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Usp18 Promotes Conventional CD11b+ Dendritic Cell Development

Xiu-Li Cong,* Miao-Chia Lo,* Brian A. Reuter,* Ming Yan,* Jun-Bao Fan,* and Dong-Er Zhang*†

Dendritic cells (DCs) represent the key cells linking innate and adaptive immune responses. It is critical to understand the molecular factors regulating DC differentiation. Usp18 is an IFN-inducible member of the ubiquitin-specific protease family, which deconjugates ubiquitin-like modifier ISG15 from target proteins and competitively inhibits IFN-α/β-induced JAK/STAT activation. This study demonstrates that the frequency of conventional CD11b+ DCs in the spleen of Usp18−/− mice was significantly reduced, whereas the frequencies of conventional CD8+ DCs and plasmacytoid DCs remained normal. In addition, Usp18−/− bone marrow (BM) cells generate DCs less efficiently in GM-CSF-supplemented culture, demonstrating a fundamental defect throughout the DC differentiation pathway. Usp18−/− BM cells were rescued by exogenous expression of either wild-type or deconjugation-inactive Usp18, and superimposition of an IFN-α/β receptor knockout returned in vivo DC populations to normal, clearly showing that the defect seen is due solely to Usp18’s effect on IFN signaling. Finally, Usp18−/− BM-derived DCs expressed high levels of SOCS1/SOCS3, known inhibitors of GM-CSF signaling, providing a mechanistic explanation for the phenotype. In conclusion, we have identified a novel role of Usp18 in modulating conventional CD11b+ DC development via its inhibitory effect on type I IFN signaling. *The Journal of Immunology, 2012, 188: 4776–4781.

It is well known that type I IFNs are essential for host defense against viral and bacterial infections. In addition, type I IFNs are also known to be involved in many immunoregulatory processes, such as NK cell activation (1) and proliferation/survival of CD8+ T cells (2, 3). Beside their well-known role in innate immunity, type I IFNs are constitutively expressed at a low level to ensure the maintenance of cellular homeostasis and may also play a role in shaping the adaptive immunity (4–6). This constitutive IFN expression may, however, have detrimental effects if not tightly controlled.

Usp18 (Ubp43) is an IFN-inducible cysteine protease of the ubiquitin-specific protease family (7) and acts as an ISG15 deconjugating protease in the ISGylation system (8). Furthermore, Usp18 functions in the type I IFN pathway by downregulating the JAK/STAT pathway independently of its isopeptidase activity through an interaction between Usp18 and the IFNAR2 subunit of the type I IFN receptor complex, whereas neither IFNAR1 nor IFNGR1 (type II IFN) receptor subunits were able to interact with Usp18 (9). Usp18-deficient cells have enhanced IFN-α/β signaling and more ISG15 modified proteins (10). As confirmed by gene expression microarray, the expression of IFN-inducible genes is increased and prolonged in the absence of Usp18 (8). In addition, Usp18−/− mice are able to restrict the growth of Salmonella typhimurium more efficiently than wild-type mice (9) and mount a more effective immune response to a number of viruses, including lymphocytic choriomeningitis virus, vesicular stomatitis virus, and Sindbis virus, via the enhanced innate immune responses (11).

Dendritic cells (DCs) play a central role of linking the innate and adaptive immune responses, making them a logical target to study Usp18 in the immune system. In the spleen and lymph nodes of mice, there are several populations of DCs, which generally fall into two major groups: conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs) (12). Within the cDCs group, CD11b+ DCs and CD8+ DCs have been identified as the two major steady-state subsets. In this report, we analyzed DC development in Usp18-deficient mice. Usp18 selectively affects the development of conventional CD11b+ DCs. The frequency of conventional CD11b+ DCs in the spleen of Usp18−/− mice was reduced by ~50%. Bone marrow-derived DCs (BMDCs) are also less efficiently generated by Usp18−/− bone marrow (BM) than by control BM in the presence of GM-CSF. Both overexpression of protease-inactive Usp18 and superimposition of a type I IFN receptor null mutation (Usp18−/−/Ifnar1−/−) rescued the defect. These results indicate that the effect of Usp18 on conventional CD11b+ DC development is independent of its role in protein modification by the ubiquitin-like protein ISG15 and occurs via the type I IFN pathway, which supports the previous finding that constitutively high IFN-α/β signaling inhibits the developmental capacity of the conventional CD11b+ DC population (13, 14). Furthermore, we detected the increased expression of SOCS1 and SOCS3 in Usp18-deficient DC precursor cells. Both of them are type I IFN-inducible inhibitors of GM-CSF signaling. Therefore, our current study has revealed a previously unknown function of Usp18.
Usp18 in DC development and provides a possible molecular mechanism in regulating DC development via the enhanced expression of SOCS1/3 and reduced GM-CSF signaling.

