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CUTTING EDGE

Cutting Edge: TLR2 Directly Triggers Th1 Effector Functions[1]

Takayuki Imanishi,[*†‡] *Hiromitsu Hara,*[2]* *Shinobu Suzuki,*[3]* *Nobutaka Suzuki,** *Shizuo Akira,*[§] *and Takashi Saito*[4]*

***Toll-like receptors recognize pathogen-associated molecular patterns, activate innate immunity, and consequently modulate adaptive immunity in response to infections. TLRs are also expressed on T cells, and it has been shown that T cell activation is modulated by TLR ligands. However, the functions of TLRs on Th1 and Th2 effector cells and the molecular mechanisms underlying TLR-mediated activation are not fully understood. We analyzed TLR functions and downstream signaling events in both effector T cells. In mouse Th1 cells the stimulation by TLR2 but not by other TLRs directly induced IFN-γ production, cell proliferation, and cell survival without TCR stimulation, and these effects were greatly enhanced by IL-2 or IL-12 through the enhanced activation of MAPKs. In contrast, no TLR affected the function of effector Th2 cells. These results identify TLR2 as a new specific activator of Th1 cell function and imply the involvement in Th1-mediated responses. The Journal of Immunology,* 2007, 178: 6715–6719.**

Toll-like receptors are primary sensor molecules of the innate immune system that function by recognizing pathogen-associated molecular patterns (1) and play important roles not only in the initiation of innate immune responses but also in the induction of T cell-mediated adaptive immune responses through the up-regulation of MHC and costimulatory molecules, as well as the induction of Th1-polarizing cytokines (2).

Several recent studies have reported that TLRs are functionally expressed not only on innate immune cells and B cells in the adaptive immune system but also on T cells (3–5). It has been shown that TLR ligands directly act on regulatory T cells and abrogate their suppressive function (6–8). Moreover, TLRs exhibit costimulatory functions in response to TCR stimulation, although TLRs alone fail to activate naive T cells (4, 9, 10). However, the influences of TLRs on T cells once differentiated into effector T cells such as Th1 and Th2 cells, which may be important for inflammation and diseases, have not yet been elucidated.

In this study, we analyzed the functions of TLRs expressed on effector Th1 and Th2 cells. Only TLR2 engagement induced direct Th1 activation in the absence of TCR stimulation as evidenced by IFN-γ production, cell proliferation, and cell survival. TLR2-mediated IFN-γ production was greatly enhanced by IL-2 or IL-12 through the enhanced activation of MAPKs. Our results suggest that TLR2 expressed on Th1 cells directly triggers the effector function of Th1 cells.

Materials and Methods

Mice

C57BL/6 mice were purchased from CLEA Japan. B6.129P2-Tlr1^{tm1Aki} (*Tlr1*$^{-/-}$) (11), B6.129P2-Tlr2^{tm1Aki} (*Tlr2*$^{-/-}$) (12), B6.129P2-Myd88^{tm1Aki} (*Myd88*$^{-/-}$) (13), and B6.129P2-Irak4^{tm1Yeh} (*Irak4*$^{-/-}$) (14) mice were generated previously and backcrossed for more than eight generations with C57BL/6 mice.

Reagents and Abs

The TLR2 ligands *N*-palmitoyl-*S*-(2,3-bis(palmitoyloxy)-(2*RS*)-propyl)-Cys-Ser-Lys$_4$ (Pam3)[5] and macrophage-activating lipopeptide 2 (MALP-2 were purchased from EMC Microcollections. Polyinosinic/polycytidylic acid, a TLR3 ligand, and LPS (*Escherichia. coli* O111:B4), a TLR4 ligand, were obtained from Amersham Biosciences and Sigma-Aldrich, respectively. Flagellin, a TLR5 ligand, and loxoribine, a TLR7 ligand, were obtained from InvivoGen. The CpG oligodeoxynucleotide (TCCATGACGTTCCTGATGCT) (15), a TLR9 ligand, was purchased from Rikaken.

Abs specific for IκB-α, phospho-JNK, phospho-p38, phospho-ERK, phospho-STAT5 (Y694), JNK, p38, and ERK were purchased from Cell Signaling Technology. Abs specific for phospho-STAT5 (S726) and actin were purchased from Santa Cruz Biotechnology and Fisher Scientific, respectively. Abs specific for IL-4 (clone 11B11), IFN-γ (clone XMG1.2), and TLR2 (clone 6C2) and TLR9 (clone M9.D6) were purchased from eBioscience.

