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Myeloid Differentiation Factor 88 Is Required for Cross-Priming In Vivo

Deborah Palliser,* Hidde Ploegh,²† and Marianne Boes†‡

We describe a role for myeloid differentiation factor 88 (MyD88) in the induction of functional CTLs in vivo, in response to exogenously administered Ag, using a heat shock fusion protein, hsp65-P1, as a model Ag. CD8 T cells transferred into MyD88-deficient animals produce normal numbers of CD8 effector cells that have normal activation marker profiles after immunization with hsp65-P1. However, these CD8 T cells produced significantly less IFN-γ and showed reduced killing activity. This reduction in activation of functional CTLs appears to be unrelated to Toll-like receptor 4 function, because in vitro hsp65-P1-experienced Toll-like receptor 4-deficient dendritic cells (DCs), but not MyD88-deficient DCs, activated CD8 T cells to a similar extent to wild-type DCs. We identify a cross-presentation defect in MyD88-deficient DCs that, when treated with hsp65-P1 fusion protein, results in surface display of fewer SIYRYYGL/class I MHC complexes. Thus, MyD88 plays a role in the developmental maturation of DCs that allows them to prime CD8 T cells through cross-presentation. The Journal of Immunology, 2004, 172: 3415–3421.

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1 Abbreviations used in this paper: hsp, heat shock protein; DC, dendritic cell; MyD88, myeloid differentiation factor 88; PI, propidium iodide; TLR, Toll-like receptor; WT, wild type.

Materials and Methods

hsp65-P1

hsp65-P1 (StressGen Biotechnologies, Victoria, British Columbia, Canada) was prepared by linking a polypeptide called P1 to the C terminus of...
cytotoxic (baccillus Calmette-Guerin) hsp65 (StressGen Biotechnologies). P1, 26 aa in length, contains the SIYRYGL octapeptide sequence flanked at its C-terminal side by 6 aa from α-ketoglutaraldehyde dehydrogenase and at its N-terminal side by 9 aa from OVA. The specific lysis of K\(^+\) cells transfected with hsp65-P1 or P1 by a CTL clone, whose TCR binds specifically to the SIYRYGL-K\(^+\) complex, indicated that the SIYRYGL sequence can be excised intracellularly and presented with K\(^+\) (6). The production of this hsp65-P1 in Escherichia coli and its purification have been described (6, 21). After passage through a 0.2-μm filter, the protein was stored in sterile solution in PBS at 4°C or frozen at −80°C. The level of LPS in this preparation of hsp65-P1 was 6 EU/mg (by Limulus amebocyte lysate assay; Cape Cod Associates, Falmouth, MA). P1 and SIYRYGL peptides were synthesized by the Massachusetts Institute of Technology Biopolymers Laboratory. Molecular weights were confirmed by amino acid analyses (Massachusetts Institute of Technology Biopolymer Laboratory).

**Mice**

C57BL/6 and C57BL/10 mice were purchased from The Jackson Laboratory (Bar Harbor, ME), C57BL/10ScNc mice were from the National Cancer Institute (National Institutes of Health). MyD88\(^−/−\) mice were kindly provided by S. Akira (Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; Ref. 22). The 2C transgenic animals, expressing a TCR specific for SIYRYGL-K\(^+\), were on a recombinant-activating gene-1-deficient background (20). All mice were maintained in a specific pathogen-free mouse facility. Studies were performed according to institutional guidelines for animal use and care.

**Immunizations**

Naive 2C T cells were isolated from spleen and lymph nodes of 2C recombinant-activating gene mice. The cells were depleted of macrophages and DCs by magnetic sorting, according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, CA), using anti-CD11c- and anti-CD11b-coated microbeads. Purity was in the range of 80–85% (1B2 CD8\(^+\) cells), as judged by FACS analysis using a clonotypic Ab (1B2) specific for the 2C αβ TCR. A total of 5 × 10\(^5\) 2C cells was injected retroorbitally. Three days later, mice were injected s.c. in the scuff of the neck and base of the tail with 50 μg of hsp65-P1, or equimolar amounts of unmodified hsp65 plus peptide (Massachusetts Institute of Technology Biopolymer Lab), in PBS. After 3 days, the frequency of 2C cells (percentage of total progeny iodide (PI)-negative cells that were 1B2\(^+\)) CD44 expression, and their ability to secrete IFN-γ were determined by flow cytometry. These cells were also tested for cytolytic activity (see below).

