



# Toll-like receptors control activation of adaptive immune responses

Markus Schnare<sup>1,2,\*</sup>, Gregory M. Barton<sup>1,2,\*</sup>, Agnieszka Czopik Holt<sup>1</sup>, Kiyoshi Takeda<sup>3</sup>, Shizuo Akira<sup>3</sup> and Ruslan Medzhitov<sup>1,2</sup>

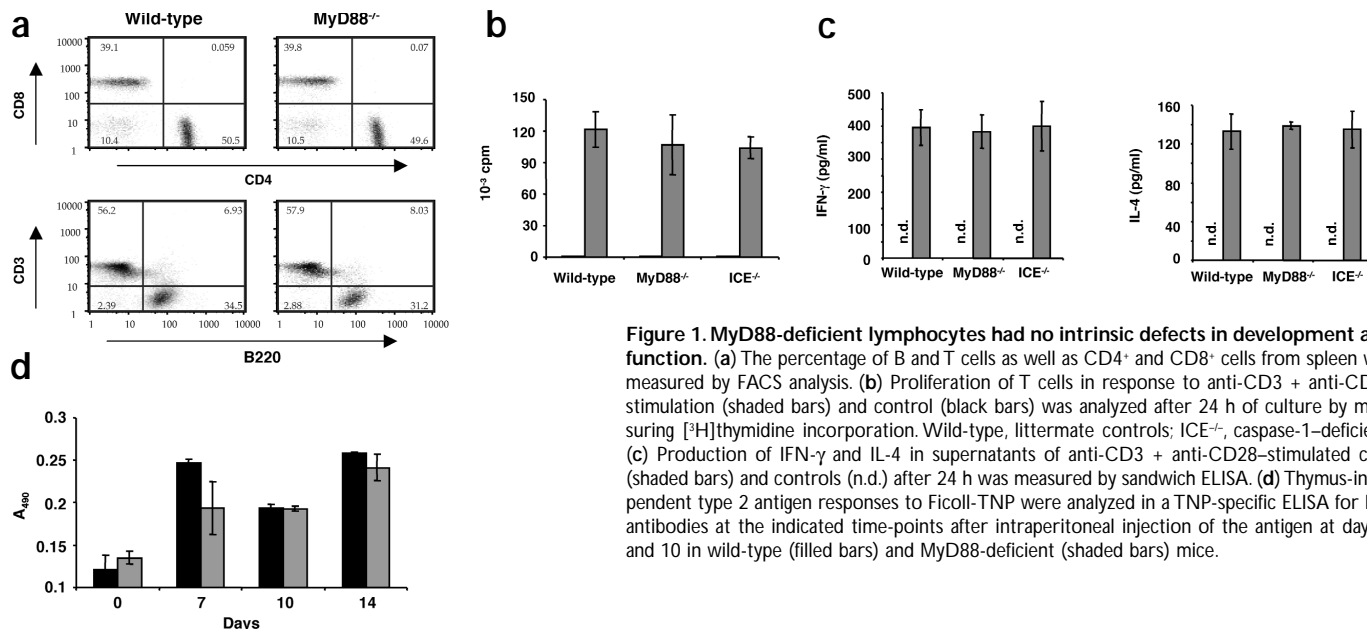
Published online: 4 September 2001, DOI: 10.1038/ni712

Mechanisms that control the activation of antigen-specific immune responses *in vivo* are poorly understood. It has been suggested that the initiation of adaptive immune responses is controlled by innate immune recognition. Mammalian Toll-like receptors play an essential role in innate immunity by recognizing conserved pathogen-associated molecular patterns and initiating the activation of NF- $\kappa$ B and other signaling pathways through the adapter protein, MyD88. Here we show that MyD88-deficient mice have a profound defect in the activation of antigen-specific T helper type 1 (T<sub>H</sub>1) but not T<sub>H</sub>2 immune responses. These results suggest that distinct pathways of the innate immune system control activation of the two effector arms of adaptive immunity.

A family of Toll-like receptors (TLRs) plays an essential role in innate immune recognition in mammalian species and in *Drosophila*<sup>1-4</sup>. The mammalian TLRs are a family of highly conserved, germline-encoded transmembrane receptors that recognize conserved products of microbial metabolism (PAMPs), such as lipopolysaccharide (LPS), peptidoglycan (PGN), lipoteichoic acids (LTA) and other components of microbial cell walls<sup>5,6</sup>. PAMPs are invariant among classes of pathogens and represent a signature of that particular class. One characteristic common to all PAMPs is that they are produced by microorganisms but not by host cells. Therefore, detection of PAMPs by TLRs may represent the immune recognition step that accounts for self-nonself discrimination<sup>4</sup>.

