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FcεRI-mediated mast cell response is modulated by TLR2 and TLR4 ligation

ALEKSANDRA SŁODKA, EWA BRZEZIŃSKA-BŁASZCZYK

Department of Experimental Immunology, Medical University of Łódź, Poland

Abstract

It is well documented that mast cells express both FcεRI and Toll-like receptors (TLRs). It is also suggested that subsequent/simultaneous mast cell activation via TLR and FcεRI may affect these cells activity. In the present study we examined the influence of lipoarabinomannan (LAM), that is TLR2 ligand, and lipopolysaccharide (LPS), that is TLR4 agonist, on mast cell degranulation and preformed mediator release and cysteinyl leukotriene (cysLT) generation mediated by FcεRI aggregation. Our experiments were performed on native rat peritoneal mast cells and on peritoneal mast cells pre-coated in vitro with IgE. We found that neither LAM- nor LPS-priming modified degranulation of native mast cells. Under the same experimental conditions LAM-induced TLR2 ligation caused statistically significant ($p < 0.01$) decrease in FcεRI-mediated histamine release from IgE-coated mast cells. Moreover, we noticed that simultaneous stimulation via TLR4 or TLR2 and FcεRI resulted in synergistic amplification of cysLT generation from IgE-coated mast cells and, to a lesser extent, from native mast cells. These observations might be a clue to understanding the mechanistic basis of allergic disease exacerbation upon coexisting infections.

Key words: *mast cells, Toll-like receptors, bacterial antigens, allergic diseases, histamine, leukotrienes.*

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Introduction

The high affinity receptor for immunoglobulin E (FcεRI) is expressed on the surface of mast cells. These cells are the principal effector cells in the classical type I hypersensitivity reaction which underlies the pathomechanism of IgE-dependent allergic diseases. In response to FcεRI cross-linking by specific IgE and antigen (allergen) various cascade signaling downstreams are activated leading to mast cell degranulation, arachidonic acid metabolite generation as well as synthesis of chemokines, cytokines and growth factors [1, 2].

It is now well documented that Toll-like receptors (TLRs), the pattern recognition receptors for pathogen components, are constitutively and highly expressed on the mast cells [3, 4]. TLR ligation by its agonists induces the highly selective mast cell response. A number of studies have indicated that neither TLR2 nor TLR4 stimulation resulted in mast cell degranulation and preformed mediator release [5-10] but it induces synthesis of newly generated lipid mediators, including cysteinyl leukotrienes (cysLTs) [5, 7-13]. TLR2 and TLR4 ligation can activate mast cells to synthesize many different cytokines and chemokines, as well [5, 7, 14-17].

Taking into account that both TLR2 and TLR4 ligation as well as FcεRI-mediated stimulation lead to mast cell activation, it may be assumed that to some extent subsequent or simultaneous stimulation *via* TLR and *via* FcεRI may affect mast cell response. Current knowledge about the influence of TLR/FcεRI network on mast cell activation is limited and the results of latest research are often ambiguous. In the present study we scheduled to examine the effect of lipoarabinomannan (LAM), that is TLR2 ligand, and lipopolysaccharide (LPS), that is TLR4 agonist, on mast cell preformed mediator release and cysLT generation mediated by FcεRI aggregation with IgE and anti-IgE. Considering that FcεRI-IgE complex formation induces upregulation of FcεRI expression [18-21], which in turn may significantly alter mast cell sensitivity and releasability, we

Correspondence: Ewa Brzezińska-Błaszczyk, Department of Experimental Immunology, Medical University of Łódź, Pomorska 251, 92-213 Łódź, Poland, tel./fax +48 42 675 73 06, e-mail: ewab@csk.umed.lodz.pl

conducted our research using native as well as mast cells previously coated with IgE.

Material and methods

Reagents

Dulbecco's Modified Eagle's Medium (DMEM), Hank's balanced salt solution (HBSS), sodium bicarbonate, fetal calf serum (FCS), gentamicin and glutamine were purchased from GIBCO, Life Technologies (Gaithersburg, MD, USA). Lipopolysaccharide from *Escherichia coli* O55:B5, NaCl, KCl, $MgCl_2$, $CaCl_2$, 2-hydroxyethylpiperazine-N'-ethanesulphonic acid (HEPES), NaOH, glucose, HCl, *o*-phthaldehyde (OPT), Percoll, trypan blue, toluidine blue and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Lipoarabinomannan from *Mycobacterium smegmatis* was purchased from InvivoGen (San Diego, CA, USA) and rat myeloma IgE was obtained from Invitrogen, Life Technologies (Gaithersburg, MD, USA). Mouse anti-rat IgE was purchased from AbD Serotec (Oxford, United Kingdom), goat IgG isotype control was obtained from Abcam Inc. (Cambridge, MA, USA), cysLT immunoassay kit was purchased from Cayman Chemical (Ann Arbor, MI, USA), and goat IgG antibodies blocking TLR2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Isolation of mast cells

