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Protective Roles of Mast Cells Against Enterobacterial Infection Are Mediated by Toll-Like Receptor 4

Volaluck Supajatura,*† Hiroko Ushio,‡ Atsuhiro Nakao,‡ Ko Okumura,‡‡ Chisei Ra,‡‡ and Hideoki Ogawa*‡

Toll-like receptors (TLRs) are mammalian homologues of the Drosophila Toll receptors and are thought to have roles in innate recognition of bacteria. We demonstrated that TLR 2, 4, 6, and 8 but not TLR5 were expressed on mouse bone marrow-derived mast cells (BMMCs). Using BMMCs from the genetically TLR4-mutated strain C3H/HeJ, we demonstrated that functional TLR4 was required for a full responsiveness of BMMCs to produce inflammatory cytokines (IL-1β, TNF-α, IL-6, and IL-13) by LPS stimulation. TLR4-mediated stimulation of mast cells by LPS was followed by activation of NF-κB but not by stress-activated protein kinase/c-Jun NH2-terminal kinase signaling. In addition, in the cecal ligation and puncture-induced acute septic peritonitis model, we demonstrated that genetically mast cell-deficient W/Wv mice that were reconstituted with TLR4-mutated BMMCs had significantly higher mortality than W/Wv mice reconstituted with TLR4-intact BMMCs. Higher mortality of TLR4-mutated BMMC-reconstituted W/Wv mice was well correlated with defective neutrophil recruitment and production of proinflammatory cytokines in the peritoneal cavity. Taken together, these observations provide definitive evidence that mast cells play important roles in exerting the innate immunity by releasing inflammatory cytokines and recruitment of neutrophils after recognition of enterobacteria through TLR4 on mast cells. The Journal of Immunology, 2001, 167: 2250–2256.

Mast cells have been preserved through evolution even among the lowest orders of animals. In addition to production and secretion of a wide spectrum of mediators and cytokines, their functional capacity, including the ability to phagocytose and process Ags, has led us to propose a potential role of mast cells in innate immune response against infectious organisms as well as in allergic diseases (1–4). As shown in two different models of acute bacterial infection, mast cell-deficient W/Wv mice were less efficient in clearing the pathogenic bacteria in the cecal ligation and puncture (CLP)3-induced peritonitis and from the lungs of mice intranasally challenged with Klebsiella pneumoniae (5, 6). It has been shown that mast cell-derived TNF-α and leukotriene-dependent recruitment of circulating leukocytes with bactericidal properties is crucial for a full response against acute infection (5–7). Although the molecular basis for the interaction between mast cells and various Gram-negative and Gram-positive bacteria is thought to be mediated by “pattern recognition receptors” that display binding specificity for structural pattern molecules common to many microorganisms (8), precise mechanisms for mast cell activation by these microorganisms are yet to be clarified.

Toll-like receptor (TLR) families are transmembrane proteins containing repeated leucine-rich motifs in their extracellular portions similar to other pattern recognition proteins of the innate immune system, and a cytoplasmic domain that is homologous to the signaling domain of the IL-1R which mediates activation of NF-κB (9). Although 10 such receptors have been identified (10), functional data are available only for two TLRs, TLR2 and TLR4. TLR2 appears to participate in the innate recognition response to soluble peptidoglycan, lipoteichoic acid, or whole Gram-positive bacteria (11–15), whereas TLR4 is implicated in cellular responses to LPS, the major constituent of the Gram-negative bacteria outer membrane (16, 17). Immunocompetent cells express a variety of specific transcripts of TLRs (18). TLR1 is expressed in all leukocytes including monocytes, polymorphonuclear cells, T and B cells, and NK cells, whereas TLR2, 4, and 5 are expressed in myelomonocytic cells. Specific expression of TLR3 is observed only in dendritic cells (18). Thus, the difference in TLR expression may reflect their specialized functions in immune responses.

Despite the assumption that mast cells and TLRs are both involved in innate immune response, no data are available regarding the expression patterns of TLRs on mast cells. Signaling pathways upstream of cytokine expression of mast cells in response to LPS also have not yet been well defined. Using TLR4-intact LPS-responsive C3H/HeN and TLR4 4-mutated LPS-hyporesponsive C3H/HeJ mice (19), we therefore first examined the TLR expression on mast cells and functional roles of TLR4 on mast cells in LPS-induced cytokine production, degranulation, and signal transduction pathways. In addition, in vivo TLR 4-mediated protective roles of mast cells were examined in an acute peritonitis model using genetically mast cell-deficient W/Wv mice that were reconstituted with bone marrow-derived-mast cells (BMMCs) from C3H/HeN or C3H/HeJ mice.