Efficient priming of adaptive immune responses requires not only the

presentation of antigen in the context of major histocompatibility complex (MHC) but also the induction of accessory signals (costimulators and cytokines) on antigen-presenting cells (APCs). TLRs expressed on APCs may regulate these accessory signals through their recognition of PAMPs and consequently control activation of antigen-specific adaptive immune responses<sup>1,3,7</sup>. To test this hypothesis, we examined adaptive immune responses in mice deficient for MyD88, an adapter protein that mediates signal transduction by TLRs<sup>8-10</sup>. MyD88-deficient mice have an impaired ability to signal through TLRs and, consequently, their APCs are unresponsive to TLR ligands<sup>11</sup>. In addition to Toll signaling, MyD88 is required for interleukin 1 (IL-1) and IL-18 signaling<sup>8,12-14</sup>. The protease caspase-1 is required for processing of IL-1 $\beta$  and IL-18 into their biologically active

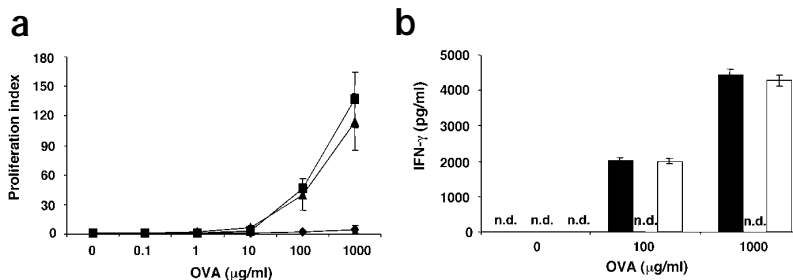


**Figure 1. MyD88-deficient lymphocytes had no intrinsic defects in development and function.** (a) The percentage of B and T cells as well as CD4<sup>+</sup> and CD8<sup>+</sup> cells from spleen was measured by FACS analysis. (b) Proliferation of T cells in response to anti-CD3 + anti-CD28 stimulation (shaded bars) and control (black bars) was analyzed after 24 h of culture by measuring [<sup>3</sup>H]thymidine incorporation. Wild-type, littermate controls; ICE<sup>-/-</sup>, caspase-1-deficient. (c) Production of IFN- $\gamma$  and IL-4 in supernatants of anti-CD3 + anti-CD28-stimulated cells (shaded bars) and controls (n.d.) after 24 h was measured by sandwich ELISA. (d) Thymus-independent type 2 antigen responses to Ficol-TNP were analyzed in a TNP-specific ELISA for IgM antibodies at the indicated time-points after intraperitoneal injection of the antigen at days 0 and 10 in wild-type (filled bars) and MyD88-deficient (shaded bars) mice.

<sup>1</sup>Section of Immunobiology, Yale University School of Medicine and <sup>2</sup>Howard Hughes Medical Institute, New Haven, CT 06520, USA. <sup>3</sup>Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan. \*These authors contributed equally to this work. Correspondence should be addressed to R. M. ([ruslan@yale.edu](mailto:ruslan@yale.edu)).



**Figure 2. Antigen-specific  $T_H1$  cell responses were impaired in MyD88-deficient mice.** Lymph node cells were collected from mice after immunization (day 8) with OVA in CFA. (a) The proliferation of antigen-specific T cells from wild-type (■), MyD88-deficient (◆) and caspase-1-deficient (▲) mice was analyzed by culture of these cells in the presence or absence of different concentrations of antigen (OVA) for 72 h. [ $^3H$ ]thymidine incorporation was then assessed and the proliferation index calculated. (b) IFN- $\gamma$  production was determined by ELISA from supernatants of antigen-stimulated cells from wild-type (filled bars), MyD88-deficient (n.d.) and caspase-1-deficient (open bars) mice. Data are mean  $\pm$  s.d. from three mice per group; they are representative of seven independent experiments. n.d., not detected.



forms<sup>15</sup>. Therefore, we used caspase-1-deficient mice as a control for the deficiency in the function of these cytokines. We have attributed differences between MyD88-deficient and caspase-1-deficient mice to the defect in Toll function.