Materials and Methods

Mice

Usp18<sup>−/−</sup> mice were back-crossed to FVB mice over 10 generations. Usp18/IFN-α/β receptor 1 (Ifnar1) double-mutant mice, IFN-α/β receptor 1 knockout mice, and Ube1L<sup>−/−</sup> mice were generated as described (15, 16). Age-matched female mice of each genotype were used for experiments. All of the mice were maintained under specific pathogen-free conditions and used at 6–8 wk of age. All experiments were performed according to institutional guidelines and approved by the institutional animal care and use committee.

Flow cytometry and Abs

Cell suspensions were double- and triple-stained by using various combinations of the following fluorochrome-conjugated Abs: FITC-mixture-anti-Lin (mixture included B220, CD3, CD4, CD8, CD19, Ter119), PE-anti-CD8 and Flt3, FITC–anti-CD11c, allophycocyanin–anti-Sirp1, PE-Cy5.5-anti-CD11b, CD4, and B220, allophycocyanin–anti-CD86 and MHC class II, and PE-anti-pSTAT5 (eBioscience). Stained cells were analyzed by a FACS Canto cytometer (BD Biosciences), and dead cells were excluded by positive propidium iodide staining. Magnetic lineage negative cell sorting was performed using Lineage cell depletion kit containing “lineage” Ag (CD5, B220, CD11b, Gr-1, 7-4, and Ter-119) Abs according to the manufacturer’s instructions (Miltenyi Biotec).

In vivo DC analysis

Cell suspensions from spleen were obtained by physical disaggregation and passage through a 40-μm cell strainer. RBCs were removed by NHCl treatment. Splenocytes were stained and analyzed for the levels of conventional CD11b<sup>+</sup> DCs and CD8<sup>+</sup> DCs and pDCs. For in vivo DC maturation, male Usp18<sup>+/+</sup> and Usp18<sup>−/−</sup> mice were i.p. injected with LPS (500 ng/g body weight) (Escherichia coli; serotype 0118; Sigma-Aldrich) dissolved in PBS. Control groups were injected with PBS alone. Sixteen hours after injection, mice were sacrificed. Single-cell suspensions were prepared from spleen, and maturation was identified by increased expressions of CD86 and MHC class II after gating CD11c<sup>high</sup> DCs from splenocytes.

In vitro BMDC cultures

BMDCs were generated as previously described (17). In brief, BM was obtained from the femurs and tibias of 6–8-wk-old Usp18<sup>+/+</sup> or Usp18<sup>−/−</sup> mice. After RBC lysis, 10<sup>6</sup> total BM cells per milliliter were seeded in 24-well tissue-culture plates and cultured with 20 ng/ml murine GM-CSF (PeproTech) in RPMI 1640 (Life Technologies) supplemented with penicillin (100 U/ml; Sigma), streptomycin (100 μg/ml; Sigma), L-glutamine (2 mM; Sigma), 2-mercaptoethanol (50 μM; Life Technologies), HEPES (100 μM, Life Technologies), and 10% heat-inactivated FCS (Omega). Some cells also received universal type I IFN (PBL Biomedical Laboratories) at 1000 U/ml. After 7 d in culture, nonadherent cells were harvested as immature BMDCs.

For FACS analysis, cells were preincubated with purified anti-mouse CD16/CD32 Fc Block (BD Pharmingen) before staining with the designed mAb or isotype control for 30 min, then washed with buffered normal saline. For staining, cells were labeled with anti-CD11b, CD11c, and CD86 Abs and analyzed by flow cytometry.

