



Revisiting the role of IRF3 in inflammation and immunity by conditional and specifically targeted gene ablation in mice

Hideyuki Yanai^{a,b}, Shiho Chiba^{a,1}, Sho Hangai^{a,b}, Kohei Kometani^c, Asuka Inoue^{a,d}, Yoshitaka Kimura^a, Takaya Abe^e, Hiroshi Kiyonari^e, Junko Nishio^a, Naoko Taguchi-Atarashi^{a,2}, Yu Mizushima^{a,3}, Hideo Negishi^a, Rudolf Grosschedl^c, and Tadatsugu Taniguchi^{a,b,4}

^aDepartment of Molecular Immunology, Institute of Industrial Science, The University of Tokyo, 153-8505 Tokyo, Japan; ^bMax Planck–The University of Tokyo Center for Integrative Inflammation, 153-8505 Tokyo, Japan; ^cDepartment of Cellular and Molecular Immunology, Max Planck Institute of Immunobiology and Epigenetics, 79108 Freiburg, Germany; ^dJapan Research and Open Innovation, 163-1488 Tokyo, Japan; and ^eAnimal Resource Development Unit and Genetic Engineering Team, RIKEN Center for Life Science Technologies, 650-0047 Kobe, Japan

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IRF regulatory factor 3 (IRF3) is a transcription regulator of cellular responses in many cell types that is known to be essential for innate immunity. To confirm IRF3's broad role in immunity and to more fully discern its role in various cellular subsets, we engineered *Irf3*-floxed mice to allow for the cell type-specific ablation of *Irf3*. Analysis of these mice confirmed the general requirement of IRF3 for the evocation of type I IFN responses in vitro and in vivo. Furthermore, immune cell ontogeny and frequencies of immune cell types were unaffected when *Irf3* was selectively inactivated in either T cells or B cells in the mice. Interestingly, in a model of lipopolysaccharide-induced septic shock, selective *Irf3* deficiency in myeloid cells led to reduced levels of type I IFN in the sera and increased survival of these mice, indicating the myeloid-specific, pathogenic role of the Toll-like receptor 4–IRF3 type I IFN axis in this model of sepsis. Thus, *Irf3*-floxed mice can serve as useful tool for further exploring the cell type-specific functions of this transcription factor.

IRF3 | infection | Bcl2l12 | interferon | inflammation

A hallmark of the innate immune response is the induction of the type I IFN (IFN- α/β) that occurs on activation of the signal-transducing pattern recognition receptors (PRRs) of the innate immune system (1–3). The transcription factor IFN regulatory factor 3 (IRF3) plays an essential role in this process (3, 4). It was also reported that IRF3 is required to protect the host from viral infections, such as encephalomyocarditis virus (EMCV) and herpes simplex virus type 1 (HSV-1) (4, 5). Furthermore, IRF3 is also critical to the suppression of dextran sodium sulfate-induced colitis (6). On the other hand, IRF3 may also be involved in unfavorable immune responses, such as proinflammatory diseases and autoimmune diseases (7). Finally, IRF3 participates in the induction of septic shock syndrome (8).

IRF3 is constitutively expressed in various types of cells, where it resides in an inactive form with the cytoplasm. On activation of PRR by pathogen infection or stimulation with a synthetic ligands, IRF3 is phosphorylated on specific serine residues by the kinases TANK-binding kinase 1 (TBK1) or inhibitor of NF- κ B kinase ϵ/i (IKK ϵ/i) (9, 10), resulting in translocation of IRF3 into the nucleus, where it induces the transcription of type I IFN genes on binding to conserved sequences known as IFN stimulated response elements (ISREs) (3, 11). Several PRRs, including the cytosolic RNA helicase retinoic acid-inducible gene-I (RIG-I)-like receptor (RLR) family members; cytosolic DNA sensors, including cGMP-AMP synthase (cGAS), stimulator of IFN genes (STING), and DNA-dependent activator of IRFs (DAI); and the transmembrane Toll-like receptor (TLR) 3 and 4, are identified as inducers of type I IFN gene expression through the phosphorylation of IRF3 (1–3, 11, 12). IRF3 directly targets several cytokine genes, including CXCL10, RANTES, IFN-stimulated gene 56, IL-12p35, IL-15, and arginase II, as well as

type I IFN genes (8, 13, 14). IRF3 also functions as a negative regulator of gene expression, where, for example, IRF3 is directly recruited to the *I12b* gene promoter and enhancer on RLR or cytosolic DNA sensor stimulation, where it suppresses *I12b* mRNA induction by competing with IRF5, the bona fide transcriptional activator for the gene induction (15, 16). Furthermore, IRF3 activated by RLR signaling interacts with a transcription factor SMAD3 to interfere with transforming growth factor- β (TGF- β)-induced gene expression (17).

It should be noted that much of the foregoing data were obtained from *Irf3*-deficient mice (*Irf3*^{-/-}) generated in our laboratory (3, 4). However, subsequent reanalysis revealed that this line of mice was also carrying a null mutation for the *Bcl2l12* gene located adjacent to the *Irf3* gene (18). Thus, these mutant mice are renamed *Irf3*^{-/-}*Bcl2l12*^{-/-} mice (6, 18). The *Bcl2l12* gene encodes Bcl2-like-12 (Bcl2L12), a protein that functions as

Significance

IFN regulatory factor 3 (IRF3) is one of the most well-characterized transcription factors involved in the regulation of innate immune responses. Many studies have used conventional *Irf3*-deficient mice to analyze the functions of IRF3 in immunity and other biological systems; however, these mice were found to carry another null mutation in the *Bcl2l12* gene, which has confounded the analyses of these mice. For this study, we generated conditional *Irf3*-deficient mice while leaving the *Bcl2l12* gene intact. Conditional ablation of IRF3 in myeloid cells showed increased survival when challenged with endotoxin-induced shock, indicating a myeloid cell-specific role of IRF3. The IRF3 conditional knockout mouse may be a useful tool to identify cell type-specific functions of this transcription factor.