Materials and Methods

Mice

WBB6F1-W/Wv, WBB6F1-cre, C3H/HeN, C3H/HeJ, BALB/c, and C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan). It has been reported that C3H/HeJ mice are hyporesponsive to LPS because of the point mutation at codon 712 within the cytoplasmic domain of...
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Tlr4 gene by substitution of highly conserved proline by histidine, which leads to the defect in the signal transduction through TLR4 (19). NC/Nga mice were originally obtained from Kowa Institute (Ibaraki, Japan) and maintained in the specific pathogen-free animal facility at Juntendo University. All animal experiments were performed under the approved manual of the Institutional Review Board of Juntendo University.

Generation of BMMCs

BMMCs were generated from the femoral bone marrow cells of mice as described previously (20). Cells were grown in the RPMI 1640 (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated FCS (Biological Industries, Hamerik, Israel), 100 U/ml penicillin, 100 µg/ml streptomycin, 10−4 M 2-ME, 10 mM sodium pyruvate, 10 µM MEM nonessential amino acids solution, and 10% pokeweed mitogen-stimulated spleen-conditioned medium as a source of mast cell growth factors (21) by replacement of half of the medium weekly. After 4–5 wk of culture, >98% of the cells were identifiable as mast cells as determined by toluidine blue staining and FACS analysis of cell surface expression of c-kit and FcεRI.

β-Hexosaminidase release assay

A total of 5 × 106 cells/ml BMMCs in Tyrode’s buffer (10 mM HEPES buffer (pH 7.4), 130 mM NaCl, 5 mM KCl, and 5.6 mM glucose) containing 10% FCS (as a source of soluble CD14), 1 mM CaCl2, and 0.6 mM MgCl2 was stimulated with the indicated concentration of LPS (Escherichia coli serotype 0111:B4; Sigma) for 1 h at 37°C. The BMMCs stimulated with IgE (1 µg/ml, BD Pharmingen, San Diego, CA) plus anti-IgE (1 µg/ml, Pharmingen) or with PMA (10 ng/ml) plus ionomycin (100 ng/ml) were used as positive controls. Cell supernatants and total cell lysates solubilized with 1% Nonidet P-40 were collected, and β-hexosaminidase in the supernatants and cell lysates was quantified by spectrophotometric analysis of hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucopyranoside (Sigma). The percentage of β-hexosaminidase release was calculated using the following formula: percent release = (OD of the stimulated supernatant - OD of the unstimulated supernatant) / (OD of the total cell lysate - OD of the unstimulated supernatant), Viability of the cells under each condition was >98% as assessed by the trypan blue dye exclusion test.

Evaluation of cytokine concentrations

BMMCs (1 × 106/ml) in complete cultured medium were stimulated at 37°C with the indicated concentration of LPS, 3 h for TNF-α, and 6 h for IL-β, IL-6, and IL-13. From preliminary experiments, these time points were optimal for production of these cytokines from BMMCs upon LPS stimulation. The levels of each cytokine in the culture supernatants were determined by ELISA kits according to the manufacturer’s instructions (Genzyme Techne, Minneapolis, MN). Viability of the cells under each condition was >98% as assessed by a trypan blue dye exclusion test.

RT-PCR analysis of TLR expressions on mast cells

Total RNA was extracted from BMMCs using STAT-60 (Tel-Test, Friendswood, TX) according to the manufacturer’s instructions. First-strand cDNA was constructed from 3 µg of total RNA with oligo(dT)12-18, as primers using Superscript II RNase H− reverse transcriptase (Life Technologies, Rockville, MD). PCR was performed using primers for mouse TLR2 (GenBank accession no. AF185284; 5'-CTT CCT GGT TCC CTG CTT CTC and 5'-CAA GAA CAA AGA AAA TGA GTC AAG), mouse TLR4 (accession no. AF110133) (22), mouse TLR5 (accession no. AF186107) (23), mouse TLR6 (accession no. NM011604) (24), mouse TLR8 (accession no. AF113795; 5'-TCT TGC CCT TGG CAG AAG AGT and 5'-GGAA GCT GAC ATT CCA GAC ACA), and species nonspecific GAPDH (5'-AGT AGT ACT CCA CTC ACG GCA A and 5'-GCT CGC TCC TGG AAA ATG GT) (25).

