Inhibitory Immunoreceptors on Mast Cells in Allergy and Inflammation

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Abstract Activation of immune cells is regulated by positive and negative signals triggered by activating and inhibitory cell surface immunoreceptors, respectively. Inhibitory receptors are characterized by the immunoreceptor tyrosine-based inhibition motif (ITIM) in their cytoplasmic domains and play an important role in immune regulation by both lymphoid and myeloid cells. Mast cells express the high-affinity receptor for IgE (FcεRI) and toll-like receptors (TLR) on the cell surface, and play a central role in allergic and non-allergic inflammations. We identified novel inhibitory immunoglobulin-like receptors, Allergin-1 and CD300a, which are expressed on mast cells. Allergin-1 inhibits mast cell degranulation via suppression of FcεRI-mediated signaling. Allergin-1-deficient mice showed significantly exacerbated IgE-associated type 1 immediate hypersensitivity reactions. On the other hand, CD300a recognizes phosphatidylserine exposed on the plasma membrane of apoptotic cells and inhibits production of chemoattractants from mast cells in response to LPS stimulation. CD300a-deficient mice showed significantly prolonged survival after cecum ligation and puncture (CLP). Together, our results suggest that Allergin-1 and CD300a may be candidates as molecular targets for the treatment of mast cell-dependent inflammatory diseases.

Keywords Mast cell • Inhibitory immunoreceptor • Allergin-1 • CD300a • Allergy • Sepsis • Immunoreceptor tyrosine-based inhibitory motif (ITIM)

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Introduction

Activation of immune cells is regulated by positive and negative signals triggered by activating and inhibitory cell surface immunoreceptors, respectively. These [1] immunoreceptors play important roles in regulation of immune responses [2, 3]. Inhibitory receptors are characterized by the immunoreceptor tyrosine-based inhibition motif (ITIM) in their cytoplasmic domains. The prototype 6–amino acid sequence for ITIM is (I/V/L/S)-x-Y-x-x-(L/V) (x denotes any amino acid), whose tyrosine is phosphorylated upon ligand binding, providing a docking site for the recruitment of Src homology 2 (SH2)-containing cytoplasmic phosphatases [4, 5] and shutting down activation signals by dephosphorylating intracellular substrates at the earliest steps of the activation response. The ITIM-bearing cell surface immunoreceptors, including certain NK receptors, Fc receptors (FcγRIIb), and others, play a central role in mediating negative signals in both lymphoid and myeloid cells [6].

Mast cells express the high-affinity receptor for IgE (FcεRI) on the cell surface, and play a central role in IgE-associated allergic responses [7, 8]. Crosslinking of FcεRI-bound IgE with multivalent antigen initiates the activation of mast cells by promoting the aggregation of FcεRI. This process results in the degranulation of mast cells, with the concomitant secretion of chemical mediators, such as histamine, tryptase, carboxypeptidase A, and proteoglycans, which are stored in the cytoplasmic granules, the de novo synthesis of pro-inflammatory lipid mediators, such as prostaglandins and leukotrienes, and platelet-activating factor (PAF) in the early phase (5–30 min after exposure to antigen), and the synthesis and secretion of cytokines and chemokines in the late phase (2–6 h after exposure to antigen) [9].

FcεRI-mediated mast cell activation is modulated by several cell surface inhibitory receptors [10], including FcγRIIB [4], paired Ig-like receptor (PIR)-B [11, 12], gp49B1 [13], mast-cell function-associated antigen (MAFA) [14, 15], and signal regulatory protein (SIRP)-α [16]. The cytoplasmic portion of these inhibitory receptors commonly contains the ITIM. When these inhibitory receptors are co-ligated with FcεRI, the tyrosine residue in the ITIM is phosphorylated and recruits *src* homology 2 (SH2) domain–containing protein tyrosine phosphatase (SHP)-1, SHP-2, and/or SH2 domain–containing inositol 5-phosphatase (SHIP), thereby blocking the early step in the activation signal mediated by FcεRI. The regulatory mechanisms of FcεRI-mediated mast cell activation have not been completely elucidated, and a molecular target that controls allergic and inflammatory responses has not been identified.

We identified Allergin-1 and CD300a, which are novel inhibitory immunoglobulin-like receptors expressed on mast cells. In this chapter, we describe the molecular and functional characteristics of these receptors and discuss the possibility of these molecules as molecular targets for the therapy of allergy and inflammation.