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¹Present address: Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI 53711.

²Present address: Laboratory for Intestinal Ecosystems, RIKEN Center for Integrative Medical Sciences, Yokohama, 230-0045 Kanagawa, Japan.

³Present address: Department of Otolaryngology and Head and Neck Surgery, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Japan.

⁴To whom correspondence should be addressed. Email: tada@m.u-tokyo.ac.jp.

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an antiapoptotic factor that suppresses DNA damage-induced apoptosis by inhibiting caspase-7 activity or p53-mediated gene transcription (19, 20). In fact, mouse embryonic fibroblasts (MEFs) from *Irf3*^{-/-}*Bcl2l12*^{-/-} mice show resistance to apoptosis induced by UV irradiation or doxorubicin treatment (18). As a result, analysis of IRF3 in these mice has been complicated by loss of the *Bcl2l12* gene.

In this study, we constructed *Irf3*-floxed (*Irf3*^{fl/fl}) mice, which retains an intact *Bcl2l12* gene, to achieve the conditional deletion of the gene in a cell- and tissue-specific manner affected by crossing these mice with a corresponding *Cre* recombinase transgenic strain. In fact, there is no mouse model with which to study cell type- or tissue-specific function of IRF3 in vivo. The contribution of IRF3 in the innate immune responses was reexamined without the influence of *Bcl2l12* nullizygosity. As exemplified by our results showing the myeloid cell-specific contribution of IRF3 in LPS-induced shock, the newly generated *Irf3*^{fl/fl} mice offers a useful tool with which to further study the cell- and tissue-specific function of this transcription factor.

Results

Generation of *Irf3*-Floxed (*Irf3*^{fl/fl}) Mice. To generate *Irf3*^{fl/fl} mice, a vector targeting exons 2, 3, and 4 flanked by loxP (floxed) sites designed to delete these exons on expression of *Cre* recombinase was constructed (*SI Appendix*, Fig. S1A). TT2 mouse embryonic stem cells (ES cells) (21) were electroporated with this vector, and the clones with the homologously recombined allele were identified by PCR and Southern blot analysis. The resulting ES clones, carrying the floxed *Irf3* gene, were used to generate chimeric mice with germ line transmission (*SI Appendix*, Fig. S1B). The FRT-flanked neomycin cassette was then removed from the chimeric mice by crossing the mice with Flippase-expressing transgenic mice. Mice homozygous for the floxed *Irf3* gene (*Irf3*^{fl/fl}) were born at the expected Mendelian ratios with no obvious abnormality. *Irf3*^{fl/fl} mice were crossed with transgenic mice with a CAG promoter-driven *Cre* (*CAG-Cre*) gene, and then further crossed with wild-type (WT) mice to eliminate the *CAG-Cre* gene to obtain systemic *Irf3*-deficient mice (*Irf3*^{-/-}). IRF3 deletion was confirmed in both the genome (*SI Appendix*, Fig. S1C) and at the protein expression level in MEFs derived from *Irf3*^{-/-} mice (Fig. 1A); it was also confirmed in bone marrow-derived dendritic cells (BM-DCs) and macrophages (BMMs) (Fig. 1B).

We next examined the status of the *Bcl2l12* gene in these mice. Since an antibody reactive to mouse Bcl2L12 protein is not available, *Bcl2l12* mRNA expression levels were monitored by quantitative reverse-transcription PCR (qRT-PCR) analysis. As shown in Fig. 1C, *Bcl2l12* mRNA expression levels in *Irf3*^{-/-} MEFs were comparable to those in WT MEFs. We further examined DNA damage-induced apoptosis in the *Irf3*^{-/-} MEFs. As expected, MEFs from *Irf3*^{-/-}*Bcl2l12*^{-/-} mice showed resistance to UV-induced apoptosis (*SI Appendix*, Fig. S1D), whereas *Irf3*^{-/-} MEFs did not (*SI Appendix*, Fig. S1E). Collectively, these results indicate that the newly generated *Irf3*^{fl/fl} mice retain the *Bcl2l12* gene.

Impairment of Type I IFN Gene Expression Evoked by Stimulation of PRRs in *Irf3*^{-/-} Cells. We next examined the role of IRF3 in the induction of type I IFN gene expression in response to PRR ligands. MEFs from WT or *Irf3*^{-/-} mice stimulated with double-stranded RNA [polyinosinic-polycytidylic acid [poly (I:C)]], double-stranded DNA [poly(deoxyadenylic-deoxythymidylic) acid (B-DNA)], or lipopolysaccharide (LPS) to activate RLRs, cytosolic DNA sensors, or TLR4 (1–3, 11, 12), respectively, and then *Irf3* mRNA expression levels examined by qRT-PCR analysis. As shown in Fig. 2A, *Irf3* mRNA expression induced by stimulation of these ligands was impaired in *Irf3*^{-/-} MEFs, confirming the sine qua non role of IRF3.