**Flow cytometry**

Fluorochrome-conjugated Abs to CD8\(^a\), CD44, CD69, CD11c, CD38, ICAM-1, I-A\(^b\), and B7.2 were purchased from BD Biosciences (San Jose, CA). Y3 cross-reacts with MHC class I from H-2b (K\(^b\)) and H-2k haplotypes and is affinity purified from culture supernatants from the Y3 hybridoma (obtained from American Type Culture Collection, Manassas, VA) and labeled with fluorescein using FITC (Pierce, Rockford, IL). The clonotypic Ab 1B2, specific for the 2C TCR (23), was isolated from supernatants of cultured 1B2 cells, purified by protein G affinity chromatography, and conjugated to FITC. In addition to the specific Abs, purified anti-FcR Ab (anti-CD16; BD Biosciences) was added to each sample to minimize nonspecific Fc-mediated binding to DCs. Cells were stained in PBS containing 0.5% BSA and 0.1% NaN\(_3\), and at least 20,000 live cells (PI-negative) were acquired on a FACSCalibur (BD Biosciences). Analysis was conducted using CellQuest software (BD Biosciences). Binding of hsp65-P1 to DCs was measured by incubation with FITC-conjugated hsp65-P1 at 4°C in PBS containing 0.5% BSA and 0.1% NaN\(_3\) for 30 min.

**IFN-γ assays**

IFN-γ was measured using a kit from Miltenyi Biotec. Briefly, 1 × 10\(^6\) cells were restimulated with PMA (25 ng/ml) and ionomycin (500 ng/ml) for 2 h. After the cells were washed, they were resuspended in cold medium containing mouse IFN-γ catch reagent on ice. Following an additional 45-min incubation at 37°C, cells were washed with mouse IFN-γ detection reagent, in addition to 1B2 and anti-CD8\(^a\) (BD Pharmingen, San Diego, CA), and analyzed by FACS, gating on PI-negative cells.

**Cell preparation and culture**

DCs were generated, as described (24). In brief, bone marrow was flushed from the femur and tibia, RBC were lysed, and the remaining cells were cultured at 10\(^7\) cells/ml in RPMI 1640 medium (supplemented with 10% heat-inactivated FCS, 2 mM l-glutamine, 10 mM HEPES, 50 μM 2-ME, penicillin, and streptomycin) containing 20 ng/ml murine GM-CSF (R&D Systems, Minneapolis, MN). The medium was replaced on days 2 and 4, and on day 6 the cells (immature DCs) were harvested. The DCs were isolated by positive sorting (MACS) using anti-CD11c Ab (purity >95%), conducted according to the manufacturer’s instructions (Miltenyi Biotec). The clone L3.100 was derived from 2C TCR transgenic mice (25). It was maintained by weekly stimulation with irradiated P815 (L\(^+\)) cells. The clone was used in assays 6–9 days following stimulation.

**In vitro Ag presentation assay**

Purified DCs derived from bone marrow were plated at 4 × 10\(^5\) cells/well of a 96-well plate in RPMI 1640 medium. Protein or peptide was added at appropriate concentrations to each well. After 2-h incubation at 37°C, cells were fixed with 0.5% paraformaldehyde for 15 min at room temperature. The cells were then washed extensively with complete medium. A total of 10\(^3\) 2C cells, either mouse lymph node or the CTL clone L3.100, was then added to each well, and the plate was incubated for 18 h, 37°C. We then assessed the activation of 2C T cells either by FACS (CD69 expression), or by ELISA (IFN-γ production).