Allergin-1

Identification of Allergin-1

By using the signal sequence trap method [17], we identified a cDNA (*MILR1*) encoding an Ig-like receptor, designated Allergin-1, which consists of a 19–amino acid (aa) leader sequence, a 208-aa extracellular region composed of two Ig-like domains, a 21-aa transmembrane domain, and a 95-aa cytoplasmic domain (Fig. 1) [18]. We also identified two cDNAs encoding Allergin-1 isoforms, which lacked the first or second Ig-like domain in the extracellular portion and were designated Allergin-1 short form 1 (Allergin-1S1) and Allergin-1 short form 2 (Allergin-1S2), respectively (Fig. 1). The extracellular portions of mouse Allergin-1 and human Allergin-1L, and those of human Allergin-1S1 and Allergin-1S2, contained six and three potential N-linked glycosylation sites, respectively. Genomic DNA database analyses demonstrated that *MILR1* consists of ten exons. The genes encoding Allergin-1 (*MILR1 and milr1)* are located on chromosome 17q23.3 in humans and chromosome 11E1 in mice, near *CD300* (or *Cd300*) family genes that encode

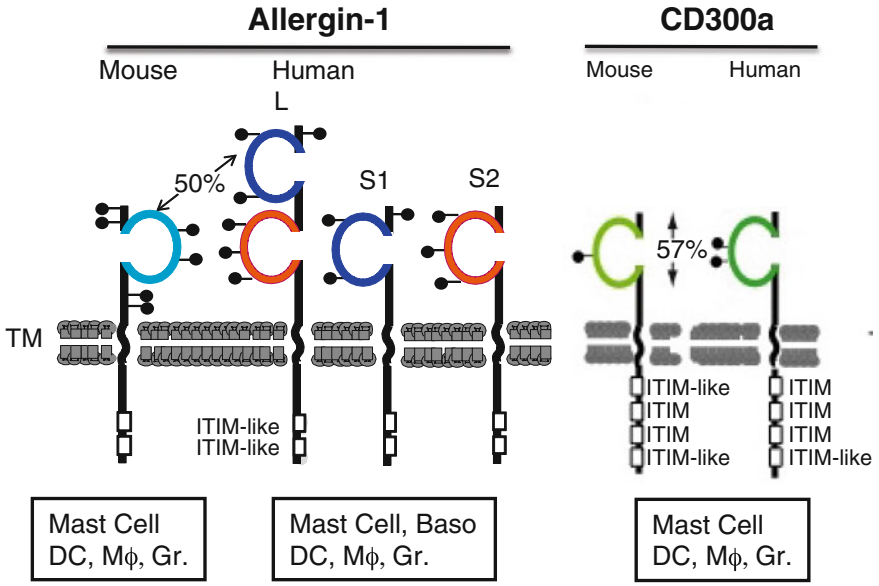

Fig. 1 Schematic diagram of mouse and human Allergin-1 and CD300a proteins. The transmembrane domain (TM) and the potential N-linked glycosylation sites are indicated. The amino acid (aa) sequence of the first Ig-like domain (*blue*) in human Allergin-1-L is 50 % identical to the Ig-like domain in mouse Allergin-1. The aa sequences of the first (*blue*) and second (*red lined*) Ig-like domain in human Allergin-1-L are identical to those of Allergin-1-S1 and Allergin-1-S2. The amino acid sequence of the Ig-like domain in human CD300a is 57 % identical to the Ig-like domain in mouse CD300a. DC (dendritc cell), MΦ (macrophage), Gr (neutrophil)

Ig-like receptors mediating positive or negative signals in myeloid cells [19, 20]. *MILR1 and milr1* were also close to the gene, which encodes PECAM-1 (CD31 or Cd31), an ITIM-bearing Ig-like receptor expressed on myeloid cells and platelets.

Expression of Allergin-1

Flow cytometry analyses of spleen cells demonstrated that Allergin-1 is expressed on dendritic cells, macrophages and neutrophils, but not on T, B, or natural killer (NK) cells in mice. However, Allergin-1 was most strongly expressed on mast cells in the peritoneal cavity. In contrast, Allergin-1 was not detected on basophils in the bone marrow. In agreement with mouse Allergin-1, human Allergin-1L and/or Allergin-1S1 were also expressed in myeloid cell lineages, including monocytes, neutrophils, and dendritic cells, in the peripheral blood. It was noted that, unlike mouse Allergin-1, human Allergin-1L and/or Allergin-1S1 were considerably expressed on peripheral blood basophiles [18].

Anti-human Allergin-1 monoclonal antibodies (mAbs) EX32 and EX33 recognize an epitope of the first and the second Ig-like domain of Allergin-1L, respectively. By using these mAbs simultaneously together with mAbs against the lineage markers (CD3, CD19, CD56, CD11b, and CD11c), c-Kit and FcεRIα, we developed a multi-color flow cytometric method to characterize the human primary mast cells in the bronchial alveolar lavage fluid (BALF). Mast cells were defined as $PI^{-}CD45^{+}Lin^{-}c\text{-}Kit^{+}Fc\varepsilon RI\alpha^{+}$cells, which comprised 0.153 % ± 0.041 % ($n = 28$) of the total cell population in BALF. We found diverse expression profiles of Allergin-1 isoform on BAL mast cells among donors; however, Allergin-1S1 was dominantly expressed on BAL-MC, compared with Allergin-1L1 or Allergin-1S2 (Fig. 2) [21].

