FEBS Letters 467 (2000) 160–164 FEBS 23292

TAK1 mediates an activation signal from toll-like receptor(s) to nuclear factor-κB in lipopolysaccharide-stimulated macrophages

Takashi Irie, Tatsushi Muta*, Koichiro Takeshige

Department of Molecular and Cellular Biochemistry, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 15 December 1999; received in revised form 7 January 2000

Edited by Shozo Yamamoto

Abstract Stimulation of monocytes/macrophages with lipopolysaccharide (LPS) results in activation of nuclear factor-κB (NF-κB), which plays crucial roles in regulating expression of many genes involved in the subsequent inflammatory responses. Here, we investigated roles of transforming growth factor-β activated kinase 1 (TGF-TAK1), a mitogen-activated protein kinase kinase kinase (MAPKKK), in the LPS-induced signaling cascade. A kinase-negative mutant of TAK1 inhibited the LPS-induced NF-κB activation both in a macrophage-like cell line, RAW 264.7, and in human embryonic kidney 293 cells expressing toll-like receptor 2 or 4. Furthermore, we demonstrated that endogenous TAK1 is phosphorylated upon simulation of RAW 264.7 cells with LPS. These results indicate that TAK1 functions as a critical mediator in the LPS-induced signaling pathway.

Key words: Lipopolysaccharide; TAK1; Toll-like receptor; Nuclear factor-κB; Macrophage; Innate immunity

1. Introduction

In innate immunity of mammal, monocytes/macrophages play key roles in detection and elimination of pathogens. These types of cells are activated by microorganism-derived molecules such as lipopolysaccharide (LPS), peptidoglycan, or lipoteichoic acid. LPS, also called endotoxin, a major component of the outer cell wall of Gram-negative bacteria, is one of the strongest activator for monocytes/macrophages [1]. The activated macrophages secrete chemical mediators including proinflammatory cytokines, chemokines and reactive oxygen species, which, in concert, lead to inflammation and the activation of adaptive immunity effective to eliminate microorganisms. On the other hand, uncontrolled activation of macrophages by severe infections of Gram-negative bacteria causes life-threatening septic-shock via overproduction of the cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, -6 or -8 [2].

*Corresponding author. Fax: (81)-92-642 6202.
E-mail: tmuta@mailserver.med.kyushu-u.ac.jp

Abbreviations: LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL, interleukin; NF-κB, nuclear factor-κB; MAP kinase, mitogen-activated protein kinase; TLR, toll-like receptor; TGF, transforming growth factor; TAK1, TGF-β activated kinase 1; IRAK, IL-1 receptor-associated kinase; TRAF, TNF receptor-associated factor; NIK, NF-κB-inducing kinase; HEK, human embryonic kidney; SDS–PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis

Upon infection of Gram-negative bacteria, LPS forms a complex with LPS-binding protein (LBP) in plasma, and is then transferred to CD14, a cell surface antigen of monocytes/macrophages [3,4]. In the LPS-stimulated macrophages, activated are two important intracellular proteins, the transcription factor nuclear factor-κB (NF-κB) [5] and p38 mitogen-activated protein kinase (MAP kinase) [6]. NF-κB is activated by degradation of its cytoplasmic inhibitor, IκB-α or IκB-β, induced by phosphorylation catalyzed by IκB-kinase (IKK)-α and -β [7]. The activated NF-κB regulates transcription of many cytokines, chemokines, nitric oxide synthase, cell adhesion molecules and co-stimulatory ligands such as B7.1 [8,9]. p38 is also activated by phosphorylation by MAP kinase kinase. Although precise roles of p38 in the cytokine production are not yet known, a specific inhibitor for p38, SB203580, was shown to inhibit tumor necrosis factor-α (TNF-α) or IL-1 production in LPS-stimulated macrophages [10].

