Negative regulation of IFN-α/β signaling by IFN regulatory factor 2 for homeostatic development of dendritic cells

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The development and cooperation of distinct subsets of antigen-presenting cells, particularly dendritic cells (DCs), may be critical for maintaining homeostatic immune responses. Recently, much attention has been focused on IFN-α/β, the cytokines induced *en masse* by virus infection or the activation of Toll-like receptors, in the context of DC activation. Here, we show that mice deficient in IFN regulatory factor 2 exhibit selective loss of CD8α^- DCs, the so-called myeloid DCs, which is accompanied by a notable increase in CD11c$^-$CD11b^{high} other myeloid lineage cells. Such deficiency is intrinsic to the bone marrow precursors, in which the abnormal induction of *IFN-*α/β genes causes excessive IFN signaling. The critical function of IFN regulatory factor 2 in the negative regulation of IFN-α/β signaling is underscored by the observation that the deficiency is rescued by introducing an additional null mutation for the IFN receptor complex. In view of accumulating evidence of the critical role of IFN-α/β signaling in DC activation, our present study offers a unique example in that the magnitude of a cytokine signal should be properly balanced in a stage-specific manner during the differentiation and activation of DCs.

The hallmark of the immune system is that hematopoietic stem cells undergo differentiation to multiple cell lineages to create cellular repertoires to rapidly evoke appropriate immune responses to a given pathogen. Although much has been studied about the diversity of lymphocyte populations in this context, the prevailing notion is that antigen-presenting cells (APCs) also create a repertoire that may be critical to maintain the homeostasis of the immune system (1–3).

The interaction of T lymphocytes with diverse sets of APCs is indeed a central aspect in triggering both the induction of adaptive immune responses and the induction and maintenance of immunological tolerance (4). Among APCs, dendritic cells (DCs) are the most potent in the activation of naïve T cells by sensing the presence of infection and expressing the MHC and costimulatory molecules at high levels (5). The functional diversity of DCs in evoking versatile immune responses may be attributed, at least in part, to the existence of distinct DC subsets (2). Two major DC subsets have been identified on the basis of CD8α expression, namely CD8α^+ and CD8α^- DCs, and much has been studied on the differentiation pathways for these DC subsets (2, 6). It has been reported that CD8α^+ DCs, termed "lymphoid DCs," are derived from CD8α^+CD11c$^-$ precursor cells in the spleen (7), whereas most of CD8α^- DCs, termed "myeloid DCs", are derived from MHC class II$^-$CD11c$^+$ peripheral blood DC precursors (8, 9). Some studies suggest that CD8α^+ DCs produce IL-12 and prime Th1 responses whereas CD8α^- DCs prime Th2 responses (10). In addition, the CD8α^+ DC subset has been shown to play a preferential role in the induction of cytotoxic T cells by cross-priming (11). In view of the functional differences between distinct DC subsets, one may envisage that a proper balance of the development of DCs and other APCs constitutes an essential aspect for maintaining the homeostasis of the immune system.

APC differentiation is tightly controlled by extracellular stimuli, particularly cytokines, as well as by their downstream effector molecules. In this context, the fms-related tyrosine kinase 3 ligand (Flt3L), granulocyte/macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor are well known positive regulators for APC development (1, 12–14). In addition, several intracellular signaling molecules and transcription factors that affect the development of individual DC subsets *in vivo* have been reported (9). Ikaros (15), RelB (16), and PU.1 (17) are essential for the development of CD8α^- myeloid DCs. In contrast, IFN consensus sequence-binding protein (ICSBP) (18) and Id2 (19) are involved in the differentiation of CD8α^+ lymphoid DCs.

IFN regulatory factors (IRFs) constitute a family of transcription factors, in which IRF-2 was originally identified as an antagonist of IRF-1 (20). In addition, IRF-2 functions as a positive regulator on some genes, such as *VCAM-1* (21), *Histone H4* genes (22), and class II transactivator (23). The gene targeting of the *IRF-2* gene revealed another function of IRF-2 as a transcriptional attenuator of IFN-α/β-activated IFN-stimulated gene factor 3 (24, 25). Mice deficient in *IRF-2* (IRF-2$^{-/-}$ mice) spontaneously develop autoimmune-like symptoms, which include the polyclonal activation of CD8$^+$ T cells, enlargement of lymph nodes, and skin inflammation (25). The development of these symptoms is due to the hyperresponsiveness of IRF-2$^{-/-}$ CD8$^+$ T cells; however, the introduction of IRF-2$^{-/-}$ CD8$^+$ T cells into irradiated WT mice did not induce these symptoms, suggesting the involvement of other types of cells (25).