We further examined the induction of mRNA for the activation of type I IFN and other cytokines in myeloid-derived BM-DCs and BMMs. BM-DCs and BMMs were stimulated with poly (I:C), B-DNA, LPS, or CpG-B oligodeoxynucleotide (ODN), a

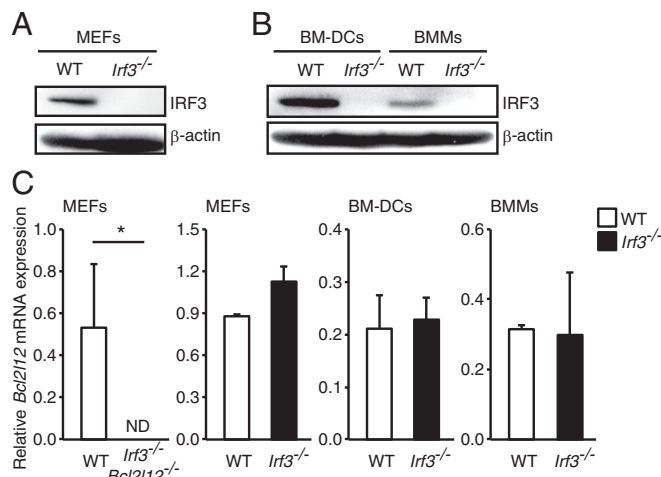


Fig. 1. *Bcl2l12* mRNA expression in WT and *Irf3*^{-/-} cells. (A) Whole-cell extracts were prepared from WT and *Irf3*^{-/-} MEFs and subjected to immunoblot analysis with anti-IRF3 and β -actin antibodies. (B) Whole-cell extracts were prepared from WT and *Irf3*^{-/-} GM-CSF-cultured BM-DCs and macrophage-CSF-cultured BMMs and subjected to immunoblot analysis. (C) MEFs were prepared from WT, *Irf3*^{-/-}*Bcl2l12*^{-/-}, or *Irf3*^{-/-} mice. Total RNA was extracted, and *Bcl2l12* mRNA expression was determined by qRT-PCR analysis. ND, not detected. * $P < 0.05$. Results shown are mean \pm SD of three independent experiments.

TLR9 agonist (2, 11), and cytokine mRNA induction levels were measured by qRT-PCR. We found that *Irf3* mRNA induction by poly (I:C), B-DNA, or LPS stimulation was significantly reduced in both BM-DCs and BMMs from *Irf3*^{-/-} mice (Fig. 2B and C). Of note, *Il6* and *Rantes* mRNA induction levels were also decreased in the *Irf3*^{-/-} BM-DCs, and BMMs, indicating the involvement of IRF3 (*SI Appendix*, Fig. S2A–D). On the other hand, the induction of these cytokine mRNAs remained unaffected when the cells were stimulated by a TLR9 agonist, CpG-B ODN (Fig. 2B and C and *SI Appendix*, Fig. S2A–D). The data are consistent with the previous observations that other IRFs are involved in gene induction by the TLR-myeloid differentiation primary response gene 88 (MyD88) signaling pathway (2, 3).

Consistent with our previous study (15, 16) in which IRF3 suppressed the *Il12b* promoter on stimulation by cytosolic nucleic acid receptors (15, 16), *Il12b* mRNA expression was enhanced when reanalyzed in *Irf3*^{-/-} BM-DCs and BMMs (*SI Appendix*, Fig. S2E and F). Since M1-type macrophages secrete larger amounts of proinflammatory cytokines, such as IL-12p40, compared with M2-type macrophages (22), we next analyzed whether IRF3 skews macrophage polarization toward the M2-type phenotype, but found no significant differences in the in vitro polarization of M1 and M2 macrophages in *Irf3*^{-/-} BMMs (*SI Appendix*, Fig. S2G).

We next evaluated the regulation of type I IFN on virus infections. In *Irf3*-deficient MEFs, BM-DCs, and BMMs infected with vesicular stomatitis virus (VSV) and EMCV, which activate RLR family members RIG-I and the melanoma differentiation-associated gene (MDA5), respectively, we observed a reduction in type I IFN mRNA expression compared with WT cells. A similar observation was made in MEFs infected by HSV-1 and *Listeria monocytogenes*, which are activated mainly by cytosolic DNA sensors (2, 12, 23, 24) (Fig. 2D–F).

Involvement of IRF3 in Antiviral Response and Tumor Growth in Vivo.

We next examined the contribution of IRF3 to the type I IFN responses induced by virus infection in vivo. WT and *Irf3*^{-/-} mice were i.v. infected with EMCV, and IFN- β production levels in serum and survival rate were monitored. As shown in Fig. 3A, *Irf3*^{-/-} mice were more vulnerable than WT mice to EMCV infection with reduced IFN- β production, indicating the critical role of IRF3 in antiviral response in vivo.