Function of Allergin-1

In Vitro Analyses of Allergin-1

Allergin-1 contains immunoreceptor tyrosine-based inhibitory motif (ITIM)-like sequences in the cytoplasmic portion, suggesting that Allergin-1 is tyrosine phosphorylated and recruits the SH2-containing phosphatases, such as SHP-1, SHP-2, or SHIP. Because Allergin-1 is strongly expressed on mast cells, we examined whether mouse Allergin-1 mediates an inhibitory signal against FcεRI-mediated degranulation, by using a rat basophil leukemia cell line, RBL-2H3. Co-ligation of Allergin-1 with FcεRI significantly decreased degranulation, as determined by β-hexosaminidase release from the transfectants, when compared with FcεRI stimulation alone, suggesting that mouse Allergin-1 inhibits IgE-mediated degranulation of mast cells (Fig. 3).

Because an insufficient number of human primary mast cells can be obtained to conduct the ELISA assay for chemical mediators such as histamine or β-hexosaminidase released from mast cells of BALF samples, we performed an

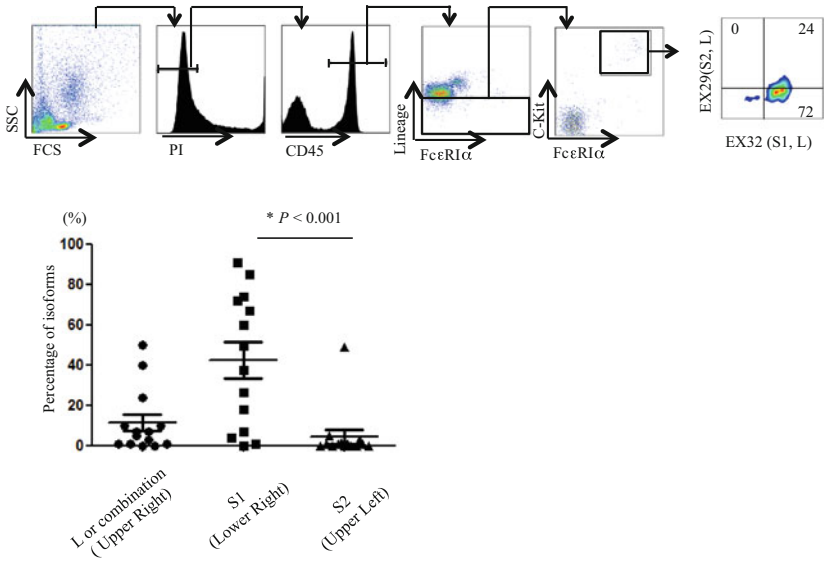

Fig. 2 Expression of Allergin-1 isoforms on human mast cells in bronchial alveolar lavage fluid (BALF). Cells obtained from the BALF of patients with pulmonary diseases ($n = 14$) were stained with PI, anti-CD45, lineage mAb cocktail containing anti-CD3, anti-CD19, anti-CD56, anti-CD11b, and anti-CD11c mAbs, anti-FcεRIα, and anti-c-Kit mAbs together with isotype control, and EX32 and/or EX29 mAb, and the BAL-MC were analyzed by using flow cytometry. Representative results are shown in the *upper panel*. "Combinations" indicates mast cells in the *upper right quadrant* that express either L + S1, L + S2, L + S1 + S2, or S1 + S2

activation assay by utilizing a multicolor flow cytometry to detect cell surface CD107a as a marker of mast cell degranulation. We detected mast cells derived from the culture of cord blood and peripheral blood stem cells that turned out to be positive for CD107a expression on the cell surface when the mast cells were stimulated with anti-TNP IgE followed by TNP-conjugated control mAb. However, $CD107a^+$ cells were significantly decreased when FcεRIα was colligated with Allergin-1 with anti-TNP IgE and TNP-conjugated anti-Allergin-1 mAb [21]. We then examined the inhibitory function of Allergin-1 on mast cells in BALF. BALF cells were also stimulated and then stained with anti-CD107a and mAbs for the identification of BAL mast cells, as described above, and analyzed by using multi-color flow cytometry. We detected a subpopulation of BAL mast cells that was positive for CD107a when the BALF cells were stimulated with anti-TNP IgE followed by TNP-conjugated control mAb. However, the population of $CD107a^+$ mast cells was significantly decreased when BAL mast cells were stimulated via anti-TNP IgE and TNP-conjugated anti-Allergin-1 mAb [21]. Since BAL mast cells also preferentially express Allergin-1S1 rather than Allergin-1L or Allergin-1S2, these results indicated that Allergin-1S1 inhibits the IgE-mediated activation of BAL mast cells.

