

Endotoxin can induce MyD88-deficient dendritic cells to support T_h2 cell differentiation

Tsune yasu Kaisho^{1,2,3}, Katsuaki Hoshino^{1,2}, Tomio Iwabe^{1,4}, Osamu Takeuchi^{1,2}, Teruhito Yasui⁵ and Shizuo Akira^{1,2}

¹Department of Host Defense and

⁵Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan

²SORST, Japan Science and Technology Corporation, Osaka 565-0871, Japan

³RIKEN Research Center for Allergy and Immunology, Osaka 565-0871, Japan

⁴Department of Obstetrics and Gynecology, Tottori University, School of Medicine, Yonago 683-8503, Japan

Keywords: dendritic cells, innate immunity, lipopolysaccharide, T_h1, T_h2, Toll-like receptor

Abstract

Toll-like receptor (TLR) signaling activates dendritic cells (DC) to secrete proinflammatory cytokines and up-regulate co-stimulatory molecule expression, thereby linking innate and adaptive immunity. A TLR-associated adapter protein, MyD88, is essential for cytokine production induced by TLR. However, in response to a TLR4 ligand, lipopolysaccharide (LPS), MyD88-deficient (MyD88^{-/-}) DC can up-regulate co-stimulatory molecule expression and enhance their T cell stimulatory activity, indicating that the MyD88-independent pathway through TLR4 can induce some features of DC maturation. In this study, we have further characterized function of LPS-stimulated, MyD88^{-/-} DC. In response to LPS, wild-type DC could enhance their ability to induce IFN- γ production in allogeneic mixed lymphocyte reaction (alloMLR). In contrast, in response to LPS, MyD88^{-/-} DC augmented their ability to induce IL-4 instead of IFN- γ in alloMLR. Impaired production of T_h1-inducing cytokines in MyD88^{-/-} DC cannot fully account for their increased T_h2 cell-supporting ability, because absence of T_h1-inducing cytokines in DC caused impairment of IFN- γ , but did not lead to augmentation of IL-4 production in alloMLR. *In vivo* experiments with adjuvants also revealed T_h2-skewed immune responses in MyD88^{-/-} mice. These results demonstrate that the MyD88-independent pathway through TLR4 can confer on DC the ability to support T_h2 immune responses.

Introduction

Toll-like receptors (TLR) are type I transmembrane proteins expressed on antigen-presenting cells (APC) including macrophages and dendritic cells (DC), and play critical roles in recognizing microbial components called as pathogen-associated molecular patterns (1,2). For example, TLR4 and TLR9 are essential for recognizing lipopolysaccharide (LPS) from Gram-negative bacteria and bacterial DNA containing CpG motifs respectively (3–5). Upon recognition of these TLR ligands, TLR can induce cytokine production and up-regulation of co-stimulatory molecule expression in APC, thereby conferring on APC the ability to activate adaptive immune responses.

A cytoplasmic adapter protein, MyD88, associates with TLR and is essential for cytokine production in response to TLR ligands including LPS (6–9). However, in response to LPS, MyD88^{-/-} DC can up-regulate surface expression of co-stimulatory molecules and T cell stimulatory activity, indicating that TLR4 signaling can lead to DC maturation in a MyD88-independent manner (10). However, it remains unknown whether MyD88^{-/-} DC are functionally equivalent to wild-type DC.

In this study, we have further characterized T cell stimulatory activity of MyD88^{-/-} DC. LPS could augment the ability of wild-type DC to support T_h1 cell differentiation in allogeneic mixed

lymphocyte reaction (alloMLR). However, LPS could enhance T_H2 cell-supporting ability of MyD88^{-/-} DC. This ability did not depend only on defective production of T_H1 -inducing cytokines such as IL-12 in MyD88^{-/-} DC. These results strongly suggest that TLR4 signaling can induce MyD88^{-/-} DC to support T_H2 cell differentiation through as yet unidentified mechanism.

Methods

Mice

C57BL/6 or BALB/c mice were purchased from SLC (Shizuoka, Japan). TLR4^{-/-} (11), MyD88^{-/-} (12) and IL-18^{-/-} (13) mice were generated as described previously. IL-12^{-/-} mice were generously provided by Dr J. Magram (14). IL-12^{-/-} mice were crossed with IL-18^{-/-} mice to generate mice heterozygous for the IL-12 and IL-18 genes. These mice were intercrossed to create mice lacking both IL-12 and IL-18 (IL-12/18^{-/-} mice). MyD88^{-/-} mice were backcrossed to C57BL/6 or BALB/c mice >6 times. IL-12^{-/-}, IL-18^{-/-} and IL-12/18^{-/-} mice were backcrossed to C57BL/6 mice >6 times. TLR4^{-/-} mice were backcrossed to C57BL/6 mice twice.

