LAT and NTAL mediate immunoglobulin E-induced sustained extracellular signal-regulated kinase activation critical for mast cell survival

Sho Yamasaki
RIKEN Research Center for Allergy and Immunology, Yokohama

Eri Ishikawa
RIKEN Research Center for Allergy and Immunology, Yokohama

Machie Sakuma
RIKEN Research Center for Allergy and Immunology, Yokohama

Osami Kanagawa
RIKEN Research Center for Allergy and Immunology, Yokohama

Alec M. Cheng
Washington University School of Medicine in St. Louis

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation
Yamasaki, Sho; Ishikawa, Eri; Sakuma, Machie; Kanagawa, Osami; Cheng, Alec M.; Malissen, Bernard; and Saito, Takashi, "LAT and NTAL mediate immunoglobulin E-induced sustained extracellular signal-regulated kinase activation critical for mast cell survival." Molecular and Cellular Biology. 27,12. 4406–4415. (2007).
https://digitalcommons.wustl.edu/open_access_pubs/3084

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Authors
Sho Yamasaki, Eri Ishikawa, Machie Sakuma, Osami Kanagawa, Alec M. Cheng, Bernard Malissen, and Takashi Saito
LAT and NTAL Mediate Immunoglobulin E-Induced Sustained Extracellular Signal-Regulated Kinase Activation Critical for Mast Cell Survival

Sho Yamasaki, Eri Ishikawa, Machie Sakuma, Osami Kanagawa, Alec M. Cheng, Bernard Malissen and Takashi Saito


Published Ahead of Print 9 April 2007.
LAT and NTAL Mediate Immunoglobulin E-Induced Sustained Extracellular Signal-Regulated Kinase Activation Critical for Mast Cell Survival

Sho Yamasaki, Eri Ishikawa, Machie Sakuma, Osami Kanagawa, Alec M. Cheng, Bernard Malissen, and Takashi Saito

Laboratory for Cell Signaling and Laboratory for Autoimmune Regulation, RIKEN Research Center for Allergy and Immunology, Yokohama 230-0045, Japan; Center for Immunology, Washington University School of Medicine, St. Louis, Missouri; and Centre d’Immunologie INSERM-CNRS de Marseille-Luminy, Marseille, France

Received 10 November 2006/Returned for modification 14 December 2006/Accepted 30 March 2007

Immunoglobulin E (IgE) induces mast cell survival in the absence of antigen (Ag) through the high-affinity IgE receptor, Fcε receptor I (FcεRI). Although we have shown that protein tyrosine kinase Syk and sustained extracellular signal-regulated kinase (Erk) activation are required for IgE-induced mast cell survival, how Syk couples with sustained Erk activation is still unclear. Here, we report that the transmembrane adaptors LAT and NTAL are phosphorylated slowly upon IgE stimulation and that sustained but not transient Erk activation induced by IgE was inhibited in LAT−/− NTAL−/− bone marrow-derived mast cells (BMMCs). IgE-induced survival requires Ras activation, and both were impaired in LAT−/− NTAL−/− BMMCs. Sos was preferentially required for FcεRI signals by IgE rather than IgE plus Ag. Survival impaired in LAT−/− NTAL−/− BMMCs was restored to levels comparable to those of the wild type by membrane-targeted Sos, which bypasses the Grb2-mediated membrane recruitment of Sos. The IgE-induced survival of BMMCs lacking Gads, an adaptor critical for the formation of the LAT–SLP-76–phospholipase Cγ (PLCγ) complex, was observed to be normal. IgE stimulation induced the membrane retention of Grb2-green fluorescent protein fusion proteins in wild-type but not LAT−/− NTAL−/− BMMCs. These results suggest that LAT and NTAL contribute to the maintenance of Erk activation and survival through the membrane retention of the Ras-activating complex Grb2-Sos and, further, that the LAT–Gads–SLP-76–PLCγ and LAT/NTAL-Grb2-Sos pathways are differentially required for degranulation and survival, respectively.