Retroviral transduction

Mouse Usp18 cDNA was amplified and cloned into the BamHI–XhoI site of a retrovirus vector MSCV-IRE-EGFP, and Usp18cDNA was used as an enzyme active site mutant control (9). The retrovirus DNA constructs and ecotropic packaging construct were transfected into 293T cells for producing retrovirus as described previously (18). For retrovirus-mediated Usp18 expression in DCs, on days 2, 3, and 4 of the GM-CSF–assisted BM culture, BM cells were incubated in the virus-containing medium in the presence of 8 μg/ml polybrene (Sigma) under 3000 rpm centrifugation for 3 h at 32°C. CD11c expression of cells was analyzed on day 7.

Quantitative RT-PCR analysis

Total RNA was isolated using RNeasy Mini kits (Qiagen). First-strand cDNA was synthesized using a Superscript II Kit (Invitrogen). Quantitative RT-PCR analysis was performed using the SYBR Green system (Bio-systems) with the following primers: SOCS3, 5′-CCCTCAGCTCCAAAAGCGG-3′ and 5′-GCTCTCTTCGCTGCTTGGC-3′; SOCS1, 5′-ACCTTTGTTGGCGACG-3′ and 5′-AAGGCATCCTTCAGCTGACG-3′; GM-CSFRα, 5′-GAGAAGCTTCACTGGAGATCC-3′ and 5′-TGGCAATG-ACGTTGATGTA-3′; GAPDH, 5′-TGATGACATCAAGAAAGGTTGGA-3′ and 5′-TCTCTGAGGCCATGAGGCCAT-3′; Usp18, 5′-TTGGGCCTCTCGAGAAACCA3′ and 5′-GCTAGTTGTTGTAACACACACA-3′. The data were normalized by the corresponding GAPDH expression level in each sample.

Results

Selective loss of the conventional CD11b<sup>+</sup> DC subset in Usp18-deficient mice

To study the role of Usp18 in DC development, various subsets of immune cells in secondary lymphoid organs of Usp18<sup>+/+</sup> and Usp18<sup>−/−</sup> mice were analyzed. The percentages of thymic CD4<sup>+</sup> and CD8<sup>+</sup> T cells and splenic and BM B220<sup>+</sup> B cells were similar in control and Usp18<sup>−/−</sup> mice (data not shown). However, although the total number of splenocytes in Usp18<sup>−/−</sup> mice was similar to that in wild-type littermate controls, the frequency of CD11c<sup>high</sup> cDCs in Usp18<sup>−/−</sup> spleen was significantly lower than that of controls (p = 0.0003) (Fig. 1A). Thus, decreased DC populations in Usp18<sup>−/−</sup> mice suggest a specific role for Usp18 in DC development. Furthermore, in comparison with control mice, the frequency of conventional CD11b<sup>+</sup> DCs in the spleen of Usp18<sup>−/−</sup> mice was reduced by ~50% (Fig. 1B, 1C), however, the conventional CD8<sup>+</sup> DC (Fig. 1D) and pDC (CD11c<sup>int</sup>, B220<sup>+</sup> CD11b<sup>−</sup>) (data not shown) populations were observed with the same frequency in the spleens of both Usp18<sup>−/−</sup> and control mice. Together, these results indicate that Usp18 selectively affects the development of conventional CD11b<sup>+</sup> DCs in vivo.

Further analysis was performed to determine the effect of Usp18 deficiency on the development of late-stage DC precursors, which are defined as Lineage-negative (CD3, CD19, B220, NK1.1, Ter119) and then CD11c<sup>−/low</sup>MHC class II<sup>−/low</sup>Flt3<sup>−/low</sup>CD11b<sup>−</sup>cells in peripheral blood (PB) (19). As shown in Fig. 1E, the late-stage DC precursor population in Usp18<sup>−/−</sup> PB was also markedly reduced (~40% reduction) (p = 0.0039). The compounding phenotype seen throughout the developmental stages suggests that the observed DC deficiency stems from a fundamental defect in the developmental pathway through late DC precursors in PB.