**IFN-γ ELISA**

IFN-γ was measured using a kit from eBioscience (San Diego, CA), according to manufacturer’s specifications. Briefly, supernatants of DC/2C T cell cultures were collected after 18 h of cocultures and stored at −80°C. Plates were coated with purified anti-mouse IFN-γ (clone XMG1.2). After blocking with normal mouse serum, undiluted supernatants were incubated for 2 h (room temperature), and the assay was developed using a biotin-conjugated anti-mouse IFN-γ (clone R4-6A2) and streptavidin.

**Cytolytic T cell assays**

\(^{3}H\)-Cr-labeled T2\(^K\) cells were used as target cells. They were incubated with effector cells derived from fusion protein-injected mice for 4 h in the presence or absence of SIYRYGL (1 μM). Specific lysis was calculated as follows: ((experimental counts − spontaneous counts)/(total counts − spontaneous counts)) × 100. Control wells, from which the SIYRYGL peptide was omitted, had background specific lysis of <10%; the background values were subtracted from the results shown. T2\(^K\) cells were a generous gift from P. Cresswell, Yale University School of Medicine (New Haven, CT).

**Results**

Hsp65 fusion proteins induce a CTL response in mice without the need for adjuvant (6). The fusion protein used in this study consists of polypeptide P1 fused to the C terminus of mycobacterial hsp65. The P1 segment itself is a composite of OVA (OVA 251–257) and α-ketoglutaraldehyde dehydrogenase (6). The polypeptide P1 includes the octapeptide SIYRYGL, which when complexed with H2\(^K\) is a strong agonist for the TCR of 2C T cells (20). The generation of these hsp (6), in mice deficient in a major TLR adaptor protein, MyD88.

We analyzed MyD88-deficient and wild-type (WT) mice for their ability to cross-present hsp65-P1 fusion protein in vivo. CD8 T cells were purified from lymph node cells of 2C transgenic mice (>98% purity) to exclude the transfer of APCs from 2C mice, and were adoptively transferred into MyD88\(^−/−\) or WT mice (5 × 10\(^6\) cells, by i.v. injection). Three days later, mice were immunized with either hsp65 + P1 peptide or hsp65-P1 fusion protein (s.c. immunization, 50 μg), or were left untreated. After another 3 days, draining lymph nodes were isolated and analyzed for 2C T cell expansion and activation (Fig. 1, A and B). The 2C T cell effector function was determined by IFN-γ secretion by 2C T cells as well as their ability to kill appropriate target cells by CTL assay (Fig. 1, C and D).

For both WT and MyD88-deficient mice, the mixture of hsp65 and P1 (hsp65 + P1) failed to evoke expansion of the transferred
The ability of naive precursors to differentiate into CTL effectors was analyzed further by IFN-γ secretion, a cytokine produced by effector CD8 T cells. Immunization with hsp65-P1 induced high levels of IFN-γ by 2C T cells that had been transferred into WT mice. In contrast, immunization with hsp65-P1 in MyD88-deficient mice resulted in less IFN-γ production by transferred 2C T cells (*p < 0.007). As expected, the mixture of hsp65 + P1 failed to induce IFN-γ in either strain of mice (Fig. 1C). When assayed for cytolytic activity, 2C T cells transferred into WT animals showed significantly higher levels of cytolytic activity than 2C T cells transferred into MyD88-deficient recipients (Fig. 1D). Therefore, immunization with hsp65-P1 results in cross-presentation of SIYRYGL. This cross-presentation leads to proliferation and CD44 up-regulation by 2C T cells at comparable levels between WT and MyD88-deficient mice. However, in the MyD88-deficient mouse, this level or mode of cross-presentation is not sufficient for the generation of fully differentiated CTL.