Although CD14 was shown to be critical for LPS recognition on the cell surface, other protein(s) that transduce LPS-signaling across the plasma membrane has been postulated since CD14 is a glycosylphosphatidylinositol (GPI)-anchored membrane protein without a cytoplasmic domain. Recently several reports showed that two human homologues of the membrane receptor toll of *Drosophila*, toll-like receptors (TLR) 2 and 4, are candidates for such a signal transducer(s) on the membrane. When transfected in LPS-unresponsive cells, TLR2 was capable of mediating the activation of NF-κB upon LPS stimulation [11,12]. Moreover it has been demonstrated that two strains of LPS-hyporesponder mice, C3H/HeJ and C57BL/10ScCr, have defects in their genes for TLR4 [13,14]. It was also shown that TLR4-knockout mice lose sensitivity to LPS [15].

Since cytoplasmic domains of TLRs are homologous to that of IL-1 receptor, similarities in their intracellular signaling pathways through these receptors are suggested [16,17]. Recently, transforming growth factor (TGF)-β activated kinase 1 (TAK1), which was originally identified as a member of MAP kinase kinase kinase (MAPKKK) family mediating TGF-β signaling, has been shown to be involved in the IL-1-induced activation of NF-κB and c-Jun NH_2-terminal protein kinase (JNK) [18,19]. In the present study, we explored the involvement of TAK1 in the LPS-mediated NF-κB activation. Our results demonstrated that TAK1 is phosphorylated concomitant with its activation in LPS-stimulated macrophages and its activity was necessary for the activation of NF-κB. Thus, TAK1 plays critical roles in the LPS-mediated cellular activation leading to the subsequent inflammation.

PII: S0014-5793(00)01146-7

2. Materials and methods

2.1. Plasmids

cDNAs for full-length human TAK1, human TNF receptor-associated factor 6 (TRAF6) and mouse MyD88 were obtained by reverse transcription-polymerase chain reaction (RT-PCR) with RNA of THP-1 cells or RAW 264.7 cells as a template. cDNAs for human TLR2 and TLR4 were amplified from cDNA derived from human leukocyte (Clontech Laboratories, Palo Alto, CA, USA). Obtained cDNAs were subcloned into pcDNA3 (Invitrogen, Carlsbad, CA, USA) with an NH_2-terminal Flag or Myc epitope. A dominant negative mutant TAK1(K63W) and a truncated MyD88 mutant (MyD88-N: amino acids 1–151) were created by a polymerase chain reaction-mediated mutagenesis. The sequences were confirmed by dideoxy sequencing. An expression vector for Flag-tagged full-length human NF-κB-inducing kinase (NIK) was kindly provided by Dr. D. Wallach. An NF-κB-luciferase reporter plasmid, pELAM1-Luc, was constructed by inserting a fragment of −730 to +52 of E-selectin gene into pGL3-Basic (Promega, Madison, WI, USA). As an internal control reporter, the plasmid pRL-TK (Promega) containing *Renilla* luciferase cDNA was used.

2.2. Cell culture and reagents

Human embryonic kidney (HEK) 293 cells were kindly provided by Dr. F. Tokunaga and RAW 264.7 cells, a murine macrophage-like cell line, were obtained from Dainippon, Osaka, Japan. RAW 264.7 cells and HEK 293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (BioWhittaker, Walkersville, MD, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO_2. LPS from *Escherichia coli* 0111:B4 was purchased from List Biological Laboratories, Campbell, CA, USA.

2.3. Transfection

RAW 264.7 cells were transfected with expression plasmids using FuGENE6 Transfection Reagent according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN, USA). HEK 293 cells were transfected by the calcium phosphate method as described previously [20].

2.4. Luciferase assay

Cells were transiently transfected with pELAM1-Luc and pRL-TK together with indicated plasmids. The total amount of DNA was kept constant with empty vector for each transfection. 48 h after transfection, cells were stimulated with LPS at 37°C for 6 h. Cells were then lysed, and their luciferase activities were measured by using the Dual-Luciferase Reporter system (Promega).