In the present study, we addressed the issue of how development of a distinct APC population is regulated by the absence of IRF-2. If any abnormality is found in APCs, it may provide a clue to the understanding of the above-described autoimmune-like symptoms of IRF-2$^{-/-}$ mice. We show here that IRF-2 is essential for the development of CD8α^- myeloid DCs. Furthermore, we provide evidence that aberrant IFN-α/β signaling is responsible for the developmental defect of CD8α^- DCs in IRF-2$^{-/-}$ mice. These findings indicate that the absence of negative regulation of IFN-α/β signaling by IRF-2 selectively affects development of the CD8α^- myeloid DC population and may have implications in the CD8$^+$ T cell-mediated immunological disorders in IRF-2$^{-/-}$ mice.

Materials and Methods

Mice. The generation of IRF-2$^{-/-}$ mice has been described (26). IFN-α/β receptor 1 (IFNAR1)$^{-/-}$ mice (27) and C57BL/6 Ly 5.1-Pep3b mice were purchased from B & K Universal (Hull, U.K.) and The Jackson Laboratory, respectively. 2C T cell receptor-transgenic mice (28) were kindly provided by K. Takeda (Juntendo University, Tokyo). All of the mice were maintained under specific pathogen-free conditions and used at 8–12 weeks of age. Until 12 weeks of age, the numbers of total splenocytes and T and B lymphocytes in the IRF-2$^{-/-}$ spleen

Abbreviations: Flt3L, fms-related tyrosine kinase 3 ligand; GM-CSF, granulocyte/macrophage colony-stimulating factor; IRF, IFN regulatory factor; DC, dendritic cell; APC, antigen-presenting cell; IFNAR1, IFN-α/β receptor 1; PE, phycoerythrin; BM, bone marrow.

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were essentially the same as those in the WT mice, as described in refs. 25 and 26.

Bone Marrow (BM) Transplantation. C57BL/6 Ly5.1-Pep3b mice were exposed to a single dose of 9.5-Gy total-body irradiation. The irradiated recipients were transplanted with 10^7 BM cells isolated from IRF-$2^{-/-}$ or WT mice.

Mixed-Lymphocyte Reaction Assay. Splenic APCs were prepared by T cell depletion with anti-CD5 microbeads (Miltenyi Biotec, Auburn, CA). A total of 5×10^4 $CD4^+$ or $CD8^+$ T cells purified with magnetic-activated cell sorting from a BALB/c spleen were used as responder cells. The responder cells were cultured with 30-Gy irradiated APCs and pulsed with 1 μCi of [^{3}H]thymidine for 15 h. [^{3}H]Thymidine incorporation was measured by β-scintillation counting.

Immunohistochemistry. After i.p. injection with lipopolysaccharide (from *Salmonella minnesota* Re-595; Sigma), spleens were harvested and embedded in an OCT compound. Cryostat sections (10 μm thick) were fixed with acetone and doubly stained with FITC-conjugated anti-B220 and biotin-conjugated anti-CD11c antibodies (BD Pharmingen), and subsequently with streptavidin–Alexa Fluor 546 (Molecular Probes). The sections were examined under a fluorescence microscope (Nikon).

Flow Cytometry. Cell suspensions were double- and triple-stained by using various combinations of the following antibodies: FITC-anti-CD11c (HL3), phycoerythrin (PE)-anti-CD8α (53-6.7), PE-anti-CD11b (M1/70), PE-anti-B220 (RA3-6B2), PE-anti-I-A^b (AF6-120.1), PE-anti-Gr-1 (RB6-8C5), biotin-anti-Ly5.2 (104) (all from BD Pharmingen), and PE-anti-F4/80 (A3-1, Caltag). Biotinylated antibody was detected with allophycocyanin-conjugated streptavidin (Molecular Probes). Stained cells were analyzed by a FACSCalibur (BD Bioscience) while being gated to exclude dying cells positive for propidium iodide (Molecular Probes).

***In Vivo* DC Analysis.** Cell suspensions from lymphoid organs were obtained as described for splenic DC purification (6). Briefly, spleens or thymi were digested with 1 mg/ml collagenase A (Roche Biochemicals) and 0.2 mg/ml DNase I for 35 min at 37°C and further treated by EDTA (20 mM final concentration) for 5 min at room temperature. To enrich DCs, immunomagnetic negative selection of T and B cells was carried out by incubation with anti-CD5 and anti-CD19 antibodies (BD Pharmingen), followed by the depletion with anti-rat IgG-coated Dynabeads (Dynal) according to the manufacturer's instructions. Recovered cells were then immunofluorescent-stained and analyzed by flow cytometry. The absolute numbers of $CD8\alpha^+CD11c^+$ or $CD8\alpha^-CD11c^+$ cells were calculated by using frequencies estimated by flow cytometry and total viable cell counts for the spleen of each animal. The total number of viable cells harvested from the spleens was determined by the trypan blue exclusion method. The *t* test was used for statistical analysis. For analysis of DC precursors, peripheral blood was overlaid onto Lymphosepar II (IBL, Gunma, Japan) and centrifuged at $400 \times g$ for 20 min at room temperature. The mononuclear cell fraction was collected and analyzed by flow cytometry.

