The molecular mechanisms by which pathogen-associated molecular patterns recognized by TLRs, such as peptidoglycan (PGN), induce homotolerance are largely unknown. It was recently reported that IRAK-M negatively regulates TLR signaling. In this study, we elucidate the molecular mechanisms of tolerance induced by PGN, with a focus on the role of IRAK-M. We demonstrate that pretreatment of macrophage RAW264.7 cells with a high concentration (30 μg/ml) of PGN for 16 h effectively induces tolerance against following stimulation with 30 μg/ml of PGN; while pretreatment with a low concentration (1 μg/ml) of PGN does not. IRAK-M is induced in cells treated with the high concentration of PGN 4–24 h after PGN stimulation, but not in cells treated with the low concentration of PGN up to 24 h after stimulation. Phosphorylation of MAPKs and IkBa is inhibited after the second PGN stimulation in tolerant cells. Kinase activity of IRAK-1 and association between IRAK-1 and MyD88 are also suppressed in PGN-induced tolerant cells. Furthermore, down-regulation of IRAK-M expression by small interfering RNAs specific for IRAK-M reinstates the production of TNF-α after PGN restimulation. These results suggest that induction of IRAK-M and inhibition of kinase activity of IRAK-1 are crucial to PGN-induced tolerance in macrophages.

Essential components of the innate immune system are TLRs, which recognize microbial products termed pathogen-associated molecular patterns (PAMPs). PAMP recognition leads to activation of the innate immune system, which in turn activates adaptive immunity. The cytoplasmic portion of TLRs is very similar to that of the IL-1 receptor family and is currently referred to as the Toll/IL-1 receptor (TIR) domain. The signaling pathway via the TLR family is highly homologous to that via the IL-1 receptor family. TLR interacts with adaptor protein MyD88, which recruits IRAK (2, 3). IRAK becomes activated and associates with TRAF6, leading to the activation of several distinct signaling pathways, including MAPKs and NF-κB (4).

Many reports have been published on the reduced capacity of circulating leukocytes from septic patients to produce cytokines as compared with those from healthy controls, a phenomenon referred to as “endotoxin tolerance.” It is an adaptive host response that may represent an essential regulatory mechanism during Gram-negative bacterial infection, but it may also promote subsequent infection in survivors of septic shock (5–11). Monocytes from septic patients have a reduced capacity to release TNF-α, IL-1β, IL-6, IL-10, and IL-12 (12–14). A defect in the activation of transcription factor NF-κB has also been reported in endotoxin tolerance (15). An understanding of the molecular mechanisms of the septic shock syndrome is critical, yet despite numerous studies little is known about these mechanisms.

One of the main characteristics of endotoxin tolerance in vitro is a change in the pattern of inflammatory gene expression in cells of myeloid lineage (16). Whereas inhibition of cell surface expression of the TLR4/MD-2 complex has been suggested to underlie LPS tolerance in mouse macrophages (17), LPS induces tolerance in CHO cells without affecting cell surface expression of transfected TLR4 and MD-2 (18). In addition, LPS-tolerant cells exhibit significantly suppressed LPS-induced activation and degradation of IRAK and diminished IRAK-MyD88 association (19). These data imply that tolerance induction may affect the expression and/or functions of intracellular intermediates downstream of TLRs (20).

Induction of an endotoxin-tolerant phenotype is not specific to the initiation action of LPS, because engagement of TLR/IL-1 receptor (IL-1R) family members other than TLR4, also results in macrophage resistance to subsequent challenge with respective ligands. However, the molecular mechanisms that induce homotolerance by PAMPs recognized by TLR2, such as PGN and LTA, are largely unknown. THP-1 cells with prolonged LTA treatment develop LTA homotolerance. Stimulation of TLR2 by LTA, although activating IRAK, does not cause IRAK degradation, indicating that molecular mechanism underlying LTA-induced tolerance is clearly distinct from that of LPS-induced tolerance (21). Furthermore, it is speculated that disruption of unique TLR2 signaling components may occur upstream of MyD88/IRAK (21).

