

Selective impairment of FcεRI-mediated allergic reaction in Gads-deficient mice

Sho Yamasaki¹, Mitsuyo Takase-Utsugi², Eri Ishikawa¹, Machie Sakuma¹, Keigo Nishida³, Takashi Saito¹ and Osami Kanagawa²

¹Laboratory for Cell Signaling, ²Laboratory for Autoimmune Regulation and ³Laboratory for Cytokine Signaling, RIKEN Research Center for Allergy and Immunology, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Keywords: Allergy, IgE, mast cells, signal transduction

Abstract

Gads is a Grb2-like adaptor protein expressed in hematopoietic cells. We demonstrated that mast cells from Gads^{-/-} mice have selective functional defects. Bone marrow-derived mast cells from Gads^{-/-} mice failed to induce Ca²⁺ mobilization, degranulation and cytokine production upon cross-linking of FcεRI. *In vivo* passive cutaneous anaphylaxis was also greatly impaired in Gads^{-/-} mice. In contrast, Gads was dispensable for Toll-like receptor-mediated cytokine production in mast cells. Accordingly, mast cell-dependent resistance to acute peritoneal bacterial infection is not reduced in Gads^{-/-} mice *in vivo*. Moreover, mature T and B cell responses and antibody production upon immunization were apparently normal in Gads^{-/-} mice. Thus, inhibition of Gads *in vivo* would suppress the IgE-mediated allergic reaction with minimum adverse effects on both innate and acquired immune responses, and Gads could be an ideal target for the control of allergic responses.

Introduction

IgE is the central mediator of allergic responses via the activation of mast cells and basophils (1, 2). The cross-linking of FcεRI, predominantly expressed on these cells, with IgE and multivalent antigens initiates an activation cascade via tyrosine phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) by a protein tyrosine kinase (PTK), Lyn. Another PTK, Syk, is then recruited to the phospho-ITAMs of Fcγ chains, where it phosphorylates adaptor molecules such as LAT and SLP-76 (3, 4). Accumulating evidence demonstrates that LAT and SLP-76 play critical roles in FcεRI signaling (5–9); however, the specific inhibition of FcεRI signaling for the treatment of allergic disease has not been achieved.

Gads is a Grb2-like SH3-SH2-SH3 adaptor protein (also called Mona, Grf40, GRID or GrpL) that is expressed in hematopoietic tissues such as T cells, B cells, mast cells, NK cells, monocytes and plasmacytoid dendritic cells (10–14). In T cells, Gads associates with phosphorylated LAT through the SH2 domain, thereby recruiting SLP-76 which is constitutively bound to the SH3 domain of Gads (15, 16). In Gads-deficient (–/–) mice, stimulation of thymocytes through the TCR leads to uncoupling of LAT and SLP-76 and decreased Ca²⁺ mobilization, resulting in impaired β-selection of T cells in the thymus (17, 18). Although Gads is also expressed in

mast cells, its precise role in mast cell function remains unclear. Since a mutant form of SLP-76 lacking Gads-binding sites could not restore FcεRI-mediated mast cell responses in SLP-76-deficient mast cells (19, 20), Gads likely contributes to the coupling of LAT and SLP-76 in IgE-mediated activation of mast cells, and it may be a possible target for the efficient inhibition of FcεRI signaling.

Because of their central role in mediating allergies, mast cells have been extensively studied as therapeutic targets for the treatment of allergic responses. However, recent studies have revealed that mast cells also play critical roles in host defense against bacterial infection through the Toll-like receptor (TLR)-mediated rapid secretion of inflammatory cytokines or chemokines (21–23). Given that signals through FcεRI and TLR can induce overlapping inflammatory cytokines in mast cells (24), the blockade of mast cell function as a means of treating allergic diseases should be targeted to FcεRI-specific pathways in order to keep defense function intact.

Here, we demonstrate that the disruption of Gads impairs FcεRI-mediated degranulation and cytokine production, with no effect on other mast cell functions such as TLR-mediated responses or on acquired immune responses through T and B cells.

Methods

Mice

Gads^{-/-} mice with B6 and 129 mixed genetic background were previously described (17). Kit^{W-sh/W-sh} mice (25) were purchased from Nihon SLC (Hamamatsu, Japan). All mice were maintained under specific pathogen-free conditions in accordance with institutional guidelines.

Antibodies

Mouse anti-dinitrophenol (DNP) IgE (SPE-7) was purchased from Sigma (St Louis, MO, USA); anti-phospho-Erk antibody from Promega (Madison, WI, USA); anti-phospho-Syk from Cell Signaling Technology (Beverly, MA, USA) and anti-actin from Sigma. Anti-mouse IgG-HRP and Protein G Sepharose were purchased from Amersham Biosciences (Piscataway, NJ, USA), and anti-rabbit Ig-HRP was from Zymed (San Francisco, CA, USA). Anti-CD63 was described previously (26). Anti-Gads was kindly provided by C. J. McGlade (Hospital for Sick Children, Toronto, Ontario, Canada).

Degranulation assays

For antigen cross-linking, mast cells were sensitized with 1 µg ml⁻¹ mouse anti-DNP IgE at 37°C for 4 h and then stimulated with the indicated amounts of DNP-HSA. Degranulation assays were performed as previously described (27). Sensitized mast cells were briefly treated with DNP-HSA in Tyrode's buffer in 96-well plates for 30 min. Supernatants and cell lysates were assayed for β-hexosaminidase activity. The percentage of specific β-hexosaminidase release was calculated as [supernatant activity/(supernatant activity + cell lysate activity)] × 100.

Western blot

Bone marrow-derived mast cells (BMMCs) were sensitized with 1 µg ml⁻¹ anti-DNP IgE (SPE-7) for 18 h and stimulated with DNP-HSA at 37°C for the times indicated. Cells were lysed in lysis buffer containing 1% Nonidet P-40 and subjected to western blot as previously described (27).

Ca²⁺ mobilization

Ca²⁺ influx was measured as previously described (27). Briefly, BMMCs were sensitized with 1 µg ml⁻¹ IgE for 18 h, followed by incubation with 10 µM indo-1-AM (Molecular Probes, Eugene, OR, USA) in the presence of F127 and 0.2% FCS at 37°C for 30 min. Cells were stimulated with various amounts of DNP-HSA or ionomycin. Ca²⁺ mobilization was analyzed with a BD-LSR (BD Biosciences) equipped with UV lasers. Ratiometric analysis of Ca²⁺-bound indo-1 (FL5)/Ca²⁺-free indo-1 (FL4) was performed using FlowJo (Tree Star, San Carlos, CA, USA) software.