*Laboratory for Cell Signaling, RIKEN Research Center for Allergy and Immunology, Yokohama, Japan; †Division of Molecular Mycology and Medicine, Center for Neurological Disease and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan; ‡Japan Society for the Promotion of Science and §Department of Host Defense, Research Institute for Microbial Disease, Osaka University, Osaka, Japan

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[2] Current address: Department of Biomolecular Sciences, Faculty of Medicine, Saga University, Saga, Japan.

[3] Current address: Nihon Schering K. K. Research Center, Kobe, Japan.

[4] Address correspondence and reprint requests to Dr. Takashi Saito, Laboratory for Cell Signaling, RIKEN Center for Allergy and Immunology, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa, Japan 230-0045. E-mail address: saito@rcai.riken.jp

[5] Abbreviations used in this paper: Pam3, *N*-palmitoyl-*S*-(2,3-bis(palmitoyloxy)-(2*RS*)-propyl)-Cys-Ser-Lys$_4$; CsA, cyclosporin A; GADD, growth arrest and DNA damage; IRAK, IL-1R-associated kinase 4; MALP-2, macrophage-activating lipopeptide 2; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium.

Recombinant mouse IL-2 and IL-4 were purchased from PeproTech, and IL-12 and IL-18 were obtained from Sigma-Aldrich and Medical and Biological Laboratories, respectively. Cyclosporin A (CsA), SB23580, and SP600125 were obtained from Calbiochem.

Cell preparation and cell stimulation

$CD4^+CD25^-CD62L^-$ (naive) and T cells were isolated from spleens using a FACSAria cell sorter. For Th1 cells, the sorted naive $CD4^+$ T cells were stimulated with plate-bound anti-CD3 (clone 2C11; 10 μg/ml) and anti-CD28 (clone PV-1; 5 μg/ml) Abs in the presence of IL-12 (10 ng/ml) and anti-IL-4 Abs (10 μg/ml) for 7 days. For Th2 cells, the purified naive $CD4^+$ T cells were similarly stimulated with plate-bound anti-CD3 and anti-CD28 Abs in the presence of IL-4 (10 ng/ml) and anti-IFN-γ Abs (10 μg/ml) for 7 days. These cells were then stimulated with various TLR ligands, IL-18, and immobilized anti-CD3 mAb (5 μg/ml) for 24 h. In a typical preparation, upon anti-CD3 mAb stimulation Th1 and Th2 cells produced 416.7 $\pm$ 22.4 and 1.9 $\pm$ 0.6 ng/ml IFN-γ and 0.5 $\pm$ 0.0 and 62 $\pm$ 7.5 ng/ml IL-4, respectively.

Functional analyses

For apoptosis analysis, cells were stained with propidium iodide and annexin V and analyzed by FACS. Cell proliferation was measured by an 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazo-lium (MTS) assay using Cell Counting Kit-8 (Dojindo Molecular Technologies). IFN-γ production in the supernatants of the same cultures for cell proliferation was determined by using ELISA kits (BD Biosciences).