***In Vitro* DC Culture.** DCs were generated from mouse BM, as described (13, 29). In brief, BM cells were cultured with 10 ng/ml mouse GM-CSF (BD Pharmingen) or 100 ng/ml human Flt3L (PeproTech, Rocky Hill, NJ) in RPMI medium 1640 supplemented with 10% FBS. Some wells also received recombinant murine IFN-α/β (Sigma) at indicated concentrations. Loosely adherent cells were harvested at indicated days of culture. For antigen presentation assay, irradiated BM-derived cells were plated at 10^4 cells per well with 5×10^4 magnetic-activated cell sorting-purified $CD8^+$ T cells from 2C T cell receptor-transgenic mice in a medium with different concentrations of a specific peptide (SIYRYYGL) for 3 days. The cultures were pulsed with 1 μCi of [^{3}H]thymidine at the final 15 h. For endocytosis assay, 2 mg/ml FITC-conjugated ovalbumin (OVA) (Molecular Probes) was added to BM-derived cells and then incubated for 20 min at 4°C or 37°C. After the washes, the uptake of FITC-conjugated OVA was measured by flow cytometry.

RNA Analysis. RNA extraction and reverse-transcription reaction were performed as described (30). Quantitative real-time RT-PCR analysis was performed by using a LightCycler and SYBR Green system (Roche), and data were normalized by the β-actin expression level for each individual sample. Primers for β-actin, IFN-α1, IFN-β, protein kinase R, IRF-7, and 2′,5′-oligoadenylate synthetase were described in refs. 25 and 30.

Results

IRF-2-Deficient Mice Present Selective Loss of $CD8\alpha^-CD11b^+$ Myeloid DC Subset. To examine whether any abnormality exists in IRF-$2^{-/-}$APCs, we first conducted a mixed-lymphocyte reaction assay by mixing splenic APC fractions from the WT or IRF-$2^{-/-}$ mice (in the C57BL/6 background) with allogeneic WT CD4 or CD8 T cells from BALB/c mice. Interestingly, IRF-$2^{-/-}$ APCs showed poor stimulatory activities for both types of allogeneic T cells (Fig. 1*a*), suggesting that splenic APCs from IRF-$2^{-/-}$ mice are functionally abnormal. Because DCs are the most potent cells for the activation of naïve T cells (5), we next carried out an immunohistochemical analysis of the spleen section of IRF-$2^{-/-}$ mice with anti-B220 and anti-CD11c antibodies to analyze DCs (Fig. 1*b*). Interestingly, the number of $CD11c^+$ cells in the T cell area of IRF-$2^{-/-}$ mouse spleen was markedly lower than in that of WT mouse spleen.

Consistent with the above observation, flow cytometric analysis showed that the number of $CD11c^+$MHC class II^+ DCs was markedly reduced in the spleen of the IRF-$2^{-/-}$ mice (Fig. 1*c*). In particular, such reduction was prominent for $CD11c^+CD8\alpha^-CD11b^+$ myeloid DCs, which are barely detectable in the IRF-$2^{-/-}$ mice, and a rather modest difference was observed for $CD11c^+CD8\alpha^+CD11b^-$ lymphoid DCs (Fig. 1 *c* and *d*). The numbers of $CD11c^{int}B220^+$, termed plasmacytoid DCs (or IFN-producing cells; ref. 31) and $CD11c^+CD8\alpha^+$ thymic DCs were found to be normal in the mutant mice (Fig. 1*e*). Thus, IRF-$2^{-/-}$ mice exhibit a selective decrease in the number of $CD8\alpha^-CD11b^+$ DCs, the so-called myeloid DC subset. Notably, $CD11c^-CD11b^{high}$ cells, which may represent other myeloid lineage cells, are dominant in the splenocytes of IRF-$2^{-/-}$ mice, suggesting the developmental skewing of BM precursors from $CD11c^+CD8\alpha^-CD11b^+$ DCs to $CD11c^-CD11b^{high}$ cells (Fig. 1*c*).

We further analyzed the effect of IRF-2 deficiency in the development of peripheral blood $CD11c^+$MHC class II^- cells, which has been characterized as a DC-committed precursor population (8, 9). As shown in Fig. 1*f*, the number of DC precursors in IRF-$2^{-/-}$ peripheral blood cells was also markedly reduced. These results are consistent with a previous report that the DC precursor in the peripheral blood mainly differentiate into myeloid DCs (8) and suggest that DC differentiation is developmentally skewed before the stage of peripheral blood precursors in IRF-$2^{-/-}$ mice.