Immunoglobulin E (IgE) binding to its high-affinity receptor, Fcε receptor I (FcεRI), is usually considered to be a passive step called sensitization. However, recent studies have shown that IgE actively promotes several mast cell responses, such as survival, adhesion, and the promotion of in vivo inflammatory reactions in the absence of antigen (Ag) (3, 6, 16, 17, 26). Furthermore, high concentrations of IgE (>10 μg/ml) in the plasma of subjects with parasitic infections or atopic diseases have often been observed (18), suggesting a possible Ag-independent contribution of IgE to the exacerbation of these diseases. Recent studies have suggested that IgE may elicit weak FcεRI aggregation to induce mast cell responses, even in the absence of multivalent Ag (22, 23). IgE-induced responses in the presence and absence of Ag are known to utilize distinct signaling pathways; by manipulating the duration of extracellular signal-regulated kinase (Erk) activation, we previously showed that sustained Erk activation is critical for mast cell survival but not for degranulation (52). Although we and others have also found that IgE-induced mast cell survival requires Fc receptor γ-ITAM, Lyn, and Syk (21, 22, 24, 40), the molecular mechanism underlying the coupling of Syk with sustained Erk activation is still unclear.

Many reports have demonstrated that the duration of Erk activation alters the quality of biological responses (29, 30, 52). In a variety of receptor systems, Erk activation is regulated by Ras through the subsequent activation of Raf and mitogen-activated protein kinase/Erk kinase (MEK). Although Ras is activated by guanine nucleotide exchange factors (GEFs) such as Sos upon growth factor stimulation (7), recent reports have suggested that another GEF, RasGRP, also activates Ras in T-cell receptor (TCR)- or B-cell receptor (BCR)-mediated signaling (10, 32). RasGRP is activated by diacylglycerol, a product of phospholipase Cγ (PLCγ)-mediated hydrolysis (11), providing a new pathway for Ras activation from Ag receptors. However, the contributions of Sos and RasGRP to FcεRI signaling through IgE in the presence and absence of Ag are still unknown.

NTAL (5), also called LAB (15), and LAT are raft-localized transmembrane adaptor molecules possessing multiple tyrosine-based activation motifs, and they are the substrates for Syk (13, 36). Although Grb2 and Gads binding motifs are conserved in both proteins, the binding site for PLCγ is present only in LAT and not in NTAL (5, 8, 14). It has been demonstrated previously that LAT is critical for mast cell activation upon IgE stimulation in the presence of Ag (38). On the other hand, NTAL has been reported to function as a negative regulator as well as a positive regulator of FcεRI signaling in mast cells upon IgE-Ag stimulation (43, 47, 57). The analysis of bone marrow-derived mast cells (BMMCs) has led to the proposal of a possible mechanism for the molecular competition between the two proteins within the lipid raft, which may...
explain how NTAL exerts a negative regulatory function in a LAT-dependent manner (47, 57).

Using BMMCs deficient in both LAT and NTAL, we showed that these two adaptors contribute to IgE-induced sustained Erk activation and survival by acting as a scaffold for the retention of the Grb2-Sos complex within the plasma membrane.

MATERIALS AND METHODS

Mice. LAT−/−, NTAL−/−, and LAT−/− NTAL−/− mice with a C57BL/6 background have been described previously (47). Syk−/− mice with a BALB/c background were provided by V. L. J. Tyulewicz (24). Gads−/− mice with a C57BL/6 and 129 mixed genetic background have been described previously (56). All mice were maintained under specific-pathogen-free conditions in accordance with institutional guidelines.

Abs. The mouse anti-dinitrophenol IgE antibody (Ab) H1-DNP-e-26 was kindly provided by F.-T. Liu and T. Kawakami (La Jolla Institute, Ontario, CA) and used for survival assays. The mouse anti-dinitrophenol IgE Ab SPE-7 was purchased from Sigma (St. Louis, MO) and used for survival assays and biochemistry analyses. For IgE stimulation, each IgE clone was ultracentrifuged at 100,000 × g for 10 min just before use in the assays in order to exclude aggregates, as described previously (24, 33). Anti-phospho-Erk Ab was purchased from Cell Signaling Technology (Beverly, MA), antiactin was obtained from Sigma, anti-phospho-PLCγ was from BioSource International (Camarillo, CA), anti-Ras was purchased from Pierce Biotechnology (Rockford, IL), anti-green fluorescent protein (anti-GFP) was obtained from Invitrogen (Carlsbad, CA), anti-GFP-horseradish peroxidase (HRP) was from Miltenyi Biotec (Bergisch Gladbach, Germany), anti-mouse IgG-HRP and protein G-Sepharose were purchased from Amersham Biosciences (Piscataway, NJ), and anti-rabbit Ig-HRP was from Zymed (San Francisco, CA), and anti-Sos Ab was obtained from BD Pharmingen (San Diego, CA), Anti-mCD63 monoclonal Ab, which has been proven to be specific to CD63 by the absence of IL-3 for 1 h and then stimulated with IgE for 1 h. RNA was isolated by using an RNeasy minikit (QIAGEN, Valencia, CA). After the removal of genomic DNA by treatment with DNase (Wako Nippon Gene, Tokyo, Japan), randomly primed cDNA strands were generated with reverse transcriptase II (Invitrogen). By using gene-specific primers, RNA expression was quantified by real-time PCR, and values were normalized to β-actin expression.