Mast cells were obtained from peritoneal cavities of female albino Wistar rats weighing 200-250 g by lavage with 50 ml of 1% HBSS supplemented with 0.015% sodium bicarbonate. After abdominal massage (90 s), the cell suspension was removed from the peritoneal cavity, centrifuged (1200 rpm, 5 min, 20°C), and washed twice in complete (c)DMEM containing DMEM supplemented with 10% FCS, 10 μg/ml gentamicin, and 2 mM glutamine (1200 rpm, 5 min, 20°C). To prepare purified mast cells, peritoneal cells were resuspended in 72.5% isotonic Percoll solution and centrifuged (1500 rpm, 20 min, 20°C). Isolated mast cells were washed twice in cDMEM by centrifugation, counted and resuspended in an appropriate volume of medium for rat mast cells containing 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES buffer, 5.6 mM glucose, and 1 mg/ml BSA (pH of the medium was adjusted to 6.9) to obtain a mast cell concentration of 1.5×10^6 cells/ml. The mast cells were prepared with purity > 98%, as determined by metachromatic staining with toluidine blue.

Histamine release assay

Native or IgE-coated mast cells (i.e. cells preincubated with 1 μg/ml of IgE for 60 min at 37°C and then washed by centrifugation) were incubated with LAM or LPS (bacterial antigens at final concentrations of 0.1 μg/ml or 10 μg/ml) or medium alone for 60 min at 37°C in a water bath with constant stirring. Mast cells were washed by centrifugation, suspended in medium and incubated with anti-IgE at final concentration of 5 μg/ml or medium alone for 30 min at 37°C. The reaction was stopped by adding 1.9 ml of cold medium and then the cell suspensions were centrifuged (1200 rpm, 5 min, 4°C). The supernatants were decanted into separate tubes and a total of 2 ml of distilled water was added to each tube with cell pellet. All samples were acidified with 3 N HCL. The histamine content was determined in both cell pellets (residual histamine) and supernatants (released histamine) by spectrofluorometric method using OPT. Histamine release was expressed as a percentage of the total cellular content of the amine after correction for the spontaneous release indicated in controls.

For time-course experiments IgE-coated mast cells were incubated with bacterial antigen at final concentration of 10 μg/ml for 1, 3, 5, 10, 20, 40 or 60 min and next treated as described above.

To determine the specificity of bacterial antigen effect on anti-IgE-induced histamine release in some experiments, before main procedure, mast cells were preincubated with anti-TLR2 antibodies or goat IgG as a isotype control at final concentration of 40 μg/ml for 15 min at 37°C. After that, mast cells were washed twice, resuspended in medium and treated as described above.

CysLT release assay

Native or IgE-coated mast cells were suspended in medium and incubated with LAM (final concentration of 2.5 μg/ml) or LPS (final concentration of 0.5 μg/ml) in the presence or absence of anti-IgE (final concentration of 5 μg/ml) in a water bath with constant stirring for 60 min at 37°C. At the same time, mast cells were incubated with anti-IgE alone, LAM alone, LPS alone, calcium ionophore A23187 at final concentration of 5 μg/ml (positive control), or medium alone (spontaneous cysLT release). The supernatans were collected by centrifugation (1200 rpm, 5 min, 20°C) and analyzed by an ELISA commercial kit that detected LTC_4 and its degradation products LTD_4 and LTE_4. The sensitivity of this assay was < 13 pg/ml.

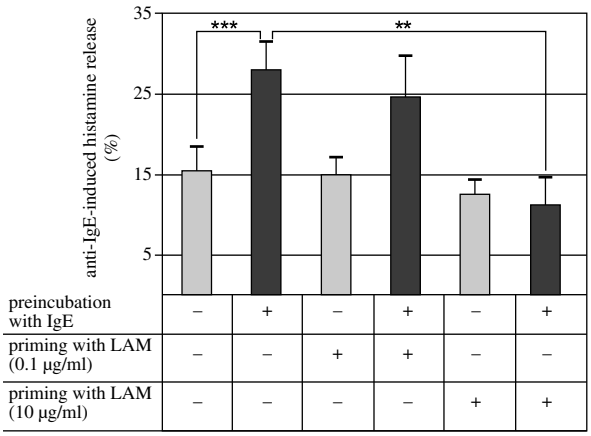
Statistical analysis

Statistical parameters included mean value ± standard error of the mean (SEM), and Student's *t*-test for "small groups". Values of $p < 0.05$ were considered statistically significant.