Developmental Defect of Myeloid DCs Is Intrinsic to the IRF-$2^{-/-}$ BM Cells. To determine whether this developmental defect found in myeloid DCs is cell-autonomous for IRF-$2^{-/-}$ precursor cells, IRF-$2^{-/-}$ BM cells or WT BM cells (both expressing the Ly5.2 antigen) were introduced into lethally irradiated, C57BL/6 Ly 5.1-Pep3b mice. After introduction, the spleens of the chimeric mice were analyzed by flow cytometry. As shown in Fig. 2, the

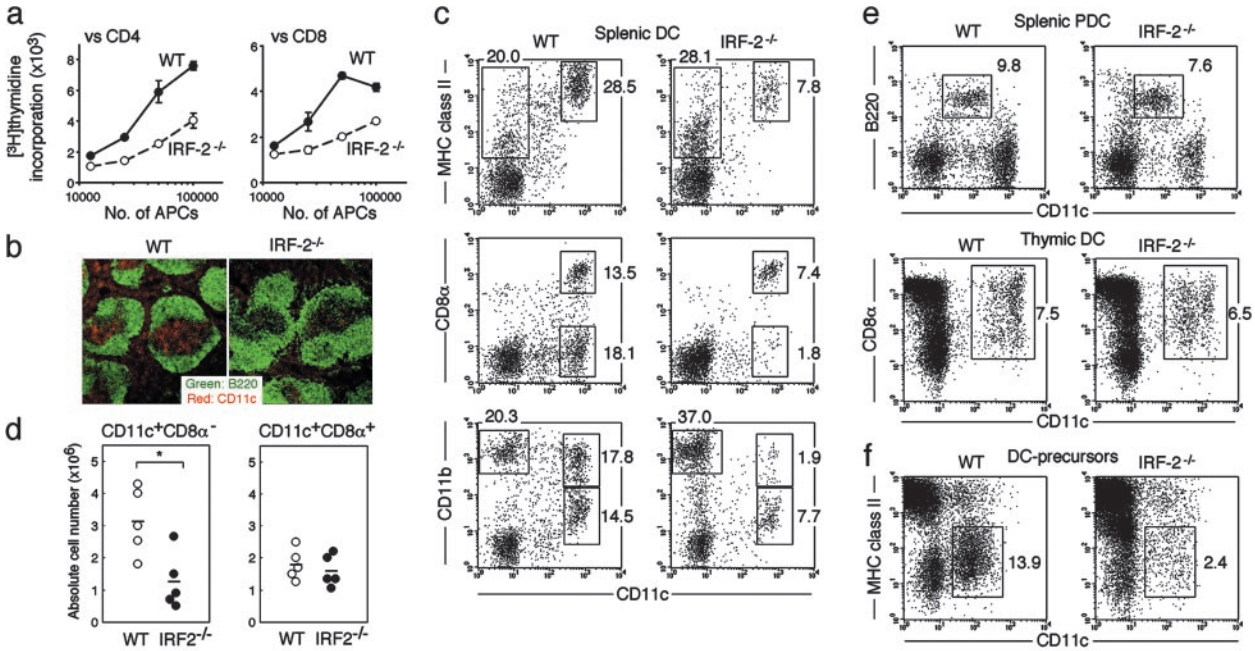

Fig. 1. IRF-$2^{-/-}$ mice lack CD8α^- DCs and DC precursors. (*a*) T cell stimulatory activity of APCs from WT or IRF-$2^{-/-}$ mouse spleen was measured by mixed-lymphocyte reaction assay. Data are expressed as mean ± SD of triplicate samples. (*b*) Immunohistochemical analysis of the spleen from WT or IRF-$2^{-/-}$ mice 6 h after i.p. injection with lipopolysaccharide (200 ng per body). Spleen sections were doubly stained with antibodies against B220 (FITC, green) and CD11c (Alexa Fluor 546, red). (*c*, *e*, and *f*) Cells from the spleen, thymus, or peripheral blood in IRF-$2^{-/-}$ and WT mice were doubly stained with an FITC-conjugated anti-CD11c antibody and a PE-conjugated antibody against IA^b, CD8α, CD11b, or B220 and analyzed by flow cytometry. (*d*) The absolute number of CD8α^+CD11c^+ or CD8α^-CD11c^+ cells per spleen of WT or IRF-$2^{-/-}$ mice was calculated. Each point represents an individual mouse ($n = 5$). *, $P < 0.01$.

CD8α^-CD11b^+B220$^-$ myeloid DCs that developed from IRF-$2^{-/-}$ BM cells were fewer than those from WT BM cells; this observation is accompanied by a significant increase in the number of CD11b^+CD11c^- cells. These results indicate that the aberrant development of APC subsets is a cell-autonomous defect of IRF-$2^{-/-}$ BM cells rather than owing to environmental cues of the mutant mice.

To gain further insights into the defects of IRF-$2^{-/-}$ BM cells, we used a GM-CSF-based *in vitro* culture system (29). The day-6 culture plates of IRF-$2^{-/-}$ or WT BM cells are shown in Fig. 3*a*. WT BM cells formed many cell aggregations, an event characteristic of growing DCs (29). In contrast, IRF-$2^{-/-}$ BM cells failed to form such aggregations. To determine which cell type could be developed in the IRF-$2^{-/-}$ BM cell culture, we analyzed the cell surface markers of APCs by flow cytometry. As reported in ref. 29, the WT BM cell culture showed patterns characteristic of myeloid DC cell differentiation, but the patterns of the IRF-$2^{-/-}$ BM culture were quite different, in that the major population is positive for CD11b, F4/80, and Gr-1 but negative or dull for CD11c (Fig. 3*b*). In fact, these cells resemble the major population of the APCs derived from the IRF-$2^{-/-}$ mouse spleen (Fig. 1*c*). We also cultured IRF-$2^{-/-}$ BM cells with Flt3L, another cytokine that induces DC differentiation *in vitro* (13). As shown in the time course analysis of developing BM cells for the expression of CD11c and MHC class II (Fig. 4), IRF-$2^{-/-}$ BM cells cultured with Flt3L also show similar skewing of differentiation from CD11c$^+$MHC class II^+ DCs to CD11c$^-$MHC class II^{int} cells.