Western blotting

A total of 5 × 106 cells/ml BMMCs was stimulated with LPS (50 ng/ml) for the indicated time period. At the indicated time points, the reaction was stopped by adding cold Tyrode’s (−) buffer. The cells were lysed with 20 µl of lysis buffer (1% Triton X-100, 150 mM NaCl, 25 mM Tris-HCl (pH 7.5), 1 mM EDTA, containing 1 µM PMSF, 10 µg/ml leupeptin, 10 µg/ml pepstatin A, 50 µg/ml aprotinin, and 2 mM sodium orthovanadate), and the lysates were subjected to SDS-PAGE (26). In brief, the mice were anesthetized by i.p. injection of 50 mg/kg sodium pentobarbital (Abbott Laboratories, Abbott Park, IL) in 200 µl of sterile PBS. A 1-cm midline incision on the anterior abdominal wall was made. The cecum was exposed and filled with feces by squeezing stool back from the ascending colon. The cecum was 50% ligated below the ileocecal valve and then punctured using a 0.9-mm needle followed by gentle squeezing of the cecum. Mice were observed for mortality at least five times daily over a period of 10 days. Before CLP was performed, the mice were coded so that the CLP was done without notifying individual groups.

Cecal ligation and puncture

CLP was performed as previously described with slight modification (5, 6, 26). In brief, the mice were anesthetized by i.p. injection of 50 mg/kg sodium pentobarbital (Abbott Laboratories, Abbott Park, IL) in 200 µl of sterile PBS. A 1-cm midline incision on the anterior abdominal wall was made. The cecum was exposed and filled with feces by squeezing stool back from the ascending colon. The cecum was 50% ligated below the ileocecal valve and then punctured using a 0.9-mm needle followed by gentle squeezing of the cecum. Mice were observed for mortality at least five times daily over a period of 10 days. Before CLP was performed, the mice were coded so that the CLP was done without notifying individual groups.

Differential cell counts and estimation of cytokine concentrations in peritoneal exudates

Peritoneal exudates were collected from CLP-induced mice at the indicated time points and total cell numbers were counted. Cytosup preparations were made from the exudates of each mouse and differential cell counts of infiltrating leukocytes were done by counting 500 leukocytes under oil immersion fields after staining with Diff-Quik (Kokusaishi, Yaku, Japan). The percentage of mast cells in the exudates was determined by toluidine blue (pH 4.0) or Alcian blue/safranin staining. The concentrations of cytokines in peritoneal fluids were determined by ELISA kits according to the manufacturer’s instruction (Genzyme Techne).

Statistical analysis

Statistical analysis of most data was performed using the Student t test. Statistical analysis of survival data in CLP experiments was performed using the log rank test.

Results

TLR mRNA expressions in mouse mast cells

TLR mRNA expressions of mouse mast cells were initially assessed by RT-PCR. cDNA from mouse bone marrow-derived dendritic cells was used as a known source for mouse TLR expression to confirm the specificity of the primers and PCR. As shown in Fig. 1, transcripts of TLR2, TLR4, TLR6, and TLR8 were detected in both C3H/HeN and C3H/HeJ BMMCs; in contrast, no transcript of TLR5 was detected in these BMMCs. Similar results were obtained in BMMCs from BALB/c, C57BL/6, and NC/Nga mice (data not shown). RT-PCR analysis of GAPDH expression confirmed the equality of all RNA preparations used for these experiments.

LPS induces cytokine production via TLR4 without causing degradation from mast cells

Significant degranulation and cytokine release were associated with mast cell activation by soluble microbial products. To investigate whether LPS-stimulated cytokine production and degranulation activities of mast cells were mediated via functional...
TLR4, we developed BMMCs from TLR4-intact C3H/HeN or TLR4-mutated C3H/HeJ mice, then assessed the cytokine production (TNF-α, IL-1β, IL-6, and IL-13) and β-hexosaminidase release from these mast cells upon LPS stimulation. As shown in Fig. 2A, C3H/HeN BMMCs could respond to produce TNF-α, IL-1β, IL-6, and IL-13 by LPS stimulation in a dose-dependent manner, whereas C3H/HeJ BMMCs failed to produce any cytokines by stimulation with LPS (1–1000 ng/ml). The viability of BMMCs from both strains of mice after LPS stimulation was >95%, indicating cytokine release was not due to cytotoxic effects of LPS.