Results

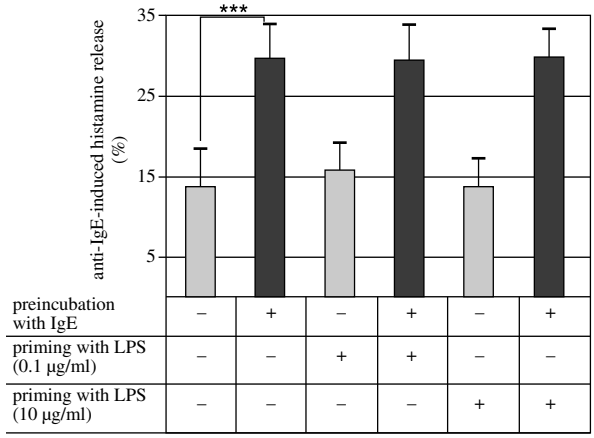
The effect of bacterial antigens on mast cell anti-IgE-induced histamine release

We first examined the effect of priming with TLR2 ligand, that is LAM, on anti-IgE-induced degranulation and



*** p < 0.01, **** p < 0.001*

Fig. 1. Effect of LAM priming on anti-IgE-induced histamine release from native (white bars) and IgE-coated (black bars) mast cells. Results are expressed as the mean ± SEM of three independent experiments and each experiment was carried out in duplicate ($n = 6$)



**** p < 0.001*

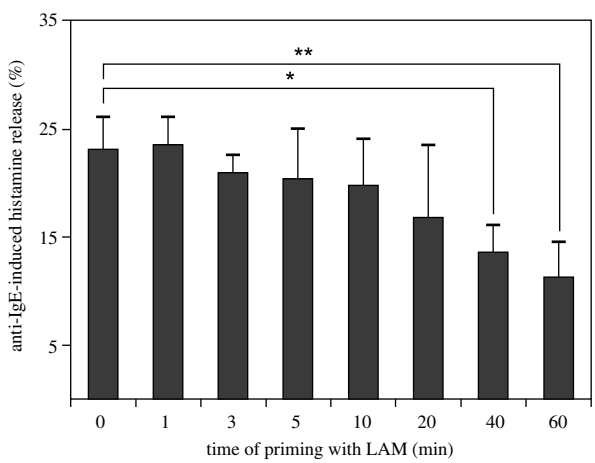
Fig. 2. Effect of LPS priming on anti-IgE-induced histamine release from native (white bars) and IgE-coated (black bars) mast cells. Results are presented as the mean ± SEM of three independent experiments and each experiment was carried out in duplicate ($n = 6$)

histamine release from mast cells. As shown in Fig. 1, pretreatment of mast cells with LAM did not affect histamine release induced by anti-IgE from native mast cells. However, priming with LAM at concentration of 10 μg/ml caused statistically significant ($p < 0.01$) decrease in histamine release from IgE-coated mast cells in response to anti-IgE stimulation. Under the same experimental conditions priming with LPS, that is TLR4 ligand, used at concentrations of 0.1 μg/ml and 10 μg/ml did not influence anti-IgE-induced histamine release from native as well as IgE-coated mast cells (Fig. 2). It should be underlined, that neither LAM nor LPS directly activated mast cells to degranulation and histamine release (data not shown).

We examined the effect of LAM-priming time on histamine release from IgE-coated mast cells in response to anti-IgE stimulation, as well. We noticed that statistically significant ($p < 0.05$) decrease of anti-IgE-induced histamine release was observed after 40 min preincubation with LAM. After 60 min of LAM-pretreatment anti-IgE-induced release of histamine decreased from 23% to 11% ($p < 0.01$) (Fig. 3). We also noticed that blocking TLR2 with related antibodies, prior to IgE-coated mast cell priming with LAM (at concentration of 10 μg/ml) and anti-IgE challenge, strongly and statistically significant abolished LAM-induced suppressive effect exerted on IgE-mediated histamine secretion (data not shown).