## Results

### Phenotype of MyD88-deficient lymphocytes

We first examined whether deletion of MyD88 resulted in defects that were intrinsic to lymphocyte development and function. Fluorescence-activated cell sorting (FACS) analysis of the thymus, spleen and lymph nodes showed normal development of B cells and CD4<sup>+</sup> and CD8<sup>+</sup> T cells (Fig. 1a and data not shown). In response to anti-CD3 + anti-CD28 cross-linking or stimulation with allogeneic dendritic cells (DCs) *in vitro*, T cells from MyD88<sup>-/-</sup> mice showed no defect in proliferation and production of interferon- $\gamma$  (IFN- $\gamma$ ) and IL-4 (Fig. 1b,c and data not shown). Caspase-1-deficient mice also showed normal responses in these assays (Fig. 1b,c and data not shown). B cells from MyD88<sup>-/-</sup> and caspase-1<sup>-/-</sup> mice proliferated normally in response to anti-immunoglobulin M (anti-IgM) cross-linking in the presence of IL-4 (data not shown). The production of antigen-specific IgM (Fig. 1d) and IgG3 (data not shown) in response to immunization with the thymus-independent type 2 antigen Ficolll-conjugated trinitrophenol (TNP) was normal. Together, these data showed that MyD88-deficient mice have no obvious intrinsic defects in T and B lymphocyte function.

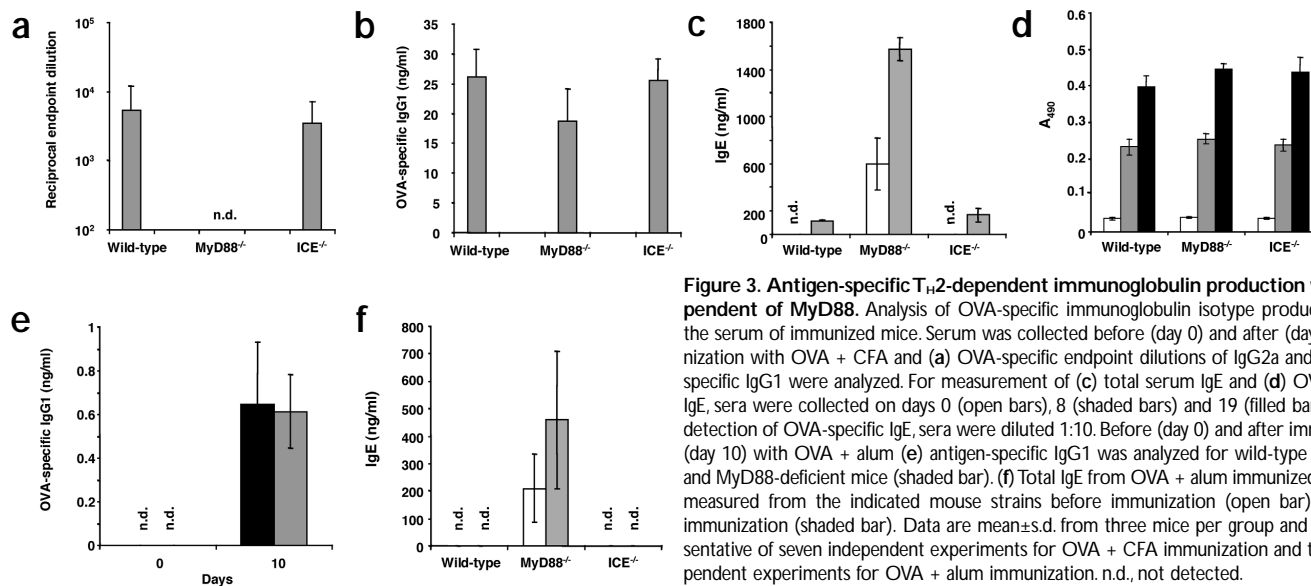
### Antigen-specific T cell responses

We next analyzed the ability of MyD88<sup>-/-</sup> mice to generate antigen-specific immune responses *in vivo*. MyD88<sup>-/-</sup>, wild-type littermate control and