Flow cytometric analysis of degranulation

Degranulation was analyzed by flow cytometry as described (26). Briefly, BMMCs were sensitized with 1 µg ml⁻¹ IgE for 4 h and then stimulated with 15 ng ml⁻¹ DNP-HSA for 30 min in Tyrode's buffer. Cells were fixed with 4% PFA for 15 min, followed by staining with anti-CD63 antibody and PE-conjugated goat anti-mouse IgG (H+L) Fab (Cedarlane,

Ontario, Canada). Surface expression of CD63 was determined by flow cytometer.

Staining of mast cells

Stomachs and pieces of dorsal skin were removed and embedded in paraffin. Sections were stained with Alcian blue and nuclear fast red. The number of mast cells in the stomach was counted as previously described (28).

Passive cutaneous anaphylaxis

Passive cutaneous anaphylaxis (PCA) was performed as previously described (29). Mice were injected intra-dermally in the ears with anti-DNP IgE (SPE-7) at 0.5 µg 20 µl⁻¹. Twenty-four hours after IgE sensitization, 250 µg of DNP-HSA and Evans blue dye (1.25 mg) in 0.25 ml saline were injected intravenously. Thirty minutes after antigen challenge, the mice were sacrificed and the ears removed. Evans blue dye that had leaked into the ear tissues was extracted and quantified by spectrophotometry at a wavelength of 650 nm.

Lymphocyte proliferation

For T cell response, 1 × 10⁵ splenic T cells were stimulated with anti-CD3. For staphylococcal enterotoxin B (SEB) stimulation, 1 × 10⁵ splenic T cells were stimulated with SEB (Toxin Technology, Sarasota, FL, USA) and 4 × 10⁵ T cell-depleted splenocytes. For B cell proliferation, B220⁺ cells were purified from splenocytes by MACS and stimulated with anti-IgM, anti-CD40 or LPS. Cells were cultured for 42 h, pulsed with [³H]thymidine and harvested after 6 h. [³H]thymidine incorporation was determined by liquid scintillation counter (Amersham Pharmacia, Uppsala, Sweden).

In vivo immunization

Mice were injected intra-peritoneally with 200 µg ovalbumin peptide (OVA) (Grade III, A5378, Sigma) emulsified in CFA. After 6 weeks, mice were injected with 200 µg OVA in incomplete Freund's adjuvant. Two weeks after the second immunization, the concentrations of OVA-specific IgM and IgG in serum were determined by ELISA. For ELISA, plates were coated with 1 mg ml⁻¹ OVA, and anti-mouse IgM-HRP (61-6820, Zymed) and anti-mouse IgG-HRP (NA931V, Amersham Pharmacia) were used as detection antibodies.

Model for bacterial peritonitis

Cecal ligation and puncture were performed as previously described (21). Mice were anesthetized and the cecum was exposed by a 1- to 2-cm midline incision in the anterior abdomen. The distal half of the cecum was ligated and then punctured twice with an 18-G needle. The mice were observed for mortality over a period of 14 days.

Results

Gads is required for FcRI-mediated mast cell activation

BMMCs from Gads^{-/-} mice were established by a standard method and found to express surface FcεRI at levels similar to those expressed on wild-type mast cells (Fig. 1A). IL-3-mediated growth of BMMCs was similar in wild-type and Gads^{-/-} mice (data not shown), indicating that Gads is

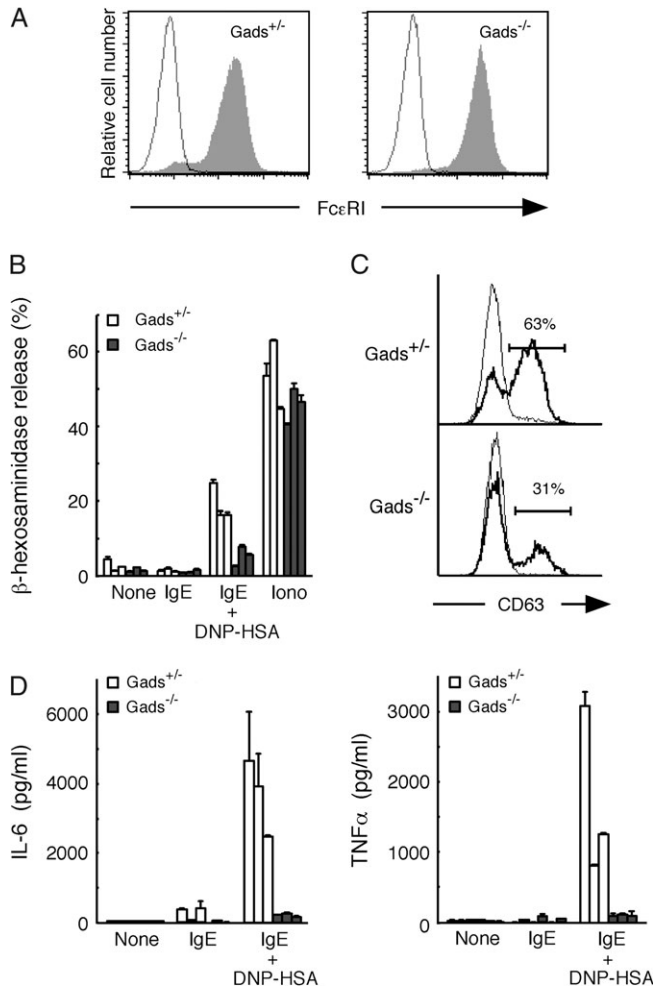


Fig. 1. Impaired FcεRI-mediated responses in *Gads*^{-/-} BMMCs. (A) Surface FcεRI expression levels of BMMCs in *Gads*^{+/-} and *Gads*^{-/-} mice. Cells were left unstained (open histograms) or stained with anti-FcεRIα-PE (filled histograms). (B) β-Hexosaminidase release. BMMCs were stimulated with IgE and antigen as described in Methods and the release of β-hexosaminidase was determined. Data are expressed as percentage of maximum release. Data from three independent mice for each group are shown. (C) Induction of CD63 expression on cell surface. BMMCs were left unstimulated (thin line) or stimulated with IgE and antigen for 30 min (thick line), stained with anti-CD63 and anti-rat IgG-PE and analyzed by flow cytometer. (D) Cytokine production of BMMCs. BMMCs from *Gads*^{+/-} and *Gads*^{-/-} mice were sensitized with 1 μg ml⁻¹ anti-DNP IgE and stimulated with 15 ng ml⁻¹ DNP-HSA for 4 h. IL-6 (left) and TNFα (right) production was determined by ELISA.

dispensable for IL-3 receptor signaling. However, when FcεRI was engaged with DNP-HSA after sensitization with anti-DNP IgE, *Gads*^{-/-} BMMCs showed markedly less degranulation than the wild-type cells as measured by β-hexosaminidase secretion (Fig. 1B). This was also confirmed by the lower cell surface CD63 expression, a cellular marker of mast cell degranulation (26), in *Gads*^{-/-} BMMCs (Fig. 1C). Production of IL-6 and tumor necrosis factor (TNF) α was also impaired in *Gads*^{-/-} BMMCs (Fig. 1D). This is not due to a general defect in the *Gads*^{-/-} BMMCs since both *Gads*^{+/-} and *Gads*^{-/-} BMMCs showed an equivalent

amount of degranulation and cytokine production upon stimulation with ionomycin which bypasses the FcεRI-proximal signaling events (Fig. 1B and D). These results suggest that *Gads* is critical for the production of allergy-related mediators by FcεRI cross-linking in mast cells.