DCs are more effective than macrophages at presenting processed hsp fusion protein to naive CD8 2C T cells (6). We therefore focused on the potential role of MyD88 in cross-presentation by DCs. Compared with WT, cytokine production by DCs is altered when MyD88-deficient DCs are stimulated with various pathogens (16). Many different molecules are required to ensure optimal T cell activation in response to a cross-presented Ag. What are the possible factors that play a role in cross-presentation, other than DC cytokine profiles, that are affected by absence of MyD88? We first examined whether binding and uptake of hsp65-P1 by DCs are influenced by the absence of MyD88. For all the in vitro assays, we used bone marrow-derived DCs. Although bone marrow-derived DCs may not be fully equivalent to the DCs involved in cross-priming in vivo, we used them, as they are a homogenous population of highly purified DCs. DCs purified from mouse tissues tend to consist of a highly variable collection of DC populations that also vary in activation status. In addition, it is currently unknown which DC population is responsible for cross-priming in vivo, and various populations have been implicated (for review, see Ref. 26). FITC-conjugated hsp65-P1 was added to DCs cultured from bone marrow of MyD88-deficient and WT mice (40 min, on ice). Equivalent levels of binding of labeled hsp65-P1 by WT and MyD88-deficient DCs were observed (Fig. 2A). Uptake of labeled hsp65-P1 (12.5 µg/ml) was also measured (30 min, 37°C), and occurred with similar or more rapid kinetics by MyD88-deficient DCs compared with WT DCs (Fig. 2B). We conclude that the absence of MyD88 does not impair the ability of DCs to bind and expand in lymph nodes or acquire an Ag-experienced surface phenotype.

FIGURE 1. Adoptive transfer of naive 2C T cells into MyD88-deficient mice results in defective CTL activation. A total of 5 x 10⁴ purified naive 2C T cells was adoptively transferred by retro-orbital injection into either B6 or MyD88−/− recipients. Three days later, mice were injected with either nothing, a mix of hsp65 plus P1, or hsp65-P1. All injections were administered in saline, without added adjuvants. After an additional 3 days, draining lymph nodes were removed and analyzed for the following: A, expansion of transferred 2C T cells; B, expression of CD44; C, IFN-γ production; D, CTL killing ability. Values of p by unpaired two-tailed t test comparing B6 and MyD88-deficient mice injected with hsp65-P1 are shown. * Indicates statistically significant difference. Results shown are representative of four experiments, with each group containing three to five mice.

2C T cells in the draining lymph nodes, and no up-regulation of the activation marker CD44 was seen. In contrast, 2C cells transferred into MyD88-deficient or WT hosts immunized with the fusion protein hsp65-P1 showed robust expansion and an Ag-experienced phenotype, characterized by high levels of CD44 (Fig. 1, A and B). Thus, the MyD88 gene product is not required for 2C T cells to cross-present hsp fusion protein to naive CD8 T cells (6). We therefore focused on the potential role of MyD88 in cross-presentation by DCs. Compared with WT, cytokine production by DCs is altered when MyD88-deficient DCs are stimulated with various pathogens (16). Many different molecules are required to ensure optimal T cell activation in response to a cross-presented Ag. What are the possible factors that play a role in cross-presentation, other than DC cytokine profiles, that are affected by absence of MyD88? We first examined whether binding and uptake of hsp65-P1 by DCs are influenced by the absence of MyD88. For all the in vitro assays, we used bone marrow-derived DCs. Although bone marrow-derived DCs may not be fully equivalent to the DCs involved in cross-priming in vivo, we used them, as they are a homogenous population of highly purified DCs. DCs purified from mouse tissues tend to consist of a highly variable collection of DC populations that also vary in activation status. In addition, it is currently unknown which DC population is responsible for cross-priming in vivo, and various populations have been implicated (for review, see Ref. 26). FITC-conjugated hsp65-P1 was added to DCs cultured from bone marrow of MyD88-deficient and WT mice (40 min, on ice). Equivalent levels of binding of labeled hsp65-P1 by WT and MyD88-deficient DCs were observed (Fig. 2A). Uptake of labeled hsp65-P1 (12.5 µg/ml) was also measured (30 min, 37°C), and occurred with similar or more rapid kinetics by MyD88-deficient DCs compared with WT DCs (Fig. 2B). We conclude that the absence of MyD88 does not impair the ability of DCs to bind and present hsp fusion protein to naive CD8 T cells.
endocytose the hsp65-P1 fusion protein. We have observed that DCs from WT and MyD88<sup>−/−</sup> mice are also equally efficient at endocytosis of fluorescein-labeled OVA (27).