Reduced frequency of the generation of DCs from Usp18-deficient BM in vitro

DCs originate from BM, and DC differentiation is tightly controlled by extracellular stimuli, particularly cytokines. GM-CSF is a well-known positive regulator of DC development (20), especially under inflammation condition, and promotes generation of CD11b<sup>+</sup> CD11c<sup>+</sup> DCs from BM cells under in vitro culture. Therefore, GM-CSF–supplemented cultures of BM cells from Usp18<sup>−/−</sup> mice were used to determine the specific role of Usp18 in BMDC development. Nonadherent CD11c<sup>+</sup> cells were generated from BM cells of Usp18<sup>−/−</sup> mice and of control mice. As shown in Fig. 2A, although total numbers of cells were similar, nearly 60% of wild-type cells were CD11c<sup>+</sup>, and only 35% of Usp18<sup>−/−</sup> cells were CD11c<sup>+</sup>. These results indicate that BMDCs are less efficiently generated by Usp18<sup>−/−</sup> than control BM in the presence of GM-CSF.

To address further the influence of Usp18 deficiency on BMDC development, Usp18 was introduced into Usp18-deficient BM cells...
via retrovirus transduction. Substantial increases in the gene expression levels of Usp18 were seen in the Usp18-deficient cells when transduced with wild-type or mutant Usp18 cDNA compared with those in wild-type cells (Supplemental Fig. 1). In GM-CSF–supplemented culture, overexpression of Usp18 restored the development of CD11b<sup>+</sup>CD11c<sup>+</sup> cells in Usp18-deficient BM cells but did not affect DC development in wild-type BM cells (Fig. 2B), which confirmed the notion that the development defect of BMDCs is intrinsic to Usp18-deficient cells.

**Usp18 activity independent of the ISGylation system**

Usp18 is a known deconjugating enzyme for the protein ISGylation system (21), and Usp18-deficient cells show high ISG15 conjugation levels even without additional type I IFN treatment (10). To determine whether the observed defect was dependent on Usp18 isopeptidase activity, full-length Usp18-deficient cells were transduced with an expression vector for Usp18 [wild-type (wt) or mutant] and cultured as above. The profiles of BMDCs (CD11b<sup>+</sup>CD11c<sup>+</sup>) are shown as a representative FACS plot (left panel). Data represent mean of value from three independent experiments ± SD (right panel). *p < 0.01, #p > 0.1.

Usp18 in BMDC development is not due to its ability to deconjugate ISG15 from target proteins (Fig. 2B).

To address further whether the DC development defect is related to the high level of protein ISG15 conjugation, in vivo analysis of conventional CD11b<sup>+</sup> DC development was performed on Ube1L<sup>−/−</sup> mice, which lack the E1 conjugating enzyme for ISG15 and lack ISGylation (16). The data showed that Ube1L-deficient mice maintain normal conventional CD11b<sup>+</sup> and CD8<sup>+</sup> DCs development in the homeostatic spleen (Fig. 3A). The efficiency of in vitro generation of BMDCs from Ube1L-deficient and control BM cells in GM-CSF–supplemented culture was also similar (Fig. 3B). Therefore, lack of protein ISGylation did not contribute to the DC development defect in Usp18<sup>−/−</sup> mice. These results, taken with the Usp18 overexpression results, strongly suggest that the role of Usp18 in DC development is independent of its role in protein ISGylation.

**Inhibition of DC development by excessive IFN-α/β signaling**

Another important function of Usp18 is the attenuation of IFN-α/β signaling by competitive inhibition of JAK/STAT signal transduction (9). In addition, low and constitutive expression levels of IFN-α have been detected in many cell types, including BM cells (15, 23), indicating that a proper balance of IFN-α/β signaling negatively effects DC development.