2.5. Detection of phosphorylated TAK1

RAW 264.7 cells were incubated with or without 1 μg/ml LPS at 37°C for the indicated time and then lysed in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 0.15 M NaCl, 1% NP-40, 2 mM EDTA, 50 mM NaF, 0.2 mM Na_3VO_4 and 2 μg/ml aprotinin. The lysate was divided into half and immunoprecipitated with anti-TAK1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). One of the immunoprecipitates was treated with 400 units of lambda protein phosphatase (New England Biolabs, Beverly, MA, USA) at 30°C for 30 min and the other was left untreated. The immunoprecipitates were subjected to 7.5% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by immunoblotting with the same antibody.

3. Results and discussion

3.1. TAK1 is required for the LPS-induced NF-κB activation

A recent report by Ninomiya-Tsuji et al. showed that TAK1 is activated by the stimulation of IL-1 and is required for the activation of NF-κB [19]. Since IL-1 receptor and TLRs have homologous cytoplasmic domains and several common intracellular signaling molecules, such as MyD88 and IL-1 receptor-associated kinase (IRAK), were shown to be involved both in the IL-1- and the LPS-signaling [16,17,21–24], we investigated whether TAK1 also participates in the signaling pathway induced by LPS.

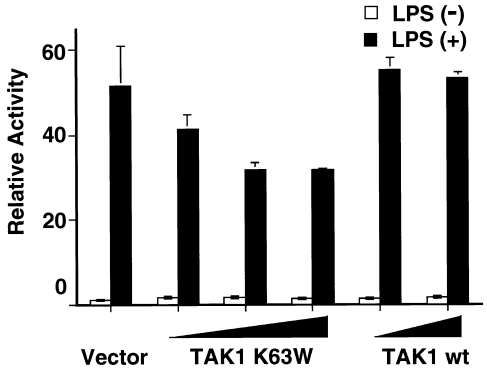

Fig. 1. Effects of the mutant TAK1 on the LPS-induced NF-κB activation. RAW 264.7 cells were transfected with an expression vector for the mutant TAK1 (TAK1 K63W; 0.1, 0.4, and 0.7 μg) or the wild type TAK1 (TAK1 wt; 0.1 and 0.7 μg) together with reporter plasmids. 48 h after transfection, the transfected cells were stimulated with or without 100 ng/ml LPS at 37°C for 6 h. Then the cells were lysed and luciferase activities were measured. Activity of the NF-κB reporter was normalized on the basis of *Renilla* luciferase activity. Data shown are the mean ± S.E.M. of two independent transfections. A representative result of two separate experiments is shown.

We constructed an expression vector for a kinase-negative mutant of TAK1 carrying a single amino acid substitution (Lys-63 to Trp) [18,19] and analyzed the effects of expression of the mutant form of TAK1 on the activation of NF-κB in a mouse macrophage-like cell line, RAW 264.7. RAW 264.7 cells transfected with an NF-κB reporter showed strong activation of NF-κB 6 h after the LPS stimulation (Fig. 1). We then transfected the wild type and the mutant form of TAK1 into the cells and examined the NF-κB activity with or without the LPS stimulation. Without the simulation, both the wild and mutant TAK1 did not affect the basal NF-κB activity. On the other hand, the mutant form of TAK1 inhibited the LPS-induced activation of NF-κB in a dose-dependent manner, whereas expression of the wild type TAK1 exhibited marginal effects. Thus, the kinase activity of TAK1 is required for the LPS-induced signaling pathway leading to the activation of NF-κB in macrophages.

3.2. TAK1 is phosphorylated upon LPS stimulation

The previous report showed that TAK1 is activated on the stimulation by IL-1 [19]. Since there is no report that examined TAK1 activation in LPS stimulated cells, we investigated

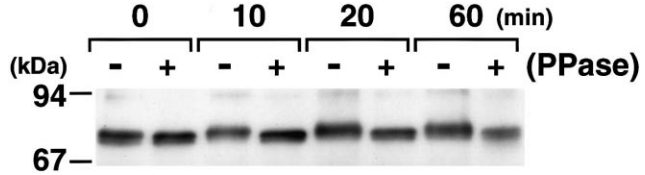

Fig. 2. Phosphorylation of TAK1 by LPS stimulation. RAW 264.7 cells were stimulated with 1 μg/ml LPS at 37°C for the indicated time. Then the cells were lysed and endogenous TAK1 was immunoprecipitated with anti-TAK1 antibody. Equal amounts of the immunoprecipitates were treated (+) or untreated (−) with protein phosphatase (PPase) and TAK1 was visualized by immunoblotting.