IRAK was originally described as a signal transducer for the proinflammatory cytokine, IL-1, and was later implicated in
signal transduction of other members of the TLR/IL-1R family. To date, four different IRAK-like molecules have been identified: two active kinases, IRAK-1 and IRAK-4, and two inactive kinases, IRAK-2 and IRAK-M. All IRAKs mediate activation of NF-κB and MAPK pathways (22). Furthermore, all IRAKs have been shown to act downstream of MyD88 and be capable of binding to TRAF6 (23–26). Among four IRAKs, IRAK-M is highly expressed in monocytes/macrophages, and initial reports indicated that IRAK-M activates NF-κB when overexpressed in 293T cells and partially restores IL-1 signaling in IRAK−/− cells (23, 27). However, recent reports state that innate immunity was strongly enhanced in IRAK-M−/− mice, and IRAK-M−/− cells have strikingly impaired endotoxin tolerance, showing that IRAK-M negatively regulates TLR signaling (28). Despite potential importance of IRAK-M in signaling via TLRs, however, the relationship between IRAK-M and TLR-mediated tolerance has been fully obscure. In this study we elucidate the molecular mechanisms of homotolerance induced by PGN, with a focus on the role of IRAK-M.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Anti-extracellular-regulated kinase 1 and 2 (anti-ERK), anti-phospho-ERK1/2, anti-IRAK-1, and anti-MyD88 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphospecific antibodies for p38, JNK, and IκBα were purchased from Cell Signaling Technology (Beverly, MA). Anti-murine TLR2 antibody was purchased from eBioscience (San Diego, CA). IRAK-M antibody was purchased from Chemicon International (Temecula, CA) (28). Antibody specific for β-actin was obtained from Abcam. Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG and HRP-conjugated anti-mouse IgG were obtained from Dako (Denmark). Peptidoglycan from Staphylococcus aureus was purchased from Fluka, and the endotoxin level in the peptidoglycan was less than 0.5 pg/ml in culture medium as determined by a Limulus amebocyte lysate (LAL) assay.

Cell Culture—RAW264.7, a murine macrophage-like cell line, was obtained from the ATCC, and was maintained in Eagle's modified minimal essential medium (EMEM) supplemented with 2 mM glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin (ICN, Aurora, OH), and 10% fetal bovine serum (Sigma). RAW264.7 cells were pretreated with 1 or 30 μg/ml of peptidoglycan (PGN) at 37 °C. PGN stimulation was stopped by addition of ice-cold phosphate-buffered saline. The cells were then lysed for immunoprecipitation and immunoblotting.

Immunoprecipitation and Immunoblotting—Immunoprecipitation and immunoblotting were performed as previously reported (29). Briefly, cells were lysed in ice-cold Nonidet P-40 lysis buffer containing 1% Nonidet P-40, 25 mM Tris-HCl (pH 7.5), 150 mM sodium chloride, 1 mM EDTA, 5 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM leupeptin, and 1 mM phenylmethylsulfonyl fluoride, and cell membrane fractions were prepared as reported previously (30). For immunoprecipitation studies, cell lysates were mixed with the indicated antibodies for 1 h. Cell lysates were then mixed with protein G and coupled Sepharose beads and rotated for 1 h at 4 °C. After the beads were washed 3 times with ice-cold Nonidet P-40 lysis buffer, the precipitated proteins were boiled for 5 min and eluted with sodium dodecyl sulfate, polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. For the precipitation of total-cell lysates, cells were lysed directly by addition of SDS-PAGE sample buffer containing 2-mercaptoethanol. Immunoprecipitated proteins and total cell lysates were separated by SDS-PAGE under reducing conditions and were electrophoresed to a polyvinylidene difluoride membrane. The membrane was blocked for 1 h at room temperature with 1% bovine serum albumin in TBS (Tris-buffered saline) buffer. The membrane was then incubated with the indicated antibody, and the reactive bands were visualized with a horseradish peroxidase-coupled secondary antibody via an enhanced chemiluminescence (ECL) detection system (Amersham Biosciences) according to the manufacturer's instructions.