Results and Discussion

TLR2 stimulation activates Th1 cells to produce IFN-γ

To elucidate the functional significance of TLRs expressed on Th1 cells, Th1 cells were developed and restimulated with each TLR ligand (specific for TLR1–7 and 9) and the cytokine production was examined. Surprisingly, we found that only TLR2 ligands specifically induced IFN-γ production from Th1 cells without TCR stimulation, similar to IL-18 stimulation that synergizes with IL-12 to induce proliferation, survival and IFN-γ production of Th1 cells (Fig. 1*A*) (16). Whereas TLR2 or TLR9 stimulation synergizes with anti-CD3 stimulation of naive T cells and the expressions of TLR2 and TLR9 were not different between Th1 cells and naive T cells as shown by cell surface (TLR2) or intracellular (TLR9) staining (data not shown), this direct stimulation of Th1 cells was induced specifically by TLR2 ligands. Importantly, TLR2-induced IFN-γ production was markedly enhanced in the presence of IL-2 or IL-12 (Fig. 1*D*), which also resembles IL-18 stimulation. We also observed that Pam3 directly induced IFN-γ production under the condition where the expression of the activation marker CD69 decreased almost to the background as in resting cells (Fig. 1*E*). These data suggest that TLR2 induced direct Th1 stimulation irrespective of the activation status before TLR stimulation. TLR2-mediated IFN-γ production was also observed in effector $CD8^+$ T cells that had been stimulated for 1 wk with plate-bound anti-CD3 and anti-CD28 Abs (data not shown). In addition to IFN-γ production, TLR2 stimulation specifically induced up-regulation of the activation markers CD69 and CD70 and enhanced the proliferation and survival of Th1 cells (Fig. 1, *B*, *C*, and *F*). Consistent with the induced proliferation and survival, TLR2 engagement strongly induced the expression of c-Myc and Bcl-X_L, in addition to IFN-γ, which was synergistically up-regulated by IL-2 (Fig. 1*G*).

To confirm that TLR2-mediated Th1 activation is indeed independent of TCR signaling, Th1 cells were treated with CsA, an inhibitor of TCR-mediated calcineurin activation. Whereas CsA inhibited TCR-mediated activation in a dose-dependent manner, it did not affect the TLR2-induced IFN-γ production, confirming that the Th1 cells are directly activated through TLR2 (Fig. 1*H*). These data also indicate that TLR2 stimulation along with IL-2 induces IFN-γ production comparable to that of TCR stimulation.

We next analyzed the intracellular signaling mechanisms responsible for TLR2-mediated direct Th1 activation. It is noteworthy that TLR2 stimulation induced weak and transient activation of NF-κB and MAPKs (JNK, p38, and ERK) in naive $CD4^+$ T cells (data not shown). In contrast, strong and sustained activation of NF-κB and MAPKs was observed in Th1 cells in response to the TLR2 ligand Pam3 but not to a TLR9 ligand CpG (Fig. 1*I*).

Because TLR2 was expressed on the cell surface of Th1 and Th2 cells at similar levels (data not shown), it seemed likely that TLR ligands would directly activate Th2 cells. However, all TLR ligands tested failed to stimulate Th2 cells for IL-4 production and the induction of activation markers (data not shown). We also observed that Pam3 failed to induce strong and sustained activation of NF-κB and MAPKs in Th2 cells, unlike Th1 cells (Fig. 1*H*). These data suggest that pathogen-associated molecular patterns directly induce the activation of Th1 but not Th2 cells to mediate effector function.

TLR2-induced Th1 cell activation is MyD88 and IL-1R-associated kinase 4 (IRAK-4) dependent

Because TLR2 functions as a heterodimer with TLR1 or TLR6 (1), we examined whether the partner TLRs are involved in TLR2-mediated Th1 activation. Whereas IFN-γ production was completely abrogated in *Tlr2*$^{-/-}$ Th1 cells, *Tlr1*$^{-/-}$ Th1 cells exhibited a normal induction of IFN-γ production (Fig. 2*A*) and the up-regulation of activation markers (data not shown) upon stimulation with TLR1/2-specific ligands. These results suggest that Pam3 stimulation and MALP-2 stimulation are mediated through TLR2 but not TLR1.

Because both MyD88 and IRAK-4 are essential for TLR2-mediated cytokine production and the activation of MAPK and NF-κB in the innate responses (13, 14), we examined whether the same signaling pathway is used for TLR-mediated activation of Th1 cells. IFN-γ production upon stimulation with the TLR2 ligands was severely impaired in both *Myd88*$^{-/-}$ and *Irak4*$^{-/-}$ Th1 cells even in the presence of IL-2 (Fig. 2*C*). In *Myd88*$^{-/-}$ or *Irak4*$^{-/-}$ Th1 cells, Pam3 induced impaired the activation of NF-κB as evidenced by IκB degradation and ERK activation (Fig. 2*D*). In contrast, Pam3-induced p38 activation showed delayed kinetics, whereas JNK activation was initially normal but quickly subsided in these mutant Th1 cells (Fig. 2*D*). These data suggest that TLR2-mediated activation signals in Th1 cells involve MyD88/IRAK4-dependent (NF-κB and ERK) and partially MyD88/IRAK4-dependent (JNK and p38) pathways that differ from those for innate immunity. This difference may be attributed to another Toll-IL-1R (TIR)-containing adaptor such as TIRAP (also known as Mal) (17, 18) or to unidentified adaptors, which play a role in the activation of JNK and p38 in Th1 cells.