Gads is critical for *in vivo* allergic reaction but not mast cell development

We next analyzed the effect of *Gads* in mast cell development and activation *in vivo*. *Gads*^{-/-} mice showed no defects in the number of mucosal mast cells in the skin and stomach (Fig. 2A) or in the number of peritoneal resident mast cells (Fig. 2B). These results indicate that although the *Gads* is expressed in mast cells, it is dispensable for the development of mast cells *in vivo*.

In order to examine the significance of *Gads* in the FcεRI-mediated allergic reaction, *Gads*^{-/-} and *Gads*^{+/-} mice were tested for their capacity to mount PCA. Mast cell-mediated *in vivo* anaphylaxis was monitored by measuring leakage of dye at the site of mast cell sensitization with specific IgE. Both *Gads*^{+/-} and *Gads*^{-/-} mice were sensitized with anti-DNP IgE in the ear and challenged with intravenous injection of DNP-HSA and the dye. Leakage of the dye was measured as a marker of increased permeability of blood vessels. *Gads*^{-/-} mice showed significantly reduced dye leakage compared with *Gads*^{+/-} mice (Fig. 2C), indicating that *Gads* is required for optimal type I allergic response.

Gads is essential for Ca²⁺ mobilization but not activation of MAP kinases

Following the initial observation that FcεRI-mediated mast cell activation and *in vivo* allergic reaction are impaired, we examined intracellular signaling in *Gads*^{-/-} mast cells. Intracellular Ca²⁺ influx upon cross-linking of FcεRI was impaired in *Gads*^{-/-} BMMCs (Fig. 3A, left panels), whereas ionomycin-induced Ca²⁺ influx in *Gads*^{-/-} BMMCs was comparable to that in *Gads*^{+/-} BMMCs (Fig. 3A, right panel).

In contrast, the phosphorylation of Erk, JNK and p38 by FcεRI stimulation was comparable in *Gads*^{-/-} and *Gads*^{+/-} BMMCs (Fig. 3B). Thus, FcεRI-induced activation of the MAP kinase (MAPK) family, which is shown to be involved in other mast cell functions such as IgE-induced survival (27), is not dependent on *Gads*.

To address the molecular mechanism of *Gads*-mediated Ca²⁺ responses, we analyzed SLP-76 that is critical regulator of PLCγ-Ca²⁺ pathway (30). Stimulation-dependent association of LAT and SLP-76 was markedly diminished in the absence of *Gads* (Fig. 3B), although phosphorylation of LAT was normally observed (Fig. 3B and C). Thus, similar to TCR-mediated signaling in T cells, *Gads* acts as an adaptor protein that couples LAT and SLP-76 in mast cells, which is required for Ca²⁺ influx possibly through SLP-76-PLCγ axis.

Gads is dispensable for acquired immune responses

Although loss of *Gads* interferes with T cell development in the thymus (17, 18), reduced but substantial number of T cells emigrate to the peripheral lymphoid organs (31). However, peripheral *Gads*^{-/-} T cells exhibited no impaired responses