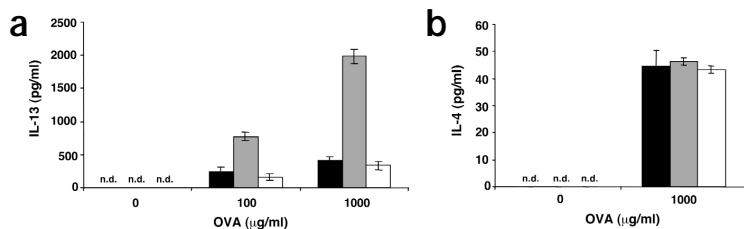
caspase-1<sup>-/-</sup> mice were immunized with ovalbumin (OVA) mixed with complete Freund's adjuvant (CFA); the adaptive immune responses were analyzed 8 and 19 days after immunization. T cells derived from the draining lymph nodes of MyD88<sup>-/-</sup> mice failed to proliferate in response to antigen, which suggested that the antigen-specific priming of T cells was deficient in these mice (Fig. 2a). The T cell response in caspase-1-deficient mice was normal (Fig. 2a). T cells from MyD88<sup>-/-</sup> mice also failed to produce detectable amounts of IFN- $\gamma$  in response to antigen stimulation (Fig. 2b). This was not due to a defect in IL-18 receptor (IL-18R) signaling, as T cells from caspase-1-deficient mice produced normal amounts of IFN- $\gamma$  in the same assay (Fig. 2b).

### TLRs control $T_H1$ responses

Analysis of antigen-specific B cell responses showed that MyD88<sup>-/-</sup> mice did not produce detectable amounts of OVA-specific IgG2a after immunization with OVA in CFA (Fig. 3a). However, MyD88-deficient mice could make OVA-specific IgG1 antibodies, although at lower amounts than the wild-type littermate control mice (Fig. 3b). We also found that MyD88-deficient mice had very high amounts of IgE, even before immunization (Fig. 3c). Immunization consistently resulted in a further increase in total serum IgE (Fig. 3c). This was due to the generation of OVA-specific IgE, which was produced in equal amounts by all three strains of mice (Fig. 3d). The failure of MyD88<sup>-/-</sup> mice to generate an antibody response of  $T_H1$ -dependent IgG2a isotype was consistent with the absence of IFN- $\gamma$  production by the antigen-specific T cells (Fig. 2b). As expected, immunization with OVA mixed with alum, a strong promoter of  $T_H2$  responses, resulted in comparable OVA-specific IgG1 responses in MyD88-deficient and wild-type mice (Fig. 3e). This result was not unexpected because the



**Figure 3. Antigen-specific  $T_H2$ -dependent immunoglobulin production was independent of MyD88.** Analysis of OVA-specific immunoglobulin isotype production from the serum of immunized mice. Serum was collected before (day 0) and after (day 8) immunization with OVA + CFA and (a) OVA-specific endpoint dilutions of IgG2a and (b) OVA-specific IgG1 were analyzed. For measurement of (c) total serum IgE and (d) OVA-specific IgE, sera were collected on days 0 (open bars), 8 (shaded bars) and 19 (filled bars); for the detection of OVA-specific IgE, sera were diluted 1:10. Before (day 0) and after immunization (day 10) with OVA + alum (e) antigen-specific IgG1 was analyzed for wild-type (filled bar) and MyD88-deficient mice (shaded bar). (f) Total IgE from OVA + alum immunized mice was measured from the indicated mouse strains before immunization (open bar) and after immunization (shaded bar). Data are mean  $\pm$  s.d. from three mice per group and are representative of seven independent experiments for OVA + CFA immunization and three independent experiments for OVA + alum immunization. n.d., not detected.



**Figure 4. Increased antigen-specific IL-13 production in MyD88-deficient lymph node cells.** Eight days after immunization, lymph node cells from wild-type (filled bars), MyD88-deficient (shaded bars) and caspase-1-deficient (open bars) mice were cultured with the indicated amounts of antigen (OVA). Production of (a) IL-13 and (b) IL-4 was analyzed by ELISA. Data are mean  $\pm$  s.d. from three mice per group and are representative of three independent experiments.

adjuvant activity of alum was not due to stimulation of TLRs but rather was due to the increased stability and local concentration that alum confers on the antigen. At the same timepoint, antigen-specific IgG2a was undetectable in both mouse strains and total IgE was elevated in MyD88-deficient mice (Fig. 3f).

Because IgE isotype switching is dependent on  $T_H2$ -derived cytokines, we tested whether, under the same conditions, T cells can produce  $T_H2$  cytokines upon antigen stimulation. We found that T cells from MyD88<sup>-/-</sup> mice produced IL-4 and IL-13 in response to antigen restimulation *in vitro* (Fig. 4). The amount of IL-4 secreted by T cells from MyD88-deficient mice was similar to that produced by wild-type and caspase-1<sup>-/-</sup> T cells (Fig. 4b). T cells from MyD88<sup>-/-</sup> mice consistently produced higher amounts of IL-13 compared to T cells from wild-type and caspase-1<sup>-/-</sup> control mice (Fig. 4a). The increased production of IL-13 by T cells in MyD88<sup>-/-</sup> mice was presumably due, at least in part, to the lack of IFN- $\gamma$  production by these cells. Together, these data suggested that the defect in Toll signaling resulted in the failure to generate  $T_H1$ -dependent immune responses, whereas  $T_H2$  responses appeared to be unaffected.