IL-2/IL-12 augment TLR2-mediated Th1 cell activation through enhanced MAPK activation

To identify the mechanism underlying the synergistic effects of TLR2-mediated Th1 activation by IL-2/IL-12 in the induction of IFN-γ production, we analyzed signaling molecules related to TLR2 and IL-2/IL-12. We found that Pam3-stimulated activation of p38 and JNK was enhanced by IL-2, whereas activation of STAT5, ERK and NF-κB was not changed (Fig. 3*A*).

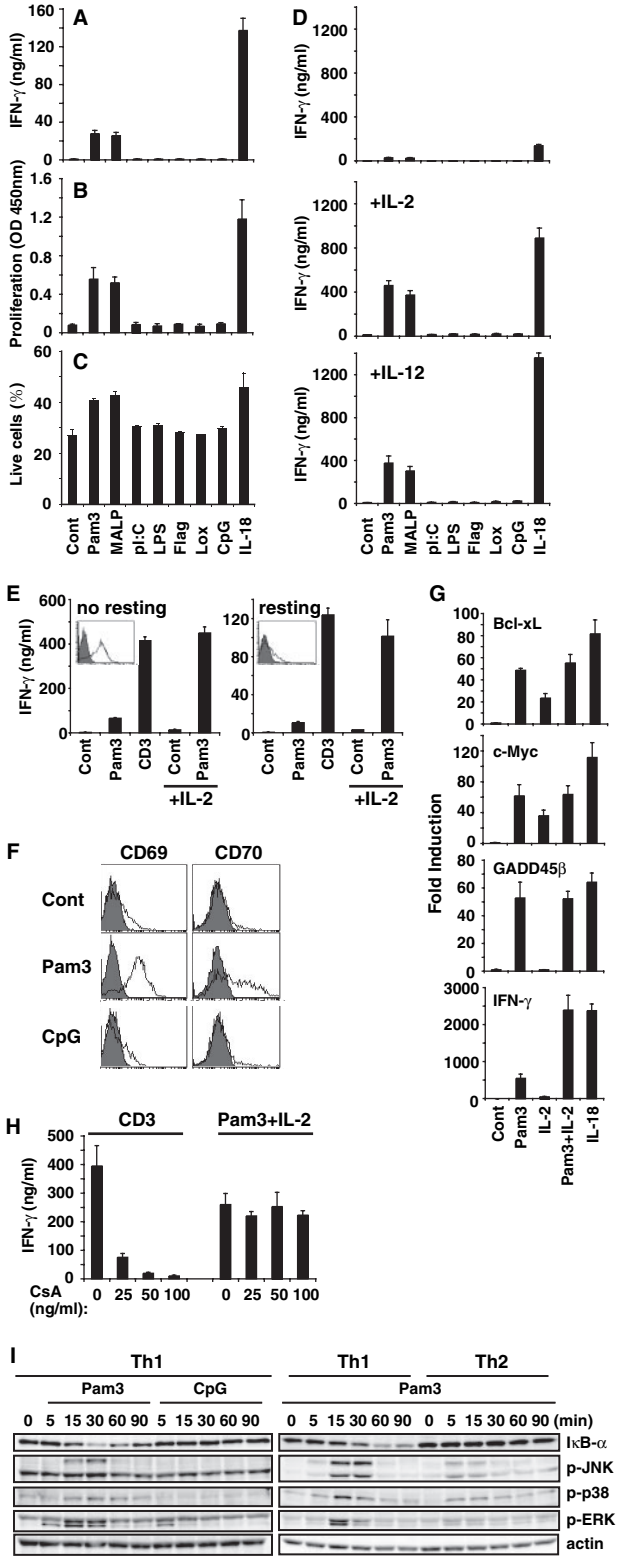

FIGURE 1. Functions of TLRs in the activation of Th1 cells. *A–C*, Activation of Th1 cells by various TLR ligands for the production of IFN-γ (*A*), cell proliferation (*B*), and cell survival (*C*). Naive $CD4^+$ T cells were stimulated with immobilized anti-CD3 mAb in the presence of IL-12 and anti-IL-4 Abs for 7 days (Th1 cells), and cells were left untreated (Cont, control) or stimulated with the indicated TLR ligands (listed below) for 24 h. IFN-γ production, proliferation, and cell survival were assessed using ELISA, an MTS assay, and the exclusion of annexin V- and propidium iodide-positive cells, respectively. *D*, IFN-γ production in Th1 cells in the presence of IL-2 or IL-12. Th1 cells were stimulated with the indicated TLR ligands in the presence or absence of IL-2 or IL-12 for 24 h. *E*, TLR2 mediated direct IFN-γ production from Th1 cells regardless of activation status. Th1 cells were further cultured in the presence (no resting) or absence (resting) of IL-2 and IL-12 for 24 h, followed by stimulation with Pam3 or anti-CD3 mAb for 24 h. Islets show the FACS profile of CD69 expression on the cell surface of the resting and no resting Th1 cells. *F*, Cell surface expression of activation markers on Th1 cells upon stimulation with various TLR ligands. Th1 cells were left unstimulated (filled histograms) or were stimulated (open histograms) with the indicated ligands for 24 h. *G*, Induction of gene