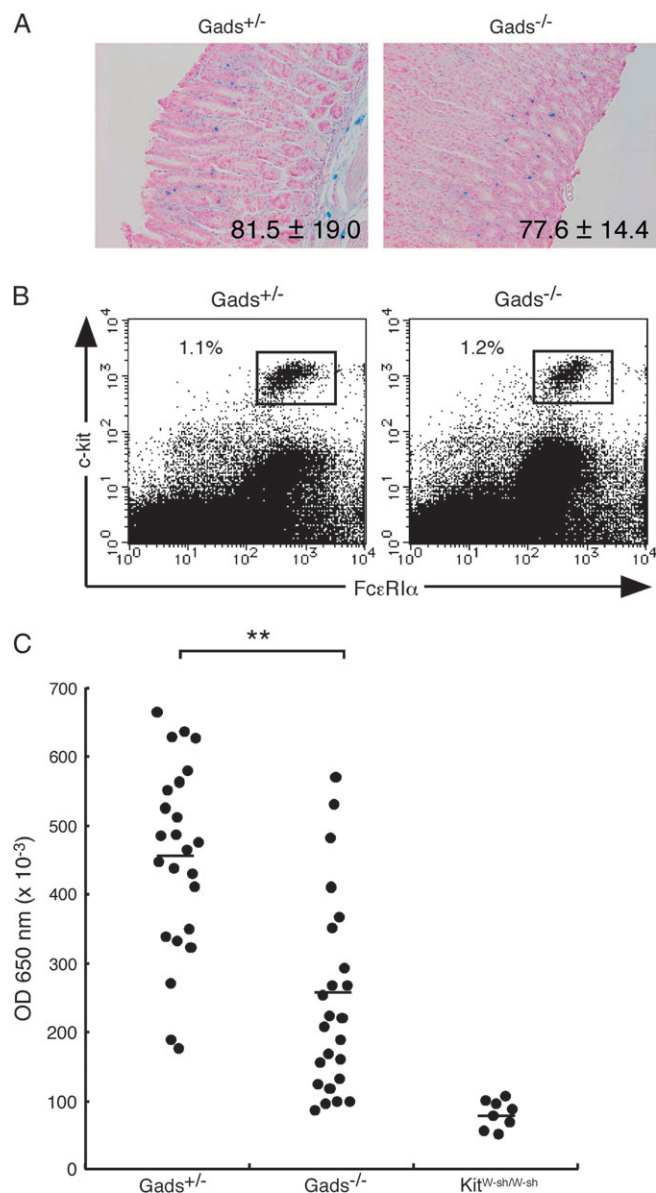


Fig. 2. Normal mast cell development and impaired allergic reaction in *Gads*^{-/-} mice. (A) Mast cells in the forestomach in *Gads*^{-/-} mice. Sections were stained with Alcian blue and nuclear fast red. Representative fields from three independent sections are shown. The average number of mast cells is shown (cells mm⁻²). Average mast cell number in the skin of *Gads*^{+/-} and *Gads*^{-/-} mice is 87.1 ± 7.3 and 107 ± 7.8 cells cm⁻¹, respectively. (B) Development of peritoneal mast cells. Cells in peritoneal cavity were counted and analyzed for the expression of c-kit and FcεRIα. The percentage of peritoneal mast cells (c-kit⁺FcεRIα⁺) within total peritoneal cells is shown. (C) *In vivo* PCA responses in *Gads*^{-/-} mice. Mice were sensitized with anti-DNP and challenged with DNP-HSA and Evans blue dye for 30 min and then dye leakage into ear tissues was quantified. Symbol indicates individual mouse of each group from three experiments. Mast cell-deficient *Kit*^{W-sh/W-sh} mice were also examined as an independent experiment and shown as negative control. Horizontal bars indicate mean values. ***P* < 0.001 (Student's *t*-test).

induced by anti-CD3 (Fig. 4A) and staphylococcal superantigen, SEB (Fig. 4B). Purified CD4⁺ T cells from *Gads*^{-/-} mice also showed normal responses upon stimulation through

TCR and CD28 (data not shown). We found no apparent difference in BCR-, CD40- and TLR-mediated responses between B cells from *Gads*^{-/-} and *Gads*^{+/-} mice *in vitro* (Fig. 4C and D). Furthermore, *in vivo* immunization with T cell-dependent antigen OVA induced similar amounts of antigen-specific IgM and IgG in the sera of *Gads*^{-/-} and *Gads*^{+/-} mice (Fig. 4E and F). These results suggest that *Gads* deficiency would not essentially affect the acquired immune responses of peripheral mature T and B cells.

Gads is dispensable for mast cell activation through TLR

Finally, we analyzed whether *Gads* is involved in TLR-mediated mast cell activation that is critical for first defense against acute infection. In the *in vitro* assay, BMMCs from *Gads*^{+/-} and *Gads*^{-/-} mice produced comparable amounts of IL-6 and TNFα upon LPS stimulation (Fig. 5A), suggesting that *Gads* plays a minimum role in the TLR-mediated activation of mast cells. For the *in vivo* assay, we used puncture-induced acute septic peritonitis to test the role of *Gads* in the TLR-mediated activation of mast cells. It has been shown that resistance to this acute infection is mediated by mast cell activation through TLR since TLR4^{-/-} mice as well as mast cell-deficient *Wv/Wv* mice exhibit extreme susceptibility and die (21, 22). *Gads*^{-/-} mice exhibited a motility curve similar to that of *Gads*^{+/-} mice after puncture (Fig. 5B). These results suggest that *Gads* is dispensable for the innate response of mast cells to pathogens through TLR, both *in vitro* and *in vivo*.

Discussion

In mast cells, the β and γ subunits of FcεRI contain ITAM and cross-linking of the receptor induces phosphorylation of the ITAM, which initiates specific signaling events through Lyn, Fyn, Syk, LAT and SLP-76 for degranulation and cytokine production (32). We have shown that the point mutation of ITAM within FcRγ results in complete abrogation of FcεRI-mediated mast cell activation *in vitro* and *in vivo* (33).

Therefore, FcRγ-ITAM or its downstream molecules could be targets for the treatment of allergic diseases (34), although the redundancy and broad expression of these molecules make it difficult to control mast cell responses in a selective manner. Deficiency of such molecules as Syk, LAT, SLP-76 and Vav1 leads to profound impairment of lymphocyte development and function (35–39) as well as FcεRI signaling (5, 9, 40, 41). Adhesion and degranulation promoting adaptor protein (ADAP) was initially reported as a positive regulator of mast cell degranulation (42), but ADAP^{-/-} mice did not show any defects in mast cell function (20). Although MIST (also called as Clnk), a SLP-76-related adaptor molecule, is selectively expressed in mast cells and NK cells (43, 44), this protein is dispensable for the normal function of mast cells (45). On the other hand, the genetic disruption of a *Gads*-related adaptor, Grb2, results in embryonic lethality (46).

Gads has been demonstrated to play a crucial role in normal TCR-mediated signaling during thymocyte development (17, 18). However, mature T cells from *Gads*^{-/-} mice are capable of responding to signals through TCR (31), and lack of the *Gads* had minor effect on B cell development and function (47). Our study also supports this idea. In contrast,