Inhibition of DC development by excessive IFN-α/β signaling

**FIGURE 1.** Impaired splenic conventional CD11b<sup>+</sup> DC subpopulation in Usp18<sup>−/−</sup> mice. (A) Total number of splenocytes, the FACS profile, and frequencies of CD11c<sup>high</sup> cells in total splenocytes in Usp18<sup>−/−</sup> and control mice. (B) The FACS profiles and the distribution of (C) conventional CD11b<sup>+</sup> DC (CD11b<sup>+</sup>CD8<sup>a</sup>CD11c<sup>high</sup>) and (D) conventional CD8<sup>+</sup> DC (CD11b<sup>+</sup>CD8<sup>a</sup>CD11c<sup>low</sup>) subsets in total splenocytes in Usp18<sup>−/−</sup> and control mice. Each dot represents the value obtained from an individual animal. (E) The number of DC precursors was markedly reduced in PB in Usp18<sup>−/−</sup> compared with control mice (defined as Lineagenegative [CD3, CD19, B220, NK1.1, Ter119] and then CD11c<sup>+</sup>MHC class II<sup>+</sup>F4/80<sup>−</sup>Sirp1<sup>−</sup>cells). Each dot represents the value obtained from an individual animal.

**FIGURE 2.** Inefficient generation of BMDCs from Usp18-deficient BM cells in vitro. (A) BMDCs were less efficiently generated from Usp18<sup>−/−</sup> BM in GM-CSF–supplemented culture. A representative result of six individual mice. (B) Retrovirus-mediated expression of Usp18 restored the development of CD11c<sup>+</sup> cells from Usp18-deficient BM cells. BM cells were transduced with an expression vector for Usp18 [wild-type (wt) or mutant] and cultured as above. The profiles of BMDCs (CD11b<sup>+</sup>CD11c<sup>+</sup>) are shown as a representative FACS plot (left panel). Data represent mean of value from three independent experiments ± SD (right panel). *p < 0.01, #p > 0.1.
and CD8+ DC subsets in spleen were analyzed in mice that were doubly deficient in Usp18 and Ifnar1, mice in which Usp18’s attenuating effect on the IFN-α/β receptor is overshadowed by the lack of IFN-α/β signaling altogether (9). As shown in Fig. 4C, the DC precursor population in PB of doubly deficient mice was increased compared with that in wild-type mice, which was opposite to the reduction in Usp182/2 PB; however, similar distribution of conventional CD11b+ and CD8+ DC subsets was shown in the spleen of doubly deficient mice and control mice (Fig. 4D). Similar splenic DC subsets were also observed in the Ifnar1-/- and control mice (Supplemental Fig. 2). These results reinforce the evidence that excessive IFN-α/β signaling results in the retarded development of conventional CD11b+ DCs. Combined with the evidence that conventional CD11b+ DCs develop independent of Usp18 isopeptidase activity, this suggests that the effect of Usp18 is a result of attenuated type I IFN signaling, which, when Usp18 is not present, hinders conventional CD11b+ DCs development.

**Upregulation of SOCS1/SOCS3 and inhibition of GM-CSF signaling with Usp18 deficiency**

Because GM-CSF promotes the development of DCs from BM but was less effective in Usp182/2 mice, the mechanism of modulation of GM-CSF signaling by Usp18 in hematopoietic cells was investigated. GM-CSF signaling is transduced through the JAK/STAT pathway, preferentially STAT5 (24), which is inhibited by the IFN-inducible proteins SOCS1 and SOCS3 (25, 26). Real-time RT-PCR was used to determine whether Usp18 could affect the expression of SOCS1 and SOCS3 in Lin- BM cells, the primitive source of DC precursors in BM. The results showed that SOCS1 and SOCS3 transcript levels were upregulated in Usp18-deficient Lin- cells, especially that of SOCS3 with more than a 6-fold in-

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**FIGURE 3.** Normal conventional CD11b+ DC development in Ube1L-deficient mice. (A) The distribution of conventional CD11b+ DC (CD11b+ CD8a+CD11chigh) and CD8+ DC (CD11b+ CD8a+CD11chigh) subsets in total splenocytes in Ube1L-/- and control mice. (B) Normal generation of BMDCs from Ube1L-deficient BM cells in vitro with GM-CSF-supplemented condition. Data represent mean of four mice ± SD.