expression in Th1 cells upon stimulation with TLR2 ligand. Th1 cells were stimulated with the indicated ligands for 3 h. mRNA levels were analyzed by real-time RT-PCRs. Data represent the relative fold changes in samples normalized to the level of GAPDH mRNA expression. *H*, TLR2-mediated Th1 stimulation is independent of TCR signaling. Th1 cells were treated with the graded concentrations of CsA for 30 min and stimulated with anti-CD3 mAb or Pam3 plus IL-2 for 24 h. *I*, IκB degradation and MAPK activation in Th1 cells (*left*) and Th2 cells (*right*) upon stimulation with Pam3 or CpG. Cell lysates after stimulated for the indicated periods were immunoblotted with the indicated Abs. The ligands used were Pam3 (5 μg/ml) for TLR1 plus TLR2, MALP-2 (MALP) (5 μg/ml) for TLR2 plus TLR6, polyinosinic/polycytidylic acid (pI:C) (100 μg/ml) for TLR3, LPS (5 μg/ml) for TLR4, flagellin (Flag) (5 μg/ml) for TLR5, loxoribine (Lox) (100 μM) for TLR7, CpG (10 μM) for TLR9, and IL-18 (50 ng/ml) for IL-18R.

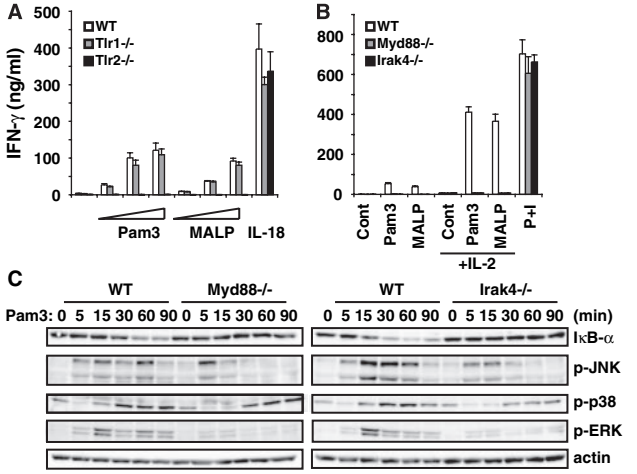

FIGURE 2. Signaling mechanism underlying TLR2-mediated Th1 activation. *A*, TLR2-mediated IFN-γ production in Th1 cells depends on TLR2 signaling, but not on TLR1 signaling. Th1 cells derived from $Tlr1^{-/-}$ (gray bars), $Tlr2^{-/-}$ (filled bars), or wild-type (WT) (open bars) mice were stimulated with graded doses of TLR2 ligands (Pam3 and MALP-2) or IL-18 for 24 h. *B*, TLR2-mediated IFN-γ production in Th1 cells depends on MyD88 and IRAK-4. Th1 cells derived from $Myd88^{-/-}$ (gray bars), $Irak4^{-/-}$ (filled bars), or WT (open bars) mice were stimulated with TLR2 ligands (Pam3 and MALP-2) in the presence or absence of IL-2 for 24 h. C, IκB degradation and MAPK activation in Th1 cells from $Myd88^{-/-}$ and $Irak4^{-/-}$ mice upon Pam3 stimulation. Th1 cell lysates from $Myd88^{-/-}$ and $Irak4^{-/-}$ mice were immunoblotted with the indicated Abs.