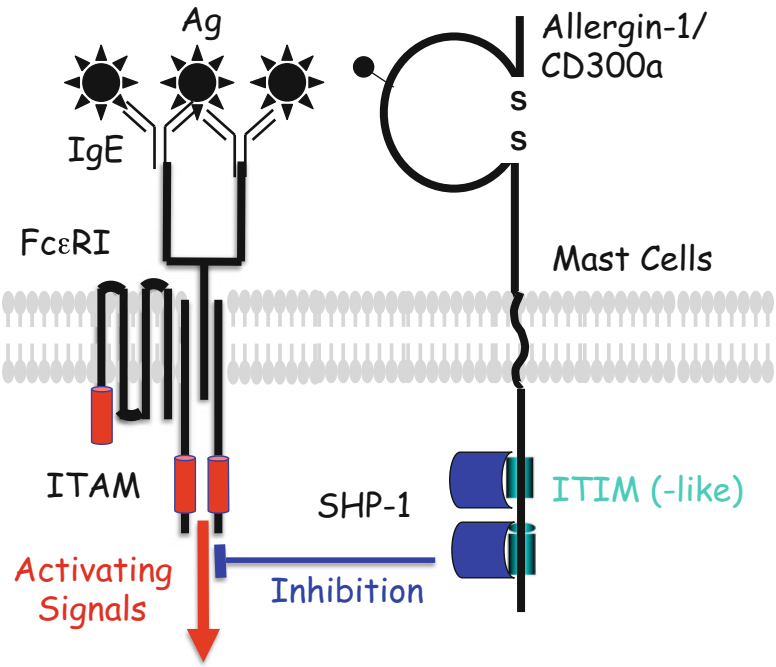

Fig. 3 Schematic model of Allergin-1 and CD300a-mediated inhibition of FcεRI signaling in mast cells. Allergin-1 and CD300a recruit tyrosine phosphatases such as SHP-1 and SHP-2 to the ITIM in the cytoplasmic region. The activated tyrosine phosphatases then dephosphorylate tyrosine phosphorylated FcεRIγ-mediated signaling molecules and shut down FcεRI-mediated mast cell activation

In Vivo Analyses of Allergin-1

Since Allergin-1 inhibited FcεRI-mediated degranulation of mast cells in vitro, we next examined whether Allergin-1 was involved in passive systemic and cutaneous anaphylaxises (PSA), an IgE-mediated type 1 immediate hypersensitivity reaction. WT and Allergin-1-deficient mice were passively sensitized with anti-TNP IgE mAb and then intravenously injected with ovalbumin (OVA) or TNP-conjugated OVA (TNP-OVA). WT mice challenged with TNP-OVA showed a progressive decrease in rectal temperature to 4 °C below the basal temperature by 18 min after injection (Fig. 4). Allergin-1-deficient mice challenged with TNP-OVA showed significantly lower rectal temperatures than the corresponding WT mice (Fig. 4) [18].

WT and Allergin-1-deficient mice were also passively sensitized by intravenous injection of anti-dinitrophenol (DNP) IgE mAb and then challenged with epicutaneous application of dinitrofluorobenzene (DNFB) in acetone/olive oil in the left ear and acetone/olive oil alone in the right ear. The ear swelling in Allergin-1-deficient mice was significantly greater than the ear swelling in WT mice during the entire 50 h