Generation of bone marrow (BM)-derived DC

BM DC were generated as described previously (15). Briefly, BM cells were obtained from wild-type (C57BL/6) or mutant mice and plated at 1×10^6 cells/ml in 24-well plates with 10% FCS/RPMI 1640 with 10 ng/ml murine granulocyte macrophage colony stimulating factor (GM-CSF; Genzyme TECHNE, Minneapolis, MN). Every 2 days, non-adherent cells were discarded and the remaining cells were fed with fresh medium containing 10 ng/ml murine GM-CSF. At day 6, loosely adherent cells were harvested by gentle pipeting and cultured with or without 100 ng/ml LPS (derived from *Escherichia coli* O55:B5; Sigma, St Louis, MO) for a further 48 h.

AlloMLR assay

AlloMLR assay was performed as described previously (10). Briefly, unstimulated or LPS-stimulated BM DC (at day 8) were irradiated and co-cultured with $0.5\text{--}1 \times 10^5$ /well of splenic CD4⁺ T cells from BALB/c background mice. CD4⁺ T cells were purified by utilizing MACS with CD4 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). For measuring T cell proliferation, cultures were performed for 3 days and [³H]thymidine was added for the last 15 h. For measuring cytokines, culture supernatants were harvested after 4-day co-culture. Amounts of IL-12 p40, IFN- γ and IL-4 were measured with ELISA kits (Genzyme TECHNE). In some experiments, co-cultures were performed at indicated concentrations of IL-12. IL-12 was kindly provided by Dr H. Tsutsui (Hyogo Medical College, Hyogo, Japan).

Flow cytometry

Cells were first incubated with anti-CD16/32 (2.4G2; BD Biosciences, Mountain View, CA) to block non-specific binding of antibodies to FcR. They were further stained with biotinylated anti-CD40 mAb (3/23; BD Biosciences) for 20 min at 4°C, washed and subsequently developed with streptavidin-phycoerythrin (BD Biosciences). Flow cytometric

analysis was performed using a FACSCalibur (BD Biosciences) with CellQuest software (BD Biosciences).

Immunization with keyhole limpet hemocyanin (KLH)

KLH (Calbiochem, San Diego, CA) was emulsified with complete Freund's adjuvant (CFA) (Iatron, Tokyo, Japan) before injection and 100 μ g/head of KLH-CFA was injected into hind footpads. Nine days later, bilateral popliteal and inguinal lymph node cells were harvested and CD4⁺ cells were purified by utilizing MACS with CD4 microbeads. For measuring T cell proliferation, 1×10^5 CD4⁺ cells were co-cultured with 1×10^5 irradiated C57BL/6 splenocytes at various concentrations of KLH for 3 days. [³H]Thymidine was added for the last 15 h. For measuring cytokines, 5×10^5 CD4⁺ cells were co-cultured with 5×10^5 irradiated C57BL/6 splenocytes for 3 days. Amounts of IFN- γ and IL-4 were measured with ELISA.

Results

LPS-stimulated MyD88^{-/-} DC support T_H2 cell differentiation

In order to evaluate the function of LPS-stimulated BM DC, we have investigated cytokine production from alloMLR (Fig. 1). Wild-type, TLR4^{-/-} or MyD88^{-/-} BM DC at day 6 were untreated or treated with LPS for further 48 h, irradiated and then co-cultured with allogeneic CD4⁺ T cells derived from BALB/c mice. LPS treatment augmented IFN- γ production, but inhibited IL-4 production from alloMLR of wild-type DC. In contrast, LPS-stimulated MyD88^{-/-} DC did not show enhancement of IFN- γ production, but exhibited significant elevation of IL-4 production from their alloMLR. Thus, in response to LPS, wild-type and MyD88^{-/-} DC enhanced their T_H1 and T_H2 cell-supporting ability, respectively. LPS treatment did not modulate either IFN- γ or IL-4 production from alloMLR of TLR4^{-/-} DC, verifying that LPS-induced effects depend on TLR4.