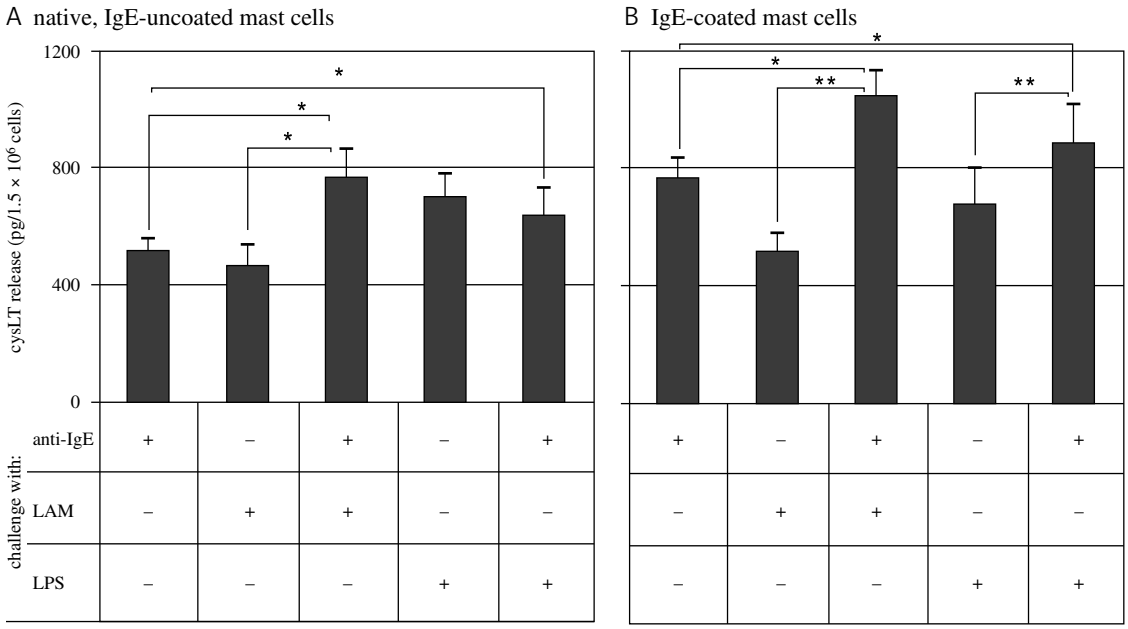
The effect of co-stimulation with bacterial antigens and anti-IgE on mast cell cysLT generation

Next we investigated whether simultaneous activation of both native and IgE-coated mast cells via TLR2 or TLR4 and FceRI affects cysLT generation and release. The results of these experiments are presented in Fig. 4. Both LAM and LPS directly activated native and IgE-coated mast cells to cysLT synthesis and release. The combination of LAM with anti-IgE markedly and statistically significant ($p < 0.05$) augmented cysLT release from native mast cells. Also, simultaneous activation of native mast cells by LPS and anti-IgE caused statistically significant ($p < 0.05$) increase in cysLT generation, as compared with anti-IgE-mediated generation by itself (Fig. 4A). Lipoarabinomannan or LPS



**p < 0.05, ** p < 0.01*

Fig. 3. Time-course of LAM-priming on anti-IgE-induced histamine release from IgE-coated mast cells. LAM was used at final concentration of 10 μg/ml. Results are presented as the mean ± SEM of three independent experiments and each experiment was carried out in duplicate ($n = 6$)



**p < 0.05, ** p < 0.01*

Fig. 4. CysLT synthesis and release from (A) native, IgE-uncoated mast cells or (B) IgE-coated mast cells in response to simultaneous stimulation with TLR ligand and anti-IgE. LAM was used at final concentration of 2.5 μg/ml and LPS was used at final concentration of 0.5 μg/ml. Results are presented as the mean ± SEM of three independent experiments and each experiment was carried out in duplicate ($n = 6$)

and anti-IgE also interacted synergistically in promoting cysLT synthesis by IgE-coated mast cells. We noticed, however, that LAM was more effective in augmenting production of cysLTs than LPS. CysLT synthesis increased up to 201% (as compared with LAM-induced cysLT generation) and up to 136% (as compared with anti-IgE-mediated cysLT generation) after co-stimulation with LAM and anti-IgE, and up to 130% and 115% (as compared with LPS-induced and anti-IgE-mediated cysLT generation, respectively) (Fig. 4B). What is more, the synergistic effect of stimulation with TLR ligands and anti-IgE on cysLT synthesis was more obvious in IgE-coated mast cells than in native mast cells.