As binding and endocytosis of hsp65-P1 by WT and MyD88-deficient DCs were similar, we examined whether Ag presentation of hsp65-P1-derived SIYRYYGL on class I MHC is affected. DCs were allowed to endocytose hsp65-P1 at various concentrations for 2 h and were fixed, and naive 2C lymph node cells were added (overnight, 37°C). Activation of 2C T cells was then assessed by expression of the early activation marker CD69 (Fig. 3A). MyD88-deficient DCs activated fewer 2C T cells, as measured by up-regulation of CD69. DCs lacking TLR4<sup>−/−</sup> obtained from C57BL/10ScNCr mice (28) show no such reduction compared with WT controls (C57BL/10) (Fig. 3B). These results suggest a role for MyD88 in cross-presentation of SIYRYYGL/class I MHC unrelated to the function of TLR4. Although these results suggest that the difference in 2C activation observed is due to the formation of fewer peptide/MHC I complexes on the surface of MyD88-deficient DC, the level of peptide/MHC I complex formation is not being measured directly.

To address whether fewer peptide/class I complexes are displayed on MyD88-deficient DCs, we repeated the assay using both naive 2C cells and a 2C CTL clone, L3.100, which requires only TCR ligation to elicit a response (20). In addition, we titrated in SIYRYYGL peptide to determine whether MHC I levels on WT and MyD88-deficient DC were comparable. Both hsp65-P1 and exogenous SIYRYYGL peptide were analyzed for their ability to activate these naive and CTL effector 2C T cells.

Exogenous SIYRYYGL peptide was added to MyD88-deficient and WT DCs (5 × 10<sup>−2</sup>–10<sup>−11</sup>M) for 2 h, then fixed (as above), and either naive 2C or 2C CTL L3.100 cells were added (18-h culture, 37°C). The 2C T cells, either naive or L3.100, were activated in a comparable manner by WT and MyD88-deficient DCs (Fig. 4A). To activate an equivalent number of the 2C CTL L3.100 when compared with naive 2C cells, 5- to 10-fold less SIYRYYGL peptide is required, demonstrating the increased sensitivity of the 2C L3.100 CTL clone (Fig. 4A). In addition, IFN-γ production was measured from the supernatants of both naive 2C and 2C CTL L3.100 cells (Fig. 4C). WT and MyD88-deficient DCs were comparable in their ability to activate 2C CTL clone cells in the presence of exogenously added SIYRYYGL peptide. Naive 2C T cells

![FIGURE 3. SIYRYYGL/Class I complex formation is impaired in DCs derived from MyD88-deficient mice, but not in DCs from TLR4-deficient mice. A and B, For Ag presentation assays, 4 × 10<sup>5</sup> DCs were incubated with various concentrations of hsp65-P1. After 2 h of incubation, the cells were fixed and washed extensively. Naive 2C cells were added and 18 h later assayed for activation using the cell surface marker CD69 (A, B6 and MyD88<sup>−/−</sup> DC; B, B10 and ScNCr DC). Only PI-negative cells were analyzed. Experiments are representative of three.](http://www.jimmunol.org/)