### Effect of MyD88-deficiency on DC maturation

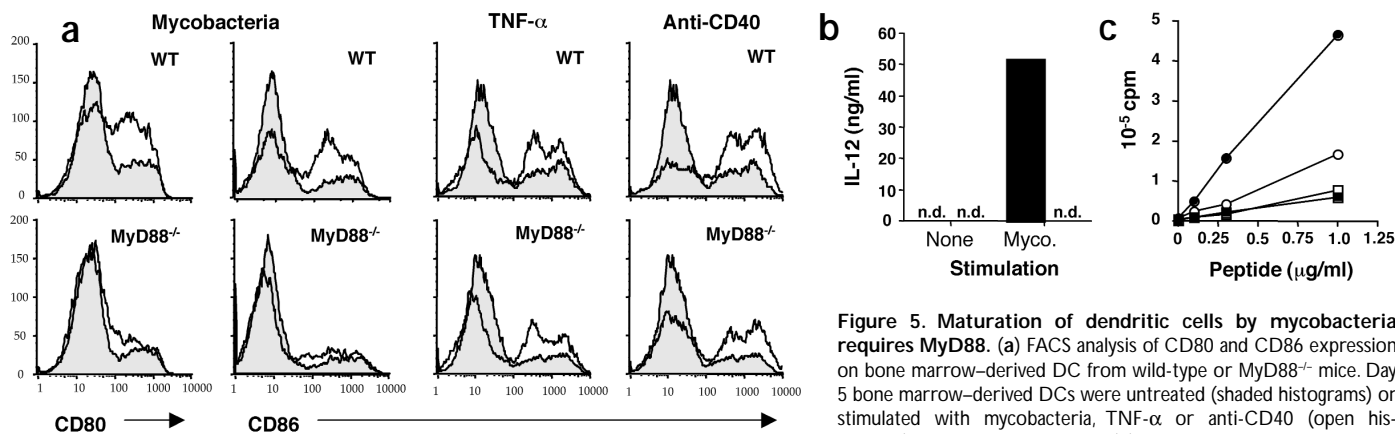
DCs are critical in the activation of naïve T cells and initiation of the adaptive immune responses<sup>16</sup>. Immature DCs express low amounts of CD80, CD86 and MHC class II and they are inefficient in activating naïve T cells. After encountering pathogens, DCs undergo a maturation program that leads to increased expression of MHC and costimulatory molecules, production of cytokines and an increased ability to prime T cells<sup>16</sup>. To define the mechanism that leads to defective  $T_H1$  responses in CFA-immunized MyD88-deficient mice, we examined the maturation of DCs from these mice in response to stimulation with *Mycobacterium tuberculosis* (heat-killed, dried and resuspended in PBS), the immunogenic component of CFA. In contrast to wild-type DCs, MyD88-deficient DCs treated with

mycobacteria did not up-regulate expression of CD80, CD86 or MHC class II and did not produce the inflammatory cytokine IL-12 (Fig. 5a,b and data not shown). In addition, the defect in the maturation of MyD88-deficient DCs after treatment with mycobacteria resulted in a reduced ability to activate naïve CD4<sup>+</sup> T cells (Fig. 5c). MyD88-deficient DCs did up-regulate CD86 in response to TNF- $\alpha$  or CD40 cross-linking (Fig. 5a). This ruled out the possibility that a lack of MyD88 prevented DC maturation in response to any stimuli. Thus, maturation of DCs by the microbial component of CFA was impaired in MyD88-deficient mice, which lead to inefficient priming of T cells.

### Discussion

We have shown that the ancient Toll-IL-1 receptor signaling pathway is essential in the control of adaptive immune responses. The results we obtained with caspase-1-deficient mice acted as a control for the possibility that IL-18 or IL-1 $\beta$  were responsible for the defect in MyD88-deficient mice. Some members of the IL-1 family (IL-1 $\alpha$ , for example) are not dependent on processing by caspase-1. However, it is unlikely that the IL-1R family is directly involved in microbial recognition. In addition, the phenotypes of mice deficient in IL-1R, IL-18 and ST2 are quite different from the one we describe here for MyD88-deficient mice<sup>17-20</sup>. These arguments, as well as our *in vitro* studies with DCs, indicate that defective induction of adaptive immune responses by CFA, in MyD88-deficient mice, was due to the defect in Toll function.