To test their APC activity, $CD8^+$ T cells were purified from 2C T cell receptor-transgenic mice (28) and cultured with GM-CSF-cultured BM cells, and *in vitro* T cell proliferation was monitored. As shown in Fig. 3*c*, the IRF-$2^{-/-}$ BM cells showed very low activity of inducing antigen-specific $CD8^+$ T cell proliferation compared with the WT BM culture cells. On the other hand, they exhibited normal phagocytotic activity, as revealed by FITC–ovalbumin endocytosis assay (Fig. 3*d*). Taken

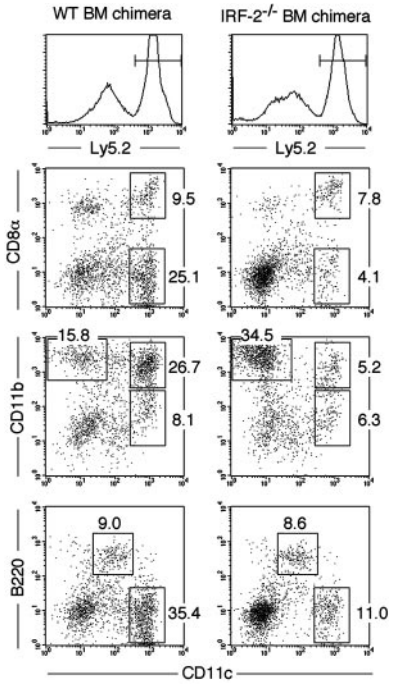

Fig. 2. Cell-autonomous defect of IRF-$2^{-/-}$ BM cells in development of the splenic CD8α^- CD11b^+ DC population. The BM chimeric mice were generated by introducing intravenously Ly5.2^+ IRF-$2^{-/-}$ or WT BM cells into irradiated Ly5.1^+ recipient mice. Four weeks after introduction, the spleens from these chimeric mice were collected and triple-stained with an FITC-conjugated anti-CD11c antibody, a PE-conjugated antibody against CD8α, CD11b, or B220, and a biotinylated anti-Ly5.2 antibody followed by allophycocyanin-conjugated streptavidin. Flow cytometry was performed on gated Ly5.2-positive cells.

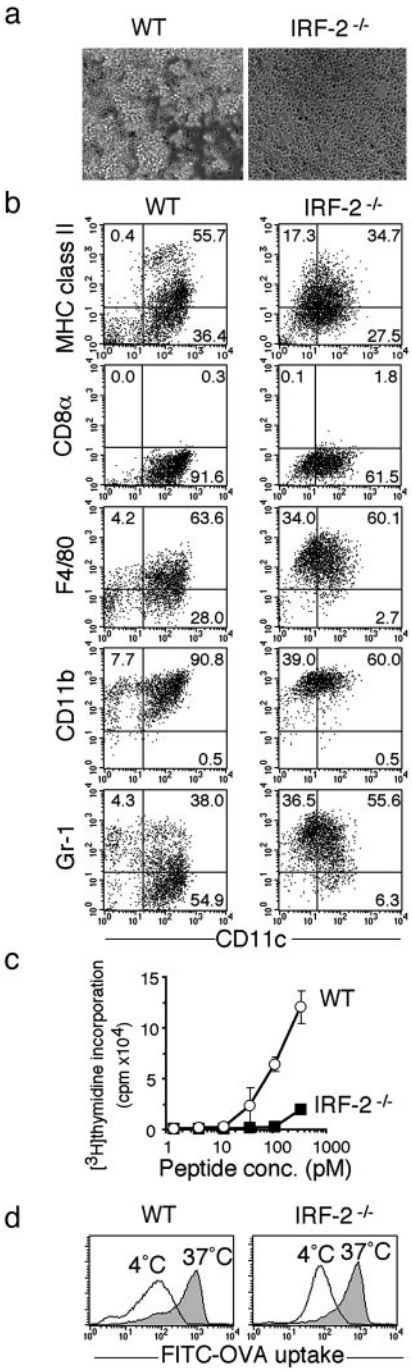

Fig. 3. Abnormal differentiation of IRF-2$^{-/-}$ BM cells cultured with GM-CSF. WT or IRF-2$^{-/-}$ BM cells cultured with GM-CSF for 6 days were analyzed. (*a*) Culture plates of WT or IRF-2$^{-/-}$ BM cells were analyzed by light microscopy. (*b*) BM cells were doubly stained with FITC- and PE-conjugated antibodies and analyzed by flow cytometry. (*c*) To measure APC activity, IRF-2$^{-/-}$ or WT BM-derived cells were incubated with CD8$^+$ T cells from the lymph nodes of 2C T cell receptor-transgenic mice. The synthesized peptide SIYRYYGL was added at the indicated concentration. Data are expressed as mean ± SD of [^{3}H]thymidine uptake. (*d*) WT or IRF-2$^{-/-}$ BM-derived cells were incubated with FITC-conjugated ovalbumin at 4°C or 37°C, and endocytic activity was measured by flow cytometry.