![FIGURE 4. Activation of costimulation-dependent naive 2C T cells and a costimulation-independent 2C CTL clone is impaired upon incubation with hsp65-P1-treated MyD88-deficient DCs. The assay was set up as in Fig. 3. For A and C, SIYRYYGL was added to DCs for 2 h before fixation. For B and D, hsp65-P1 was added to DCs for 2 h before fixation. Upper panel, SIYRYYGL; lower panel, hsp65-P1. A and B, CD69 up-regulation; C and D, IFN-γ production.](http://www.jimmunol.org/)
were not fully activated by SIYRYYGL addition, as demonstrated by decreased ability to produce IFN-γ (Fig. 4C). The low level of IFN-γ production by naive 2C T cells may reflect the fact that naive T cells require the ligation of costimulatory molecules for complete activation, which in the absence of an inflammatory stimulus may not be sufficiently expressed on the DCs (29). There was no significant difference between MyD88-deficient and WT DCs to activate 2C T cells when treated with exogenously added SIYRYYGL peptide. We conclude that MyD88 is unlikely to affect the display of peptide-receptive class I MHC molecules at the cell surface.

The ability to restimulate 2C cells by MyD88-deficient DCs is normal when exogenous SIYRYYGL peptide is provided, but is MyD88 required to generate SIYRYYGL/class I complexes derived from hsp65-P1 fusion protein? The activation of naive 2C and 2C CTL L3.100 T cells was measured after uptake of hsp65-P1 (20, 10, or 5 μg/ml, 18 h at 37°C). Activation of both naive 2C T cells and 2C CTL L3.100 T cells was impaired, as assessed by their ability to up-regulate the activation marker CD69 (Fig. 4B). We also determined the level of secreted IFN-γ. The 2C CTL L3.100 T cells that were activated by WT DCs produced more IFN-γ than 2C T cells activated by MyD88-deficient DCs (Fig. 4D). Thus, the activation of both the naive 2C T cells and the 2C CTL L3.100 via cross-presentation of SIYRYYGL derived from hsp65-P1 fusion protein is less effective in the absence of MyD88.

As mentioned above, the impaired activation of naive 2C T cells (Figs. 3 and 4) could be explained by a decrease in expression of costimulatory molecules on the MyD88-deficient DCs. We therefore analyzed the expression of known costimulatory molecules on WT and MyD88-deficient DCs after uptake of hsp65-P1 fusion protein. Upon activation of DCs, the expression of a number of activation markers, such as ICAM-1, I-Aα, Kβ, CD38, and CD86, is increased. When we compared DCs from WT animals with DCs from MyD88-deficient animals, the relative increase in expression of these markers was comparable (Fig. 5). Thus, MyD88 is not involved in the cell surface expression of these costimulatory molecules for cross-presentation of SIYRYYGL from hsp65-P1 fusion protein. It cannot be ruled out that other, as yet unidentified cell surface molecules could show differences in expression and thus play a role.

DCs can adjust their Ag presentation machinery during maturation, as was shown using in vitro culture systems (17–19). In this study, we examined the requirement for MyD88 in H2-Kb-restricted cross-presentation of SIYRYYGL peptide derived from hsp65-P1. Injection of hsp65-P1 resulted in similar levels of SIYRYYGL-specific expansion of adoptively transferred 2C T cells (on C57BL/6 background) and similar levels of CD44 surface expression in WT and MyD88-deficient recipients (Fig. 1, A and B). However, in MyD88-deficient animals, effector functions of these CD8 T cells were impaired, as assessed by their ability to produce IFN-γ and acquire cytolytic capacity (Fig. 1, C and D).