Our results suggest that, first, activation of adaptive immune responses and induction of  $T_H1$  effector responses require Toll-mediated recognition and signaling, whereas  $T_H2$  effector responses appear to be independent of Toll function. Second, at least some adjuvants, including CFA, confer immunogenicity on the otherwise nonimmunogenic antigens by stimulating Toll receptors. Indeed, CFA contains a complex mixture of mycobacterial components, some of which are recognized by different members of



**Figure 5. Maturation of dendritic cells by mycobacteria requires MyD88.** (a) FACS analysis of CD80 and CD86 expression on bone marrow-derived DC from wild-type or MyD88<sup>-/-</sup> mice. Day 5 bone marrow-derived DCs were untreated (shaded histograms) or stimulated with mycobacteria, TNF- $\alpha$  or anti-CD40 (open histograms) for 24 h. WT, wild-type. (b) Quantification of IL-12 production by wild-type (filled bar) and MyD88<sup>-/-</sup> (n.d.) DCs after stimulation with mycobacteria. (c) Mycobacteria-treated MyD88-deficient DCs did not efficiently stimulate T cell proliferation. TCR-transgenic CD4<sup>+</sup> T cells were incubated with fixed wild-type (filled symbols) or MyD88-deficient (open symbols) DCs, treated with (circles) or without (squares) mycobacteria and pulsed with different concentrations of cognate peptide. After 24 h, proliferation was measured, by [<sup>3</sup>H]thymidine incorporation, for an additional 24 h.

with mycobacteria. Supernatants were diluted 1:100. n.d., not detected. (c) Mycobacteria-treated MyD88-deficient DCs did not efficiently stimulate T cell proliferation. TCR-transgenic CD4<sup>+</sup> T cells were incubated with fixed wild-type (filled symbols) or MyD88-deficient (open symbols) DCs, treated with (circles) or without (squares) mycobacteria and pulsed with different concentrations of cognate peptide. After 24 h, proliferation was measured, by [<sup>3</sup>H]thymidine incorporation, for an additional 24 h.



the Toll family, including TLR2 and TLR4<sup>21,22</sup>. In the future it will be important to analyze the role of TLRs in the activation of adaptive immune responses by other adjuvants and by pathogens themselves.

The results presented here support the theory that innate immune recognition is required for the initiation of adaptive immune responses<sup>1</sup>. Tolls and other pattern recognition receptors detect the presence of infection because they evolved to recognize molecular structures that are of distinct microbial origin. Recognition of pathogens by Tolls leads to the induction of accessory signals (costimulators and cytokines) that are necessary for the activation of naïve T cells. We believe that this mechanism accounts for self and nonself discrimination by the immune system because it ensures that only pathogen-specific T cells can be activated. Our data suggest, however, that Toll-mediated recognition may only be relevant for the generation of T<sub>H</sub>1, but not T<sub>H</sub>2, responses, at least under the experimental conditions used in this study. T<sub>H</sub>2 responses may be dependent on other, as yet uncharacterized, pattern-recognition receptors. Alternatively, T<sub>H</sub>2 responses may occur "by default", in the absence of Toll triggering and IL-12 production by DCs. This possibility is consistent with our finding that MyD88-deficiency leads to highly increased production of IgE, presumably in response to environmental antigens. Thus, it appears that immune responses that lead to IgE production can occur independently of Toll signaling. It is tempting to speculate that IgE responses to allergens and multicellular eukaryotic parasites may occur in the absence of Toll triggering because neither allergens nor eukaryotic parasites are associated with PAMPs. Further studies will be required to investigate the possible role of TLRs in the control of allergic responses and to examine whether the observed inverse correlation between the incidence of microbial infections and allergic disorders<sup>23</sup> is related to our observation that a lack of Toll signaling leads to increased IgE responses.