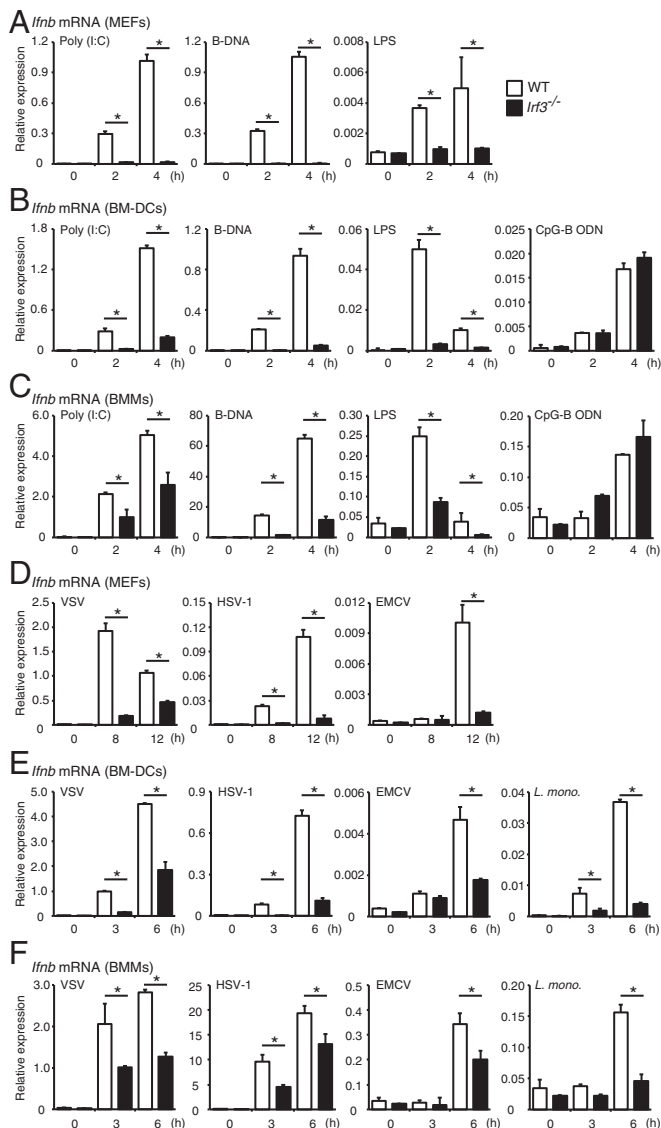


Fig. 2. *Ifnb* mRNA induction in WT and *Irf3*^{-/-} cells in response to PRR ligands or virus infection. (A–C) MEFs (A), BM-DCs (B), and BMMs (C) obtained from WT and littermate *Irf3*^{-/-} mice were stimulated with poly (I:C) (10 μg/mL), B-DNA (10 μg/mL), LPS (200 ng/mL), or CpG-B ODN (1 μM) for the indicated times. Total RNA was extracted, and *Ifnb* mRNA expression was determined by qRT-PCR analysis. **P* < 0.05. Results shown are mean ± SD from three independent experiments. (D–F) WT and littermate *Irf3*^{-/-} MEFs (D), BM-DCs (E), and BMMs (F) were infected with VSV, HSV-1, EMCV, or *L. monocytogenes* (*L. mono.*) at a multiplicity of infection of 1 for the indicated times. Total RNA was extracted, and *Ifnb* mRNA expression was determined by qRT-PCR analysis. **P* < 0.05. Results are mean ± SD of three independent experiments.

We also examined tumor growth in *Irf3*^{-/-} mice (Fig. 3B), since the STING-IRF3-type I IFN axis is implicated in antitumor immunity (25, 26). In this context, it has been shown that *Sting*^{-/-} mice are more susceptible to tumor growth compared with WT mice when s.c. inoculated with B16F10 tumor cells (25, 26). Somewhat unexpectedly, tumor size was smaller in *Irf3*^{-/-} mice compared with WT mice, indicating a tumor-promoting function of IRF3 (Fig. 3B). We then examined the involvement of myeloid cells or DCs in the IRF3-mediated tumor growth by crossing *Irf3*^{fl/fl} mice with mice expressing the *Cre* recombinase gene under the promoter of *LysM* or *CD11c* (27). As shown in Fig. 3C and D, the B16F10 tumor growth was not affected in *Irf3*^{fl/fl}-*LysM-Cre*⁺ or *Irf3*^{fl/fl}-*CD11c-Cre*⁺ mice (Fig. 3C and D). Therefore, IRF3 may

be involved in tumor progression by an as-yet unknown mechanism, but likely is not involved in STING-mediated antitumor immune responses, at least in this experimental setting. Of note, no significant difference was observed in the frequencies of tumor-infiltrating immune cells (SI Appendix, Fig. S3).

B Cell-Specific Ablation of *Irf3* and Its Effect on the Differentiation of B Cells and Antibody Production. Recent reports have raised the question of whether IRF3 modulates adaptive immune responses (7, 16, 17, 28, 29). To delineate functions of IRF3 in B cells, we first generated mice with B-cell-specific *Irf3* ablation by crossing *Irf3*^{fl/fl} mice with *Mb1-Cre* knockin mice. The *Mb1-Cre* mice express *Cre* recombinase during early B cell development (30). We examined the distribution of bone marrow B cell lineage subsets by flow cytometry analysis. The percentages of B220⁺ B cells (Fig. 4A), B220^{lo}CD43⁻IgM⁺ immature B cells, B220^{lo}CD43⁻IgM⁻ pre-B cells, B220^{lo}CD43⁺IgM⁻ pro-B cells (Fig. 4B), or B220⁺CD43⁺ (Hardy fraction ABC) (31) pro-B cells, including B220⁺CD43⁺CD24⁻BP-1⁻ (prepro-B cells; fraction A), B220⁺CD43⁺CD24⁺BP-1⁻ (early pro-B cells; fraction B), and B220⁺CD43⁺CD24⁺BP-1⁺ (late pro-B and large pre-B cells; fraction C), were not affected by the IRF3 deficiency (Fig. 4C).