Similar to IL-2, Pam3-stimulated activation of p38 was enhanced by IL-12 without affecting ERK or NF-κB activation (Fig. 3*B*), while IL-12 did not costimulate JNK activation (Fig. 3*B*).

To directly test whether the synergistic responses resulted from the activation of the p38 and JNK signal transduction pathway, the effects of the p38 inhibitor SB23580 and the JNK inhibitor SP600125 were analyzed. The enhanced effect of the TLR2 ligand and IL-12 on IFN-γ production in Th1 cells was severely reduced by the p38 inhibitor, whereas the effect of the TLR2 ligand and IL-2 was modestly reduced. (Fig. 3*C*). We also observed that the JNK inhibitor SP600125 (Fig. 3*C*) and the NF-κB essential modulator (NEMO)-binding domain, a

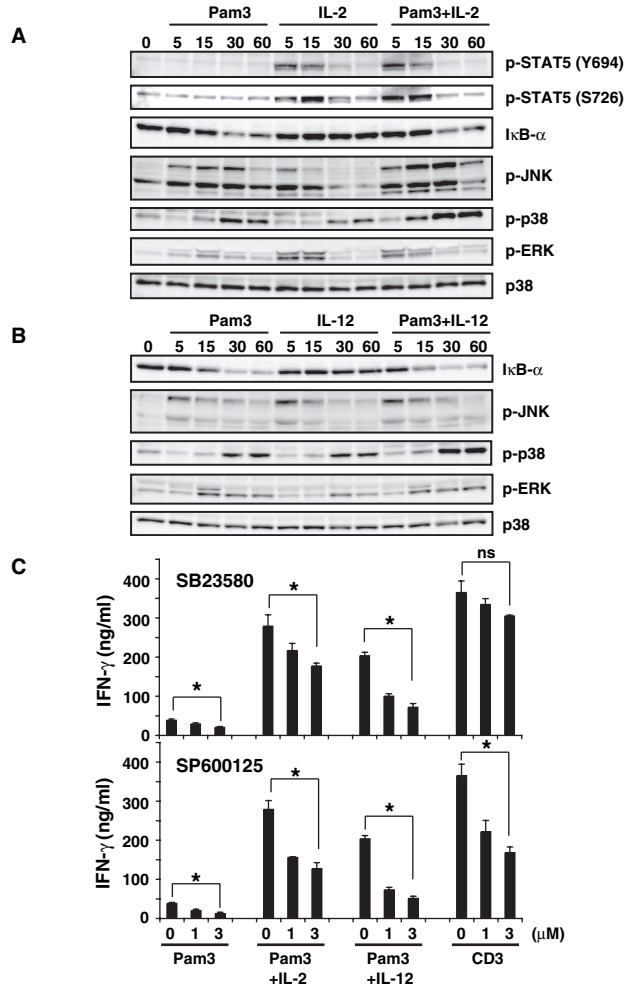

FIGURE 3. Synergistic augmentation of TLR2-mediated Th1 activation by IL-2 or IL-12 through increased MAPK activation. *A*, Augmentation of JNK and p38 activation by TLR2 stimulation in the presence of IL-2. Th1 cells were stimulated with Pam3 (5 μg/ml), IL-2 (10 ng/ml), or both Pam3 and IL-2. Cell extracts were analyzed by Western blotting using the indicated Abs. *B*, Augmentation of p38 activation by TLR2-stimulation in the presence of IL-12. Th1 cells were stimulated with Pam3 (5 μg/ml), IL-12 (10 ng/ml), or both Pam3 and IL-12. Cell lysates were analyzed by Western blots using the indicated Abs. *C*, Inhibition of Th1 activation by a p38 inhibitor. Th1 cells were re-stimulated with Pam3 or anti-CD3 mAb in the presence or absence of different concentrations of the p38 inhibitor SB203580 and the JNK inhibitor SP600125 for 12 h. IFN-γ levels in the supernatants were analyzed by ELISA. *, $p < 0.005$. ns, not significant.