## Methods

**Mice and immunizations.** The mouse strains used were as follows: MyD88-deficient mice and wild-type control littermates (F<sub>2</sub> generations from 129/SvJ × C57/BL6) and caspase-1-deficient mice (F<sub>2</sub> generations from 129/SvJ × C57/BL6). Mice were immunized *via* injections into each hind footpad with 50 µg of OVA (chicken egg albumin Fraction V, Sigma, St. Louis, MO) in 25 µl of saline emulsified 1:1 in CFA containing 1 mg/ml of *Mycobacterium tuberculosis* (H37RA, heat-killed and dried, Sigma) or 50 µg of OVA in 25 ml of saline emulsified in alum (Imject alum, Pierce, Rockford, IL). Eight days after immunization, the draining popliteal lymph nodes were isolated and serum collected from the mice before (day 0) and after immunization (day 8 for immunization with OVA + CFA and days 0 and 10 for immunization with OVA + alum). For the analysis of antigen-specific IgE, as well as total IgE, serum from some immunized mice was also collected after 19 days.

**Lymphocyte proliferation and cytokine production.** Cells from the draining lymph nodes were prepared after immunizing mice with OVA + CFA. Lymph node cells (3 × 10<sup>6</sup>) were cultured in the presence of various amounts of OVA for 72 h. Incorporation of [<sup>3</sup>H]thymidine after a 16-h culture period was determined. The proliferation index was calculated as the quotient of cpm at a given concentration divided by cpm for medium control. For T cell proliferation, 10<sup>6</sup> lymph node cells from unimmunized mice were stimulated with a mixture of monoclonal anti-mouse CD3 + anti-mouse CD28 (both from BD PharMingen, San Diego, CA) for 24 h; [<sup>3</sup>H]thymidine incorporation was analyzed thereafter. Production of IL-4, IL-13 and IFN-γ by lymph node cells into the supernatant was analyzed by standard sandwich ELISA assays, as recommended by the manufacturer (matched antibody pairs were from BD PharMingen and R&D Systems, Minneapolis, MN, respectively).

**Responses to thymus-independent type 2 antigen.** Mice were injected intraperitoneally with 25 µg of Ficol-TNP on days 0 and 10. Serum was collected on days 0 (before immunization), 7, 10 and 14, and analyzed for TNP-specific IgM and IgG3 antibodies; this was done with an ELISA by coating a microtiterplate with bovine serum albumin-conjugated TNP and detecting bound immunoglobulins with specific secondary antibodies (biotinylated anti-mouse IgM and anti-mouse IgG3 were from Southern Biotechnology, Birmingham, AL).

**Determination of immunoglobulin isotypes.** Serum was collected from the mice before and after immunization (days 0 and 8 for immunization with OVA + CFA and days 0 and 10 for immunization with OVA + alum) and OVA-specific IgG1 and IgG2a were analyzed. Total IgE and OVA-specific IgE were analyzed from serum collected at days 0, 8 and 19 and, in the case of immunization with OVA + alum, total IgE was analyzed before (day 0) and after (day 10) immunization. Briefly, 10 µg/ml of OVA was used to coat a microtiterplate and bound immunoglobulin isotypes were detected with specific secondary antibodies (biotinylated

anti-mouse IgG1 and anti-mouse IgG2a were from Southern Biotechnology, biotinylated anti-mouse IgE was from Biosource, Camarillo, CA). Total serum IgE was measured by standard ELISA with the use of sheep anti-mouse IgE as capture antibody (The Binding Site, Birmingham, England) and detection antibody was as described for the OVA-specific IgE ELISA.

**Dendritic cell cultures.** Bone marrow was isolated from femurs and tibia, depleted of erythrocytes and cultured in RPMI 1640 supplemented with 5% fetal calf serum, 10 mM HEPES, 10 mM sodium pyruvate, 50 µM β-mercaptoethanol, 100 U/ml of penicillin, 100 µg/ml of streptomycin and granulocyte-macrophage-colony-stimulating factor. Media were replaced every 2 days. On day 5, DCs were matured with 50–100 µg/ml of *Mycobacterium tuberculosis* resuspended in PBS, 50 ng/ml of TNF-α or 5 µg/ml of anti-CD40 (both from BD PharMingen), which was followed by incubation with goat anti-hamster sera (Caltag, Burlingame, CA) for 24 h to induce maturation. On day 6, cells were collected and incubated on ice with fluorescein isothiocyanate-conjugated anti-CD11c, phycoerythrin (PE)-conjugated anti-CD80 or anti-CD86 and biotinylated anti-I-A<sup>b</sup>, followed by cychrome C-conjugated streptavidin (all from BD PharMingen). Stained cells were analyzed with a FACScan flow cytometer (Becton Dickinson, San Jose, CA). IL-12 production was measured in supernatants by sandwich ELISA with matched antibody pairs (BD PharMingen).