Discussion

In the present study we demonstrated that subsequent or simultaneous mast cell stimulation *via* TLR and FcεRI may influence mast cell activity. However, this effect seemed to be dependent on the kind of TLR and FcεRI/IgE complex formation, as well. We established that TLR4 ligation mediated by LPS priming did not modify degranulation and histamine release from native as well as IgE-coated mast cells following FcεRI cross-linking. Under the same experimental conditions LAM-induced TLR2 ligation caused statistically significant ($p < 0.01$) decrease in FcεRI-mediated histamine release from IgE-coated mast cells. What is more, simultaneous stimulation *via* TLR4 or TLR2 and FcεRI resulted in synergistic amplification of cysLT generation from IgE-coated mast cells and, to a lesser extent, from native mast cells.

The data available so far, concerning the effect of TLR2 or TLR4 ligation on FcεRI-dependent mast cell response, are sparse. It should be emphasized that the majority of research were carried out only on mast cell lines coated in vitro with IgE. It has been established that TLR2 ligands, i.e. peptidoglycan (PGN) and lipoteichoic acid (LTA), reduced FcεRI-mediated degranulation of human mast cell line LAD2, and only LTA-priming significantly inhibited FcεRI-dependent degranulation of human pulmonary mast cells, as assessed by β-hexosaminidase release [22]. It was also stated that there was no significant change in degranulation of bone marrow-derived mast cells (BMMCs) following stimulation through TLR4 with LPS and FcεRI cross-linking [23]. Substantial enhancement of FcεRI-mediated degranulation of connective as well as mucosal like mast cells was noticed after prolonged exposure (up to 96 h) to LPS. Under the same experimental conditions amplified secretion of cysLT was observed [24]. Moon *et al*. [25] established that TLR4 ligation induced by LPS caused significant increase in IgE-dependent prostaglandin (PG) D_2 generation from BMMC. On the contrary, some data clearly demonstrated no influence of LPS stimulation on mast cell arachidonic acid metabolite synthesis following FcεRI aggregation [17, 26]. Interestingly, it was documented that

the combination of TLR2 or TLR4 stimulation and FcεRI cross-linking resulted in synergistically amplified secretion of many, especially Th2-skewed, cytokines and chemokines from mast cells [14, 23, 26-28].

The underlying mechanisms by which TLR ligands in combination with FcεRI-mediated activation influence mast cell response remain unclear. TLR2 and TLR4 agonists may modulate the surface expression of FcεRI and in consequence can alter mast cell FcεRI-dependent response [17, 22, 29]. Since it is well documented that TLR ligation stimulates mast cell to various cytokine secretion [5, 7, 14-17] it may be assumed that these secreted cytokines indirectly affect FcεRI-dependent mast cell activity by autocrine feedback loop mechanism [22, 23, 25]. Little is known about impact of mast cell stimulation mediated *via* both TLR- and FcεRI-dependent pathway on intracellular signaling events. On the one hand, mast cell co-treatment through both FcεRI and TLR2 or TLR4 receptors resulted in the attenuation of mitogen activated protein kinase (MAPK) phosphorylation [23, 30] as well as in suppression of intracellular calcium mobilization with no change in calcium influx [26, 30, 31]. On the other hand, Qiao *et al*. [26] observed the synergistic enhancement of the MAP kinases (p38, JNK) and transcriptional factors phosphorylation (ATF-2, c-Jun, c-Fos) after mast cell activation *via* TLR2 or TLR4 and FcεRI. An intriguing observation was made by Mertsching *et al*. [32] that mast cell response to LPS and FcεRI aggregation, which resulted in synergistic increase of cytokine secretion, may be regulated *via* inhibitory receptor FcεRIIB.

In the course of our study we also demonstrated that native mast cells isolated from rat peritoneal cavity and cells that were coated *in vitro* with IgE may respond differently to sequential/simultaneous TLR ligation and FcεRI cross-linking. There is a compelling evidence that IgE alone augments cell surface expression of FcεRI on mast cells. The main mechanism of this upregulation involves IgE-induced stabilization and accumulation of receptors at the plasma membrane and, in consequence, lower FcεRI internalization and degradation [18-21]. This phenomenon might be partially implicated in different response to TLR- and FcεRI-mediated activation of IgE-coated in comparison with native mast cells observed in our study.

In summary, more and more data indicate that putative *cross-talk* between TLR- and FcεRI-dependent stimulation modulates mast cell response. Since mast cells are regarded to be a crucial effector cells in allergic processes [1, 2] further research in this field may provide us with better understanding of mechanistic basis for allergic disease exacerbation upon coexisting infections.

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The authors declare no conflict of interest.

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