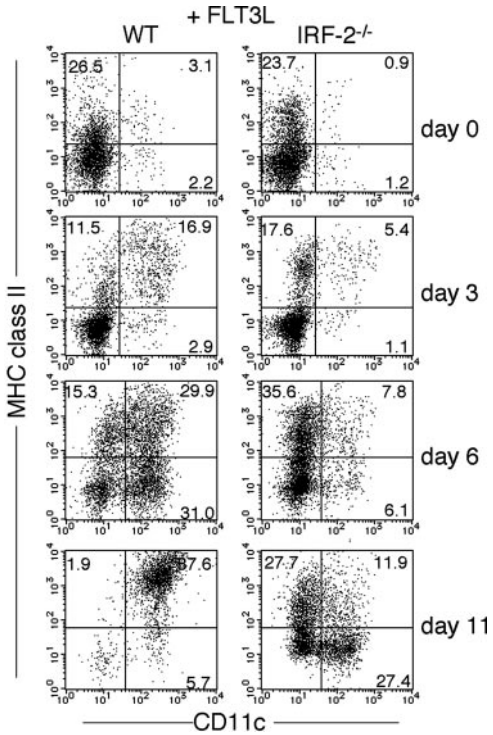

Fig. 4. Defective DC development in Flt3L-supplemented IRF-2$^{-/-}$ BM cells. IRF-2$^{-/-}$ or WT BM cells were cultured with Flt3L. On the indicated days of culture, cells were harvested and analyzed by flow cytometry.

together, the IRF-2$^{-/-}$ BM cells cultured with GM-CSF differentiate into other myeloid lineage cells, which show normal phagocytic activity but poor APC activity. These cells may well reflect the *in vivo* conditions of the IRF-2$^{-/-}$spleen, i.e., the loss of CD8α^- DCs and the increase in the number of CD11c$^-$CD11b^{high} cells.

It is worth noting that the IRF-2$^{-/-}$ BM cells differentiate into CD11b$^+$ macrophages in the presence of macrophage colony-stimulating factor as effectively as the WT BM cells (Fig. 7, which is published as supporting information on the PNAS web site), indicating that impaired DC generation is not due to the reduced number and/or survival of myeloid precursors in BM culture.

Inhibition of DC Development by Excessive IFN-α/β Signaling. One of the functions of IRF-2 is in the attenuation of IFN-α/β signaling to suppress the excessive transcription of its target genes (25). In fact, a low and constitutive expression level of IFN-α/β mRNAs was detected in many cell types, including WT BM cells (data not shown), indicating that a proper balance of IFN-α/β signaling may affect DC development. In fact, recombinant IFN-α/β exerted inhibitory effects on the development of myeloid DCs in both GM-CSF- and Flt3L-supplemented culture (Fig. 5*a*). In this regard, it is interesting that the DC development is notably enhanced in BM cells from mice lacking the IFN-α/β receptor component IFNAR1 (27) when cultured with Flt3L (Fig. 5*b*), an observation congruent with the above notion that a weak IFN signal negatively affects DC development.

The above observations prompted us to examine DC development in mice doubly deficient in *IRF-2* and *IFNAR1*, in which the IRF-2's function of attenuating the IFN signal is not required (25). Thus, we analyzed myeloid DC development *in vitro* and *in vivo* in *IRF-2/IFNAR1* doubly deficient mice. As shown in Fig. 6*a*, the CD8α^- DC subset, which is scarce in the IRF-2$^{-/-}$ spleen, significantly increased in number in the spleen of these mice. In addition, BM cells from these doubly deficient mice, cultured with either GM-CSF or Flt3L, showed a quasi-normal development of CD11c$^+$MHC class II$^+$ DCs *in vitro* (Fig. 6*b*). These results provide genetic evidence that excessive IFN-α/β signaling results in developmental arrest in myeloid DCs and that IRF-2 is necessary to

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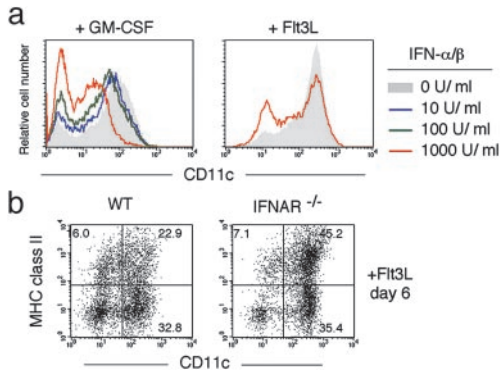

Fig. 5. IFN-α/β inhibits DC development *in vitro*. (*a*) BM cells from WT mice were cultured with GM-CSF or Flt3L in the presence or absence of recombinant IFN-α/β. After 6 or 11 days of culture, cells were analyzed by flow cytometry. (*b*) BM cells from WT or IFNAR1$^{-/-}$ mice were cultured with Flt3L. After 6 days of culture, the cells were analyzed by flow cytometry.

attenuate the signaling. Of note, the IRF-2 mRNA level is higher by ≈3-fold in CD8α^- DCs than in CD8α^+ DCs (data not shown).