For *in vitro* T cell stimulation assays, day 5 bone marrow DC cultures were treated overnight with 50 µg/ml of mycobacteria in the presence of different concentrations of Eα peptide. After 16–20 h, cells were collected, washed once with PBS, fixed for 5 min in 0.2% paraformaldehyde, washed twice with media and plated at 10,000 cells per well in a 96-well plate. Purified 1H3.1 TCR-transgenic CD4<sup>+</sup> T cells (3 × 10<sup>6</sup>, specific for Eα peptide bound to I-A<sup>b</sup>) were added to each well. CD4<sup>+</sup> T cells were isolated to >95% purity by first incubating 1H3.1 lymph node and spleen cells with anti-CD4 magnetic beads (Miltenyi Biotec), followed by separation with an AutoMACS magnetic sorter (Miltenyi Biotec). After 24 h, proliferation was quantified by measuring the incorporation of [<sup>3</sup>H]thymidine for an additional 12–24 h.

## Acknowledgements

We thank C. A. Janeway and R. A. Flavell for the 1H3.1 TCR-transgenic and caspase-1-deficient mice, respectively. R. M. thanks C. A. Janeway for critical reading of the manuscript and continuous support. Supported by Howard Hughes Medical Institute, NIH (AI44220-01) and Searle (to R. M.) and by Deutsche Forschungsgemeinschaft (to M. S.).

Received 30 April 2001; accepted 2 July 2001.

- Janeway, C. A. Jr Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* **54**, 1–13 (1989).
- Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. & Hoffmann, J. A. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983 (1996).
- Medzhitov, R., Preston-Hurlburt, P. & Janeway, C. A. Jr A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **388**, 394–397 (1997).
- Medzhitov, R. & Janeway, C. A. Jr Innate immunity: the virtues of a nonclonal system of recognition. *Cell* **91**, 295–298 (1997).
- Poltorak, A. et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085–2088 (1998).
- Takeuchi, O. et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* **11**, 443–451 (1999).
- Fearon, D. T. & Locksley, R. M. The instructive role of innate immunity in the acquired immune response. *Science* **272**, 50–53 (1996).
- Medzhitov, R. et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol. Cell* **2**, 253–258 (1998).
- Muzio, M., Natoli, G., Saccani, S., Levrero, M. & Mantovani, A. The human toll signaling pathway: divergence of nuclear factor κB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). *J. Exp. Med.* **187**, 2097–2101 (1998).
- Kawai, T., Adachi, O., Ogawa, T., Takeda, K. & Akira, S. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* **11**, 115–122 (1999).
- Kaisho, T., Takeuchi, O., Kawai, T., Hoshino, K. & Akira, S. Endotoxin-induced maturation of MyD88-deficient dendritic cells. *J. Immunol.* **166**, 5688–5694 (2001).
- Wesche, H., Henzel, W. J., Shillinglaw, W. V., Li, S. & Cao, Z. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* **7**, 837–847 (1997).
- Muzio, M., Ni, J., Feng, P. & Dixit, V. M. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* **278**, 1612–1615 (1997).
- Adachi, O. et al. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* **9**, 143–150 (1998).
- Fantuzzi, G. & Dinarello, C. A. Interleukin-18 and interleukin-1β: two cytokine substrates for ICE (caspase-1). *J. Clin. Immunol.* **19**, 1–11 (1999).
- Banchereau, J. & Steinman, R. M. Dendritic cells and the control of immunity. *Nature* **392**, 245–252 (1998).
- Satoskar, A. R. et al. Enhanced Th2-like responses in IL-1 type 1 receptor-deficient mice. *Eur. J. Immunol.* **28**, 2066–2074 (1998).
- Takeda, K. et al. Defective NK cell activity and Th1 response in IL-18-deficient mice. *Immunity* **8**, 383–390 (1998).
- Townsend, M. J., Fallon, P. G., Matthews, D. J., Jolin, H. E. & McKenzie, A. N. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J. Exp. Med.* **191**, 1069–1076 (2000).
- Hoshino, K. et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J. Exp. Med.* **190**, 1541–1548 (1999).
- Underhill, D. M., Ozinsky, A., Smith, K. D. & Aderem, A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. USA* **96**, 14459–14463 (1999).
- Means, T. K. et al. Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J. Immunol.* **163**, 3920–3927 (1999).
- Erb, K. J. Atopic disorders: a default pathway in the absence of infection? *Immunol. Today* **20**, 317–322 (1999).