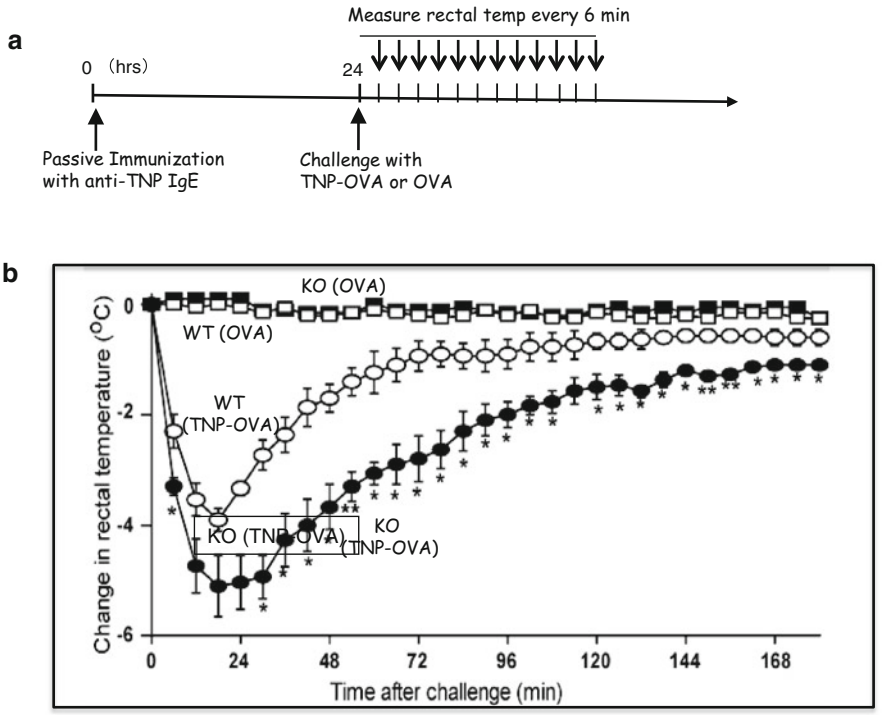

Fig. 4 Enhanced systemic anaphylaxis in Allergin-1-deficient mice. (**a**) *Allergin-1*$^{+/+}$ mice (WT) and *Allergin-1*$^{-/-}$ (KO) mice were intravenously injected with 20 μg of anti-TNP mouse IgE mAb. (**b**) After 24 h, mice were challenged with 1 mg of OVA or TNP_6-conjugated OVA, and rectal temperatures were measured every 6 min. The data are the mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ for the comparison of WT versus KO mice challenged with TNP-OVA

observation period after the antigen challenge [18]. Taken together, these results suggest that Allergin-1 negatively regulates IgE-mediated mast cell activation in vivo and suppresses the type 1 immediate hypersensitivity reactions.

CD300a

Identification and Expression of CD300a

To identify novel genes involved in immune responses by myeloid cells, we performed representative differential analysis (RDA), which is a PCR-based subtractive hybridization, using day 14 fetal livers from PU.1$^{-/-}$ mice lacking myeloid cells and control littermates, followed by screening of a macrophage cDNA library. We cloned a cDNA encoding a type-1 transmembrane protein with one Ig-like domain in the extracellular portion, indicating that the protein, designated myeloid-associated immunoglobulin-like receptor (MAIR)-I (CD300a), is a member of the

Ig superfamily (Fig. 1). The cytoplasmic region contains the consensus sequence for ITIMs, and the ITIM-like sequences. The CD300a gene is located in the proximal region of the E2 band of mouse chromosome 11 and consists of six exons, as determined by fluorescence in situ hybridization (FISH) and a genomic DNA sequence database. Biochemical analyses demonstrated that the molecular weight of CD300a is ~50 KDa protein when analyzed under both reducing and non-reducing conditions [22].

CD300a is expressed on the majority of myeloid cells, including macrophages, dendritic cells, granulocytes, and bone-marrow-derived cultured mast cells, and a subset of B cells, but neither on T nor on NK cells.

Identification of a Ligand for CD300a

To identify the ligand for CD300a, we generated a chimeric fusion protein of the extracellular portion of CD300a with the Fc portion of human IgG (CD300a-Fc) (Fig. 5a). Although the CD300a-Fc did not stain any viable cells tested, we found that CD300a-Fc bound apoptotic cells (Fig. 5b). A neutralizing monoclonal antibody (mAb)

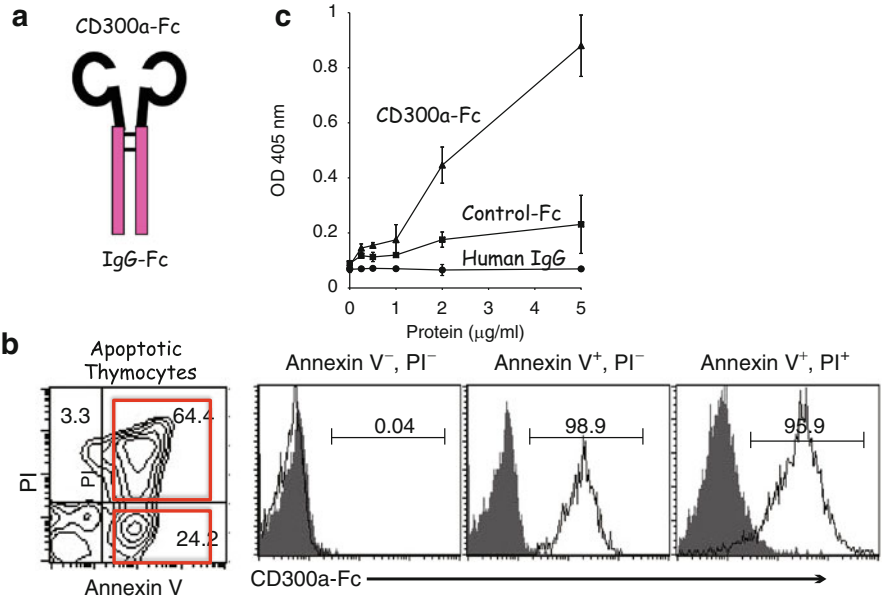

Fig. 5 CD300a-Fc binds phosphatidylserine on apoptotic cells. (**a**, **b**) Dexamethasone-treated mouse thymocytes were stained with APC-conjugated annexin V and mouse CD300a-Fc followed by an FITC-conjugated antibody against human IgG and PI in the presence of $CaCl_2$ and analyzed by flow cytometry. (**c**) Microtiter plates coated with PS were incubated with increasing concentrations of CD300a-Fc, control protein-Fc, or human IgG, and PS binding was analysed by ELISA. *Error bars* show SDs

against mouse CD300a (TX41) specifically inhibited the CD300a-Fc binding to apoptotic cells. Solid-phase ELISA confirmed that CD300a directly bound PS in a CD300a dose-dependent manner (Fig. 5c). These results suggested that CD300a is a PS receptor [23].