Analysis of TNF-α Production—The production of TNF-α was analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Cells were seeded at a density of 10^5/ml in 24-well plates, and the supernatants collected 24 h after LPS stimulation. The concentration of TNF-α was measured by ELISA according to the manufacturer’s instructions (BIOSOURCE International, Camarillo, CA).

In Vitro IRAK-1 Kinase Assay—The IRAK-1 kinase assay was conducted as described previously (19, 20). Briefly, the immunoprecipitated IRAK-1 complexes were washed four times with lysis buffer and twice with kinase buffer (20 mM HEPES at pH 7.6, 20 mM MgCl2, 20 mM glyceraldehyde, 20 mM para-nitrophenylphosphate, 1 mM EDTA, 1 mM sodium orthovanadate, and 1 mM benzamidine). Fifty microliters of kinase buffer were then added to each sample, supplemented with 5 μM ATP, 1 μg of myelin basic protein (MBP, Sigma), and 1 μl of [γ-32P]ATP, and incubated at 37 °C for 30 min. Ten microliters of SDS sample buffer was added, and the samples incubated at 50 °C for 10 min and subjected to SDS-PAGE analysis. The gel was dried and exposed to x-ray film.

RESULTS

Tolerance Induced by Pretreatment with High Concentrations of PGN—Fig. 1A shows the dose response curve of TNF-α production by PGN. Levels of TNF-α in the supernatants increased in a dose-dependent manner: a small amount of TNF-α production was observed in cells stimulated with less than 3 μg/ml PGN, while maximal TNF-α production was obtained by stimulation with more than 30 μg/ml PGN. To determine the threshold of tolerance induction by PGN, RAW264.7 cells were pretreated with graded concentrations of PGN for 16 h and then restimulated with 30 μg/ml PGN for additional 16 h. TNF-α production was augmented in cells pretreated with less than 3 μg/ml PGN. In contrast, prior exposure to more than 30 μg/ml PGN markedly suppressed the ability of cells to produce TNF-α in response to PGN (Fig. 1B). These data showed the existence of threshold of PGN concentration in the tolerance induction; pretreatment of cells with higher concentrations of PGN (≥30 μg/ml) for 16 h effectively induced PGN tolerance, while pretreatment with low concentrations of PGN (≤3 μg/ml) did not. In the subsequent experiments, we chose 1 μg/ml and 30 μg/ml of PGN as the low and high concentration of stimulation, respectively.

Phosphorylation of MAPKs and IκBα Was Inhibited in PGN-induced Tolerant Cells—In the next series of experiments, we compared the time kinetics of phosphorylation of MAPKs (ERK, p38, and JNK) and IκBα between cells treated with 1 and 30 μg/ml PGN. As shown in Fig. 2A, phosphorylation of these molecules was observed in cells treated with 30 μg/ml PGN 15–30 min after addition. PGN-induced phosphorylation of the MAPKs and IκBα was also observed in cells treated with 1 μg/ml PGN, although the intensity of the phosphorylation was much weaker in these cells. Fig. 2B shows time kinetics of PGN-induced phosphorylation of MAPKs and IκBα in cells pretreated for 16 h with 1 μg/ml PGN (PGN-non-tolerant cells) and 30 μg/ml PGN (PGN-tolerant cells). Phosphorylation of MAPKs and IκBα was observed in non-tolerant cells after the second stimulation with 30 μg/ml PGN, but hardly detected in tolerant cells. These results demonstrate that activation of intracellular signaling molecules such as MAPKs and IκBα is exclusively inhibited after the second PGN stimulation in tolerant cells.