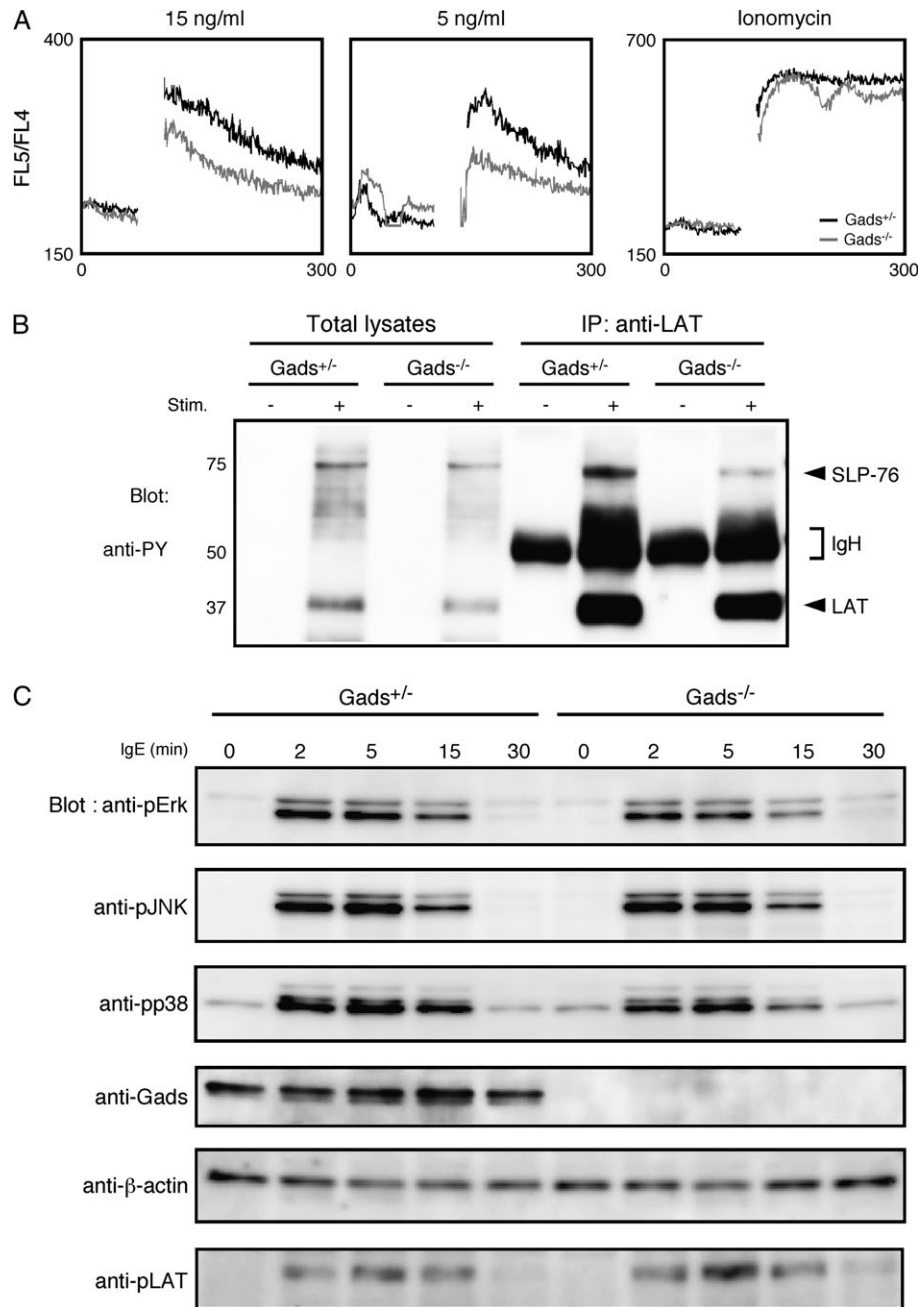


Fig. 3. Ca^{2+} influx but not MAPK activation is defective in *Gads*^{-/-} BMMCs. (A) Ca^{2+} influx. BMMCs from *Gads*^{+/+} (dark lines) and *Gads*^{-/-} (light lines) mice were sensitized with $1 \mu\text{g ml}^{-1}$ IgE for 18 h. Cells were loaded with indo-1, followed by stimulation with indicated concentrations of DNP-HSA or $1 \mu\text{M}$ ionomycin. (B) Phosphorylation-dependent association of SLP-76 and LAT. BMMCs from *Gads*^{+/+} and *Gads*^{-/-} mice were left unstimulated (–) or stimulated with pervanadate for 5 min (+) and cell lysates were immunoprecipitated with anti-LAT, followed by western blot with anti-phosphotyrosine (PY). The molecular mass markers (in kD) are shown on the left. (C) Tyrosine phosphorylation of signaling molecules. IgE-sensitized BMMCs were stimulated with $15 \mu\text{g ml}^{-1}$ of DNP-HSA for indicated periods, and total lysates were analyzed for phosphorylation using anti-phospho-specific antibodies, anti-Gads and anti-β-actin.

mast cells from *Gads*-deficient mice exhibited significant impairment in their response to IgE-mediated signaling. Furthermore, it has been recently reported that blockade of *Gads*–SLP-76 interaction by the *Gads*-binding fragment of SLP-76 efficiently suppresses $\text{Fc}\epsilon\text{RI}$ -mediated mast cell activation *in vitro* (48).

The differential effect of *Gads* on different cells in the immune system can be explained as follows. For B cells, BLNK/SLP-65, a B cell homologue of SLP-76, possesses its own membrane-localizing capacity in a LAT-independent manner (30, 49), and therefore its signaling pathway does not require *Gads*, as a linker of LAT and SLP-76. The modest effect of