β-Hexosaminidase is released in parallel with histamine by mast cell activation and is, thus, a marker of mast cell degranulation (27). Although PMA-ionomycin or FceRI cross-linking triggered similar amounts of β-hexosaminidase release from both C3H/HeN and C3H/HeJ BMMCs, LPS failed to trigger any release of β-hexosaminidase from these mast cells (Fig. 2B). Similar results were obtained in BMMCs derived from BALB/c, C57BL/6, and NC/Nga mice (data not shown).

These results suggested that cytokine productions from BMMCs by LPS stimulation were highly dependent on the functional expression of TLR4 and mechanisms of cytokine and β-hexosaminidase release from BMMCs were different.

LPS-TLR4-mediated pathways activate NF-κB signaling in mast cells

Signaling through the TLRs has been focused on NF-κB activation that is required for the transcription of many immune responsive genes including cytokine genes. It has been reported that LPS can activate NF-κB in human intestinal epithelial cells expressing TLRs (28) and activate NF-κB and c-Jun/activating transcription factor 2/T cell-specific factor in human leukocytes (29). We determined whether NF-κB and stress-activated protein kinase (SAPK)/JNK cascade were also triggered in mast cells upon LPS stimulation. The phosphorylation of IκB-α at Ser32, essential for release of active NF-κB, is a marker of NF-κB activation. As shown in Fig. 3, LPS (50 ng/ml) strongly up-regulated the phosphorylated IκB-α in C3H/HeN BMMCs but not in C3H/HeJ BMMCs. The activity was at maximum 15 min after stimulation and then gradually decreased within 60 min (Fig. 3A). However, the cell lysates of both BMMCs did not exhibit specific induction of SAPK/JNK activity at any indicated time, although positive control provided by the manufacturer showed significant phosphorylation of SAPK/JNK (Fig. 3B). Although we confirmed that an equal amount of protein was loaded in each lane, total amount

FIGURE 1. TLR mRNA expressions in BMMCs. Gene expressions of TLR2 (385 bp), TLR4 (540 bp), TLR5 (540 bp), TLR6 (696 bp), and TLR8 (250 bp) in C3H/HeN and C3H/HeJ BMMCs were analyzed by PCR following RT. Bone marrow-derived mouse dendritic cells (DC) were used as positive controls. Equality of the RT reaction of isolated RNA was confirmed by amplification of the housekeeping gene GAPDH. The result shown is representative of three independent experiments that had similar results.

FIGURE 2. Cytokine production and β-hexosaminidase release from BMMCs upon LPS stimulation. A, BMMCs from C3H/HeN (■) or C3H/HeJ (□) were stimulated with the indicated concentration of LPS, 3 h for TNF-α, and 6 h for IL-1β, IL-6, and IL-13. The levels of cytokines in the supernatants were estimated by ELISA kits. Data shown are mean ± SD of three independent experiments. B, Percent release of β-hexosaminidase from BMMCs of C3H/HeN (■) or C3H/HeJ (□) was evaluated as described in Materials and Methods upon stimulation with the indicated concentration of LPS for 1 h. IgE and anti-IgE- or PMA-ionomycin-stimulated BMMCs were used as positive controls. The result shown is representative of three independent experiments that had similar results.
The W/W C3H/HeJ strain showed a significant increase for phospho-IκB in the lysis buffer. Proteins were resolved by SDS-PAGE, electroblotted, and immunoblotted with Abs specific for phospho-IκB-α (A) and phospho-SAPK/JNK (B). The same blots were stripped and reblotted with Abs for the nonphosphorylated form of each respective signaling protein. LPS-stimulated Raw 264.7 was used as positive controls. Positive and negative controls provided by the kits were also used as control. The result shown is a representative of three independent experiments that had similar results.

of IκB-α from C3H/HeJ BMMCs was always lower than that from C3H/HeN BMMCs.