We have next tested whether MyD88 in T cells is also critical for T_H cell differentiation (Fig. 2). To assess this, wild-type or MyD88^{-/-} DC were co-cultured with CD4⁺ T cells from

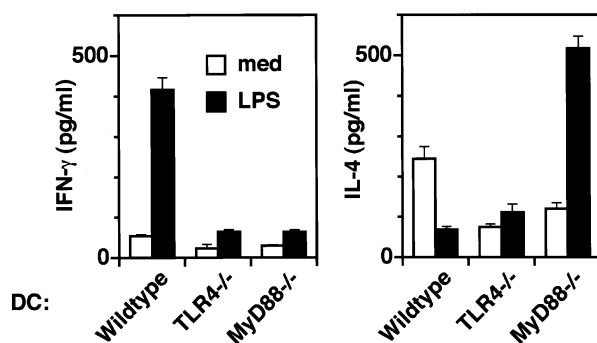


Fig. 1. LPS enhances the ability of wild-type and MyD88^{-/-} BM DC to support T_H1 and T_H2 cell differentiation respectively. Wild-type, TLR4^{-/-} or MyD88^{-/-} BM DC at day 6 were untreated (med) or stimulated with LPS for 48 h, irradiated, plated at 1×10^4 /well and co-cultured with allogeneic BALB/c CD4⁺ T cells for a further 4 days. IFN- γ and IL-4 production in supernatants of alloMLR was measured by ELISA. The data indicate means \pm SD of triplicate samples of one representative experiment.

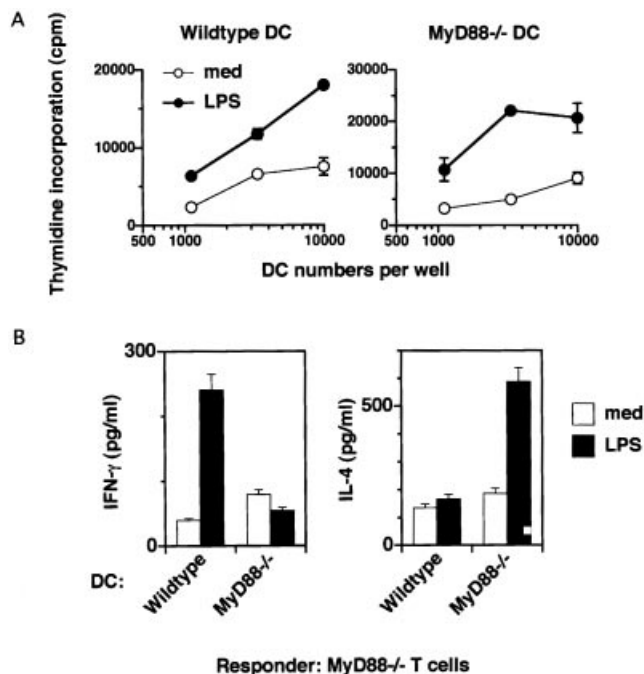


Fig. 2. MyD88^{-/-} T cells can differentiate into Th1 or Th2 cells in a similar manner to wild-type T cells. Wild-type or MyD88^{-/-} BM DC were untreated (med) or stimulated with LPS for 48 h, irradiated, plated at indicated DC numbers per well and co-cultured with allogeneic MyD88^{-/-} CD4⁺ T cells with BALB/c background. (A) Proliferation of allogeneic MyD88^{-/-} CD4⁺ T cells co-cultured with wild-type or MyD88^{-/-} DC. (B) Cytokine production from alloMLR of wild-type or MyD88^{-/-} DC. DC were plated at 1×10^4 /well. The data indicate means \pm SD of triplicate samples of one representative experiment.

MyD88^{-/-} mice with a BALB/c background. In response to LPS, both wild-type and MyD88^{-/-} DC showed enhanced ability to support proliferation of MyD88^{-/-} CD4⁺ T cells (Fig. 2A). LPS-stimulated wild-type DC induced augmentation of IFN- γ production when co-cultured with MyD88^{-/-} CD4⁺ T cells (Fig. 2B). Furthermore, LPS conferred on MyD88^{-/-} DC the ability to augment IL-4 production from their co-culture with MyD88^{-/-} CD4⁺ T cells. Thus, MyD88^{-/-} CD4⁺ T cells can differentiate into Th1 or Th2 cells in a similar manner to wild-type CD4⁺ T cells (Fig. 1) when co-cultured with LPS-stimulated wild-type or MyD88^{-/-} DC. These results suggest that MyD88 in DC, but not in T cells, is critical for decision of Th cell differentiation pathway.