Expression of mRNAs for *IFN-α/β* and IFN-Inducible Genes by the Absence of IRF-2. We also addressed the issue of how the absence of IRF-2 in fact affects the expression of *IFN-α/β* genes and IFN-inducible genes in BM cells cultured with Flt3L. Interestingly, IFN-α/β mRNAs are induced during the *in vitro* culture of BM cells by Flt3L in the absence of IRF-2. Concomitantly, IFN-inducible genes, such as 2′,5′-oligoadenylate synthetase, protein kinase R, and IRF-7, were up-regulated in IRF-2$^{-/-}$ BM cells, an observation not made in the WT BM cells (Fig. 6*c*). Furthermore, the abnormal mRNA induction pattern found in IRF-2$^{-/-}$ BM cells was not observed in the IRF-2$^{-/-}$/IFNAR1$^{-/-}$ BM cells. These results suggest operation of a unique mechanism of IFN-mediated IFN production in BM cells, wherein the loss of attenuation of the IFN signal causes a significant induction of *IFN-α/β* genes and excessive IFN signaling.

Discussion

DCs are heterogeneous populations on the basis of their phenotype, function, and tissue distribution (2). CD8α^+ and CD8α^- DCs have been identified as the main DC subsets in the mouse lymphoid organs and are called "lymphoid DCs" and "myeloid DCs," respectively (2, 6). Much has been studied on the role of cytokines in the positive regulation of DC development and activation (13, 14, 29), but little is known about the negative regulation of cytokine signaling pathways for the homeostatic development of DC subsets. In this study, we provide evidence that IRF-2 plays a selective role in the CD8α^- myeloid DC development through the negative regulation of IFN-α/β signaling. In fact, the developmental defect of CD8α^- myeloid DCs was rescued by superimposing a null mutation in the IFN receptor (IRF-2$^{-/-}$/IFNAR1$^{-/-}$ mice). We further found that the loss of IRF-2 in BM cells results in a marked up-regulation of *IFN-α/β* and IFN-inducible genes and that this abnormal gene expression is completely abolished in IRF-2$^{-/-}$/IFNAR1$^{-/-}$ BM cells. After completion of this work, we learned that similar observations were independently made by another group (S. Taki, personal communication).

These series of our results have interesting implications with respect to previous findings on the regulation of IFN-α/β induction and action in the immune system. It was previously shown that IFN-α/β signaling contributes positively to the maturation of DCs (30, 32), the process critical to mount adaptive immune responses. Indeed, IFN-α/β treatment of immature DCs induces the expression of costimulatory molecules, such as CD40 and CD86, and the functional maturation of these cells (33). IFN-α/β signaling positively regulates the expression of pattern recognition receptors, such as protein kinase R, TLR3, and TLR7 (34, 35), to amplify the DC signaling for its maturation. During viral infection, IFN-α/β are induced *en masse* and the IFN signal is essential for the full maturation of DCs, and it is also required for the cross-priming (36) of antigens on MHC-class I molecules, the expression of which is also IFN-inducible (37). Thus, in addition to their role in the innate antiviral immune response, the positive regulation of IFN-α/β signal constitutes an essential aspect of antigen presentation in DCs. On the other hand, our present study demonstrates that the negative regulation of IFN-α/β signaling is a prerequisite for these DC