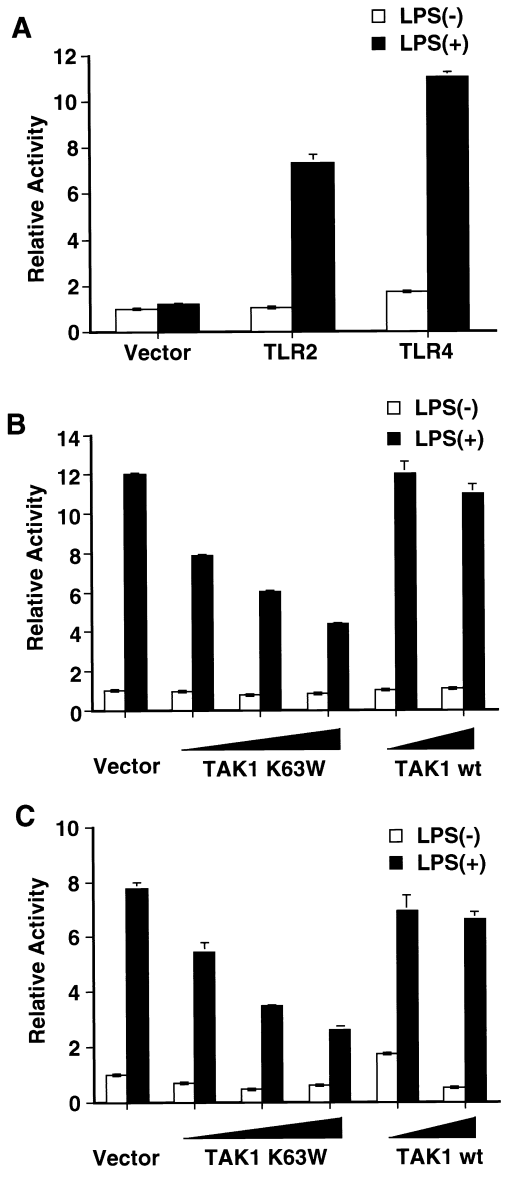

Fig. 3. Effects of the mutant TAK1 on the LPS-induced NF-κB activation in TLR-transfected HEK 293 cells. HEK 293 cells were transfected with an empty vector or an expression vector for TLR2 or TLR4 together with reporter plasmids (A). In B and C, the same cells were transfected with an expression vector for the kinase-negative TAK1 (TAK1 K63W; 0.1, 0.5, 1.0 μg) or the wild type TAK1 (TAK1 wt; 0.1, 1.0 μg) together with reporter plasmids and an expression vector for TLR2 (B) or TLR4 (C), respectively. 48 h after transfection, cells were stimulated with or without 1 μg/ml LPS at 37°C for 6 h. Then the cells were lysed and luciferase activities were measured. Activity of the NF-κB reporter was normalized on the basis of *Renilla* luciferase activity. Data shown are the mean ± S.E.M. of two independent transfections. A representative result of at least two separate experiments is shown.

activation of TAK1 in the macrophage-like cell line in which the NF-κB activity is strongly induced by LPS.

When TAK1 is activated, it is phosphorylated and the phosphorylation correlates well with the kinase activity [19,25]. Accordingly, we analyzed phosphorylation of TAK1 by the LPS stimulation. RAW 264.7 cells were incubated with 1 μg/ml LPS for the indicated time and then endogenous TAK1 was visualized by immunoprecipitation followed by immunoblotting with anti-TAK1 antibody. On 7.5% SDS–PAGE, TAK1 in the stimulated cells reproducibly showed slightly slower mobility than in the resting cells (Fig. 2). Treatment of the TAK1 immunoprecipitate with protein phosphatase abolished the mobility shift, indicating that the shift is caused by phosphorylation. Phosphorylation of TAK1 is evident at 10 min after the stimulation and continued at least for 1 h.