FIG. 1. LAT and NTAL are phosphorylated by IgE (without Ag) and are required for sustained Erk activation. (A) Western blot of total lysates from WT and Syk−/− BMMCs (Syk KO) fetal liver-derived mast cells were stimulated with 10 μg of IgE/ml for the indicated periods, and total cell lysates were blotted with antibodies to phospho-Erk (anti-pErk) and Erk (A) and phosphotyrosine (anti-pY) and phospho-LAT (anti-pLAT) (B). Brackets on the right indicate LAT (upper) and NTAL (lower). Numbers on the left are molecular size markers. (C) C57BL/6 (WT) and NTAL−/− (NTAL KO) BMMCs were stimulated as described above. Total cell lysates were blotted with antiphosphorytrosine. (D) IgE (in the presence of Ag) induces transient phosphorylation and Erk activation. BMMCs were sensitized with 1 μg of IgE/ml for 4 h and then stimulated with 15 ng of DNP-HSA/ml. Cell lysates were blotted with antibodies to phospho-Erk, phospho-Erk, and Erk.

FIG. 1. LAT and NTAL are phosphorylated by IgE (without Ag) and are required for sustained Erk activation. (A) Western blot of total lysates from WT and Syk−/− BMMCs (Syk KO) fetal liver-derived mast cells were stimulated with 10 μg of IgE/ml for the indicated periods, and total cell lysates were blotted with antibodies to phospho-Erk (anti-pErk) and Erk (A) and phosphotyrosine (anti-pY) and phospho-LAT (anti-pLAT) (B). Brackets on the right indicate LAT (upper) and NTAL (lower). Numbers on the left are molecular size markers. (C) C57BL/6 (WT) and NTAL−/− (NTAL KO) BMMCs were stimulated as described above. Total cell lysates were blotted with antiphosphorytrosine. (D) IgE (in the presence of Ag) induces transient phosphorylation and Erk activation. BMMCs were sensitized with 1 μg of IgE/ml for 4 h and then stimulated with 15 ng of DNP-HSA/ml. Cell lysates were blotted with antibodies to phospho-Erk, phospho-Erk, and Erk.
Gene-specific primer sequences were as follows: β-actin gene, 5′-TGGAAT CCTGTGGCATCCATGAAAC-3′ (forward) and 5′-TCACGAGCTCCCAGA ACCTGAACTC-3′ (reverse); IL-3 gene, 5′-ATAGGGAGCTCCCAGA ACCTGAACTC-3′ (forward) and 5′-AGACCCCTGGCAGCGCAGAGTCA TTC-3′ (reverse); and histidine decarboxylase gene, 5′-AGTCTGGCGAGA AGGGAAGG-3′ (forward) and 5′-TCTGGGCACTCATAGGCACA-3′ (reverse).

**Pulldown assay.** BMMCs were lysed with lysis buffer (Ras activation kit; Pierce, Rockford, IL). Cell lysates were incubated with GST-Raf-RBD-bound glutathione-Sepharose for 1 h at 4°C. After the samples had been washed five times with lysis buffer, Sepharose-bound protein was eluted with 10 mM glutathione and analyzed by Western blotting with anti-Ras Ab.

**Flow cytometric analysis of degranulation.** Degranulation was analyzed by flow cytometry as described previously (31). Briefly, BMMCs were sensitized with 1 μg of IgE (SPE-7)/ml for 4 h and then stimulated with 50 ng of dinitrophenol-conjugated human serum albumin (DNP-HSA)/ml for 30 min in Tyrode’s buffer. Cells were fixed with 4% paraformaldehyde for 15 min and then stained with anti-CD63 Ab and phycoerythrin-conjugated goat anti-mouse IgG (heavy- and light-chain) Fab (Cedarlane, Ontario, Canada). The surface expression of CD63 was determined by flow cytometry.