To address how MyD88 is involved in cross-presentation, bone marrow-derived DCs were treated with SIYRYYGL peptide or hsp65-P1, followed by incubation with 2C T cells (either naive or a 2C CTL clone). Compared with activation of naive CD8 T cells, the CTL clone was more readily activated due to its lack of requirement for costimulation. Indeed, naive 2C T cells required at least 5-fold more added peptide for activation, and produced little IFN-γ compared with cloned 2C CTL cells. MyD88-deficient DCs were no less competent than WT DCs in activating 2C T cells when exogenous peptide was added, thereby excluding a role for MyD88 in transport of class I MHC molecules to the cell surface (Fig. 4, A and C). Incubation of MyD88-deficient DCs with hsp65-P1 resulted in decreased activation of naive 2C T cells and cloned 2C CTLs when compared with WT DCs (Fig. 4B). The production of IFN-γ was also diminished in the 2C CTL clone after culture with MyD88-deficient DCs when compared with WT naive 2C T cells did not produce any IFN-γ after culture with hsp65-P1-loaded WT or MyD88-deficient DCs. The number of cell surface SIYRYYGL/class I complexes is thus much reduced in hsp65-P1-treated MyD88-deficient DCs compared with WT DCs. Previous studies show that, in vivo, CD4 T cell priming is affected in MyD88-deficient mice (33). Therefore, although we have shown that surface display of SIYRYYGL/class I complex is affected by the absence of MyD88 in vitro, we cannot rule out that additional factors, such as altered CD4 T cell activation, may be contributing factor in CD8 T cell priming in vivo.

MyD88 functions as an adapter molecule for several TLRs besides TLR4. Therefore, the fusion protein hsp65-P1 may ligate TLRs other than TLR4. Such a scenario would explain the more severe phenotype of MyD88-deficient DCs when analyzed for cross-presentation when compared with TLR4-deficient DCs. The more severe defect could also be explained by a role for MyD88 in DC development. Indeed, MyD88 was originally described as a differentiation factor in myeloid cell development (34). We have excluded LPS contamination of the hsp65-P1 fusion protein as a cause for activation, as our preparations contain less than 6 EU/mg (see Materials and Methods), far below the minimally stimulatory concentration of LPS.

Two pathways have been described for TLR4-mediated signaling; one requires the MyD88 adapter protein, whereas the other does not. Specifically, the MyD88-dependent pathway is required for cytokine production by DCs and subsequent T cell activation,
whereas the MyD88-independent pathway is involved in up-regulation of costimulatory molecules and activation of the transcription factor IFN regulatory factor-3 (13, 35, 36). Earlier studies in MyD88-deficient mice have demonstrated a defect in CD4 T cell activation upon immunization as well as a skewing from Th1 to Th2 cytokine production, thereby linking the lack of MyD88 to an inability to fully support Th1 responses (16, 37, 38). We observed a significant decrease in IFN-γ production by the CD8 T cells in vivo, which was previously reported for CD4 T cells in MyD88-deficient mice (16). We further show that expression of costimulatory molecules by DC from B6 or MyD88−/− is similar upon treatment with hsp65-P1, in concordance with previous studies (15), thereby confirming that MyD88 is not required for this aspect of DC activation. Therefore, it is not clear at present whether the reduction of CTL activation in vivo is attributable to the display of fewer K6/MIYRYGL complexes at the DC surface, or whether other factors required for optimal CD8 T cell activation, such as decreased production of DC cytokines, are responsible.

For the activation of naïve T cells, multiple interactions between peptide/MHC and TCR are required (39—42). Naïve CD8 T cells that recognize peptides presented by class I MHC molecules may require further costimulatory signals for their activation (43—46). Once properly primed, such CD8 T cells need to recognize only 1–10 peptides/class I MHC combinations to become functional (47). We observed a significant decrease in IFN-γ production by the CD8 T cells in vivo, which was previously reported for CD4 T cells in MyD88-deficient mice (16). We further show that expression of costimulatory molecules by DC from B6 or MyD88−/− is similar upon treatment with hsp65-P1, in concordance with previous studies (15), thereby confirming that MyD88 is not required for this aspect of DC activation. Therefore, it is not clear at present whether the reduction of CTL activation in vivo is attributable to the display of fewer K6/MIYRYGL complexes at the DC surface, or whether other factors required for optimal CD8 T cell activation, such as decreased production of DC cytokines, are responsible.

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References

fail to default to a Th2 pattern and are host protective in an IL-10−/− setting. Immunity 16:429.