**Lack of functional TLR4 on mast cells leads to increased mortality in a model of acute septic peritonitis**

The role of TLR4 in mast cell-dependent innate immunity was examined by studying genetically mast cell-deficient W/W mice to CLP, a mouse model of acute septic peritonitis. Deficiency of mast cells in W/W mice was reconstituted with BMMCs either from TLR4-intact C3H/HeN or TLR4-mutated C3H/HeJ mice. The CLP peritonitis was induced 5 wk after i.p. transfer of BMMCs. As shown in Fig. 4, in unreconstituted W/W mice, all of the animals died within 3 days. In contrast, the lethal effect of acute bacterial peritonitis was greatly diminished by reconstitution of W/W mice with BMMCs from the C3H/HeN strain (p < 0.05 from days 3 to 10). Interestingly, among the mast cell-reconstituted groups, the W/W mice reconstituted with BMMCs from the C3H/HeJ strain showed a significantly higher mortality rate than the W/W mice reconstituted with BMMCs of the C3H/HeN strain, 80% in the C3H/HeJ BMMC-reconstituted group vs 20% in the C3H/HeN BMMC-reconstituted group at day 7 (p < 0.05). Even though it appeared that W/W mice reconstituted with BMMCs from C3H/HeJ mice had a better survival rate after CLP than that of W/W mice without reconstitution of BMMCs, there were no statistically significant differences in the mortality rate between these two groups of mice during the experimental periods (from days 3 to 10). During the study period (2 mo), the recipient mice were all healthy and showed no sign of graft rejection. The cytospin preparations of peritoneal exudates from both groups 5 wk after the reconstitution of BMMCs had a similar number of peritoneal mast cells (1.23 ± 0.14 × 10^5 cell/mouse in W/W-C3H/HeN vs 1.22 ± 0.18 × 10^5 cell/mouse in W/W-C3H/HeJ). These numbers of mast cells were slightly less but not significantly different from those of W/W that received BMMCs from control WBB6F1/+ mice (1.48 ± 0.12 × 10^5 cells/mouse). The staining properties of peritoneal mast cells derived from W/W that received BMMCs from C3H/HeN, C3H/HeJ, and +/+ were similar; all cells exhibited positive staining with both Alcian blue and safranin, a prominent feature of connective tissue-type mast cells. Also, we confirmed that there were no functional differences in the peritoneal mast cells derived from W/W that received C3H/HeN BMMCs or C3H/HeJ BMMCs by measuring β-hexosaminidase release upon FceRI cross-linking (W/W-C3H/HeN vs W/W-C3H/HeJ, 31 vs 33%) or calcium ionophore stimulation (W/W-C3H/HeN vs W/W-C3H/HeJ, 42 vs 34%). Thus, the difference in the mortality rate between W/W-C3H/HeN and W/W-C3H/HeJ was not due to the differences in development of transferred cells in the W/W environment.

**Defective neutrophil recruitment and inflammatory cytokine production in W/W mice reconstituted with TLR 4-mutated BMMCs**

Next, we determined whether defective neutrophil recruitment in the W/W mice contributes to impaired resistance of animals to microbial agents as previously reported (5, 6). The peritoneal exudates were examined for leukocyte infiltration after CLP induction. The majority of infiltrating cells in the peritoneal cavity were neutrophils, 3 and 6 h after CLP induction (Fig. 5A). Interestingly, W/W mice that did not receive BMMCs or that received C3H/HeJ BMMCs showed significantly less neutrophil influx than W/W mice reconstituted with C3H/HeN BMMCs (p < 0.01). Although, W/W mice that received C3H/HeJ BMMCs showed significantly more leukocytes (neutrophils) influx than unreconstituted W/W, 6 h after CLP (p < 0.01), the value was still significantly lower than that of W/W mice reconstituted with C3H/HeN BMMCs (p < 0.01). Also, the levels of cytokines (TNF-α, IL-1β, IL-6, and IL-13) in peritoneal fluids, especially 6 h after CLP, were significantly higher in W/W mice reconstituted with C3H/HeN BMMCs than in W/W mice with C3H/HeJ BMMCs or those unreconstituted (p < 0.01, Fig. 5B). These results again suggest that functional TLR4 on mast cells is required for in vivo production of proinflammatory cytokines and for recruitment of neutrophils into the peritoneal cavity after CLP.

**Discussion**

The present study was based on the observation that mast cells and TLRs are both involved in innate immunity. The effective responses of mast cells against infection by means of TLR activation were not previously known. This study first characterized the TLR expression pattern of mast cells and showed that the existence of functional TLR4 was essential for mast cell-mediated innate immune responses.