We have also tried in vitro kinase assay of TAK1 by using MKK6 as a substrate. Unfortunately, we failed to detect the increased kinase activity of TAK1 even in the IL-1-treated 293 cells. In the previous report [19], they used 293 cells stably transfected with IL-1 receptor; therefore the cells are strongly stimulated by IL-1. Since immunoprecipitated TAK1 is autophosphorylated and activated rapidly in vitro when incubated in the presence of ATP during kinase assay, it would be difficult to detect the activation by kinase assay in normal cells.

TRAF6 was reported to be associated with TAK1 on the stimulation with IL-1 [19]. In our preliminary experiment, however, we failed to detect TRAF6 in the TAK1 immunoprecipitates from the LPS-stimulated cells. TAK1 has also been reported to be required for the NF-κB activation by the TNF-α stimulation [25]. Since the TNF-α signaling utilizes TRAF2 but not TRAF6 [26,27], and TAK1 does not associate with TRAF2 [19], the association of TAK1 with TRAF6 might not be essential for the phosphorylation and activation of TAK1 in the TNF-α- and/or LPS-signaling.

3.3. TAK1 participates in the NF-κB activation by the LPS stimulation through TLR2 and 4

The results shown in Figs. 1 and 2 indicated that TAK1 is activated and is required for the LPS-induced NF-κB activation in a macrophage-like cell line. Since TLR2 and/or 4 is involved in the LPS signaling [11–15], we next used HEK 293 cells transiently transfected by cDNAs for TLR2 and 4 to analyze the TLR-dependent LPS signaling pathway.

Several groups reported that TLR4-transfected HEK 293 cells showed constitutive activation of NF-κB and were not responsive to LPS [11,12,28]. In our experimental system, however, TLR4-transfected HEK 293 cells exhibited the acti-

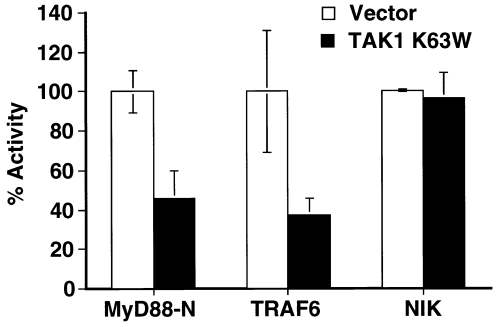

Fig. 4. Effects of the mutant TAK1 mutant on the MyD88-, TRAF6- and NIK-induced NF-κB activation. HEK 293 cells were transfected with an expression vector for MyD88-N, TRAF6 or NIK (0.65 μg), respectively and empty vector or the kinase-negative mutant of TAK1 (TAK1 K63W; 1 μg) together with reporter plasmids. 48 h after transfection, cells were lysed and luciferase activities were measured. Activity of the NF-κB reporter was normalized on the basis of *Renilla* luciferase activity. The NF-κB activity was represented as percent of the activities of the cells transfected with MyD88-N, TRAF6, and NIK with empty vector, respectively. Data shown are the mean ± S.E.M. of two independent transfections. A representative result of two separate experiments is shown.

vation of NF-κB reporter activity upon stimulation with 1 μg/ml LPS as well as TLR2-transfected cells, in the presence of fetal calf serum as a source of soluble form of CD14. The possible reason for this apparent discrepancy will be described elsewhere (T.M., unpublished). Mock-transfected HEK 293 cells did not respond to LPS (Fig. 3A). We co-transfected the kinase-negative form of TAK1 (K63W) into these cells to examine its effects on the LPS-mediated activation of NF-κB. Whereas the wild type TAK1 was without effects, expression of the kinase-negative mutant of TAK1 dose-dependently inhibited the LPS-mediated NF-κB activation both in the TLR2- and TLR4-expressing cells (Fig. 3B,C). The results indicate that TAK1 is required for the TLRs-mediated activation of NF-κB by LPS.