Function of CD300a

In Vitro Analyses of CD300a

Several receptors for PS are expressed on phagocytes and are involved in clearing apoptotic cells in physiological and pathological settings [24–29]. However, we found that CD300a does not mediate phagocytosis of apoptotic cells by macrophages [23].

CD300a was tyrosine phosphorylated and co-immunoprecipitated with SHP-1, SHP-2, and SHIP after stimulation with pervanadate in the RBL-2H3 transfectant upon stimulation with pervanadate. We demonstrated that cross-linking CD300a and FcεRI with anti-CD300a mAb and IgE antibody, followed by co-ligation with a common secondary antibody, induced inhibition of IgE-mediated degranulation from RBL-2H3 transfectant expressing CD300a and BM-derived cultured mast cells [30].

To address whether apoptotic cells affect mast cell activation via CD300a, we generated bone marrow (BM)-derived mast cells (BMMCs) from CD300a-deficient or WT mice. WT or CD300a-deficient BMMCs were cocultured with apoptotic cells in the presence of LPS. Although we did not detect any cytokines or chemokines in the culture supernatants in the absence of stimuli, stimulation with LPS induced both WT and CD300a-deficient BMMCs to produce TNF-α, IL-13, and MCP-1; however, CD300a-deficient BMMCs produced them at significantly higher concentrations, suggesting that CD300a-PS interaction inhibits these cytokines and chemokines production from BMMCs [23].

In Vivo Analyses of CD300a

TNF-α, IL-13, and MCP-1 produced by mast cells are chemoattractants for neutrophils and play an important role in bacterial clearance in a CLP peritonitis model in mice [31–34]. Therefore, we hypothesized that CD300a affects immune regulation by mast cells at the site of peritonitis, where large numbers of cells undergo apoptosis in the peritoneal cavity [35]. In fact, we observed that more than 10 % of cells in the peritoneal cavity 4 h after CLP were apoptotic cells, as determined by staining with annexin V. To test the hypothesis, we subjected WT and CD300a-deficient mice to CLP and observed that *Cd300*a$^{-/-}$ mice survived significantly longer than did WT mice after CLP [23].

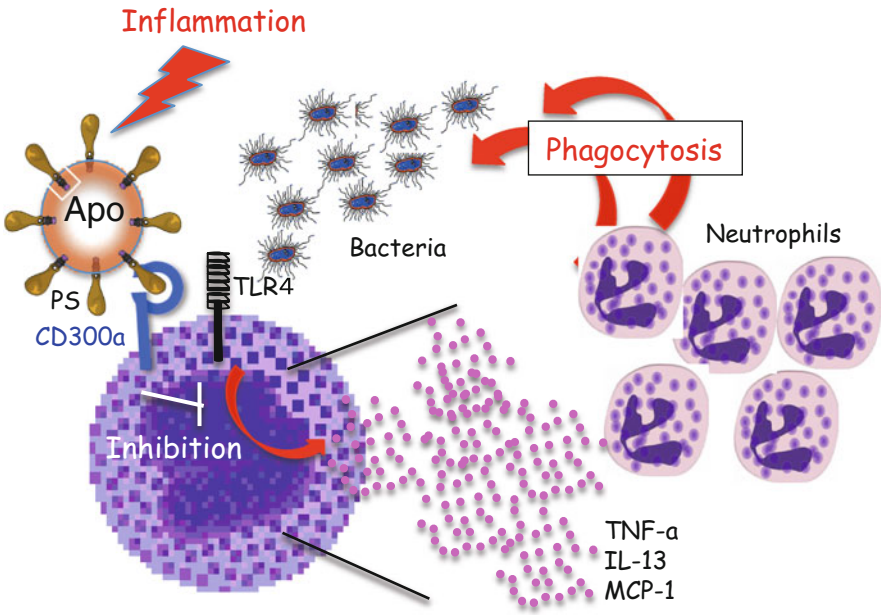

Fig. 6 CD300a inhibits the TLR4-mediated signal for production of chemoattractants for neutrophils. Upon binding to phosphatidylserine (PS) on apoptotic cells, CD300a mediates an inhibitory signal, resulting in the suppression of the TLR4-mediated signal for production of chemoattractants, including TNF-α, IL-13, and MCP-1, for neutrophils, which are involved in phagocytosis of Gram-negative bacteria