**Microscopic analysis.** BMMCs were infected with pMX-Grb2/GFP-IREShCD8. After being sorted with anti-human CD8 (anti-hCD8), the cells were stimulated with immobilized IgE (SPE-7; 10 μg/ml) on a glass-coated dish (Mat-Tech, Ashland, MA). After 30 min of incubation at 37°C, the cells were analyzed.
by wide-field fluorescence microscopy with an IX-81 instrument (Olympus, Tokyo, Japan). The membrane localization of Grb2-GFP was assessed by total internal reflection fluorescence (TIRF) microscopy with an IX81-ARCEVA system (Olympus, Tokyo, Japan).

RESULTS

LAT and NTAL are required for sustained Erk activation by IgE in the absence of Ag. IgE induces sustained Erk activation in mast cells in the absence of Ag (16, 52), whereas Erk activation in Syk−/− mast cells was totally abolished (Fig. 1A, right lanes). To assess how Syk couples with sustained Erk activation upon IgE stimulation in the absence of Ag, we first examined the total tyrosine phosphorylation pattern induced in mast cells by IgE treatment without Ag. The phosphorylation of two major proteins, pp36 and pp30-32, was observed upon IgE treatment with slow kinetics (Fig. 1B, upper panel). These phospho-proteins were not detected in Syk−/−/−/− mast cells (Fig. 1A, right lanes), suggesting that they are substrates or are located downstream of Syk. pp36 was likely to be LAT, because the anti-phospho-LAT blot exhibited similar results (Fig. 1B, upper panel), whereas pp30-32 seemed to correspond to NTAL in the basis of its gel mobility pattern (5, 15, 47) and the anti-NTAL immunoblot (data not shown). Indeed, pp30-32 was completely absent in NTAL−/−/−/− BMMCs (Fig. 1C). These results are consistent with the report that LAT and NTAL are expressed in mast cells and are substrates for Syk (5). The phosphorylation of these two adaptors was also elicited, albeit to a lesser extent in the case of NTAL, upon IgE stimulation in the presence of Ag (Fig. 1D) as reported previously (47, 57). However, the levels of phosphorylation of these adaptors rapidly declined after 5 min (Fig. 1D). Thus, LAT and NTAL are phosphorylated with slow kinetics upon IgE stimulation in the absence of Ag, in sharp contrast to the transient phosphorylation upon IgE stimulation in the presence of Ag (57).

Next, to examine the role of LAT and NTAL in IgE-induced sustained Erk activation, we stimulated BMMCs from wild-type (WT), LAT−/−/−/−, NTAL−/−/−/−, and LAT−/−/−/− NTAL−/−/−/− (dou-
induced by IgE in the presence of Ag (47, 57). The level of Syk phosphorylation, as an upstream event, in dKO BMMCs was not decreased (Fig. 2C). These results indicate that IgE-induced Erk activation can be triggered but not sustained in the absence of LAT and NTAL even when FcεRI engagement and Syk activation are prolonged.

**LAT and NTAL are required for IgE-induced mast cell survival and IL-3 induction.** We then analyzed the contribution of LAT and NTAL to IgE-induced survival. The survival of LAT<sup>−/−</sup> BMMCs was slightly impaired, but that of LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs was almost completely inhibited (Fig. 3A). An observation that was well correlated with the degree of sustained Erk activation (Fig. 2). We have previously shown that autocrine IL-3 production plays a critical role in IgE-induced mast cell survival (24). Indeed, the induction of IL-3 mRNA in LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs was greatly impaired (Fig. 3B). Furthermore, other events elicited by IgE stimulation in the absence of Ag, such as IL-6 production and histidine decarboxylase induction, were also blocked in LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs (data not shown). Taken together, these results show that LAT and NTAL are crucial for IgE-mediated IL-3 induction and mast cell survival in the absence of Ag. The results were consistent when another IgE clone, SPE-7, was used (data not shown).

**Ras activation through LAT and NTAL is critical for mast cell survival.** We next examined how sustained Erk activation was maintained through LAT and NTAL. The activation of MEK, a kinase upstream of Erk, in LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs was also impaired (data not shown). Since both LAT and NTAL are capable of binding Grb2, an event that potentially leads to MEK activation through Sos and Ras (5), we investigated Ras activation upon IgE stimulation in the absence of Ag. IgE binding induced sustained Ras activation in WT BMMCs, whereas the activation in LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs was greatly diminished (Fig. 4A). To examine the role of Ras activation in IgE-induced survival, a dominant negative form of Ras (Ras<sup>N17</sup>) was introduced into WT BMMCs. Ras<sup>N17</sup> markedly suppressed IgE-induced BMMC survival and IL-3 production (Fig. 4B and C), although the rates of exogenous IL-3-induced survival did not differ between mock- and Ras<sup>N17</sup>-transduced cells (data not shown). These results suggest that LAT and NTAL regulate IgE-induced survival through sustained Ras activation.