Impaired IL-12 production in alloMLR of LPS-stimulated MyD88^{-/-} DC

IL-12 is produced by APC and act on T cells as a potent activator for Th1 cell differentiation (16). LPS-induced induction of IL-12 is abolished in MyD88^{-/-} macrophages and DC (10, 17). Therefore, we have investigated IL-12 production also from alloMLR. LPS treatment could enhance the ability of wild-type DC to produce IL-12 from alloMLR (Fig. 3A). However, IL-12 production was severely impaired in alloMLR of both TLR4^{-/-} and MyD88^{-/-} DC (Fig. 3A). We have next investigated effects of exogenous IL-12 on alloMLR of LPS-stimulated MyD88^{-/-} DC (Fig. 3B). Exogenous IL-12 significantly

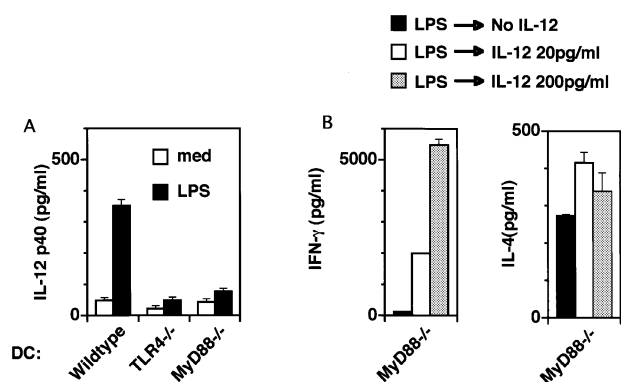


Fig. 3. Exogenous IL-12 cannot suppress IL-4 production from alloMLR of LPS-stimulated MyD88^{-/-} DC. AlloMLR of wild-type, TLR4^{-/-} or MyD88^{-/-} DC was analyzed as described in the legend of Fig. 1. Amounts of IL-12 p40 in the supernatants (A) were measured with ELISA. (B) Effect of IL-12 on allo MLR of LPS-stimulated MyD88^{-/-} DC. First, MyD88^{-/-} DC were stimulated with LPS for 48 h and irradiated. Then, the DC were co-cultured with allogeneic CD4⁺ T cells for a further 4 days in the absence or presence of indicated concentrations of IL-12. The data indicate means \pm SD of triplicate samples of one representative experiment.

enhanced IFN- γ production from alloMLR of LPS-stimulated MyD88^{-/-} DC (Fig. 3B). However, IL-12 could not inhibit IL-4 production from alloMLR of LPS-stimulated MyD88^{-/-} DC (Fig. 3B).

Th cell-supporting ability of DC lacking Th1-inducing cytokines

In addition to IL-12, IL-18 can also stimulate T and NK cells to produce IFN- γ (18, 19). Notably, IL-12 and IL-18 can synergistically enhance IFN- γ production. Therefore, although IL-18 concentration in alloMLR of LPS-stimulated wild-type DC was < 10 pg/ml (data not shown), we have analyzed the function of IL-12^{-/-}, IL-18^{-/-} and IL-12/18^{-/-} DC (Fig. 4). In response to LPS, all mutant DC showed up-regulation of CD40 expression at comparable levels with wild-type DC (Fig. 4A). Furthermore, LPS could augment the ability of not only wild-type, but also all mutant DC to support allogeneic T cell proliferation (Fig. 4B).

Next, Th cell-supporting ability of DC was analyzed (Fig. 4C). LPS treatment could enhance the ability of IL-12^{-/-} and IL-18^{-/-} DC to produce IFN- γ from their alloMLR. However, mutant DC lacking both cytokines did not augment their ability to induce IFN- γ production in response to LPS. Thus, the results indicate that Th1 cell-supporting ability of LPS-stimulated DC requires either IL-12 or IL-18. However, IL-4 production from alloMLR of all LPS-stimulated DC including IL-12/18^{-/-} DC was not elevated, but suppressed. Taken together, although defective production of Th1-promoting cytokines, IL-12 and IL-18, in DC can cause impaired IFN- γ production from alloMLR, it alone cannot result in enhanced IL-4 production as observed in alloMLR of LPS-stimulated MyD88^{-/-} BM DC.

Th2-deviated immune responses of MyD88^{-/-} mice

We have next tested whether MyD88 is critical for Th1/Th2 balance *in vivo*. KLH emulsified with CFA was s.c. injected into footpads of wild-type, TLR4^{-/-} and MyD88^{-/-} mice. Nine days