In the early phase of CLP peritonitis model, mast cells play an important role in recruitment of neutrophils into the peritoneal cavity by secretion of chemoattractants for neutrophils such as TNF-α and prolong the survival [32, 36]. We demonstrated that CD300a-deficient BMMCs produced a greater amount of chemoattractants for neutrophils than did WT BMMCs in the peritoneal cavity after CLP by transfer experiments into $Kit^{W-sh/W-sh}$ mice, which are deficient in mast cells, before CLP [23]. Our results suggested that CD300a on mast cells was primarily responsible for the phenotype of prolonged survival of CD300a-deficient mice after CLP (Fig. 6).

Blockade of CD300a-PS Interaction Prolonged Survival of Mice After CLP

Because CD300a-deficient mice survived longer after CLP, we examined whether an antibody against mouse CD300a (TX41) could have a prophylactic effect on CLP-induced sepsis. TX41 does not deplete myeloid cells, including mast cells. Intraperitoneal injection of mice with TX41 1 h before and 18 h after CLP significantly increased neutrophil numbers in the peritoneal cavity, improved bacterial clearance 4 h after CLP [23], and prolonged survival compared with treatment with

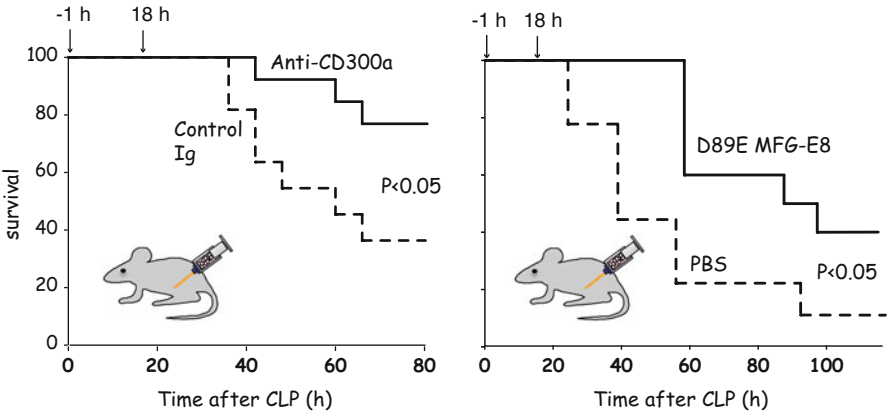

Fig. 7 The blockade of the CD300a-phosphatidylserine interaction prolongs survival of mice after CLP. Mice were injected intraperitoneally with control antibody, anti-CD300a monoclonal antibody (TX41), D89E MFG-E8, or PBS 1 h before and 18 h after CLP, and the survival rates are shown

a control antibody (Fig. 7). Similarly, intraperitoneal injection with mutated milk fat globule EGF factor 8 at residue 89 (D89E MFG-E8), which is able to block the interaction between PS and CD300a, also prolonged survival of WT mice compared with treatment with PBS. Moreover, D89E MFG-E8 improved bacterial clearance of WT mice [23]. These results provided the formal evidence that PS on apoptotic cells affected the bacterial clearance. Thus, blocking the interaction between PS and its receptor CD300a is potentially a useful therapy for prophylaxis against peritonitis-induced sepsis.

Conclusion

Mast cells express pathogen-associated molecular patterns (PAMPs) as well as FcεRI, which mediate activation signal in mast cells, resulting in various chemokines' and cytokines' secretion and degranulation from mast cells. Thereby, they are involved in a variety of allergic and non-allergic inflammatory diseases. Here, we have described molecular and functional characteristic of Allergin-1 and CD300a, which are expressed on mast cells and inhibit activating signals mediated by FcεRI and TLR-4 in vitro and in vivo. Thus, these inhibitory immunoreceptors play an important role in regulation of mast cell-dependent inflammatory responses. In fact, we showed that anti-CD300a neutralizing antibody prolonged survival in a sepsis mouse model. Further studies on regulation of mast cell activation by using these inhibitory immunoreceptor as targets for the therapy for allergy and inflammation might be promising.