Compared with the results on TLR2/4-transfected HEK 293 cells, the expression of the mutant TAK1 in RAW 264.7 cells was less effective in inhibiting the NF-κB activation. It remains to be determined if this is due to low transfection efficiency or existence of other LPS-signaling pathways independent of TLR2/4 in macrophages. It is to be noted that dominant negative mutants for MyD88, IRAK and TRAF6 only partially inhibited the LPS-induced NF-κB activation (∼50%) in human monocytic THP-1 cells [24].

3.4. TAK1 acts downstream of MyD88 and TRAF6 and upstream of NIK

In order to dissect the TAK1-mediated LPS signaling pathway, we co-expressed the TAK1 mutant with several constitutive active signaling molecules. We transfected cDNAs for an N-terminal portion of MyD88, full-length TRAF6 or NIK into HEK 293 cells together with the NF-κB reporter [27,29–31]. As expected, the NF-κB activities were activated in these cells without stimulation (Fig. 4). We then co-transfected empty vector or the dominant negative form of TAK1 cDNA with each constitutively active molecule. In contrast to empty vector, co-expression of the kinase-negative TAK1 functioned as a dominant negative mutant, inhibiting the NF-κB activity induced by the expression of MyD88 and TRAF6. The results indicate that TAK1 functions downstream of MyD88 and TRAF6 as in the IL-1 signaling (Fig. 4) [19]. On the other hand, the TAK1 mutant exhibited marginal effects on the NF-κB activity induced by NIK, suggesting that TAK1 is most likely to transduce signals upstream of NIK, which then activates IKKα/β. This is confirmed by the finding that the activation of NF-κB by co-expressing TAK1 and TAB1 is inhibited by expression of dominant negative mutants of NIK [19,25].

In conclusion, TAK1 is activated by phosphorylation in the LPS stimulated macrophages via TLR2 and/or 4-mediated pathway, whose kinase activity is required for the NF-κB activation. TAK1 regulates NIK activity that activates IKKα/β downstream of MyD88 and TRAF6. TAK1 is also known to be a MAP kinase kinase kinase for p38 [32] and the activity of p38 is critical for the production of proinflammatory cytokines by activated monocytes/macrophages [10]. Therefore, TAK1 is one of the key enzymes for the regulation of the activation of these cells in the innate immunity.

Acknowledgements: We would like to express our thanks to Dr. Soh Yamazaki (Kyushu University) for the isolation of TAK1 cDNA and helpful discussions. We are also grateful to Dr. David Wallach (Weizmann Institute of Science) for providing an expression plasmid for NIK, to Dr. Fuminori Tokunaga (Himeji Institute of Technology) for HEK 293 cells, to Drs. Hiroaki Sakurai (Tanabe Seiyaku Co., Ltd.) and Giichi Takaesu (Nagoya University) for their advices on the in vitro kinase assay, and to Yuko Sunakawa for technical assistance. This work is supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan to T.M. and K.T.