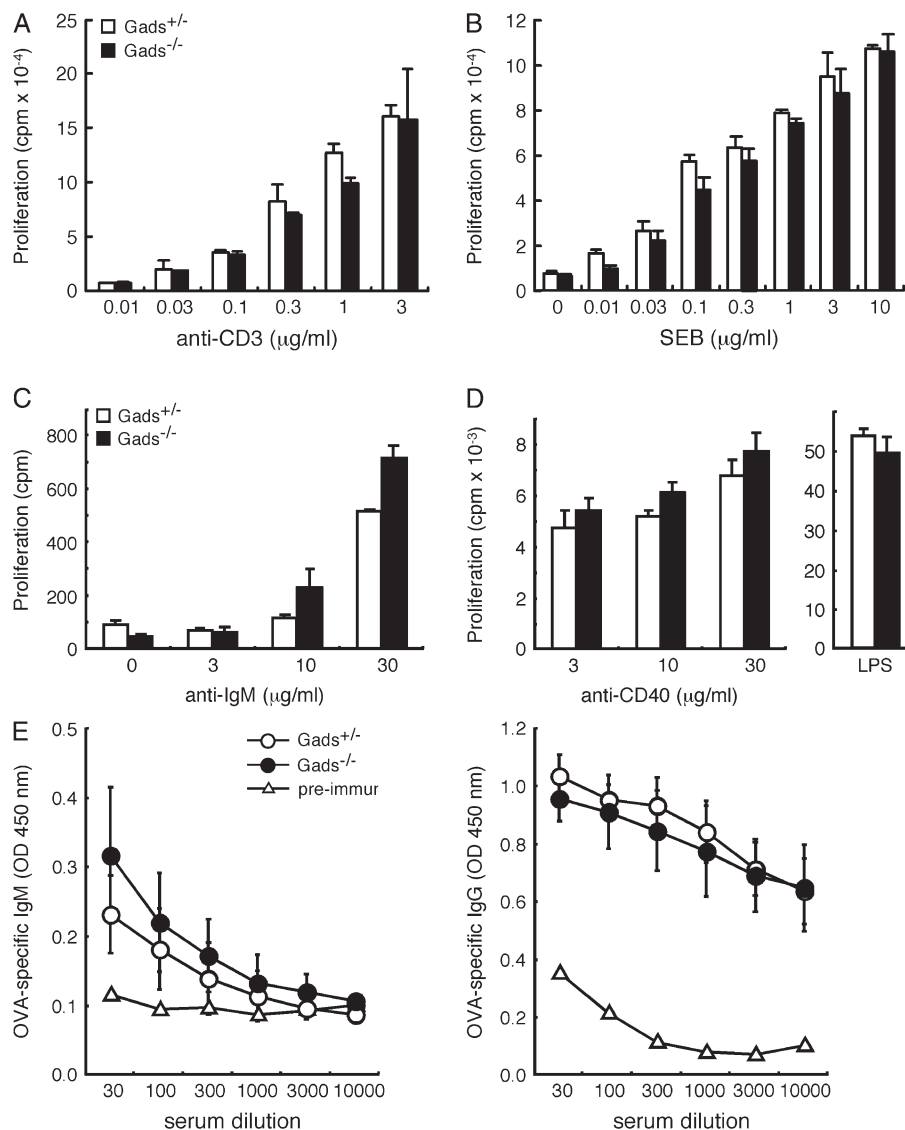


Fig. 4. Normal T and B cell responses in Gads^{-/-} mice. (A–B) T cell proliferative responses upon TCR stimulation. Splenic T cells were stimulated with anti-CD3 mAb (A) or SEB (B) for 42 h, pulsed with [³H]thymidine and harvested after 6 h. [³H] incorporation was determined by liquid scintillation counter and expressed as count per minutes (c.p.m.). (C–D) B cell proliferative responses upon stimulation through BCR, CD40 and TLR. B220⁺ splenic B cells were purified and stimulated with anti-IgM (C), anti-CD40 and 10 $\mu\text{g ml}^{-1}$ LPS (D). Cells were cultured for 42 h, pulsed with [³H]thymidine and harvested after 6 h. (E) *In vivo* antibody production. Mice were immunized with OVA twice. Two weeks after second immunization, serum concentration of OVA-specific IgM (left) and IgG (right) was determined by ELISA. No OVA-specific IgE was detected (data not shown). Data were means $\pm$ SD for four mice in each group.

Gads deficiency on mature T cells was also supported by the analysis using a mutant form of SLP-76 which lacks the Gads-binding site. The Gads-binding site of SLP-76 is absolutely required for Fc ϵ RI-mediated mast cell degranulation or cytokine production (19, 20), whereas the same mutant is able to partly restore TCR signaling in SLP-76-deficient mature T cell lines (50, 51). This could be explained by the fact that Gads-independent recruitment of SLP-76 to the signaling complex might be operating in mature T cells as proposed previously (30). It is also possible that T cells that matured in the thymus without Gads are unique and capable of responding to TCR-mediated stimuli in the absence of

Gads. Although IgG receptors, such as Fc γ RI or Fc γ RIII, also utilize ITAM-containing Fc γ chain, the recent finding that SLP-76 is dispensable for Fc γ R-mediated macrophage activation implies the minimum contribution of Gads in this pathway (52, 53).

Thus, among a number of signaling molecules shared by ITAM-containing receptors, Gads has a preferential role in mast cells. The limited role of Gads in mast cell function and its dispensable role in mature lymphocytes make this molecule an ideal target for therapeutic interventions aimed at inhibiting the allergic response. Gads may also play a critical role in allergic responses induced by basophils, since

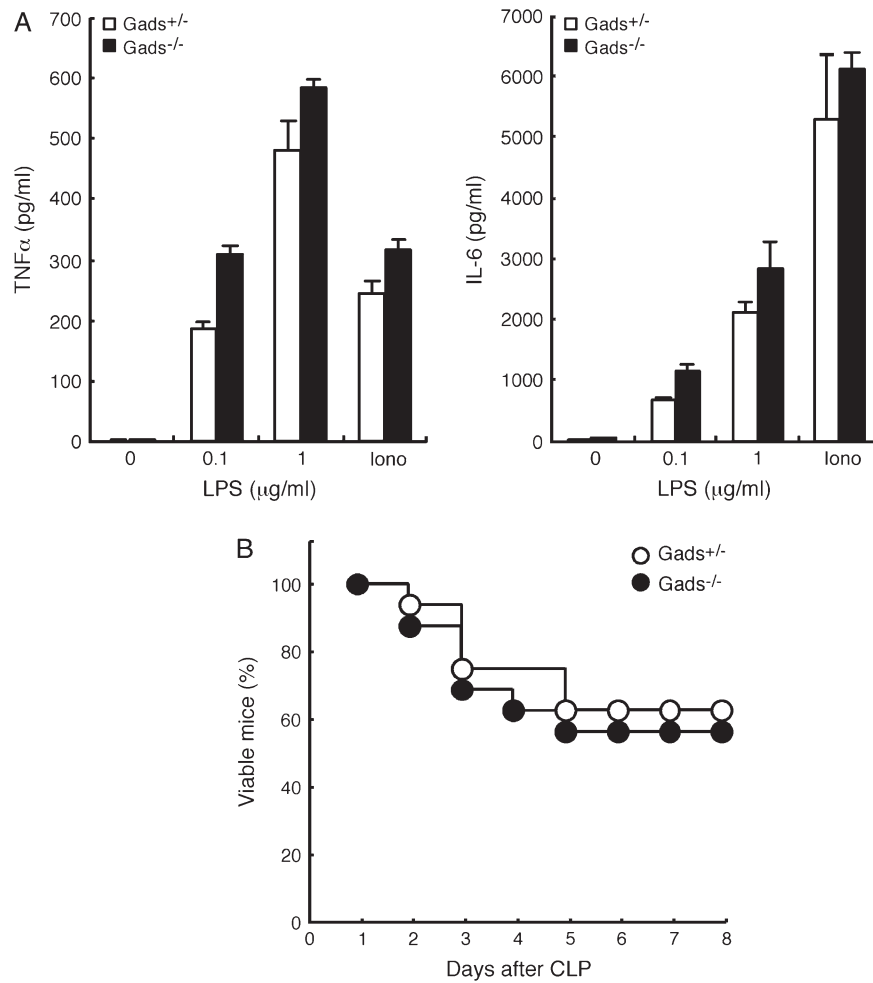


Fig. 5. *Gads* is dispensable for mast cell-mediated innate immune responses. (A) LPS-induced cytokine production in BMMCs. BMMCs were stimulated with indicated amounts of LPS or 1 μ M ionomycin for 24 h, and TNF α (left) and IL-6 (right) concentrations were determined by ELISA. (B) Survival in acute septic peritonitis. Lines are mortality curves of Gads^{+/-} (open circles) and Gads^{-/-} (closed circles) after cecal ligation and puncture ($n = 16$ per genotype).

they do express Fc ϵ RI and trigger IgE-mediated chronic allergic inflammation (54).

The Fc ϵ RI-mediated signal has two separate effects on mast cell function (55). Cross-linking of the receptor induces degranulation and cytokine production that is dependent on Ca²⁺ influx, whereas IgE binding without cross-linking results in sustained activation of Erk to maintain the survival of mast cells (27, 56, 57). Our results clearly demonstrate the critical role of *Gads* in the Ca²⁺ pathway and degranulation, but not in MAPK activation. Indeed, IgE-induced survival of mast cells via sustained Erk activation is not dependent on *Gads* (58).