We next analyzed the involvement of Sos in survival through Ras activation. If LAT and NTAL were to serve as a platform for the sustained localization of the Grb2-Sos complex within the plasma membrane, the existence of a membrane-targeted form of Sos would bypass the requirement for LAT and NTAL. Indeed, the expression of farnesylated Sos was able to abolish the defect in IgE-induced survival among LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs and restore the survival rates to levels comparable to those of WT BMMCs (Fig. 4D). On the other hand, if Sos was required for IgE-induced survival, then Sos-Pro, which is known to block Grb2-Sos interaction (46), would suppress this event. Indeed, Sos-Pro suppressed mast cell survival induced by IgE in the absence of Ag (Fig. 4E). Collectively, sustained Ras activation by the prolonged membrane targeting of Sos through LAT and NTAL seems to be necessary and sufficient for mast cell survival.

To verify if Sos also contributes to the IgE-mediated signal in the presence of Ag, we examined the effect of Sos-Pro on degranulation induced by IgE in the presence of Ag by analyzing CD63 expression on the cell surface as a marker for degranulation (31). Surprisingly, the levels of induction of surface CD63 expression in GFP-negative (control) and GFP-positive (Sos-Pro) cells were similar (Fig. 4F). Thus, in the absence of Ag the Sos-Ras pathway is preferentially required for IgE-induced events as a downstream component of LAT and NTAL.

**The Gads-mediated pathway is not essential for IgE-induced mast cell survival.** In addition to Grb2, LAT and NTAL can bind Gads, an adaptor molecule that is similar to Grb2 and contains Src homology 3 (SH3)-SH2-SH3 domains. Gads is essential for linking LAT and SLP-76 (2) and is critical for TCR-mediated PLCγ activation and Ca<sup>2+</sup> influx (56). It was recently reported that PLCγ also contributes to Ras activation through RasGRF, a diacylglycerol-dependent GEF. To examine the contribution of the Gads-mediated pathway to IgE-induced responses in the absence of Ag, we analyzed Gads<sup>−/−</sup> BMMCs. PLCγ activation in Gads<sup>−/−</sup> BMMCs was impaired, as expected (Fig. 5A). However, levels of sustained Erk activation, Ras activation, and mast cell survival in Gads<sup>−/−</sup> BMMCs were observed to be comparable to those in WT BMMCs (Fig. 5). Thus, although LAT and NTAL
LAT and NTAL are required for membrane targeting of Grb2. Finally, to confirm the contribution of LAT and NTAL to the retention of the Grb2-Sos complex close to the inner leaflet of the plasma membrane, we visualized the intracellular trafficking of Grb2 by IgE by using Grb2-GFP fusion protein. Grb2, through its SH3 domain, is constitutively associated with Sos (35), and Grb2-GFP retained a strong affinity for endogenous Sos in mast cells (Fig. 6A). WT and LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs were retrovirally transfected with Grb2-GFP, and intracellular trafficking was analyzed by fluorescence microscopy. We have previously shown that plate-coated IgE stimulation can deliver a sustained signal similar to that of soluble IgE in the absence of Ag (52). Stimulation on a plate coated with IgE induced drastic membrane localization of Grb2-GFP within 30 min (Fig. 6B). To analyze the cell surface area more precisely on an evanescent excitation field, we employed TIRF microscopy, because it enables a selective visualization of the plasma membrane (4, 44). The recruitment of Grb2-GFP to the plasma membrane was clearly observed upon IgE stimulation (Fig. 6C, upper panels). In sharp contrast, Grb2-GFP in LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs did not show membrane translocation (Fig. 6C, lower panels). Importantly, equal levels of Grb2-GFP expression in WT and LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs were confirmed by flow cytometry (data not shown). These results suggest that LAT and NTAL provide an essential scaffold for the IgE-induced membrane retention of the Grb2-Sos complex.