We next analyzed B cell distributions in peripheral tissues. CD19⁺ B cells were observed to be normal in the spleen, blood, mesenteric lymph nodes, and Peyer's patches of the gut from *Irf3*^{fl/fl}-*Mb1-Cre*⁺ mice (SI Appendix, Fig. S4A). There was no significant difference in the frequencies of CD93/AA4.1⁺B220⁺IgM^{high}CD23⁻ (T1 cells), CD93/AA4.1⁺B220⁺IgM^{high}CD23⁺ (T2 cells), CD93/AA4.1⁺B220⁺IgM^{low}CD23⁺ (T3 cells), B220⁺CD21^{high}CD23^{low} (marginal zone B cells), B220⁺CD21^{low}CD23^{high} (follicular B cells), B220^{low}CD138⁺ (plasma cells), or B220⁺GL7⁺CD95/Fas⁺ (germinal center B cells) subsets in the spleen (SI Appendix, Fig. S4B). Expression of IgD, Igλ, or Igκ light chain on splenic B220⁺ cells was also unaffected (SI Appendix, Fig. S4C). In addition, there was no significant changes in CD19⁺CD23^{low} (B1 cells) or CD19⁺CD23⁺ (B2 cells) subsets in peritoneal cavity and germinal center B cells in mesenteric lymph nodes or Peyer's patches between WT and *Irf3*^{fl/fl}-*Mb1-Cre*⁺ mice (SI Appendix, Fig. S4D). Thus, B cells developed normally in the absence of IRF3 in B cells.

We further addressed the contribution of IRF3 in B cells during humoral immune responses. WT and *Irf3*^{fl/fl}-*Mb1-Cre*⁺

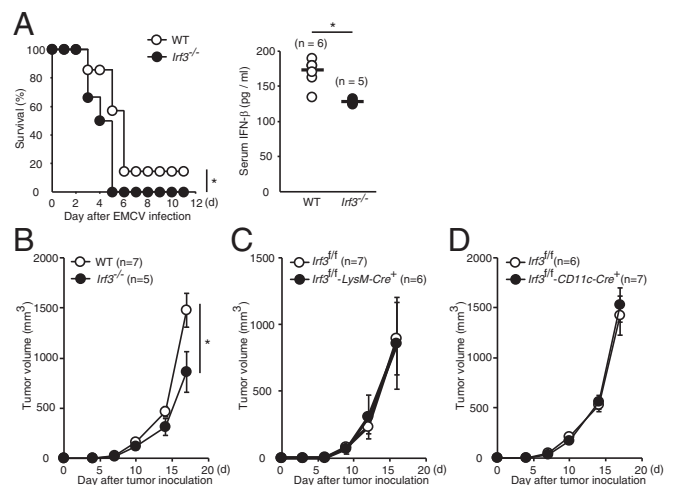


Fig. 3. In vivo role of IRF3 in EMCV infection and tumor progression. (A) WT (*n* = 7) and *Irf3*^{-/-} (*n* = 6) mice were i.v. infected with EMCV (10⁵ pfu/mouse). (Left) Mouse survival was monitored every 24 h. (Right) IFN-β levels in serum were measured by ELISA at 6 h after EMCV infection. **P* < 0.05. (B–D) 1 × 10⁵ of B16F10 cells were inoculated s.c. into *Irf3*^{-/-} (B), *Irf3*^{fl/fl}-*LysM-Cre*⁺ (C), and *Irf3*^{fl/fl}-*CD11c-Cre*⁺ mice (D). Tumor volume was measured every 3 or 4 d.