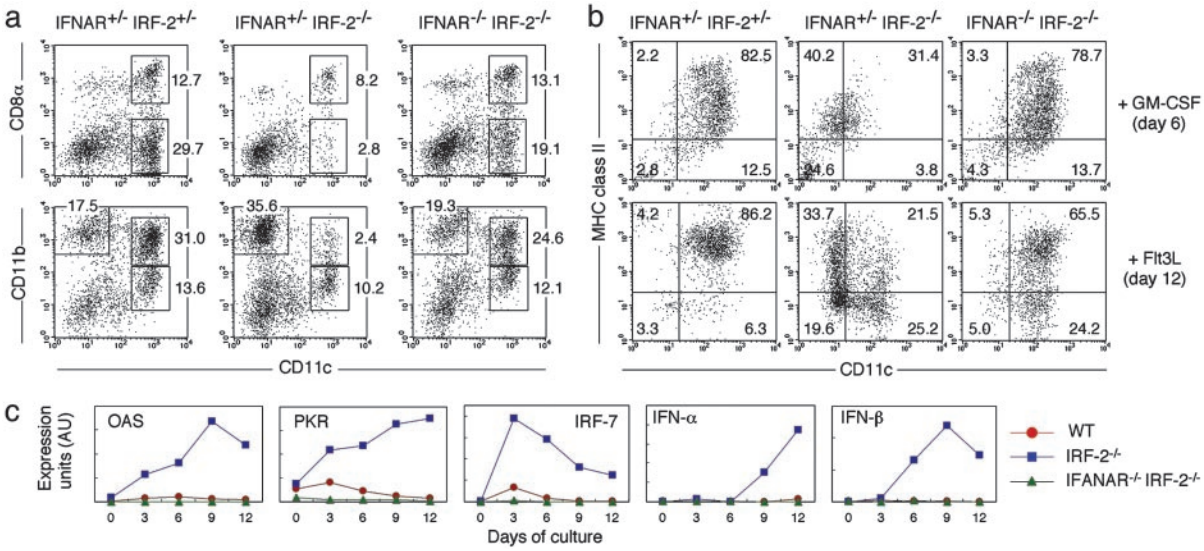

Fig. 6. Mutation of IFNAR1 rescues DC development in IRF-2$^{-/-}$ mice *in vivo* and *in vitro*. (*a*) Splenocytes from the indicated mice were analyzed by flow cytometry. (*b*) After 6 or 12 days of culture with GM-CSF or Flt3L, BM cells from the indicated mice were analyzed by flow cytometry. (*c*) Total RNA was prepared from BM cells cultured with Flt3L at the indicated days of culture and analyzed by quantitative real-time RT-PCR for *IFN-α1*, *-β*, and IFN-α/β-inducible genes (2′,5′-*OAS*, *PKR*, or *IRF-7*).

functions. Such regulation is essential for the development of CD8α^- myeloid DCs. Thus, our study offers a unique example of how stage-specific, positive and negative regulations of a cytokine system, i.e., balancing the beneficial and harmful effects of IFN-α/β signaling, is critical for the dynamic fate of DCs during an immune response. It is worth noting that IRF-2$^{-/-}$ mice also show developmental defects in NK cells in the spleen (38) and B220^{high}IgM$^+$ B cells in the BM (26). Interestingly, however, this defect is not due to excessive IFN signaling as revealed in this study for DCs, because the defect cannot be rescued in IRF-2$^{-/-}$/IFNAR1$^{-/-}$ mice, further pointing to the attenuation of the IFN-α/β signal by IRF-2 for DCs (Fig. 8, which is published as supporting information on the PNAS web site). Although it has been reported that IRF-2 forms a complex with another IRF-family member, IFN consensus sequence-binding protein (ICSBP) (39), the function of IRF-2 is distinct from that of ICSBP, the loss of which affects other DC subsets, i.e., lymphoid (18) and plasmacytoid (40) DCs.

It is interesting that, in the absence of IRF-2, IFN-α/β mRNAs, which are otherwise expressed constitutively at low levels (25), become inducible during the BM culture *in vitro*, although this induction is far weaker than that of the cells induced by a virus (K. H., unpublished data). Such an observation was not made in other cell types such as embryonic fibroblasts (T.T., unpublished data). In view of the fact that this induction requires the IFN signal, which induces the transcription factor IRF-7 that acts on *IFN-*α/β promoters, we infer the operation of a weak, positive-feedback regulation of IFN-dependent IFN gene induction as described in other cells (41). It remains to be clarified whether IRF-7 is indeed activated by GM-CSF or Flt3L.

Our present study raises an interesting issue of how the DC abnormality, found in the present study, relates to our previous study showing the development of autoimmune-like symptoms in IRF-2$^{-/-}$ mice, in which the polyclonal activation of CD8$^+$ T cells becomes prominent after $\approx$12 weeks of age (25). It was also shown that cells other than CD8$^+$ T cells are also responsible for these symptoms (25). Although the hyperactivation of DCs may be a more favorable idea for the generation of autoreactive T cells, our present study may suggest that a skewing of the DC population may perturb the homeostasis of T cells. In this regard, it is interesting to note that mice lacking the RelB transcription factor show somewhat overlapping phenotypes with IRF-2$^{-/-}$ mice, in that both mice show a defect in myeloid DCs (16) and develop autoimmune-like symptoms (42). In this regard, it may also be worth noting that the CD11c$^+$CD8α^+ DC population that developed in the IRF-2$^{-/-}$ spleen have defects in their maturation in response to pathogen-associated molecular pattern (PAMPs), such as lipopolysaccharide or poly(I:C) (Fig. 9, which is published as supporting information on the PNAS web site). Therefore, it is possible that the CD8α^+ DCs that developed in the absence of IRF-2 is anergic to these PAMPs in terms of their maturation.

Although further work will be required to clarify how these phenotypes are associated with each other, an intriguing possibility is that certain developmental defects (or skewing) in DC populations may affect homeostatic T cell responses.

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