**DISCUSSION**

We investigated the molecular mechanism of IgE-induced sustained Erk activation and mast cell survival. Many reports have demonstrated that the duration of Erk activation alters the quality of the biological response (29, 30, 52). By manipulating the duration of Erk activation, we showed previously that sustained, not transient, Erk activation is critical for mast cell survival (52). However, how the signal duration is physiologically regulated remains to be elucidated. It has been reported previously that the duration of Erk activation is determined by multiple mechanisms, such as receptor internalization and the operation of a feedback inhibitory mechanism (34, 41). The weak cross-linking of FcεRI without internalization may induce sustained Erk activation through prolonged FcεRI ligation (23, 52, 54). Nevertheless, even when FcεRI engagement and Syk activation were prolonged, sustained Erk activation in LAT<sup>−/−</sup> NTAL<sup>−/−</sup> BMMCs was almost completely inhibited, suggesting that the two adaptors LAT and NTAL constitute some kind of timer for downstream signaling. Note that transient Erk activation was not dramatically impaired by IgE stimulation in the absence of Ag (Fig. 2A and B) and in the presence of Ag (Fig. 2D) (57) in the absence of LAT and NTAL. This result suggests that transient Erk activation via FcεRI is LAT- and NTAL-independent and that the weak and prolonged cross-linking of FcεRI may preferentially utilize LAT and NTAL for sustained Erk activation, eventually leading to mast cell survival. However, so far we cannot directly exclude the possible contribution of another pathway to sustained Erk activation, such as the activation of Rap1, B-Raf, or protein kinase C, as reported previously (19, 41, 45).

Oh-hora et al. showed the differential requirements for Ras-
existence of similar divergence patterns in FcεRI-induced degranulation in the presence of Ag implies the press of IgE-mediated survival in the absence of Ag but not NTAL. Our observation that dominant negative Sos suppresses IgE-mediated survival in the absence of Ag. Considering that LAT+/− mice exhibited severe impairment of IgE-induced mast cell degranulation in the presence of Ag (38, 57), NTAL appears to compensate for LAT function preferentially under stimulation via IgE without Ag. This finding may be explained by the fact that NTAL possesses binding sites for Grb2 but not for PLCγ, which is crucial for degranulation (48, 49). Consistent with this suggestion, Y136, a PLCγ binding site of LAT, has been shown to be essential for degranulation (28, 39).

Recently, accumulating evidence has suggested that the quantity and duration of signals through immune receptors determine distinct immune responses (29, 50). It is attractive to speculate that, likewise, the differential contributions of Sos and RasGRP are also operative in Ras activation during sustained Ag receptor triggering, for example, during the tonic signal of BCR (25). TCR engagement for homeostatic proliferation (12), or autonomous pre-TCR signaling (53). Indeed, RasGRP1-deficient thymocytes display no apparent defect in autonomous pre-TCR signaling, a process that is known to depend on Ras activation (10, 37).

The augmentation of mast cell function by the genetic deletion of NTAL alone has been explained by an increase in the amount of available LAT (47, 57), in light of the possible competition between the two transmembrane adaptors within the lipid raft. In line with these speculations, IgE-induced events in the absence of Ag are also upregulated in NTAL−/− BMMCs. As an additional possible explanation, we found that specific chemokine receptors are constitutively upregulated in NTAL−/− BMMCs (S. Yamasaki and T. Saito, unpublished data); this upregulation may also contribute to the augmented mast cell responses seen in NTAL-deficient single-knockout mice.

Taking these findings together, we propose herein a possible molecular mechanism by which Syk couples with sustained Erk activation upon IgE stimulation in the absence of Ag. IgE without Ag induces the delayed phosphorylation of LAT and NTAL. Phosphorylated LAT and NTAL are critical adaptors for the raft-localized membrane adaptors LAT and NTAL are the critical transducers for timing the duration of signals from FcεRI to trigger downstream events. These results suggest that it may be possible to differentially modulate signaling events through FcεRI in order to block specific allergic reactions.

ACKNOWLEDGMENTS

We thank F.-T. Liu, T. Kawakami, V. Horejsi, and K. Nishida for Abs; V. L. J. Tybulewicz for providing mice; M. Malissen, T. Kurosaki, A. Veillette, and T. Iwahara for discussion; and H. Yamaguchi for secretarial assistance.
The development and characterization of NTAL-deficient mice were supported by FRM (Defis de la Recherche en Allergologie). This study was supported by a grant-in-aid for priority area research (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES


