Murine TOLL-like Receptor 4 Confers Lipopolysaccharide Responsiveness as Determined by Activation of NFκB and Expression of the Inducible Cyclooxygenase*

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Genetic evidence indicating that TOLL-like receptor 4 (Tlr4) is the lipopolysaccharide (LPS) receptor in mice was reported. However, biochemical evidence that murine Tlr4 confers LPS responsiveness has not been convincingly demonstrated. Inducible cyclooxygenase (COX-2) is selectively expressed in LPS-stimulated macrophages in part mediated through the activation of NFκB. Thus, we determined whether murine Tlr4 confers LPS responsiveness as evaluated by the activation of NFκB and COX-2 expression. Transfection of a murine macrophage-like cell line (RAW264.7) with the constitutively active form (ΔTlr4) of Tlr4 is sufficient to activate NFκB and COX-2 expression. However, the truncated form (ΔTlr4(P712H)) of the missense mutant Tlr4(P712H) found in LPS-hyposensitive mouse strain (C3H/HeJ) inhibits LPS-induced NFκB and COX-2 expression. The inability of ATlr4(P712H) to activate NFκB and induce COX-2 expression is rescued by a constitutively active adapter protein myeloid differentiation factor 88 (MyD88), which interacts directly with the cytoplasmic domain of Tlr proteins. Furthermore, MyD88 is co-immunoprecipitated with the wild-type ΔTlr4 but not with the ΔTlr4(P712H) mutant. Together, these results indicate that Tlr4 confers LPS responsiveness to RAW264.7 cells and suggest that hyposensitivity of C3H/HeJ mice to LPS is attributed to the disruption of Tlr4-mediated signaling pathways that results from the inability of the mutant Tlr4(P712H) to interact with MyD88.

The pathogenesis of Gram-negative septic shock is presumed to be due to immune system characteristics of host cells by bacterial lipopolysaccharide (LPS) endotoxin (1–3). Such stimulation leads to the expression and release of a plethora of pro-inflammatory marker gene products and lipid mediators, which in turn can initiate a chain of events leading to systemic toxicity (4, 5). However, the initial recognition of LPS by cells of the

innate immune system is required to defend the host from a Gram-negative infection before it becomes widely disseminated (6, 7).

Identifying the downstream signaling pathways derived from LPS stimulation is of fundamental importance to understanding the cellular mechanism of Gram-negative septic shock. CD14, a glycosylphosphatidylinositol-linked membrane protein, is shown to be widely expressed in mononuclear cells, is considered a high affinity receptor for LPS (8, 9). However, CD14 lacks a cytoplasmic domain, and there has been a puzzling question as to how CD14 transmits extracellular signals into downstream cytoplasmic signaling pathways. Recently, it was demonstrated that human TOLL-like receptor-2 (Tlr2) mediates the LPS-induced cellular signaling pathway (10, 11). Human Tlr4 can constitutively activate NFκB but fails to confer LPS responsiveness (10). However, results from studies using Tlr2- or Tlr4-deficient mice and from in vitro transfection studies suggest that Tlr4 recognizes LPS, but Tlr2 recognizes Gram-positive bacterial cell wall components (41, 42). The human TOLL protein is a transmembrane protein with an extracellular domain consisting of leucine-rich repeats and a cytoplasmic domain homologous to that of the IL-1 receptor (12).

Genetic evidence indicating that murine Tlr4 receptor was demonstrated using two mouse strains (C3H/HeJ and C57BL/10ScCr), which are hyposensitive to LPS (13). The former strain has Tlr4 with a missense mutation to replace proline with histidine at position 712, whereas the latter strain is homozygous for a null mutation of Tlr4. This genetic evidence is confirmed by another independent investigation (14). It is also demonstrated that the activation of NFκB and the expression of certain NFκB-induced gene products in LPS-stimulated macrophages derived from the C3H/HeJ mouse strain are impaired (15, 16). In addition, the overexpression of mutant Tlr4 derived from C3H/HeJ mice into human dermal endothelial cells results in the inhibition of LPS-induced NFκB activation (17). However, biochemical evidence that murine Tlr4 indeed confers LPS responsiveness has not been conclusively demonstrated.

Activation of TOLL proteins and IL-1 receptor induces recruitment of the adapter molecule, myeloid differentiation factor 88 (MyD88) (18–20), which in turn leads to the activation of NFκB and the expression of NFκB-induced gene products (10, 11, 21). Results from our previous studies indicate that LPS induces selective expression of the mitogen-induced cyclooxygenase (COX-2) in murine macrophages (22). LPS activates NFκB through TOLL-like receptors in macrophages (23–26). However, the role of NFκB in LPS-induced COX-2 expression in macrophages is not clearly established; there were contradicting reports regarding the role of NFκB in LPS-induced COX-2 expression in macrophages (27, 28). COX-2 is shown to be overexpressed in sites of inflammation and in tissues of

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‡The abbreviations used are: LPS, lipopolysaccharide; Tlr, TOLL-like receptor; NFκB, nuclear factor-κB; IL, interleukin; MyD88, myeloid differentiation factor 88; COX-2, cyclooxygenase; HA, hemagglutinin; PCR, polymerase chain reaction; NIK, NFκB-inducing kinase; ICαβ, inhibitor of β; TIR, TOLL/IL-1R homology.

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many types of tumors (29–33). Elucidating the signaling pathways is the key to understanding why COX-2 is overexpressed in such pathological states and can provide crucial information for identifying the potential targets for pharmaceutical and dietary modulation.

In this study, we have addressed three important issues in elucidating LPS-stimulated signaling pathways in murine macrophages. First, we determined whether the activation of NFκB is sufficient and required for LPS-induced COX-2 expression. Second, we determined whether the activation of Tlr4 confers LPS responsiveness as evaluated by the activation of NFκB and the expression of COX-2. Third, we determined why the Tlr4 mutant with a missense mutation at position 712 fails to transmit the LPS-induced signal to downstream signaling pathways.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**RAW264.7 cells (murine macrophage-like cell line, ATCC TIB-71) were cultured in LPS-free Dulbecco’s modified Eagle’s medium containing 10% (v/v) heat-inactivated fetal bovine serum (Intergen) and 10 units/ml penicillin and 100 μg/ml streptomycin (Life Technologies, Inc.) at 37 °C in a 5% CO2 air environment. Human embryonic kidney cells (293T cells) were provided by Sam Lee (Beth Israel Hospital, Boston, MA) and cultured in the same medium used for the RAW264.7 cells.

**DNA Constructs—**Flag epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) tagged murine Tlr4 cDNA constructs (both wild-type and mutant pFlag-CMV1-Tlr4(wt) and pFlag-CMV1-Tlr4(P712H), respectively) were kindly provided by Bruce Beutler (Southwestern Medical Center, Dallas, TX). These constructs lack DNA sequences for the first 20 amino acids representing the signal peptide. The mutant Tlr4 found in LPS-hyporesponsive mouse strain (C3H/HeJ) has a missense mutation at position 712 to replace proline with histidine.

The Flag-Tlr4 constructs were subcloned into pcDNA3.1/zeo(+) vector (Invitrogen, San Diego, CA) and the Flag epitope tag was replaced with the HA tag sequence (Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala) by polymerase chain reaction (PCR) cloning. The first PCR reaction was performed with the primers named Xho-SS-FOR and SS-HA-REV. The second PCR reaction was performed with the primers named Xho-SS-FORB and Not-HA-REV using the first PCR product as a template. The final PCR product was inserted in the XhoI and NotI sites of pcDNA-3.1/zeo(-) Flag-Tlr4 (wt) and pcDNA3.1/zeo(-) Flag-Tlr4(P712H) plasmids resulting in pcDNA3.1/zeo(-) HA-Tlr4(wt) and pcDNA3.1/zeo(-) HA-Tlr4(P712H) constructs, respectively. The primers used are as follows: Xho-SS-FOR, 5'-GTTAAACCTGAGCCACATGCTGCACTTCTGATCC-3'; SS-HA-REV, 5'-ACCGATGATCTGGGACCTGCTGTGATTGTAAGCAACTGCAGCTCCAACAAG-3'; Not-HA-REV, 5'-GGGATCCGCGGCGCAGCTGATCTGGGGACCTGCTGAATTG AACAGTATAGC-3' for amplifying the N-terminal region; BmTfr2092, 5'-GAAGGGGATCACCAAGAACATCTACGATGTCCTGCTCC-3'; Tlr-REV, 5'-CTGGAAGGATCCCTCTAGGTGCCAGGATGGTGCAGCAGAGTGTAAGCAACTGCAGCTCCAACAAG-3' for amplifying the extracellular region.

For truncated Tlr4 constructs (Flag/HA epo-taged), ΔTlr4(wt) and ΔTlr4(P712H) lacking the extracellular leucine-rich repeat domain, DNA sequences spanning the epitope tag plus N-terminal 21–79 amino acids and transmembranes plus the cytoplasmic domain (amino acids 629–835) were amplified by PCR using Flag/HA-Tlr4(wt) and pcDNA3.1/zeo(-) Flag-Tlr4 (wt) and pcDNA3.1/zeo(-) Flag-Tlr4(P712H) as templates. The primers used for the PCR cloning are as follows: Xho-Tlr4(wt), 5'-GTTAAACCTGAGCCACATGCTGCACTTCTGATCC-3'; ΔTlr4(wt), 5'-GTTAAACCTGAGCCACATGCTGCACTTCTGATCC-3'; Tlr4(P712H), 5'-GTTAAACCTGAGCCACATGCTGCACTTCTGATCC-3'; and Flag/HA-Tlr4, 5'-GTTAAACCTGAGCCACATGCTGCACTTCTGATCC-3'; Xho-REV, 5'-GAAGGGGATCACCAAGAACATCTACGATGTCCTGCTCC-3'; Tlr-REV, 5'-CTGGAAGGATCCCTCTAGGTGCCAGGATGGTGCAGCAGAGTGTAAGCAACTGCAGCTCCAACAAG-3'.

**Fig. 1.** Schematic illustration of different murine Tlr4 cDNA constructs used in these studies. Tlr4(wt), HA or Flag-tagged full-length wild-type murine Tlr4, ΔTlr4(wt), truncated HA- or Flag-tagged wild-type Tlr4, ΔTlr4(P712H), truncated HA- or Flag-tagged full-length mutant Tlr4 with the missense mutation (substitution of proline with histidine) at position 712. This mutant is found in LPS-hyporesponsive mouse strain (C3H/HeJ). ΔTlr4(P712H), truncated HA- or Flag-tagged full-length Tlr4 lacking the extracellular leucine-rich repeat domain (LRR). Cloning strategy for these constructs is described under "Experimental Procedures." TM, transmembrane domain.

**Fig. 2.** Suppression of LPS-induced activation of NFκB and COX-2 expression by a dominant-negative mutant of NIK or IκBα. RAW264.7 cells were co-transfected with a luciferase (Luc) reporter plasmid for NFκB response element (A and C) or COX-2 promoter (B and D) and the expression plasmid containing the dominant-negative mutant of NIK (IKKα) or IκBα (ΔN) cDNA. Transfected cells were treated with LPS (20 ng/ml) for 24 h. Luciferase activity was measured as described under "Experimental Procedures." Panels are representative data from more than three different analyses. Values are mean ± S.E. (n = 3). RLAs, relative luciferase activity.

pRK-NIK(wt), and a dominant-negative mutant NIK (pRK-NIK(KA)) were gifts from Mike Rothe (Tularik, South San Francisco, CA). Wild-type murine MyD88 (Flag-MyD88(wt)), dominant-negative mutant (Flag-MyD88(ΔDD)), and the constitutively active mutant (Flag-MyD88(ΔTOLL)) were kindly provided by Jurg Tschopp (University of Lausanne, Switzerland) (34). The Flag-MyD88 variant constructs were subcloned into pcDNA3 expression vector (Invitrogen). Expression plasmids for wild-type inhibitor xB (pCMV4-IκBα(wt)) and a dominant-negative mutant (pCMV4-IκBα(ΔN)) were provided by Dean Ballard (Vanderbilt University, Nashville, TN). 2xNFκB-luciferase reporter construct was a gift from Frank Mercurio (Signal Pharmaceuticals, San Diego, CA). HSF70-β-galactosidase reporter construct was from Robert Modlin (University of California, Los Angeles, CA) (35). Mouse COX-2 promoter (~3,200 base pairs) luciferase reporter construct was from David DeWitt (Michigan State University, East Lansing, MI). All DNA constructs were prepared in a large scale using EndoFree Plasmid Maxi kit (Qiagen, Chatsworth, CA) for transfection.
RESULTS AND DISCUSSION

LPS-induced Expression of COX-2 Is Suppressed by Inhibition of NFκB in RAW264.7 Cells—To determine whether LPS-induced expression of COX-2 is mediated through the activation of NFκB, we investigated whether or not the inhibition of LPS-induced activation of NFκB leads to the suppression of COX-2 expression. Cells were co-transfected with the luciferase reporter plasmid for NFκB (A) or COX-2 promoter (B) and an expression plasmid containing a dominant-negative mutant of IκBα or IκBα cDNA. The results show that LPS-induced COX-2 expression is significantly inhibited by the co-transfection of cells with a dominant-negative mutant of IκBα or IκBα (Fig. 2). These results indicate that the activation of NFκB is required for the full expression of COX-2 in LPS-stimulated RAW264.7 cells.

Activation of NFκB by NIK Leads to Expression of COX-2, and This Expression Is Significantly Suppressed by a Dominant-Negative Mutant of IκBα—RAW264.7 cells were co-transfected with a luciferase (Luc) reporter plasmid for NFκB (A) or COX-2 promoter (B) and an expression plasmid containing a dominant-negative mutant of IκBα (Fig. 3). The results show that LPS-induced COX-2 expression is significantly inhibited by the co-transfection of cells with a dominant-negative mutant of IκBα (Fig. 3). Together, these results indicate that the activation of NFκB is required for the full expression of COX-2 in LPS-stimulated macrophages.
There were two conflicting reports regarding the role of NFkB in LPS-induced COX-2 expression in RAW264.7 cells. The requirement for NFkB was demonstrated in our previous study using the pharmacological inhibitors of IκBα degradation or nuclear translocation of NFkB (27). However, the results from another study using the luciferase-reporter gene assay showed that NFkB may not be required for the full expression of COX-2 in LPS-stimulated RAW264.7 cells (28).

It is not clear what causes the discrepancy between these two studies (27, 28) in requirements of NFkB for the full expression of COX-2. We used the murine COX-2 luciferase construct containing 3.2-kilobase upstream promoter sequences, whereas the COX-2 construct used in another study showing the results that were different from our study contains only 700-base pair upstream promoter sequences (28). Although the κB binding site in the murine COX-2 promoter is located within −400 kilobases, it is possible that other enhancer elements located further upstream of the 5′-flanking region of COX-2 gene are required for the full expression of COX-2 in LPS-stimulated RAW264.7 cells.

FIG. 8. The constitutively active form of the adapter protein MyD88 lacking TOLL/IL-1R homology domain (MyD88ΔTOLL) can override the dominant-negative effect of the mutant ΔTlr4(P712H). RAW264.7 cells were co-transfected with a luciferase (Luc) reporter plasmid for NFkB (A) or COX-2 promoter (B), expression plasmids containing the truncated wild-type Flag-ΔTlr4(wt) or the truncated mutant Flag-ΔTlr4(P712H), or respective vector. Panels are representative data from more than three different analyses. Values are mean ± S.E. (n = 3). RLA, relative luciferase activity.

Kirschning et al. (10) showed that human Tlr2, when co-transfected with CD14 into 293T cells, conferred LPS inducibility of NFkB. However, the overexpression of human Tlr4 constitutively activated the NFkB reporter gene, and the treatment of these cells with LPS did not enhance the reporter gene activity. Based on these results, they concluded that human Tlr2 but not Tlr4 confers LPS responsiveness. Our results show that transfection of RAW264.7 cells with the truncated ΔTlr4...
but not the full-length wild-type murine Tlr4 elicits a constitutive activation of the NF-κB reporter gene (Fig. 4).

The Constitutively Active ΔTlr4-induced Activation of NFκB and COX-2 Expression Are Suppressed by the Inhibition of NFκB with a Dominant-negative Mutant of NIK or IkBa in RAW264.7 Cells—To determine whether the ΔTlr4-induced expression of COX-2 is mediated through activation of NFκB, we investigated whether the inhibition of NFκB by a dominant-negative mutant of NIK or IkBa results in the suppression of ΔTlr4-induced COX-2 expression. The results show that inhibition of ΔTlr4-induced NFκB activation by the dominant-negative mutant of NIK or IkBa leads to significant suppression of COX-2 expression (Fig. 5), indicating that ΔTlr4-induced COX-2 expression is at least in part mediated through NFκB.

The Truncated Mutant (ΔTlr4(P712H)) of Tlr4(P712H) Found in LPS-hyporesponsive Mouse Strain (C3H/HeJ) Inhibits LPS-induced Activation of NFκB and COX-2 Expression in RAW264.7 Cells—To establish whether activation of Tlr4 confers LPS responsiveness, we next determined whether the expression of the mutant Tlr4(P712H) found in C3H/HeJ mouse strain inhibits LPS-induced activation of NFκB and COX-2 expression. The results show that the truncated mutant ΔTlr4(P712H) inhibits whereas the truncated wild-type ΔTlr4 enhances LPS-induced activation of NFκB and COX-2 expression (Fig. 6). LPS responsiveness is slightly enhanced in cells transfected with the full-length Tlr4 as compared with the vector-transfected cells. These results suggest that ΔTlr4 (P712H) acts as a dominant-negative mutant. Together, these results indicate that the activation of Tlr4 confers LPS responsiveness in RAW264.7 cells. However, our results do not permit ruling out the possibility that other TOLL-like receptors also mediate LPS responsiveness in RAW264.7 cells.

The Adapter Protein MyD88 Is Co-immunoprecipitated with the Wild-type ΔTlr4 but Not with the Mutant ΔTlr4(P712H) in 293T Cells—Next, we investigated whether the missense mutation to replace histidine with proline at position 712 of Tlr4 resulted in loss of function. It was demonstrated that MyD88 is an adapter protein directly interacting with the cytoplasmic TOLL/IL-1R homology (TIR) domain of human Tlr4 (40) and considered as one of the most upstream components of the human Tlr4-mediated signaling cascade. This TIR domain is also present near the N-terminal death domain of MyD88. Such a sequence homology is also present among murine counterparts. Proline at position 712 lies within this TIR domain of murine Tlr4, which is critical for binding the MyD88 adapter protein. Thus, we determined whether the mutation at position 712 interferes with the binding of MyD88 to Tlr4, thereby resulting in failure of the signal transmission.

Human embryonic kidney cells (293T cells) were co-transfected with an epitope Flag-tagged MyD88 and HA-tagged wild-type ΔTlr4 or the mutant ΔTlr4(P712H) cDNA. When cell lysates from these cells were immunoprecipitated with anti-HA antibody and immunoblotted using anti-Flag or Tlr4 antibodies, MyD88 was co-immunoprecipitated with the wild-type HA-ΔTlr4 (Fig. 7, lane 1) but not with the mutant HA-ΔTlr4(P712H) (Fig. 7, lane 2). These results suggest that the mutant Tlr4(P712H) is unable to interact with MyD88 and thus fails to activate downstream signaling pathways.

The Constitutively Active Form of the Adapter Protein MyD88 Rescues Inability of the Dominant-negative Mutant Tlr4 to Activate the Downstream Signaling Pathway in RAW264.7 Cells—The adapter molecule MyD88 is known to be an immediate downstream signaling molecule interacting directly with the TIR domain of Tlr4 (18–20). Proline at position 712 is located in this TIR domain. Therefore, it would be interesting to determine whether the substitution of proline with histidine resulting from the missense mutation interferes with the binding of MyD88 to the TIR domain of Tlr4. If the failure of the mutant Tlr4 to activate downstream signaling pathways is because of its inability to recruit the adapter molecule MyD88, then transfecting cells with a constitutively active form of MyD88 should restore signal transmission. Indeed, the co-transfection of RAW264.7 cells with the dominant-negative mutant (ΔTlr4(P712H)) and a constitutively active form of MyD88 lacking TOLL/IL-1R domain (MyD88(ΔTOLL)) results in the restoration of NFκB activation and COX-2 expression (Fig. 8). Taken together these results suggest that hyporesponsiveness of the mouse strain (C3H/HeJ) to LPS is because of the disruption of Tlr4-mediated signaling pathways resulting from the inability of the mutant (Tlr4(P712H)) to recruit the downstream signaling molecule MyD88.

In summary, the results presented here indicate that activation of Tlr4 confers LPS responsiveness and that disruption of Tlr4-mediated signaling pathways leads to hyporesponsiveness to LPS in murine macrophage-like cell line (RAW264.7) as determined by the activation of NFκB and the expression of COX-2. Transcription factor NFκB regulates the expression of a diverse array of genes including inflammation marker gene products that are involved in innate immune responses and pathogenesis of Gram-negative septic shock. Therefore, our results underscore the importance of Tlr-mediated signaling pathways in these processes.