**FIGURE 4.** IFN-α/β inhibits BMDC development in vitro, and mutation of Ifnar1 rescues conventional CD11+ DC development in Usp18-/- mice in vivo. (A) BM cells from wild-type mice were cultured with GM-CSF in the presence or absence of recombinant IFN-α/β. After 7 d of culture, cells were analyzed by flow cytometry for CD11c expression. (B) A significant increase in the number of CD11b+CD11c- cells in IFN-α/β-supplemented culture. (C) Similar percentage of DC precursors in PB in Usp18-/-/Ifnar1-/- (double-knockout; DKO) and wild-type mice. (D) Splenic conventional CD11b+ DC and CD8+ DC subsets from DKO and control mice were analyzed. Data represent mean of five mice ± SD.

**FIGURE 5.** IFN-α/β signaling induces SOCS1/SOCS3 expression and inhibits GM-CSF signaling. (A) Quantitative RT-PCR detection for SOCS1 and SOCS3 mRNA expression in freshly sorted Lin- cells using magnetic lineage cell depletion kit from Usp18-/- and control mice from three independent experiments (total of 12 mice). (B) SOCS1 and SOCS3 relative transcript levels in day 7 BMDCs from Usp18-/- and control mice from five independent experiments. (C) Primary Lin- cells were treated with 20 ng/ml GM-CSF for 15 min before Stat5 phosphorylation was monitored by FACS with specific anti-phosphotyrosine Ab. Data represent mean ± SD from three independent experiments (total of 12 mice).
crease compared with that in control cells (Fig. 5A). In addition, SOCS mRNAs in primary murine DCs from culture supplemented with GM-CSF showed a similar increase in transcript levels within Usp18-deficient BMDCs over those of controls (Fig. 5B).

To confirm the effect of the increase in SOCS1 and SOCS3 in the DC lineage and determine if these inhibitory proteins could block cytokine signaling in Lin− BM cells, cells were stimulated with GM-CSF for 15 min, followed by FACS analysis of pStat5. The analysis showed a reduction in GM-CSF–induced Stat5 phosphorylation in Usp18-deficient cells compared with that in control cells (Fig. 5C). GM-CSFR levels were also analyzed in BMDCs and were similar in Usp18−/− and controls (data not shown). Therefore, a difference in receptor transcription in response to GM-CSF signaling is not responsible for the observed inhibition of GM-CSF signaling in Usp18-deficient mice. Thus, these data suggest that reduced GM-CSF signaling due to the enhanced expression of two negative regulators of this pathway (SOCS1 and SOCS3) in Usp18 knockout mice may contribute to the deficiency of cDC development.

Discussion

The balanced action of cytokines is known to be critical for the maintenance of the immune system. DCs play a crucial role in linking innate and adaptive immunity, and little is known about the molecular mechanisms that differentially regulate their development. This study demonstrates that Usp18 positively regulates homeostatic development of the conventional CD11b+ DCs subset independent of its enzyme activity and via the attenuation of IFN-α/β signaling. In addition, SOCS1/SOCS3 expression was up-regulated, and GM-CSF was less active in Usp18−/− cells, suggesting a reasonable mechanistic explanation for the observed results.

It has been demonstrated that Usp18 is a negative regulator of the type I IFN signaling pathway, and the most pronounced phenotype of Usp18 deficiency is hypersensitivity to type I IFN, with enhanced and prolonged activation of JAK/STAT signaling (10). The current study shows that Usp18−/−, but not Ube1L−/− or Usp18−/−/Ifnar1−/−, mice exhibit a marked decrease in the number of conventional CD11b+ DCs in the spleen compared with that in wild-type controls. In accordance with the positive effect of Usp18 on conventional CD11b+ DC development in vivo, the in vitro generation of BMDCs from Usp18-deficient BM cells was also impaired. In addition, type I IFN treatment reduced the generation of BMDCs from wild-type BM, whereas both Ube1L−/− BM and Usp18−/− BM overexpressing isopeptidase-inactive Usp18 (which retains its IFN-suppressive function) showed normal BMDC development, confirming the evidence that Usp18 affects DC development based on its suppressive role on the type I IFN signaling pathway and independent of its role in the ISGylation system. In contrast to the evidence regarding conventional CD11b+ DCs, other DC subsets were present at normal frequencies in Usp18−/− mice, suggesting that Usp18 is required for the efficient development of conventional CD11b+ DCs but not conventional CD8+ DCs. Recently, IFN regulatory factor 2, which also acts by inhibiting IFN-α/β signaling, was reported to be critical for the development of conventional CD11b+ DCs (13, 27), which is consistent with our results.