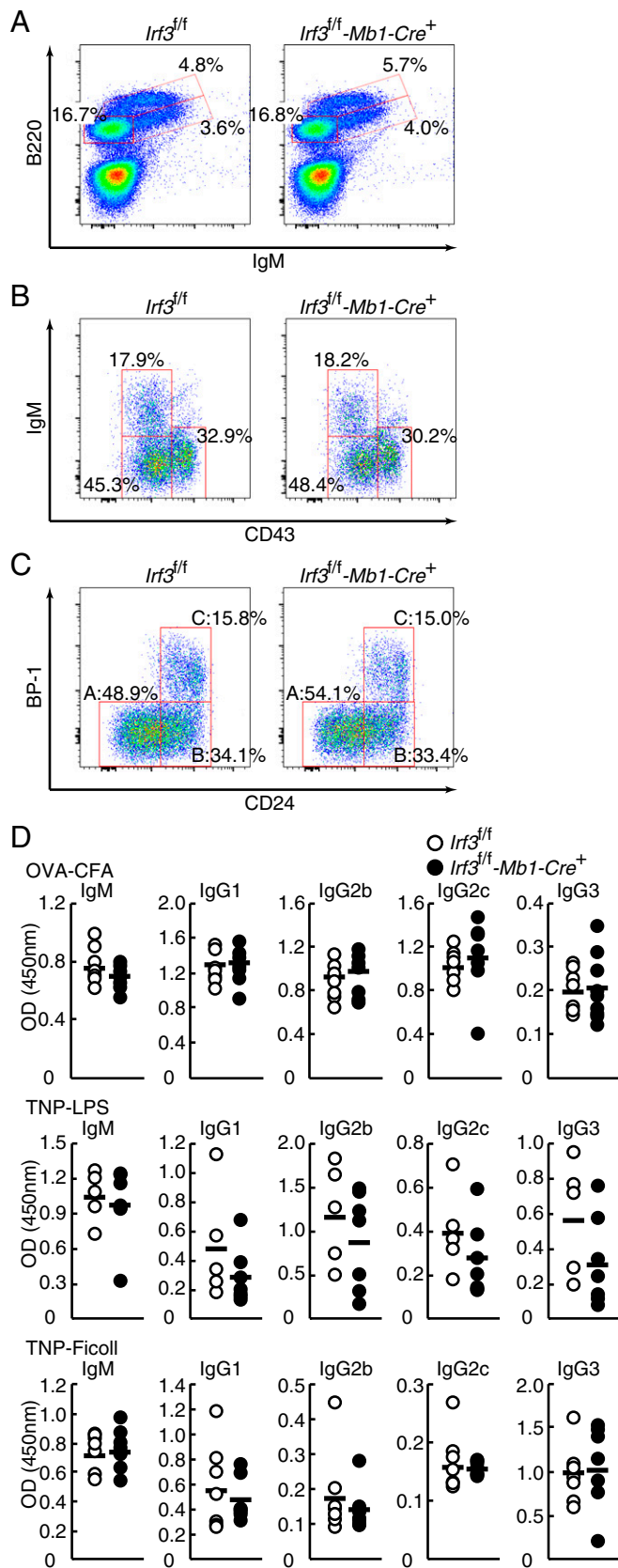


Fig. 4. Normal B-cell development in bone marrow and antibody production in B cells in IRF3-ablated mice. (A–C) Suspension of bone marrow cells were prepared from *Irf3^{fl/fl}* and *Irf3^{fl/fl}-Mb1-Cre⁺* mice, stained with antibodies, and analyzed by flow cytometry. B220^{lo} cells (B) and B220^{lo}CD43⁺

mice were immunized with T cell-dependent antigen ovalbumin (OVA) in combination with complete Freund's adjuvant, T cell-independent (TI)-1 antigen 2,4,6-trinitrophenyl-coupled LPS (TNP-LPS), or TI-2 antigen TNP-Ficoll, and OVA- or TNP-specific antibody subclasses in sera were examined. As shown in Fig. 4D, there were no significant differences in the levels of anti-OVA or anti-TNP IgM, IgG1, IgG2b, IgG2c, or IgG3 antibody subclasses in the sera between WT and *Irf3^{fl/fl}-Mb1-Cre⁺* mice. Thus, these data indicate that IRF3 in B cells is dispensable for B cell development and function, although it remains possible that IRF3 is involved in aspects other than those examined here.

T Cell-Specific Ablation of *Irf3* Gene and Its Effect on T Cell Differentiation. We then addressed whether IRF3 regulates T cell differentiation. Constitutive expression of IRF3 in T cells was ablated by crossing *Irf3^{fl/fl}* mice with *Lck-Cre* transgenic mice. We prepared naïve CD4⁺ T cells from spleen of WT and *Irf3^{fl/fl}-Lck-Cre⁺* mice and performed in vitro T cell differentiation assays. IRF3 deficiency did not impact the differentiation of T helper (Th) cells, such as Th1, Th2, and Th17 cells, or induced regulatory T cells (SI Appendix, Fig. S5A).

When we examined populations of T cells in the thymus and spleen of *Irf3^{fl/fl}-Lck-Cre⁺* mice, the CD4⁺CD8⁻ double-negative population was increased in the thymus of *Irf3^{fl/fl}-Lck-Cre⁺* mice (SI Appendix, Fig. S5B). In addition, CD44^{int-high}CD62L⁻ and CD44⁺CD62L⁺ effector/memory T cells were increased in the spleen or peripheral blood from *Irf3^{fl/fl}-Lck-Cre⁺* mice, whereas CD44⁻CD62L⁺ naïve T cells were decreased in *Irf3^{fl/fl}-Lck-Cre⁺* mice (SI Appendix, Fig. S5 C and D). However, similar phenotypes have been reported with *Lck-Cre* transgenic mice, showing decreased numbers of naïve T cells and increased frequencies of CD44⁺CD62L⁻ effector/memory T cells in the spleen and lymph nodes, as well as increased numbers of thymic CD4/CD8 double-negative cells (32). Although transgenic mice expressing Cre recombinase are widely used to generate conditional KO mice, recent publications have indicated that some models have undesirable disadvantages (33, 34).

In fact, when we analyzed systemic *Irf3^{-/-}* mice, the CD4/CD8 double-negative cells or double-positive cells in the thymus were found to be normal (Fig. 5A), as were frequencies of naïve and effector/memory T cells in the spleen or peripheral blood (Fig. 5 B and C). We further examined T cell subsets in colonic lamina propria, in which IRF3 is inherently activated with commensal bacteria through RLRs or cytosolic DNA sensors (6, 17); however, we found no overt abnormalities in T cell populations such as CD4⁺, CD8⁺, and Treg cells in the colonic lamina propria of *Irf3^{-/-}* mice (Fig. 5D).

Pathogenic Contribution of the IRF3-Type I IFN Axis in Myeloid Cells. There is strong evidence that type I IFNs function as critical inflammatory mediators in high doses of LPS-induced endotoxin lethal shock (8, 35, 36). Previous reports including our own have demonstrated that *Irf3^{-/-}-Bcl2l12^{-/-}* and *Ifnb^{-/-}* mice are resistant to the lethal endotoxemia (8, 35). As such, we examined the susceptibility of *Irf3^{-/-}* mice in the same septic shock model. We found that the production of serum IFN- β in response to i.v. LPS injection was abrogated in *Irf3*-deficient mice, which also had a higher survival rate than the WT mice (Fig. 6A).

cells (C) were gated, respectively. Percentages of cells in the total or gated population are shown. Hardy fractions (fractions A, B, and C) are also shown. (D) Mice were immunized i.p. with OVA (100 μ g) emulsified in complete Freund's adjuvant (*Irf3^{fl/fl}*, $n = 7$; *Irf3^{fl/fl}-Mb1-Cre⁺*, $n = 7$; Upper), TNP-AECM-Ficoll (50 μ g; *Irf3^{fl/fl}*, $n = 5$; *Irf3^{fl/fl}-Mb1-Cre⁺*, $n = 7$; Middle), or TNP-LPS (50 μ g; *Irf3^{fl/fl}*, $n = 7$; *Irf3^{fl/fl}-Mb1-Cre⁺*, $n = 7$; Lower). Sera were collected at 3 wk after injections. IgM, IgG1, IgG2b, IgG2c, and IgG3 titers in sera were determined by ELISA. Thick bars represent mean values.