References

1. Bachelet I et al (2005) The inhibitory receptor IRp60 (CD300a) is expressed and functional on human mast cells. J Immunol 175(12):7989–7995
2. Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. Science 290(5489):84–89
3. Lanier LL (2001) Face off–the interplay between activating and inhibitory immune receptors. Curr Opin Immunol 13(3):326–331
4. Malbec O et al (1998) Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. J Immunol 160(4):1647–1658
5. Smith KG (1998) Decisions to remove asymptomatic third molars require rational criteria. Br Dent J 184(11):546
6. Daeron M et al (2008) Immunoreceptor tyrosine-based inhibition motifs: a quest in the past and future. Immunol Rev 224:11–43
7. Galli SJ (2000) Mast cells and basophils. Curr Opin Hematol 7(1):32–39
8. Schwartz LB (1994) Mast cells: function and contents. Curr Opin Immunol 6(1):91–97
9. Kawakami T, Galli SJ (2002) Regulation of mast-cell and basophil function and survival by IgE. Nat Rev Immunol 2(10):773–786
10. Li L, Yao Z (2004) Mast cell and immune inhibitory receptors. Cell Mol Immunol 1(6):408–415
11. Maeda A et al (1998) Requirement of SH2-containing protein tyrosine phosphatases SHP-1 and SHP-2 for paired immunoglobulin-like receptor B (PIR-B)-mediated inhibitory signal. J Exp Med 187(8):1355–1360
12. Yamashita Y, Ono M, Takai T (1998) Inhibitory and stimulatory functions of paired Ig-like receptor (PIR) family in RBL-2H3 cells. J Immunol 161(8):4042–4047
13. Katz HR et al (1996) Mouse mast cell gp49B1 contains two immunoreceptor tyrosine-based inhibition motifs and suppresses mast cell activation when coligated with the high-affinity Fc receptor for IgE. Proc Natl Acad Sci U S A 93(20):10809–10814
14. Jurgens L et al (1996) Proximity relationships between the type I receptor for Fc epsilon (Fc epsilon RI) and the mast cell function-associated antigen (MAFA) studied by donor photobleaching fluorescence resonance energy transfer microscopy. Eur J Immunol 26(1):84–91
15. Xu R et al (2001) SH2 domain-containing inositol polyphosphate 5′-phosphatase is the main mediator of the inhibitory action of the mast cell function-associated antigen. J Immunol 167(11):6394–6402
16. Lienard H et al (1999) Signal regulatory proteins negatively regulate immunoreceptor-dependent cell activation. J Biol Chem 274(45):32493–32499
17. Jacobs KA et al (1997) A genetic selection for isolating cDNAs encoding secreted proteins. Gene 198(1–2):289–296
18. Hitomi K et al (2010) An immunoglobulin-like receptor, Allergin-1, inhibits immunoglobulin E-mediated immediate hypersensitivity reactions. Nat Immunol 11(7):601–607
19. Nakahashi-Oda C et al (2012) Apoptotic cells suppress mast cell inflammatory responses via the CD300a immunoreceptor. J Exp Med 209(8):1493–1503
20. Clark GJ et al (2009) The CD300 family of molecules are evolutionarily significant regulators of leukocyte functions. Trends Immunol 30(5):209–217
21. Nagai K et al (2013) Expression and function of Allergin-1 on human primary mast cells. PLoS One 8(10):e76160

22. Yotsumoto K et al (2003) Paired activating and inhibitory immunoglobulin-like receptors, MAIR-I and MAIR-II, regulate mast cell and macrophage activation. J Exp Med 198(2):223–233
23. Nakahashi-Oda C et al (2012) Identification of phosphatidylserine as a ligand for the CD300a immunoreceptor. Biochem Biophys Res Commun 417(1):646–650
24. Miyanishi M et al (2007) Identification of Tim4 as a phosphatidylserine receptor. Nature 450(7168):435–439
25. Hanayama R et al (2002) Identification of a factor that links apoptotic cells to phagocytes. Nature 417(6885):182–187
26. Kobayashi N et al (2007) TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. Immunity 27(6):927–940
27. Park D et al (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature 450(7168):430–434
28. Park SY et al (2008) Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. Cell Death Differ 15(1):192–201
29. Scott RS et al (2001) Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 411(6834):207–211
30. Okoshi Y et al (2005) Requirement of the tyrosines at residues 258 and 270 of MAIR-I in inhibitory effect on degranulation from basophilic leukemia RBL-2H3. Int Immunol 17(1):65–72
31. Marshall JS (2004) Mast-cell responses to pathogens. Nat Rev Immunol 4(10):787–799
32. Echtenacher B, Mannel DN, Hultner L (1996) Critical protective role of mast cells in a model of acute septic peritonitis. Nature 381(6577):75–77
33. Matsukawa A et al (1999) Endogenous monocyte chemoattractant protein-1 (MCP-1) protects mice in a model of acute septic peritonitis: cross-talk between MCP-1 and leukotriene B4. J Immunol 163(11):6148–6154
34. Baumhofer JM et al (1998) Gene transfer with IL-4 and IL-13 improves survival in lethal endotoxemia in the mouse and ameliorates peritoneal macrophages immune competence. Eur J Immunol 28(2):610–615
35. Hotchkiss RS, Nicholson DW (2006) Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol 6(11):813–822
36. Malaviya R et al (1996) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. Nature 381(6577):77–80