Surface Expression Levels of TLR2 Were Not Affected by PGN Stimulation—We then compared the expression levels of TLR2 between cells treated with a low and high concentrations of PGN. The expression levels of TLR2 in the membrane fraction in cells stimulated with a high concentration of PGN were similar to those in cells stimulated with a low concentration up.
to 16 h after PGN stimulation (Fig. 3). These results indicated that levels of TLR2 expression in the tolerant cells were not different from those in the non-tolerant cells.

**IRAK-1 Kinase Activity Was Inhibited in PGN-induced Tolerant Cells**—By using the kinase assay, we examined IRAK-1 activation after PGN stimulation. As shown in Fig. 4A, in resting cells stimulation with both 1 and 30 μg/ml PGN increased IRAK-1 kinase activity, which was markedly up-regulated at 15 min and decreased at 60 min after stimulation. However, kinase activity of IRAK-1 was much stronger in cells treated with 30 μg/ml PGN than that in cells treated with 1 μg/ml PGN. We also examined whether IRAK-1 is phosphorylated by stimulation with each concentration of PGN. However, IRAK-1 phosphorylation was not observed even in cells treated with 30 μg/ml PGN (date not shown). Fig. 4B exhibited the induction of kinase activity of IRAK-1 in PGN-induced non-tolerant and tolerant cells. We observed that kinase activity of IRAK-1 was augmented in non-tolerant cells 15 min after second PGN stimulation, but suppressed in PGN tolerant cells (Fig. 4B). Phosphorylation of IRAK-1 was also examined, however, it was not observed for up to 60 min in both cells (date not shown). These results demonstrate that activation of IRAK-1 is exclusively inhibited after the second PGN stimulation in tolerant cells.

**Induction of IRAK-M and Inhibition of the Association between MyD88 and IRAK-1 in PGN Tolerant Cells**—Previous studies have reported that IRAK-M negatively regulates TLR signaling (28); however, the role of IRAK-M in TLR-mediated tolerance remains fully obscure. Therefore, we compared the time kinetics of expression of IRAK-M in cells treated with 1 and 30 μg/ml PGN. As shown in Fig. 5A, the bands corresponding to IRAK-M were not observed in unstimulated cells, indicating little constitutive expression of IRAK-M in resting cells. IRAK-M expression was also not observed in cells stimulated with a low concentration of PGN up to 24 h after stimulation. However, weak but significant expression of IRAK-M was ob-


Fig. 4. IRAK-1 kinase activity is inhibited in PGN tolerant cells. A, cells stimulated with PGN for the indicated times. Cell lysates were immunoprecipitated with an antibody specific for IRAK-1. The immunoprecipitated IRAK-1 complexes in kinase buffer were added to each sample, supplemented with 5 μM ATP, 1 μg myelin basic protein, and 1 μl of [γ-32P]ATP, and incubated at 37 °C for 30 min. The samples were eluted in SDS-PAGE sample buffer and subjected to SDS-PAGE analysis. The gel was dried and exposed to x-ray film. B, cells pretreated with 1 or 30 μg/ml of PGN for 16 h were stimulated with 30 μg/ml of PGN for the indicated times, and the cell lysates were immunoprecipitated with an antibody specific for IRAK-1. Kinase activity of IRAK-1 was evaluated as described above.

Fig. 5. IRAK-M is induced by stimulation with a high concentration of PGN and the association between MyD88 and IRAK-1 is inhibited in PGN tolerant cells. A, RAW264.7 cells were stimulated with PGN for the indicated times. Total cell lysates were blotted with an antibody specific for IRAK-M or IRAK-1 and probed with anti-β-actin. B,pretreated with 1 or 30 μg/ml of PGN for 16 h were stimulated with 30 μg/ml of PGN for the indicated times. Total cell lysates were immunoprecipitated with an antibody specific for MyD88 and probed with anti-IRAK-1 (upper lane) or MyD88 (lower lane).