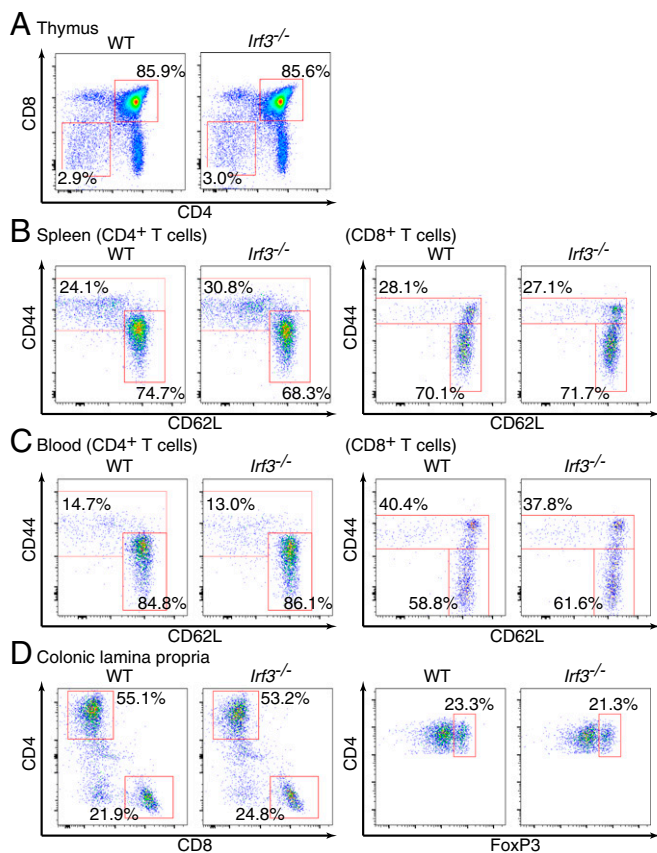


Fig. 5. Normal T cell subsets in *Irf3*-deficient mice. (A) Thymocytes from WT or *Irf3*^{-/-} mice were analyzed by flow cytometry. Percentages of CD4⁺CD8⁺ and CD4⁺CD8⁻ cells in the total thymocytes are shown. (B) Splenocytes were prepared from WT or *Irf3*^{-/-} mice and analyzed by flow cytometry. CD4⁺TCR β ⁺ T cells (Left) and CD8⁺TCR β ⁺ T cells (Right) were gated. Percentages of effector/memory T cells and naïve T cells in the gated population are shown. (C) Whole-blood cells were prepared from WT or *Irf3*^{-/-} mice. Red blood cells were lysed, and the remaining cells were analyzed by flow cytometry as in B. (D) Colonic lamina propria cells were obtained from WT or *Irf3*^{-/-} mice and subjected to flow cytometry analysis. TCR β ⁺ T cells (Left) and CD4⁺TCR β ⁺ T cells (Right) were gated. The results shown are representative of three independent experiments.

The availability of *Irf3*^{f/f} mice allowed us to further examine the cell type-specific contribution of IRF3 to LPS-induced lethality. TLR4 is highly expressed in antigen-presenting cells, such as macrophages and DCs. Interestingly, *Irf3*^{f/f}-*LysM-Cre*⁺ mice showed prolonged survival accompanied by impaired IFN- β expression in serum in the LPS-induced shock model (Fig. 6B), whereas *Irf3*^{f/f}-*CD11c-Cre*⁺ mice did not display such phenotypic abnormalities (Fig. 6C). Thus, IRF3 in myeloid cells, but not in DCs, plays a major role in the induction of type I IFN responses and mortality induced by high-dose LPS administration.

Discussion

In recent years, IRF3 has gained recognition as a key transcriptional regulator of type I IFN gene expression induced in response to pathogenic infection. Many of the discoveries on the role of IRF3 in immunity and other biological systems were made using conventional *Irf3*-null mice. However, these mice were recently discovered to also carry a null mutation in the *Bcl2l12* gene, and hence have been renamed *Irf3*^{-/-}-*Bcl2l12*^{-/-} (18). In this study, we successfully engineered *Irf3*^{f/f} mice in which the *Bcl2l12* gene remains functionally intact (Fig. 1C). This strain has enabled us to study the function of IRF3 without

considering the effect of loss of function of *Bcl2l12* (*SI Appendix, Fig. S1 D and E*). The present results, taken together with our previous reports (4, 18), confirm that IRF3 is critical for the activation of the type I IFN responses induced by innate PRRs or pathogen infections (Figs. 2, 3A, and 6).

It is interesting that the growth of s.c. transplanted B16F10 melanoma cells is significantly suppressed in *Irf3*^{-/-} mice, suggesting a tumor-promoting function of IRF3 (Fig. 3B). Our results indicate that IRF3 in myeloid cells and DCs is not involved in this process (Fig. 3C and D); therefore, further work is needed to clarify cell type(s) and mechanism(s), wherein IRF3 is functioning for the promotion of tumor growth. It is interesting that the STING-IRF3-type I IFN axis is known to inhibit tumor growth (25, 26); thus, we infer that IRF3 is bifunctional, in that it contributes to tumor suppression via the STING pathway and tumor progression by as-yet unknown mechanism(s).

