Interestingly, it has been demonstrated that human skin MCs secrete an active form of CD26 (Gschwandtner et al., 2017), which would suggest the possibility of release of an endogenous competitor against MERS coronavirus binding to MCs.

Also of interest and in line with the topic of this viewpoint, the glycoprotein 120 (gp120) of human HIV-1 is an immunoglobulin superantigen, which interacts with the heavy chain variable 3 (V$_H$3) regions of human immunoglobulins, and was shown to activate IgE-bound human lung MCs to the release of proinflammatory factors (Marone et al., 2020). FcεRI-mediated MC activation can be induced by shed gp120, and gp120 is suggested to crosslink V$_H$3-positive IgE-loaded FcεRI in its virus-bound form (Marone et al., 2001).

FcεRI-Associated Proteins as Boosters of Mast Cell Activation

MC activation by use of antibodies against non-FcεRI transmembrane proteins as well as enhancement of antigen-triggered MC effector functions by such antibodies was found for the T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), a protein described as an immune checkpoint receptor on various immune cells as well as leukemic stem cells (Wolf et al., 2020). TIM-3 is constitutively expressed on mouse peritoneal MCs (PMCs) and BMMCs (Nakae et al., 2007), and its expression has been demonstrated to be upregulated in human cord blood-derived MCs upon TGF-β stimulation (Wiener et al., 2007). Interestingly, whereas in two studies polyclonal antibodies against TIM-3 have been found to promote the production of various cytokines (IL-4, IL-6 and IL-13) (Nakae et al., 2007; Phong et al., 2015), only in the study by Phong et al. also monoclonal anti-TIM-3 antibodies were capable of promoting antigen-triggered production of cytokines. Of note, different clones of monoclonal antibodies were used in these studies, indicating that location of the respective epitope within the TIM-3 molecule and antibody-evoked conformational changes, amongst others are crucial for their stimulatory capacity. Further analyses demonstrated that TIM-3 constitutively interacted with the FcεRI signaling subunits and was co-internalized with the FcεRI upon antigen stimulation, consistent with a close physical and functional association between TIM-3 and the FcεRI (Phong et al., 2015). As mentioned above, TIM-3 can act as an immune checkpoint receptor, and the co-blockade of TIM-3 and the transmembrane protein programmed cell death protein 1 (PD-1) has been shown to attenuate tumor progression in different preclinical models (Acharya et al., 2020). Meanwhile different clinical trials using anti-TIM-3 antibodies have been initiated, either using TIM-3-specific antibodies as monotherapy or as combined therapy with anti-PD-1 antibodies or further chemotherapeutic agents (Acharya et al., 2020). It will be interesting to see whether MC activation-associated effects will be measured, particularly in allergic patients.

While anti-CD13 antibodies were able to activate BMMCs in an FcεRI-dependent but antigen-independent manner and cause considerably enhanced activation when co-applied with antigen (Zotz et al., 2016), anti-TIM-3 antibodies only showed MC stimulating effects when used in combination with antigen. Though treatment with anti-TIM-3 antibodies alone did not impact on phosphorylation/activation of PLC-γ, a significant effect was measured upon co-stimulation with antigen and anti-TIM-3 antibodies. This suggests that TIM-3 integrates its signals into the signaling processes triggered by the FcεRI (Phong et al., 2015). Interestingly, a comparable situation was found

by Sibilano *et al.* (2016) who investigated the functional interaction between FcεRI and the transmembrane protein TNF receptor superfamily 14 (TNFRSF14). The co-stimulation of MCs with antigen and the TNFRSF14 ligand, TNFSF14, resulted in strongly augmented MC signaling and production of various mediators, though TNFSF14 alone was without effect in these respects. In agreement with these data, a thorough confocal laser scanning microscopy analysis revealed a drastically increased number and size of FcεRI-containing clusters on the MC surface upon FcεRI and TNFRSF14 co-activation compared to activation of the FcεRI alone (Sibilano *et al.*, 2016). This clearly defined TNFRSF14 as a co-receptor of the FcεRI. The ligand, TNFSF14, is assembled into a trimer, which is the basis for the clustering of its receptor, probably contributing to the enhanced aggregation of the co-activated FcεRI.

Conclusion

Few but thorough studies have demonstrated that antibody-mediated crosslinking of raft-localized, FcεRI-proximal molecules, like transmembrane proteins or glycolipids, can result in FcεRI-dependent MC activation. Though "crosslinking" is a concept comprehensible to many, productive crosslinking that results in successful receptor activation requires more than uncontrolled receptor aggregation. Allergens/antigens have to exert an optimal oligo-/polyvalency to form conjugates containing antigen, IgE, and FcεRI of proper size and geometry. Studies using synthetic multivalent antigens of defined distance between their epitopes have demonstrated the importance of antigen size to promote MC activation (Tesfaye *et al.*, 2021). Thus, most likely not every FcεRI-proximal transmembrane protein bound by an antibody or a virus might have the right conformation or distance to the FcεRI sufficient to induce mediator release. Moreover, the presence of IgE prebound to the FcεRI or the co-stimulation with respective antigen can make a difference. Such mechanism of indirect FcεRI-mediated MC activation, as described in this Viewpoint, might not be observable in every case where an FcεRI-proximal transmembrane protein is recognized by a virus or an auto-antibody. Nevertheless, these situations in which FcεRI crosslinking is induced by auto-antibodies against FcεRI-proximal transmembrane proteins or infections with viruses using such proteins as entry receptors, might result in pro-inflammatory MC activation that could contribute to the development of detrimental cytokine storms.

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