Mast cells also play a critical protective role in acute bacterial infection by producing TNF and other cytokines through TLR-mediated stimulation (21). The importance of these cytokines as a first defense against infection was recently reaffirmed by the evidence of an increasing risk of serious infections in patients treated with anti-TNF antibody therapy (59). In targeting mast cells for the treatment of allergic diseases, it is necessary to preserve the viability and antibacterial function of these cells. As demonstrated in both *in vitro* and *in vivo* assays, lack of *Gads* function in adult

mice seems to inhibit the mast cell or basophil function responsible for allergic reactions without affecting other functions such as TLR-mediated inflammatory responses.

From these results, it would be of great interest to develop a specific inhibitor of *Gads* function and to test its effect on allergic responses *in vivo*.

Acknowledgements

We thank C. J. McGlade for providing antibody; H. Kitamura, O. Ohara, D. Kamimura, M. Kohno and K. Oboki for technical help and discussion; and H. Yamaguchi for secretarial assistance.

Abbreviations

ADAP	adhesion and degranulation promoting adaptor protein
BMMC	bone marrow-derived mast cells
DNP	dinitrophenol
ITAM	immunoreceptor tyrosine-based activation motif
MAPK	MAP kinase
OVA	ovalbumin peptide
PCA	passive cutaneous anaphylaxis
PTK	protein tyrosine kinase
SEB	staphylococcal enterotoxin B

TLR Toll-like receptor
TNF tumor necrosis factor

References

- Ishizaka, K. 1989. Basic mechanisms of IgE-mediated hypersensitivity. *Curr. Opin. Immunol.* 1:625.
- Kinet, J. P. 1999. The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu. Rev. Immunol.* 17:931.
- Rivera, J. 2002. Molecular adapters in Fc(epsilon)RI signaling and the allergic response. *Curr. Opin. Immunol.* 14:688.
- Gilfillan, A. M. and Tkaczky, C. 2006. Integrated signalling pathways for mast-cell activation. *Nat. Rev. Immunol.* 6:218.
- Saitoh, S., Arudchandran, R., Manetz, T. S. *et al.* 2000. LAT is essential for Fc(epsilon)RI-mediated mast cell activation. *Immunity* 12:525.
- Malbec, O., Malissen, M., Isnardi, I. *et al.* 2004. Linker for activation of T cells integrates positive and negative signaling in mast cells. *J. Immunol.* 173:5086.
- Volna, P., Lebduška, P., Drabero, L. *et al.* 2004. Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. *J. Exp. Med.* 200:1001.
- Zhu, M., Liu, Y., Koonpaew, S., Granillo, O. and Zhang, W. 2004. Positive and negative regulation of FcepsilonRI-mediated signaling by the adaptor protein LAB/NTAL. *J. Exp. Med.* 200:991.
- Pivniouk, V. I., Martin, T. R., Lu-Kuo, J. M., Katz, H. R., Oettgen, H. C. and Geha, R. S. 1999. SLP-76 deficiency impairs signaling via the high-affinity IgE receptor in mast cells. *J. Clin. Invest.* 103:1737.
- Law, C. L., Ewings, M. K., Chaudhary, P. M. *et al.* 1999. GrpL, a Grb2-related adaptor protein, interacts with SLP-76 to regulate nuclear factor of activated T cell activation. *J. Exp. Med.* 189:1243.
- Liu, S. K. and McGlade, C. J. 1998. Gads is a novel SH2 and SH3 domain-containing adaptor protein that binds to tyrosine-phosphorylated Shc. *Oncogene* 17:3073.
- Bourette, R. P., Arnaud, S., Myles, G. M., Blanchet, J. P., Rohrschneider, L. R. and Mouchiroud, G. 1998. Mona, a novel hematopoietic-specific adaptor interacting with the macrophage colony-stimulating factor receptor, is implicated in monocyte/macrophage development. *EMBO J.* 17:7273.
- Asada, H., Ishii, N., Sasaki, Y. *et al.* 1999. Grf40, a novel Grb2 family member, is involved in T cell signaling through interaction with SLP-76 and LAT. *J. Exp. Med.* 189:1383.
- Ellis, J. H., Ashman, C., Burden, M. N., Kilpatrick, K. E., Morse, M. A. and Hamblin, P. A. 2000. GRID: a novel Grb-2-related adapter protein that interacts with the activated T cell costimulatory receptor CD28. *J. Immunol.* 164:5805.
- Jordan, M. S., Singer, A. L. and Koretzky, G. A. 2003. Adaptors as central mediators of signal transduction in immune cells. *Nat. Immunol.* 4:110.
- Samelson, L. E. 2002. Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. *Annu. Rev. Immunol.* 20:371.
- Yoder, J., Pham, C., Iizuka, Y. M. *et al.* 2001. Requirement for the SLP-76 adaptor GADS in T cell development. *Science* 291:1987.
- Zeng, L., Dalheimer, S. L. and Yanke, T. M. 2007. Gads-/- mice reveal functionally distinct subsets of TCRbeta+ CD4-CD8-double-negative thymocytes. *J. Immunol.* 179:1013.
- Kettner, A., Pivniouk, V., Kumar, L. *et al.* 2003. Structural requirements of SLP-76 in signaling via the high-affinity immunoglobulin E receptor (Fc epsilon RI) in mast cells. *Mol. Cell. Biol.* 23:2395.
- Wu, J. N., Jordan, M. S., Silverman, M. A., Peterson, E. J. and Koretzky, G. A. 2004. Differential requirement for adapter proteins Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa and adhesion- and degranulation-promoting adapter protein in FcepsilonRI signaling and mast cell function. *J. Immunol.* 172:6768.
- Echtenacher, B., Mannel, D. N. and Hultner, L. 1996. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* 381:75.
- Supajatura, V., Ushio, H., Nakao, A., Okumura, K., Ra, C. and Ogawa, H. 2001. Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4. *J. Immunol.* 167:2250.
- Fukao, T., Yamada, T., Tanabe, M. *et al.* 2002. Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. *Nat. Immunol.* 3:295.
- Akira, S., Takeda, K. and Kaisho, T. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol.* 2:675.
- Yamazaki, M., Tsujimura, T., Morii, E. *et al.* 1994. C-kit gene is expressed by skin mast cells in embryos but not in puppies of Wsh/Wsh mice: age-dependent abolishment of c-kit gene expression. *Blood* 83:3509.
- Nishida, K., Yamasaki, S., Ito, Y. *et al.* 2005. Fc[epsilon]RI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane. *J. Cell Biol.* 170:115.
- Yamasaki, S., Ishikawa, E., Kohno, M. and Saito, T. 2004. The quantity and duration of FcR[gamma] signals determine mast cell degranulation and survival. *Blood* 103:3093.
- Nishida, K., Wang, L., Morii, E. *et al.* 2002. Requirement of Gab2 for mast cell development and KitL/c-Kit signaling. *Blood* 99:1866.
- Kabu, K., Yamasaki, S., Kamimura, D. *et al.* 2006. Zinc is required for Fc[epsilon]RI-mediated mast cell activation. *J. Immunol.* 177:1296.
- Koretzky, G. A., Abtahian, F. and Silverman, M. A. 2006. SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. *Nat. Rev. Immunol.* 6:67.
- Yankee, T. M., Yun, T. J., Draves, K. E. *et al.* 2004. The Gads (GrpL) adaptor protein regulates T cell homeostasis. *J. Immunol.* 173:1711.
- Rivera, J. and Gilfillan, A. M. 2006. Molecular regulation of mast cell activation. *J. Allergy Clin. Immunol.* 117:1214quiz 1226.
- Sakurai, D., Yamasaki, S., Arase, K. *et al.* 2004. FcepsilonRIgamma-ITAM is differentially required for mast cell function *in vivo*. *J. Immunol.* 172:2374.
- Oliver, J. M., Kepley, C. L., Ortega, E. and Wilson, B. S. 2000. Immunologically mediated signaling in basophils and mast cells: finding therapeutic targets for allergic diseases in the human FcvarepsilonRI1 signaling pathway. *Immunopharmacology* 48:269.
- Turner, M., Mee, P. J., Costello, P. S. *et al.* 1995. Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. *Nature* 378:298.
- Finco, T. S., Kadlec, T., Zhang, W., Samelson, L. E. and Weiss, A. 1998. LAT is required for TCR-mediated activation of PLCgamma1 and the Ras pathway. *Immunity* 9:617.
- Clements, J. L., Yang, B., Ross-Barta, S. E. *et al.* 1998. Requirement for the leukocyte-specific adapter protein SLP-76 for normal T cell development. *Science* 281:416.
- Tarakhovsky, A., Turner, M., Schaal, S. *et al.* 1995. Defective antigen receptor-mediated proliferation of B and T cells in the absence of Vav. *Nature* 374:467.
- Zhang, R., Alt, F. W., Davidson, L., Orkin, S. H. and Swat, W. 1995. Defective signalling through the T- and B-cell antigen receptors in lymphoid cells lacking the vav proto-oncogene. *Nature* 374:470.
- Costello, P. S., Turner, M., Walters, A. E. *et al.* 1996. Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. *Oncogene* 13:2595.
- Manetz, T. S., Gonzalez-Espinosa, C., Arudchandran, R., Xirasagar, S., Tybulewicz, V. and Rivera, J. 2001. Vav1 regulates phospholipase cgamma activation and calcium responses in mast cells. *Mol. Cell. Biol.* 21:3763.
- Geng, L., Pfister, S., Kraeft, S. K. and Rudd, C. E. 2001. Adaptor FYB (Fyn-binding protein) regulates integrin-mediated adhesion and mediator release: differential involvement of the FYB SH3 domain. *Proc. Natl Acad. Sci. USA* 98:11527.
- Goitsuka, R., Kanazashi, H., Sasanuma, H. *et al.* 2000. A BASH/SLP-76-related adaptor protein MIST/Clnk involved in IgE receptor-mediated mast cell degranulation. *Int. Immunol.* 12:573.
- Cao, M. Y., Davidson, D., Yu, J., Latour, S. and Veillette, A. 1999. Clnk, a novel SLP-76-related adaptor molecule expressed in cytokine-stimulated hemopoietic cells. *J. Exp. Med.* 190:1527.
- Utting, O., Sedgmen, B. J., Watts, T. H. *et al.* 2004. Immune functions in mice lacking Clnk, an SLP-76-related adaptor expressed in a subset of immune cells. *Mol. Cell. Biol.* 24:6067.
- Cheng, A. M., Saxton, T. M., Sakai, R. *et al.* 1998. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. *Cell* 95:793.