DISCUSSION

Multiple microbial stimulants can induce a wide array of gene expressions through the TLRs. Activation of TLRs subsequently lead to common downstream signaling events, including MyD88/IRAK kinase, MAPKs and NF-κB activation, which contribute to the production of TNF-α. With regard to the common signaling pathway that leads to TNF-α production, prolonged treatment with microbial stimulants has been shown to induce a state of tolerance characterized by decreased TNF-α production. Among PAMP-induced tolerance, LPS-mediated homotypic and heterotypic stimulation has been most extensively studied. Although molecular mechanisms of TLR4-mediated tolerance have still remained to be fully clarified, numerous studies have revealed the involvement of down-regulation of multiple TLR signaling components, including down-regulation of TLR4 receptor expression, disruption and degradation of IRAK, and reduced activation of
MAPKs, in LPS-mediated homotolerance. On the other hand, little information was available for TLR2-mediated homotolerance. Previous reports state that prolonged LTA treatment of murine monocytes/macrophages and human THP-1 cells can develop similar hyporesponsiveness in TNF-α/H9251 production to subsequent LTA stimulation (21, 31). In this study, we elucidated the molecular mechanisms of PGN homotolerance. We found that pretreatment with low concentrations of PGN (≤3 μg/ml) for 16 h did not induce PGN homotolerance against following addition of 30 μg/ml PGN as assessed by TNF-α/H9251 production (Fig. 1B). In fact, TNF-α production was rather promoted in these cases. On the other hand, pretreatment with high concentrations of PGN (≥10 μg/ml) for 16 h did clearly induce PGN homotolerance (Fig. 1B). Furthermore, cells treated with high concentrations of PGN (≥10 μg/ml) for 4 h also became tolerant against subsequent PGN stimulation (date not shown). When phosphorylation of intracellular signaling molecules was compared between non-tolerant and tolerant cells, phosphorylation of MAPKs and IκBα was exclusively suppressed in PGN-induced tolerant cells (Fig. 2B). These results indicate that the intensity of phosphorylation of MAPKs and IκBα is well correlated with TNF-α production, implying that phosphorylation of these molecules is also a good indicator for estimating the tolerant state.

LTA-tolerant THP-1 cells are not totally unresponsive to subsequent LTA challenge. Instead, LTA-tolerant cells are capable of responding to further LTA treatment, indicating that the TLR2 receptor is still functional in LTA-tolerant cells (21). It is speculated that the decreased expression of inflammatory proteins such as TNF-α is likely due to disruption of intracellular signaling components downstream of the TLR2 receptor (21). We show that the expression level of TLR2 protein localized in the membrane fraction in tolerant cells is similar to that in non-tolerant cells (Fig. 3), indicating that the expression levels of receptors do not account for the tolerant state.

A previous report showed that IRAK kinase activation and TNF-α protein production were inhibited in LTA-tolerant cells (31). LPS treatment induces rapid degradation as well as inactivation of IRAK (19, 36). Reduced IRAK-1 kinase activity in LPS-tolerant human and murine macrophages has been described previously (17, 19). However, the IRAK protein level does not decrease following prolonged LTA treatment (21). Our presented results demonstrated that stimulation with a high concentration of PGN induced strong IRAK-1 kinase activation in resting cells (Fig. 4A). However, the same stimulation causes the suppression of kinase activity of IRAK-1 in PGN-induced tolerant cells, while it was retained in non-tolerant cells (Fig. 4B). We examined protein levels of IRAK-1 after PGN stimulation, and found that no significant differences occur in cells treated with either a low or high concentration of PGN (Fig. 5A), suggesting that IRAK-1 is activated without degradation, results that agree with previous reports on LTA-induced tolerance (21).