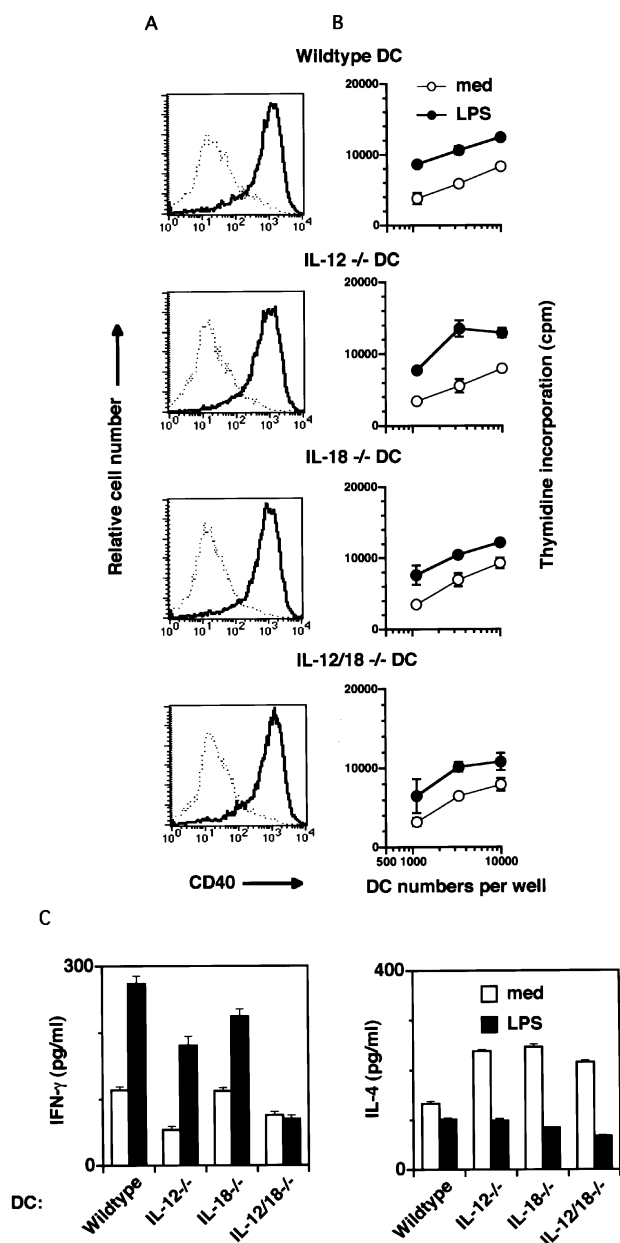


Fig. 4. AlloMLR of DC lacking T_H1 -inducing cytokines. (A) CD40 expression of BM DC in response to LPS. Wild-type, IL-12^{-/-}, IL-18^{-/-} or IL-12/18^{-/-} BM DC at day 6 were untreated (broken lines) or stimulated with LPS (solid lines) for 48 h and analyzed for CD40 expression with flow cytometry. AlloMLR of DC with allogeneic BALB/c CD4⁺ T cells was analyzed as described in the legend of Fig. 2. Data on T cell proliferation (B) and cytokine production (C) are shown. The data indicate means \pm SD of triplicate samples of one representative experiment.

after immunization, CD4⁺ T cells were prepared from draining lymph nodes and their responses to KLH were evaluated (Fig. 5). CD4⁺ T cells from immunized wild-type mice showed KLH-dependent proliferative responses. They also produced IFN- γ in an KLH dose-dependent manner. However, CD4⁺ T cells from immunized MyD88^{-/-} mice showed impaired proliferation and decreased production of IFN- γ in response to KLH.

In contrast, their IL-4 production was prominently enhanced compared with CD4⁺ T cells from immunized control mice. TLR4^{-/-} CD4⁺ T cells also showed impairment of antigen-dependent proliferative responses, which were concomitant with diminished production of both IFN- γ and IL-4. Taken together, not only *in vitro*, but also *in vivo* TLR4 signaling can induce augmented T_H2 cell differentiation in the absence of MyD88.

Discussion

Accumulating lines of evidence indicates that T cell differentiation into T_H1 or T_H2 is regulated not only by T cells themselves but also by DC (20,21). First, maturation stimuli DC receive regulate DC function. For example, TLR ligands such as *E. coli* LPS can stimulate the ability of DC to support T_H1 cell differentiation, while certain microbial products including a soluble extract of the helminth eggs can enhance T_H2 cell-supporting ability of DC (22–24). Second, DC activation status is also important, because DC can prime T_H1 responses soon after stimulation, but the same cells can prime T_H2 responses at later time points (25). Furthermore, the T_H cell-supporting ability also depends on DC subsets (26,27). However, the molecular mechanism involved in the T_H cell-supporting ability of DC still remains largely unknown. In this study we have analyzed the function of LPS-stimulated, MyD88^{-/-} DC. In response to LPS, wild-type DC augmented T_H1 cell-supporting ability, whereas MyD88^{-/-} DC lacked T_H1 , but enhanced instead T_H2 cell-supporting ability. The results indicate that MyD88 is a critical adaptor for regulating T_H1/T_H2 balance.