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REFERENCES

1. Morrison, D. C., and Ulevitch, R. J. (1978) Am. J. Pathol. 93, 526–617
2. Bone, R. C. (1991) Ann. Intern. Med. 115, 457–469
3. Morrison, D. C., and Ryan, J. L. (1987) J. Exp. Med. 165, 2091–2097
4. Dinarello, C. A. (1994) FASEB J. 8, 1314–1325
5. Tracey, K. J., and Lowry, S. F. (1990) Adv. Surg. 23, 21–56
6. O’Brien, A. D., Rosenstreich, D. L., Scher, I., Campbell, G. H., MacDermott, R. P., and Formal, S. B. (1980) J. Immunol. 124, 20–24
7. Rosenstreich, D. L., Weilblatt, A. C., and O’Brien, A. D. (1982) Crit. Rev. Immunol. 3, 263–330
8. Ingalls, R. R., Heine, H., Lien, E., Yoshiumura, A., and Golenbock, D. (1999) Infect. Dis. Clin. North Am. 13, 341–353
9. Ulevitch, R. J., and Tobias, P. S. (1995) Annu. Rev. Immunol. 13, 437–457
10. Kozak, M. (1992) J. Biol. Chem. 267, 398–403
11. Yang, R. B., Mark, M. R., Gurney, A. L., and Godowski, P. J. (1999) J. Biol. Chem. 274, 2829–2835
12. O’Neill, L. A., and Greene, C. (1998) J. Leukocyte Biol. 63, 639–643
13. Vogeli, S. N., Johnson, D., Perera, P. Y., Medvedev, A., Lariviere, L., Qureshi, S. T., Loeveque, G., Clermont, S., Moore, K. J., Gros, P., and Malo, D. (1999) J. Exp. Med. 189, 615–625
14. Beutler, B., Krohn, C., Nilsark, I. W., Luukde, C., and Cerami, A. (1986) Science 232, 977–980
15. Faure, E., Equilbey, O., Stelzig, P. A., Thomas, L., Zhang, F. X., Kirschning, C. J., Polentarutti, N., Muzio, M., and Arditi, M. (2000) J. Biol. Chem. 275, 11058–11063
16. Medzhitov, R., and Janeway, C. A., Jr. (1997) Cell 91, 295–298
17. Muzio, M., Natoli, G., Saccani, S., Levrero, M., and Mantovani, A. (1998) J. Exp. Med. 187, 2097–2101
18. O’Neill, L. A., and Greene, C. (1998) J. Leukocyte Biol. 63, 650–657
19. Medzhitov, R., Preston-Hurlburt, P., and Janeway C. A., Jr. (1997) Nature 388, 394–397
20. Lee, S. H., Soyoola, E., Channugam, P., Hart, S., Sun, W., Zhong, H., Liao, S., Simons, D., and Hwang, D. (1992) J. Biol. Chem. 267, 25834–25838
21. Qureshi, S. T., Gros, P., and Malo, D. (1999) Trends Genet. 15, 291–294
22. Yang, R. B., Mark, M. R., Gurney, A. L., and Godowski, P. J. (1999) J. Immunol. 163, 639–643
23. Chow, J. C., Young, D. W., Golenbock, D. T., Christ, W. J., and Gusovsky, F. (1999) J. Biol. Chem. 274, 10689–10692
24. Zhang, F. X., Kirschning, C. J., Mancinelli, R., Xu, X. P., Jin, Y., Faure, E., Mantovani, A., Rotte, M., Muzio, M., and Arditi, M. (1999) J. Biol. Chem. 274, 7611–7614
25. Kwak, J., Yang, B., Ku, Y., and Boudreau, M. (1997) Biochem. Pharmacol. 54, 87–96
26. Wadleigh, D. J., Reddy, S. T., Kopp, E., Ghosh, S., and Herschman, H. R.
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(2000) J. Biol. Chem. 275, 6259–6266
29. Crofford, L. J. (1997) J. Rheumatol. 24, 15–19
30. Kutchera, W., Jones, D. A., Matsunami, N., Groden, J., McIntyre, T. M.,
Zimmerman, G. A., White, R. L., and Prescott, S. M. (1996) Proc. Natl. Acad.
Sci. U. S. A. 93, 4816–4820
31. Eberhart, C. E., Coffey, R. J., Radhika, A., Giardiello, F. M., Ferrenbach, S.,
and Dulbois, R. N. (1994) Gastroenterology 107, 1183–1188
32. Sano, H., Kawahito, Y., Wilder, R. L., Hashiramoto, A., Mukai, S., Asai, K.,
Kimura, S., Kato, H., Kondo, M., and Hla, T. (1995) Cancer Res. 55,
3785–3789
33. Kargman, S. L., O'Neill, G. P., Vickers, P. J., Evans, J. F., Mancini, J. A., and
Jothy, S. (1995) Cancer Res. 55, 2556–2559
34. Burns, K., Martinon, F., Eskling, C., Pahl, H., Schneider, P., Bodmer, J. L.,
Di Marco, P., French, L., and Tschopp, J. (1998) J. Biol. Chem. 273,
12203–12209
35. Brightbill, H. D., Libraty, D. H., Krutzik, S. R., Yang, R. B., Belisle, J. T.,
Bleierski, J. R., Maitland, M., Norgard, M. V., Plevy, S. E., Smale, S. T.,
Brennan, P. J., Bloom, B. R., Godowski, P. J., and Modlin, R. L. (1999)
Science 285, 732–736
36. Paik, J. H., Ju, J. H., Lee, J. Y., Boudreau, M. D., and Hwang, D. H. (2000)
J. Biol. Chem. 275, 28173–28179
37. Laemmli, U. K. (1970) Nature 227, 680–685
38. Chandmugam, P., Feng, L., Liou, S., Jang, B. C., Boudreau, M., Yu, G., Lee,
J. H., Kwon, H. J., Beppo, T., Yoshida, M., Xia, Y., Wilson, C. B., and
Hwang, D. (1995) J. Biol. Chem. 270, 5418–5426
39. Winans, K. A., and Hashimoto, C. (1995) Mol. Biol. Cell 6, 587–596
40. Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh,
S., and Janeway, C. A., Jr. (1998) Mol Cell 2, 253–258
41. Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T.,
Takeda, K., and Akira, S. (1999) Immunity 11, 443–451
42. Yoshimura, A., Lien, E., Ingalls, R. R., Tuomanen, E., Dziarski, R., and
Golenbock, D. (1999) J. Immunol. 163, 1–5