The current model showed a reduced percentage of DC precursors (CD11c+ MHC class II+) in PB, in addition to the mature DC phenotype, in Usp18−/− mice compared with that in control mice. One crucial cytokine for hematopoietic progenitors and conventional CD11b+ DC differentiation is GM-CSF. It has been reported that GM-CSF− and GM-CSFRβc–deficient mice have a decrease in numbers of DC populations, with the most significant impact being on the steady-state development of myeloid DCs (current term is conventional CD11b+ DCs) (28). In addition, treatment of mice with GM-CSF preferentially expands conventional CD11b+ DCs in the spleen (29). GM-CSF can signal through the JAK/STAT pathway with specific activation of JAK2, STAT3, and STAT5 (30), preferentially STAT5A and STAT5B (24), which are controlled by the expression of the inhibitory protein SOCSs (25). A recent study showed that within hours, IFN-α induces the transcriptional upregulation of SOCS1 and SOCS3 (26). In the current model, SOCS1 and SOCS3 mRNA levels were constitutively upregulated in Usp18−/− primitive Lin− cells and BMDCs compared with those in controls, which is consistent with the above observation. The upregulation of SOCS proteins matched an observed reduction in Stat5 activation, which showed low phosphorylation level upon GM-CSF stimulation in Usp18-deficient primitive Lin− cells (Fig. 5C). These results demonstrate that the negative modulation of the GM-CSF/Stat5 signaling pathway by SOCS proteins is enhanced in Usp18−/− mice, which have high constitutive IFN-α/β signaling, and leads to a diminished conventional CD11b+ DCs subset throughout the stages of DC development.

It contrast, differentiated Usp18−/− BMDCs were fully susceptible to conventional maturation stimuli. They responded normally to LPS, polyinosinic-polycytidylic acid, and TNF-α, becoming activated, mature, CD86+MHC class II+ DCs in vitro at similar frequencies as those of control BMDCs. Furthermore, LPS also induced the normal maturation in Usp18−/− spleen DCs in vivo (Supplemental Fig. 3). Thus, the differentiation pathway, as opposed to the maturation pathway, of DCs appears to be more sensitive to IFN-α/β, making the negative regulatory effect of Usp18 on IFN-α/β signaling essential to the development of DCs, but not their functional activation. This could be explained by the possibility that during inflammation resulting from infection or other insults, a prominent rise in GM-CSF levels may overpower the ability of IFN-induced SOCS1 and SOCS3 to inhibit the GM-CSF response, even in hypersensitive Usp18−/− mice. Furthermore, the current examination of conventional CD11b+ DCs and their direct precursors in Usp18/Ifnar1 double-knockout mice provided evidence that excessive IFN-α/β signaling is the cause of the developmental delay in conventional CD11b+ DCs and, in combination with the previous results, showed that the attenuating effect of Usp18 on IFN-α/β signaling is essential for normal conventional CD11b+ DC development.

In conclusion, a novel role has been identified for Usp18 as an immunoregulatory factor independent of its deconjugating activity. Usp18 positively regulates homeostatic development of the conventional CD11b+ DC subset along with its direct DC precursors in PB through attenuation of IFN-α/β signaling. These data suggest that IFN-α/β are potent inhibitors of GM-CSF responses in primary hematopoietic cells and block the differentiation of functional DCs, which may be a result of the induction of SOCS1 and SOCS3. In recent years, IFN-α has been widely used for the treatment of many diseases and is generally considered to enhance the innate immune response. This study demonstrates that a possible side-effect of long-term type I IFN treatment could be a reduction in the ability to stimulate the adaptive immune response, and that Usp18−/− mice provide an appropriate model with which to study those effects.

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Disclosures
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