MyD88^{-/-} DC lack the ability to produce a T_H1 -inducing cytokine, IL-12, in response to LPS (8,10). AlloMLR of LPS-stimulated, MyD88^{-/-} DC also showed impaired production of IL-12 (Fig. 3). Therefore, it is possible that the T_H2 cell-supporting ability of MyD88^{-/-} DC is due to defective production of T_H1 -inducing cytokines. However, this possibility seems unlikely. First, exogenous IL-12 could augment IFN- γ , but could not inhibit IL-4 production from alloMLR of LPS-stimulated, MyD88^{-/-} DC. Furthermore, analysis of mutant DC lacking T_H1 -inducing cytokines, IL-12 and IL-18, also argues against this possibility. While T_H1 cell-supporting ability of LPS-stimulated IL-12/18^{-/-} DC was abolished, their T_H2 cell-supporting ability was not augmented. Proliferative response of allogeneic T cells co-cultured with IL-12/18^{-/-} DC was comparable with that of those co-cultured with wild-type DC (Fig. 4B), excluding the possibility that failure to support T_H2 cell differentiation is not due to diminished proliferation of T cells. These results demonstrate that either IL-12 or IL-18 is required for generating T_H1 cell-supporting ability of DC, but also indicate that defective production of T_H1 -inducing cytokines alone cannot fully account for T_H2 skewing ability of LPS-stimulated MyD88^{-/-} DC.

IL-18 can induce IL-4 production from T cells in the absence of IL-12 (28). Furthermore, LPS-induced IL-18 secretion is independent of MyD88 in liver macrophages (29). These facts suggest the possibility that IL-18 produced by MyD88^{-/-} DC induce allogeneic T cells to differentiate into T_H2 cells. IL-18 plays a significant role in IFN- γ production from alloMLR, which was revealed when alloMLR of IL-12^{-/-} DC was compared with

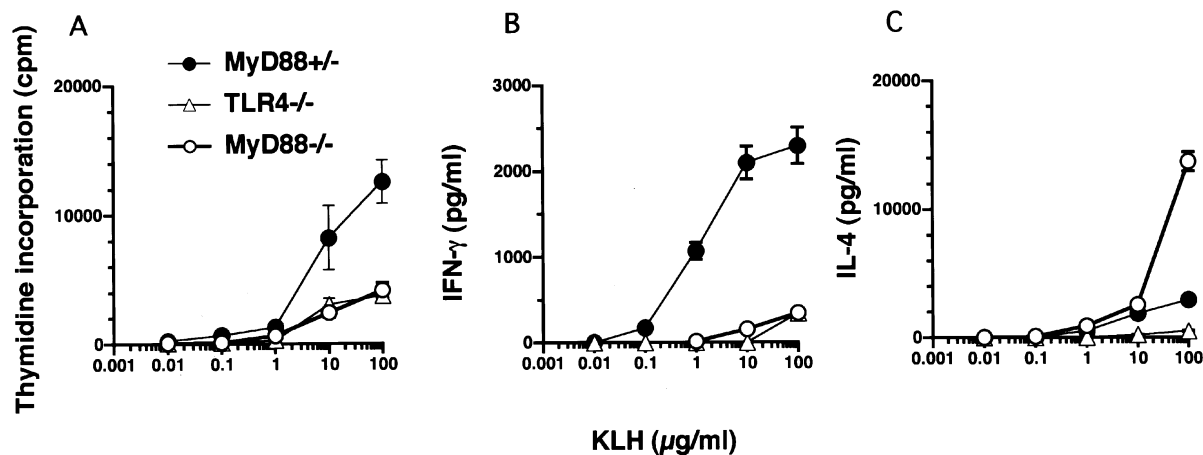


Fig. 5. Immune responses of wild-type, TLR4^{-/-} and MyD88^{-/-} mice. Mice were immunized with KLH-CFA. Nine days later, CD4⁺ T cells were purified from draining lymph nodes and co-cultured with indicated concentrations of KLH in the presence of APC. Data on T cell proliferation (A) and cytokine production (B and C) are shown. The data indicate means \pm SD of triplicate samples of one representative experiment.

that of IL-12/IL-18^{-/-} DC (Fig. 4). However, it is unlikely that IL-18 is involved in IL-4 production from alloMLR of LPS-stimulated MyD88^{-/-} DC. First, alloMLR of LPS-stimulated DC lacking IL-12 did not show increased production of IL-4 (Fig. 4C). Importantly, allogeneic T cells lacking MyD88, which is essential for IL-18 signaling (12), still augmented their IL-4 production co-cultured with MyD88^{-/-} DC treated with LPS (Fig. 2). These results strongly suggest that IL-18 is not involved in enhanced production of IL-4 in alloMLR of LPS-stimulated, MyD88^{-/-} DC.