IRAK-M is preferentially expressed in monocytes/macrophages (23), and recent studies have reported that IRAK-M...
plays a critical negative regulatory role in signaling via TLRs in these cells. IRAK-M/IRAK-1 macrophages stimulated with known TLR agonists such as LPS or CpG DNA displayed increased NF-kB and MAPK activation. IRAK-M/IRAK-1 mice showed increased inflammatory responses to bacterial infection (28). In the present study, we for the first time examined IRAK-M in PGN tolerance. We showed that IRAK-M protein is not detected in cells treated with a low concentration of PGN before and up to 24 h after stimulation (Fig. 5a), suggesting that IRAK-M expression is very low. However, IRAK-M was induced in cells treated with a high concentration of PGN 4–24 h after stimulation, implying that induction of IRAK-M is correlated with PGN-induced tolerance. Furthermore, down-regulation of IRAK-M expression by siRNAs specific for IRAK-M reinstated the production of TNF-α (Fig. 6). These results suggest that induction of IRAK-M is crucial to PGN-induced tolerance in macrophages.

MyD88 is essential for TNF-α secretion in response to TLR agonists (37), and association of MyD88 with TLR has been acknowledged as an essential step in signaling via various TLRs. Although the role of MyD88 is fully unknown in TLR-2 agonists (37), and association of MyD88 with TLR has been demonstrated by either preventing phosphorylation of IRAK and IRAK-4 or stabilizing the TLR/MyD88/IRAK complex (28). Nevertheless, our novel finding that IRAK-M is specifically induced in PGN-mediated tolerant state may be important for understanding of the molecular mechanisms of tolerance. Prevention of IRAK-1 activation is an important molecular mechanism of PGN-induced tolerance. An understanding of the molecular mechanisms of tolerance may aid development of improved treatments for inflammatory responses that lead to sepsis.