The *Irf3*^{f/f} mice also allow us to analyze the function of IRF3 in a cell type-specific manner. Indeed, we have demonstrated that

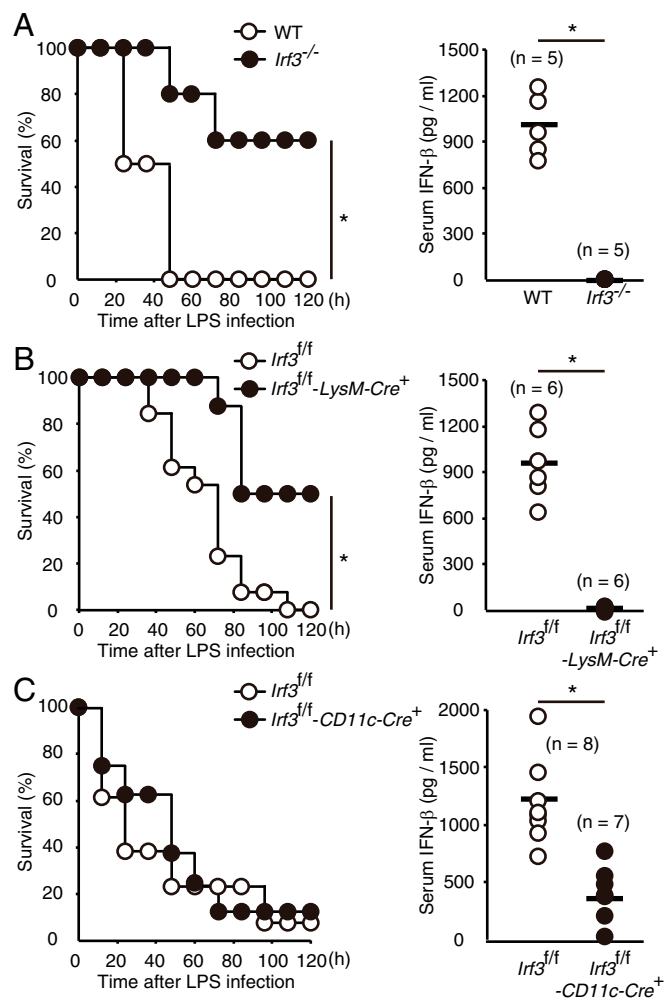


Fig. 6. The critical role of IRF3 in myeloid cells in the induction of type I IFN and mortality induced by LPS administration. IRF3 in myeloid cells, but not in DCs, plays a major role in the induction of type I IFN responses and mortality induced by high-dose LPS administration. (A) WT (n = 6) and *Irf3*^{-/-} (n = 5) mice were administered i.v. LPS (8 mg/kg). Mice survival was monitored every 12 h (Left) and IFN- β levels in sera were determined by ELISA at 2 h after LPS administration (Right). (B and C) LPS was administered to *Irf3*^{f/f} (n = 13) and *Irf3*^{f/f}-*LysM-Cre*⁺ (n = 8) mice (B) and to *Irf3*^{f/f} (n = 8) and *Irf3*^{f/f}-*CD11c-Cre*⁺ (n = 7) mice (C) as in A. Mouse survival was monitored (Left), and IFN- β levels in serum were measured (Right). *P < 0.05.

IRF3 in myeloid cells has a profound impact on LPS-induced IFN- β production and survival (Fig. 6B). Since several reports have indicated the involvement of IRF3 in the regulation of adaptive immune responses (7, 16, 17, 28, 29), we also analyzed the potential role of IRF3 in the development of and antibody production by cells of the B cell lineage using *Irf3^{fl/fl}-Mb1-Cre⁺* mice. However, no significant differences in B cell subset frequency or antibody production were observed, at least in these experimental settings (Fig. 4 and *SI Appendix*, Fig. S4). T cell subsets in tissues were also normal in *Irf3^{-/-}* mice (Fig. 5). Despite such observations, additional studies may yet elucidate a function of IRF3 in B cell- and T cell-mediated immune responses.

In conclusion, the conditional deletion of the *Irf3* gene using the *Irf3^{fl/fl}* mice described here will allow for more detailed and extensive investigation for the cell- and tissue-specific roles of IRF3 in the regulation of the immune and other biological processes.

Materials and Methods

Mice. The *Irf3^{-/-}Bcl2l12^{-/-}* mice have been described previously (4, 18). C57BL/6 mice were purchased from CLEA Japan. CAG-Cre transgenic mice, *LysM-Cre* knockin mice, *CD11c-Cre* transgenic mice, and *Lck-Cre* transgenic mice were used (27). *Mb1-Cre* knockin mice were kindly provided by Michael Reth, Max Planck Institute of Immunobiology and Epigenetics, Freiburg im Breisgau, Germany. *FLPe* transgenic mice (RBRC01834) were provided by the

RIKEN BioResource Research Center through the National BioResource Project of the Ministry of Education, Culture, Sports, and Technology of Japan. All animal experiments were done in accordance with guidelines of The University of Tokyo and RIKEN Kobe Branch.

Reagents and Cells. CpG-B ODN (ODN 1668) and other oligodeoxynucleotides were purchased from FASMAC. LPS O55:B4 was purchased from Sigma-Aldrich. B-DNA (Sigma-Aldrich) or poly (I:C) (GE Healthcare Biosciences) was mixed with Lipofectamine 2000 (Invitrogen), and cells were stimulated with the mixture as described previously (16, 37). Bone marrow cells, splenocytes, thymocytes, colonic lamina propria cells, B16F10 melanoma cells, MEFs, BM-DCs, and BMMs were prepared as described previously (4, 6, 27, 37).

Additional information is provided in *SI Appendix, Materials and Methods*.

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