NF-κB inhibitor (data not shown), substantially suppressed IFN-γ production upon stimulation either with Pam3 in the presence or absence of IL-2/IL-12 or with anti-CD3 mAb. In contrast, the ERK inhibitor PD98059 had a minimum effect on the IFN-γ production upon Pam3 stimulation, whereas IFN-γ production upon anti-CD3 mAb stimulation was partially inhibited (data not shown). Collectively, these data suggest that enhanced MAPK activation is critical for the induction of TLR2-mediated Th1 activation by IL-2 or IL-12.

Although costimulatory functions of TLRs expressed on T cells have been described for the activation of naive and memory T cells (4, 9, 10), in this study we describe a direct stimulatory function of TLR2 for Th1 activation. It is widely believed that IFN-γ production in Th1 cells is induced by two types of stimulation: TCR signals and IL-18-mediated signals (plus IL-12). Unlike the costimulatory function of TLR2 on naive T cells, which functions only with TCR stimulation, we have identified TLR2 signals as the third stimulus to induce IFN-γ production from Th1 cells independently of TCR. Similar to IL-18 stimulation, we also found that IFN-γ production induced through TLR2 is strongly enhanced by IL-12 (or IL-2) mainly through the synergistic augmentation of p38 activation, which has been shown to be critical for IFN-γ production (19). Several studies demonstrated that growth arrest and DNA damage inducible (GADD) 45β and GADD45γ induce sustained p38 activation in response to TCR signaling or IL-12 plus IL-18 (20, 21). Because TLR2 induces the expression of GADD45β (Fig. 1*F*) and GADD45γ (data not shown) in Th1 cells, it is likely that GADD45β or GADD45γ is involved in the enhanced activation of p38 by TLR2 signaling and IL-2 or IL-12.

It is noteworthy that cells expressing TLR2 and IL-18R are differentially distributed. IL-18Rα is selectively expressed on the cell surface of Th1 cells, but not Th2 cells, because of the up-regulation of IL-18Rα in response to IL-12 (22). In contrast, TLR2 is constitutively expressed on both Th1 and Th2 cells. Nevertheless, Th2 cells did not respond to any of the tested TLR ligands. Collectively, these data indicate that TLR2 mediates strong Th1 responses via two mechanisms: direct stimulation of Th1 cells to induce effector functions and through the production of IL-12 following the stimulation of APCs. Th1-mediated IFN-γ production has been shown to be critical for protective immunity against several infectious pathogens such as *Listeria monocytogenes* (23) and *Leishmania major* (24). In response to these infections, TLR2-mediated IFN-γ production by Th1 cells might be particularly critical and, therefore, the susceptibility of mice lacking TLR2 in their T cells to these pathogens should be investigated to clarify the in vivo function of TLR2 on T cells.

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Disclosures

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References

1. Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. *Annu. Rev. Immunol.* 21: 335–376.
2. Medzhitov, R. 2001. Toll-like receptors and innate immunity. *Nat Rev Immunol.* 1: 135–145.
3. Caramalho, I., T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury, and J. Demengeot. 2003. Regulatory T cells selectively express Toll-like receptors and are activated by lipopolysaccharide. *J. Exp. Med.* 197: 403–411.
4. Komai-Koma, M., L. Jones, G. S. Ogg, D. Xu, and F. Y. Liew. 2004. TLR2 is expressed on activated T cells as a costimulatory receptor. *Proc. Natl. Acad. Sci. USA* 101: 3029–3034.
5. Gelman, A. E., J. Zhang, Y. Choi, and L. A. Turka. 2004. Toll-like receptor ligands directly promote activated $CD4^+$ T cell survival. *J. Immunol.* 172: 6065–6073.
6. Peng, G., Z. Guo, Y. Kiniwa, K. S. Voo, W. Peng, T. Fu, D. Y. Wang, Y. Li, H. Y. Wang, and R. F. Wang. 2005. Toll-like receptor 8-mediated reversal of $CD4^+$ regulatory T cell function. *Science* 309: 1380–1384.
7. Sutmuller, R. P., M. H. den Brok, M. Kramer, E. J. Bennink, L. W. Toonen, B. J. Kullberg, L. A. Joosten, S. Akira, M. G. Netea, and G. J. Adema. 2006. Toll-like receptor 2 controls expansion and function of regulatory T cells. *J. Clin. Invest.* 116: 485–494.
8. Liu, H., M. Komai-Koma, D. Xu, and F. Y. Liew. 2006. Toll-like receptor 2 signaling modulates the functions of $CD4^+CD25^+$ regulatory T cells. *Proc. Natl. Acad. Sci. USA* 103: 7048–7053.
9. Caron, G., D. Duluc, I. Fremaux, P. Jeannin, C. David, H. Gascan, and Y. Delneste. 2005. Direct stimulation of human T cells via TLR5 and TLR7/8: flagellin and R-848 up-regulate proliferation and IFN-γ production by memory $CD4^+$ T cells. *J. Immunol.* 175: 1551–1557.