- 47 Yankee, T. M., Draves, K. E. and Clark, E. A. 2005. Expression and function of the adaptor protein Gads in murine B cells. *Eur. J. Immunol.* 35:1184.
- 48 Silverman, M. A., Shoag, J., Wu, J. and Koretzky, G. A. 2006. Disruption of SLP-76 interaction with Gads inhibits dynamic clustering of SLP-76 and FcepsilonRI signaling in mast cells. *Mol. Cell. Biol.* 26:1826.
- 49 Ishiai, M., Kurosaki, M., Inabe, K., Chan, A. C., Sugamura, K. and Kurosaki, T. 2000. Involvement of LAT, Gads, and Grb2 in compartmentation of SLP-76 to the plasma membrane. *J. Exp. Med.* 192:847.
- 50 Singer, A. L., Bunnell, S. C., Obstfeld, A. E. *et al.* 2004. Roles of the proline-rich domain in SLP-76 subcellular localization and T cell function. *J. Biol. Chem.* 279:15481.
- 51 Yablonski, D., Kadlec, T. and Weiss, A. 2001. Identification of a phospholipase C-gamma1 (PLC-gamma1) SH3 domain-binding site in SLP-76 required for T-cell receptor-mediated activation of PLC-gamma1 and NFAT. *Mol. Cell. Biol.* 21:4208.
- 52 Myung, P. S., Clements, J. L., White, D. W. *et al.* 2000. *In vitro* and *in vivo* macrophage function can occur independently of SLP-76. *Int. Immunol.* 12:887.
- 53 Nichols, K. E., Haines, K., Myung, P. S. *et al.* 2004. Macrophage activation and Fcgamma receptor-mediated signaling do not require expression of the SLP-76 and SLP-65 adaptors. *J. Leukoc. Biol.* 75:541.
- 54 Mukai, K., Matsuoka, K., Taya, C. *et al.* 2005. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* 23:191.
- 55 Kawakami, T. and Galli, S. J. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* 2:773.
- 56 Asai, K., Kitaura, J., Kawakami, Y. *et al.* 2001. Regulation of mast cell survival by IgE. *Immunity* 14:791.
- 57 Kalesnikoff, J., Huber, M., Lam, V. *et al.* 2001. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 14:801.
- 58 Yamasaki, S., Ishikawa, E., Sakuma, M. *et al.* 2007. LAT and NTAL mediate immunoglobulin E-induced sustained extracellular signal-regulated kinase activation critical for mast cell survival. *Mol. Cell. Biol.* 27:4406.
- 59 Bongartz, T., Sutton, A. J., Sweeting, M. J., Buchan, I., Matteson, E. L. and Montori, V. 2006. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 295:2275.