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REFERENCES
1. Medzhitov, R., and Janeway, C. A., Jr. (1997) Cell 91, 295–298
2. Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., Takeda, K., and Akira, S. (1999) Immunity 11, 443–451
3. Hashimoto, C., Hukin, K. L., and Anderson, K. V. (1998) Cell 92, 269–279
4. Lemaître, B., Nicolas, E., Michaud, L., Reichhart, J. M., and Hoffmann, J. A. (1996) Cell 86, 573–583
5. Zeiherberger, E., and Roth, J. (1998) Ann. N. Y. Acad. Sci. 856, 116–131
6. Ziegler-Heitbrock, H. W. (1995) J. Inflamm. 45, 13–26
7. Gustafson, G., Rhodes, M. J., and Hegel, T. (1995) Prog. Clin. Biol. Res. 392, 567–579
8. Henricson, B. E., Benjamin, W. R., and Vogel, S. N. (1990) Infect. Immun. 58, 2429–2437
9. Salkowski, C. A., Detore, G., Franks, A., Falk, M. C., and Vogel, S. N. (1998) Cell 96, 3569–3578
10. Bone, R. C. (1993) Clin. Microbiol. Rev. 6, 57–68
11. Ertel, W., Kremmer, J. P., Kenney, J., Steckholzer, U., Jarrar, D., Trentz, O., and Schildberg, F. W. (1995) Blood 85, 1341–1347
12. Munoz, C., Carlet, J., Fitting, C., Misset, B., Blériot, J. P., and Cavaillon, J. M. (1991) J. Clin. Invest. 88, 1747–1754
13. van Deuren, M., van der Ven-Jongekrijg, J., Emacker, P. N., Bartelink, A. K., van Dalen, R., Sauerwein, R. W., Gallati, H., Vannice, J. L., and van der Meer, J. W. (1994) J. Infect. Dis. 169, 157–161
14. Ertel, W., Keel, M., Neudarth, R., Steckholzer, U., Kremmer, J. P., Ungethüm, U., and Trentz, 0. (1997) Blood 89, 1612–1620
15. Adda-Conquy, M., Adrie, C., Moine, P., Asehnoune, K., Fitting, C., Pinsky, M. R., Dhainaut, J. F., and Cavaillon, J. M. (2000) Am. J. Respir. Crit. Care Med. 162, 1877–1883
16. Henricson, B. E., Neta, R., and Vogel, S. N. (1991) Infect. Immun. 59, 1188–1191
17. Nomura, F., Akashi, S., Sakao, Y., Sato, S., Kawai, T., Matsumoto, M., Nakashiki, K., Kimoto, M., Miyake, K., Takeda, K., and Akira, S. (2000) J. Immunol. 164, 3476–3479
18. Medvedev, A. E., Henzecke, P., Schromm, A., Lien, E., Ingalls, R., Fenton, M. J., Golenbock, D. T., and Vogel, S. N. (2001) J. Immunol. 167, 2257–2267
19. Li, L., Couxart, S., Hu, J., and McCaile, C. E. (2000) J. Biol. Chem. 275, 23340–23345
20. Medvedev, A. E., Lentschach, A., Wahl, L. M., Golenbock, D. T., and Vogel, S. N. (2002) J. Immunol. 169, 5209–5216
21. Jacinto, R., Hartung, T., McCaile, C., and Li, L. (2002) J. Immunol. 168, 6136–6141
22. Jannssen, S., and Beyaert, R. (2003) Mol. Cell 11, 293–302
23. Wescbe, H., Gao, X., Li, X., Karching, C. J., Stank, G. K., and Cao, Z. (1999) J. Biol. Chem. 274, 19403–19410
24. Li, S., Strelow, A., Fontana, E. J., and Wescbe, H. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5567–5572
25. Medvedev, A., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh, S., and Janeway, C. A., Jr. (1998) Mol. Cell 2, 253–268
26. Wescbe, H., Henzel, W. J., Shillinglaw, W., Li, S., and Cao, Z. (1997) Immunity 7, 837–847
27. Muzio, M., Ni, J., Feng, P., and Dixit, V. M. (1997) Science 276, 1612–1615
28. Kobayashi, K., Hernandez, L. D., Galan, J. E., Janeway, C. A., Jr., Medzhitov, R., and Flavell, R. A. (2002) Cell 110, 191–202
29. Okugawa, S., Ota, Y., Kitazawa, T., Nakayama, K., Yanagimoto, S., Tsukada, K., Kawada, M., and Kimura, S. (2003) Am. J. Physiol. Cell Physiol. 285, C399–408
30. Bhat, N., Perera, P. Y., Carboni, J. M., Blanco, J., Golenbock, D. T., Mayadas, T. N., and Vogel, S. N. (1999) J. Immunol. 162, 7335–7342
31. Lehner, M. D., Morath, S., Michelen, K. S., Schumann, R. R., and Hartung, T. (2001) J. Immunol. 166, 5161–5167
32. McCall, C. E., Grosse-Wilmot, L. M., LaRue, K., Guzman, R. N., and Coisart, S. L. (1993) J. Clin. Invest. 91, 855–861
33. Kraatz, J., Clair, L., Rodriguez, J. L., and West, M. A. (1999) Shock 11, 58–63
34. Medvedev, A. E., Kopylowskii, K. M., and Vogel, S. N. (2000) J. Immunol. 164, 5564–5574
35. Sato, S., Nomura, F., Kawai, T., Takeuchi, O., Muhradt, P. F., Takeda, K., and Akira, S. (2000) J. Immunol. 165, 7086–7101
36. Maeda, S., Akamuna, M., Mitsu, Y., Hirota, Y., Oogura, K., Yoshida, H., Shiratori, Y., and Omata, M. (2001) J. Biol. Chem. 276, 44856–44864
37. Kawai, T., Adachi, O., Ogawa, T., Takeda, K., and Akira, S. (1999) Immunity 11, 115–122
Involvement of IRAK-M in Peptidoglycan-induced Tolerance in Macrophages
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