10. Cottalorda, A., C. Verschelde, A. Marcais, M. Tomkowiak, P. Musette, S. Uematsu, S. Akira, J. Marvel, and N. Bonnefoy-Berard. 2006. TLR2 engagement on CD8 T cells lowers the threshold for optimal antigen-induced T cell activation. *Eur. J. Immunol.* 36: 1684–1693.
11. Takeuchi, O., S. Sato, T. Horiuchi, K. Hoshino, K. Takeda, Z. Dong, R. L. Modlin, and S. Akira. 2002. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J. Immunol.* 169: 10–14.
12. Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda, and S. Akira. 1999. Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. *Immunity* 11: 443–451.
13. Kawai, T., O. Adachi, T. Ogawa, K. Takeda, and S. Akira. 1999. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 11: 115–122.
14. Suzuki, N., S. Suzuki, G. S. Duncan, D. G. Millar, T. Wada, C. Mirtsos, H. Takada, A. Wakeham, A. Itie, S. Li, et al. 2002. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. *Nature* 416: 750–756.
15. Hemmi, H., O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, and S. Akira. 2000. A Toll-like receptor recognizes bacterial DNA. *Nature* 408: 740–745.
16. Nakanishi, K., T. Yoshimoto, H. Tsutsui, and H. Okamura. 2001. Interleukin-18 regulates both Th1 and Th2 responses. *Annu. Rev. Immunol.* 19: 423–474.
17. Yamamoto, M., S. Sato, H. Hemmi, H. Sanjo, S. Uematsu, T. Kaisho, K. Hoshino, O. Takeuchi, M. Kobayashi, T. Fujita, et al. 2002. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* 420: 324–329.
18. Horng, T., G. M. Barton, R. A. Flavell, and R. Medzhitov. 2002. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* 420: 329–333.
19. Rincon, M., H. Enslen, J. Raingeaud, M. Recht, T. Zapton, M. S. Su, L. A. Penix, R. J. Davis, and R. A. Flavell. 1998. Interferon-γ expression by Th1 effector T cells mediated by the p38 MAP kinase signaling pathway. *EMBO J.* 17: 2817–28u9.
20. Lu, B., A. F. Ferrandino, and R. A. Flavell. 2004. Gadd45β is important for perpetuating cognate and inflammatory signals in T cells. *Nat. Immunol.* 5: 38–44.
21. Chi, H., B. Lu, M. Takekawa, R. J. Davis, and R. A. Flavell. 2004. GADD45β/GADD45γ and MEKK4 comprise a genetic pathway mediating STAT4-independent IFNγ production in T cells. *EMBO J.* 23: 1576–1586.
22. Yoshimoto, T., K. Takeda, T. Tanaka, K. Ohkusu, S. Kashiwamura, H. Okamura, S. Akira, and K. Nakanishi. 1998. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-γ production. *J. Immunol.* 161: 3400–3407.
23. Pamer, E. G. 2004. Immune responses to *Listeria* monocytogenes. *Nat. Rev. Immunol.* 4: 812–823.
24. Louis, J., H. Himmelrich, C. Parra-Lopez, F. Tacchini-Cottier, and P. Launois. 1998. Regulation of protective immunity against *Leishmania major* in mice. *Curr. Opin. Immunol.* 10: 459–464.