Schnare *et al.* have also shown that MyD88 deficiency can cause defective T_H1 immune responses (30). Because MyD88^{-/-} T cells retained the ability to differentiate into T_H1 or T_H2 cells, they also argue that MyD88 plays critical roles in APC. The adjuvant they used could not up-regulate costimulatory molecule expression on MyD88^{-/-} DC (30). The adjuvant, CFA, contains killed mycobacteria extract, which is rich in TLR2 and TLR4 ligands. All effects through TLR2 signaling are dependent on MyD88, while TLR4 signaling can induce maturation of MyD88^{-/-} DC (10). Therefore, it can be assumed that their adjuvant contains TLR2 ligands much more abundantly than TLR4 ligands. In this context, impaired immune responses in their study can be ascribed to abrogated maturation of MyD88^{-/-} DC. In the present study, TLR4^{-/-} mice exhibited impaired immune responses to KLH-CFA (Fig. 5), indicating that our adjuvant contains large amounts of TLR4 ligands. As shown in this study, TLR4 signaling can make MyD88^{-/-} DC differentiate into T_H2-inducing DC *in vitro*. Therefore, DC activated through TLR4 can likely contribute to T_H2-skewed immune responses in MyD88^{-/-} mice (Fig. 5).

At present, it remains unknown how TLR4 signaling can actively instruct MyD88^{-/-} DC to support T_H2 cell differentiation. Several genes including IFN-inducible ones such as a member of CXC chemokine, IFN- γ -inducible chemokine, IP-10, have been found to be TLR4 dependent, but MyD88 independent (17). However, none of these molecules can fully account for the T_H2 cell-supporting ability of MyD88^{-/-} DC. Another TLR4-associating molecule, TIRAP/MAL, is likely

involved in expression of such T_H2 cell-supporting molecule(s) (31,32). It should be important to clarify the signaling pathway downstream of TIRAP/MAL.

LPS can instruct MyD88-expressing DC to support T_H1-inducing ability, indicating that the MyD88-dependent pathway is predominant over the MyD88-independent one in wild-type DC. However, in the absence of MyD88, the MyD88-independent pathway makes DC competent to support T_H2 cell differentiation. It remains unknown whether MyD88 expression in DC decreases in response to certain stimuli or whether there exist DC subsets lacking MyD88. In any cases, the balance between MyD88-dependent and -independent pathway in DC should be critical for determining the quality of T_H cell responses.

Acknowledgements

We thank Ms Nana Iwami for excellent technical assistance. We are also grateful to Dr Hiroko Tsutsui for useful discussions and suggestions. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology in Japan, and SORST of Japan Science and Technology Corporation.

Abbreviations

alloMLR	allogeneic mixed lymphocyte reaction
APC	antigen-presenting cell
BM	bone marrow
CFA	complete Freund's adjuvant
DC	dendritic cell
GM-CSF	granulocyte macrophage colony stimulating factor
KLH	keyhole limpet hemocyanin
LPS	lipopolysaccharide
TLR	Toll-like receptor

References

- Medzhitov, R. and Janeway, C. A., Jr. 1997. Innate immunity: the virtues of a nonclonal system of recognition. *Cell* 91:295.
- Akira, S., Takeda, K. and Kaisho, T. 2001. Toll-like receptors:

- critical proteins linking innate and acquired immunity. *Nat. Immunol.* 2:675.
- 3 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. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282:2085.
 - 4 Qureshi, S. T., Lariviere, L., Leveque, G., Clermont, S., Moore, K. J., Gros, P. and Malo, D. 1999. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J. Exp. Med.* 189:615.
 - 5 Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K. and Akira, S. 2000. A Toll-like receptor recognizes bacterial DNA. *Nature* 408:740.
 - 6 Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh, S. and Janeway, C. A., Jr. 1998. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol. Cell* 2:253.
 - 7 Muzio, M., Natoli, G., Saccani, S., Levrero, M. and Mantovani, A. 1998. The human toll signaling pathway: divergence of nuclear factor κ B and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). *J. Exp. Med.* 187:2097.
 - 8 Kawai, T., Adachi, O., Ogawa, T., Takeda, K. and Akira, S. 1999. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 11:115.
 - 9 Muzio, M., Ni, J., Feng, P. and Dixit, V. M. 1997. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* 278:1612.
 - 10 Kaisho, T., Takeuchi, O., Kawai, T., Hoshino, K. and Akira, S. 2001. Endotoxin-induced maturation of MyD88-deficient dendritic cells. *J. Immunol.* 166:5688.
 - 11 Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Takeda, K. and Akira, S. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. *J. Immunol.* 162:3749.
 - 12 Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K. and Akira, S. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 9:143.
 - 13 Takeda, K., Tsutsui, H., Yoshimoto, T., Adachi, O., Yoshida, N., Kishimoto, T., Okamura, H., Nakanishi, K. and Akira, S. 1998. Defective NK cell activity and T_H1 response in IL-18-deficient mice. *Immunity* 8:383.
 - 14 Magram, J., Connaughton, S. E., Warriar, R. R., Carvajal, D. M., Wu, C. Y., Ferrante, J., Stewart, C., Sarmiento, U., Faherty, D. A. and Gately, M. K. 1996. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. *Immunity* 4:471.
 - 15 Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. and Steinman, R. M. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 176:1693.
 - 16 Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251.
 - 17 Kawai, T., Takeuchi, O., Fujita, T., Inoue, J.-I., Muhlradt, P. F., Sato, S., Hoshino, K. and Akira, S. 2001. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. *J. Immunol.* 167:5887.
 - 18 Okamura, H., Tsutsi, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., *et al.* 1995. Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 378:88.
 - 19 Nakanishi, K., Yoshimoto, T., Tsutsui, H. and Okamura, H. 2001. Interleukin-18 regulates both T_H1 and T_H2 responses. *Annu. Rev. Immunol.* 19:423.
 - 20 Banchereau, J. and Steinman, R. M. 1998. Dendritic cells and the control of immunity. *Nature* 392:245.
 - 21 Moser, M. and Murphy, K. M. 2000. Dendritic cell regulation of T_H1 T_H2 development. *Nat. Immunol.* 1:199.
 - 22 Whelan, M., Harnett, M. M., Houston, K. M., Patel, V., Harnett, W. and Rigley, K. P. 2000. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of T_H2 cells. *J. Immunol.* 164:6453.
 - 23 MacDonald, A. S., Straw, A. D., Bauman, B. and Pearce, E. J. 2001. CD8-dendritic cell activation status plays an integral role in influencing T_H2 response development. *J. Immunol.* 167:1982.
 - 24 Pulendran, B., Kumar, P., Cutler, C. W., Mohamadzadeh, M., Van Dyke, T. and Banchereau, J. 2001. Lipopolysaccharides from distinct pathogens induce different classes of immune responses *in vivo*. *J. Immunol.* 167:5067.
 - 25 Langenkamp, A., Messi, M., Lanzavecchia, A. and Sallusto, F. 2000. Kinetics of dendritic cell activation: impact on priming of T_H1 , T_H2 and nonpolarized T cells. *Nat. Immunol.* 1:311.
 - 26 Liu, Y. J., Kanzler, H., Soumelis, V. and Gilliet, M. 2001. Dendritic cell lineage, plasticity and cross-regulation. *Nat. Immunol.* 2:585.
 - 27 Pulendran, B., Palucka, K. and Banchereau, J. 2001. Sensing pathogens and tuning immune responses. *Science* 293:253.
 - 28 Yoshimoto, T., Mizutani, H., Tsutsui, H., Noben-Trauth, N., Yamanaka, K., Tanaka, M., Izumi, S., Okamura, H., Paul, W. E. and Nakanishi, K. 2000. IL-18 induction of IgE: dependence on CD4⁺ T cells, IL-4 and STAT6. *Nat. Immunol.* 1:132.
 - 29 Seki, E., Tsutsui, H., Nakano, H., Tsuji, N., Hoshino, K., Adachi, O., Adachi, K., Futatsugi, S., Kuida, K., Takeuchi, O., Okamura, H., Fujimoto, J., Akira, S. and Nakanishi, K. 2001. Lipopolysaccharide-induced IL-18 secretion from murine Kupffer cells independently of myeloid differentiation factor 88 that is critically involved in induction of production of IL-12 and IL-1 β . *J. Immunol.* 166:2651.
 - 30 Schnare, M., Barton, G. M., Holt, A. C., Takeda, K., Akira, S. and Medzhitov, R. 2001. Toll-like receptors control activation of adaptive immune responses. *Nat. Immunol.* 2:947.
 - 31 Horng, T., Barton, G. M. and Medzhitov, R. 2001. TIRAP: an adaptor molecule in the Toll signaling pathway. *Nat. Immunol.* 2:835.
 - 32 Fitzgerald, K. A., Palsson-McDermott, E. M., Bowie, A. G., Jefferies, C. A., Mansell, A. S., Brady, G., Brint, E., Dunne, A., Gray, P., Harte, M. T., McMurray, D., Smith, D. E., Sims, J. E., Bird, T. A. and O'Neill, L. A. 2001. Mal (MyD88-adaptor-like) is required for Toll-like receptor-4 signal transduction. *Nature* 413:78.