We analyzed the expression of five TLRs in mouse mast cells. Transcripts of TLR2, 4, 6, and 8 were present in mast cells but no transcript of TLR5 was detectable. Although several reports have suggested that the expressions of TLR4 and TLR2 on macrophages...
and T cells could be regulated after interaction with bacterial products such as LPS, lipoparabinomannan, or proinflammatory cytokines (18, 30), we could not detect any modulation of the expression of TLR2, 4, 5, 6, and 8 on BMMCs by LPS, lipoteichoic acid (Gram-positive bacterial product), or FcεRI cross-linking (data not shown). Since it has been reported that different immunocompetent cells express specific transcripts of TLRs (18), it would be interesting to see whether mast cells in different tissues (e.g., mucosal vs connective tissue) have different expression patterns of TLRs.

One of the biological responses of mast cells to stimuli is release of preformed mediators in their granules and production of cytokines that are not necessarily stored within the cells as preformed substances (31, 32). We could detect the production of TNF-α, IL-1β, IL-6, and IL-13 from TLR4-intact BMMCs but not from TLR4-mutated BMMCs through stimulation with LPS. We also observed similar impairment of proinflammatory cytokine productions in peritoneal fluids of W/Wv mice reconstituted with C3H/HeJ BMMCs after CLP. Although the levels of cytokines were much less than those in W/Wv reconstituted with C3H/HeN BMMCs, a slight but significant increase of some cytokines (IL-1β at 3 and 6 h, IL-6 at 6 h) was observed in peritoneal fluids of W/Wv reconstituted with C3H/HeJ BMMCs. This suggested that not all cytokine production in the peritoneal cavity after CLP was dependent on TNF-α and IL-1β are the potent monocyte/macrophage activator (33), and TNF-α production from mast cells is thought to be critical for some of the acute inflammatory events, including the local influx of neutrophils (34). IL-6 also plays a role in local inflammatory reactions by amplifying leukocyte (monocyte, PMN, lymphocyte) recruitments (35). In contrast, since IL-13 has been reported to show anti-inflammatory properties by modulating the production of macrophage/monocyte-derived TNF-α, IL-1- and IL-8, it might have some roles for sweeping exaggerated inflammatory responses (36, 37). Compared with other cytokines (TNF-α, IL-1β, IL-6), which were produced more in vivo than in vitro after CLP (Fig. 5B), production of IL-13 seemed to be much less in vivo than in vitro. Although we do not know the precise reason for this, it may be due to the different ability to produce IL-13 upon stimulation with microorganisms between BMMCs and peritoneal mast cells or due to different stimulants. LPS for BMMCs vs whole microorganisms for peritoneal mast cells.

Our data demonstrated that mast cells responded to LPS for these cytokine productions in a dose-dependent manner, but failed to degranulate in response to LPS at concentrations that induced cytokine production. This result was consistent with a previous study using rat peritoneal mast cells in which LPS could induce substantial IL-6 production without releasing a significant amount of histamine (38). It is therefore suggested that mast cells are capable of releasing cytokines into the extracellular environment, independent of the classical degranulation pathway, in response to infection.

LPS elicits several immediate proinflammatory responses in peripheral blood leukocytes through described pathways involving serine-threonine kinases and NF-κB transcription factor (28). However, the functional responses of mouse mast cells to stimulation with LPS via TLR4 were unknown. Our results demonstrated that LPS-stimulated TLR4 on mast cells led to phosphorylation of IκB-α but not of SAPK/JNK. These results were in agreement with previous studies that LPS stimulation of some peripheral blood leukocytes or other cell lines did not always result in the activation of p42/p44 mitogen-activated protein kinase, JNK or P38 (28, 39). LPS-induced signal transduction in a different manner might be due to the distinct cell origin, the state of differentiation, or idiosyncratic alterations of the signal transduction pathway. It is possible that, in mast cells, TLR4 signal for NF-κB activation is not via the mitogen-activated protein kinase cascade and that the signaling cascade of mast cells upon LPS...
C3 reduced the activation of mast cells by means of TNF-roles in these responses, since it has been reported that the lack of (e.g., TLR6 and 8) whose functions have not yet been well char-
yzing that TLRs other than TLR4 expressed on mast cells lower than that of unreconstituted W/W.

Our results again support the proposed roles of mast cells in the inflam-

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