References

[1] Ulevitch, R.J. and Tobias, P.S. (1999) Curr. Opin. Immunol. 11, 19–22.
[2] Galanos, C. and Freudenberg, M.A. (1993) Immunobiology 187, 346–356.
[3] Schumann, R.R., Leong, S.R., Flaggs, G.W., Gray, P.W., Wright, S.D., Mathison, J.C., Tobias, P.S. and Ulevitch, R.J. (1990) Science 249, 1429–1431.
[4] Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J. and Mathison, J.C. (1990) Science 249, 1431–1433.
[5] Goldfeld, A.E., Doyle, C. and Maniatis, T. (1990) Proc. Natl. Acad. Sci. USA 87, 9769–9773.
[6] Han, J., Lee, J.D., Bibbs, L. and Ulevitch, R.J. (1994) Science 265, 808–811.
[7] Karin, M. (1999) J. Biol. Chem. 274, 27339–27342.
[8] Mercurio, F. and Manning, A.M. (1999) Curr. Opin. Cell Biol. 11, 226–232.
[9] Muller, J.M., Ziegler-Heitbrock, H.W. and Baeuerle, P.A. (1993) Immunobiology 187, 233–256.
[10] Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Heys, J.R., Landvatter, S.W., Strickler, J.E., McLaughlin, M.M., Siemens, I.R., Fisher, S.M., Livi, G.P., White, J.R., Adams, J.L. and Young, P.R. (1994) Nature 372, 739–746.
[11] Yang, R.-B., Mark, M.R., Gray, A., Huang, A., Xie, M.H., Zhang, M., Goddard, A., Wood, W.I., Gurney, A.L. and Godowski, P.J. (1998) Nature 395, 284–288.
[12] Kirschning, C.J., Wesche, H., Ayres, T.M. and Rothe, M. (1998) J. Exp. Med. 188, 2091–2097.
[13] Poltorak, A., He, X., Smirnova, I., Liu, M.-Y., Huffel, C.V., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B. and Beutler, B. (1998) Science 282, 2085–2088.
[14] Qureshi, S.T., Lariviere, L., Leveque, G., Clermont, S., Moore, K.J., Gros, P. and Malo, D. (1999) J. Exp. Med. 189, 615–625.
[15] Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Takeda, K. and Akira, S. (1999) J. Immunol. 162, 3749–3752.
[16] Medzhitov, R., Preston-Hurlburt, P. and Janeway Jr., C.A. (1997) Nature 388, 394–397.
[17] Rock, F.L., Hardiman, G., Timans, J.C., Kastelein, R.A. and Bazan, J.F. (1998) Proc. Natl. Acad. Sci. USA 95, 588–593.
[18] Yamaguchi, K., Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E. and Matsumoto, K. (1995) Science 270, 2008–2011.
[19] Ninomiya-Tsuji, J., Kishimoto, K., Hiyama, A., Inoue, J., Cao, Z. and Matsumoto, K. (1999) Nature 398, 252–256.
[20] Chen, C. and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752.
[21] Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh, S. and Janeway Jr., C.A. (1998) Mol. Cell 2, 253–258.
[22] Muzio, M., Natoli, G., Saccani, S., Levrero, M. and Mantovani, A. (1998) J. Exp. Med. 187, 2097–2101.
[23] Kawai, T., Adachi, O., Ogawa, T., Takeda, K. and Akira, S. (1999) Immunity 11, 115–122.
[24] Zhang, F.X., Kirschning, C.J., Mancinelli, R., Xu, X.-P., Jin, Y., Faure, E., Mantovani, A., Rothe, M., Muzio, M. and Arditi, M. (1999) J. Biol. Chem. 274, 7611–7614.
[25] Sakurai, H., Miyoshi, H., Toriumi, W. and Sugita, T. (1999) J. Biol. Chem. 274, 10641–10648.
[26] Rothe, M., Wong, S.C., Henzel, W.J. and Goeddel, D.V. (1994) Cell 78, 681–692.
[27] Cao, Z., Xiong, J., Takeuchi, M., Kurama, T. and Goeddel, D.V. (1996) Nature 383, 443–446.

[28] Hirschfeld, M., Kirschning, C.J., Schwandner, R., Wesche, H., Weis, J.H., Wooten, R.M. and Weis, J.J. (1999) J. Immunol. 163, 2382–2386.
[29] Muzio, M., Ni, J., Feng, P. and Dixit, V.M. (1997) Science 278, 1612–1615.
[30] Wesche, H., Henzel, W.J., Shillinglaw, W., Li, S. and Cao, Z. (1997) Immunity 7, 837–847.
[31] Malinin, N.L., Boldin, M.P., Kovalenko, A.V. and Wallach, D. (1997) Nature 385, 540–544.
[32] Moriguchi, T., Kuroyanagi, N., Yamaguchi, K., Gotoh, Y., Irie, K., Kano, T., Shirakabe, K., Muro, Y., Shibuya, H., Matsumoto, K., Nishida, E. and Hagiwara, M. (1996) J. Biol. Chem. 271, 13675–13679.