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Augmentation of Antigen-Presenting and Th1-Promoting Functions of Dendritic Cells by WSX-1(IL-27R) Deficiency

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WSX-1 is the α subunit of the IL-27R complex expressed by T, B, NK/NKT cells, as well as macrophages and dendritic cells (DCs). Although it has been shown that IL-27 has both stimulatory and inhibitory effects on T cells, little is known on the role of IL-27/WSX-1 on DCs. LPS stimulation of splenic DCs in vivo resulted in prolonged CD80/CD86 expression on WSX-1-deficient DCs over wild-type DCs. Upon LPS stimulation in vitro, WSX-1-deficient DCs expressed Th1-promoting molecules higher than wild-type DCs. In an allogeneic MLR assay, WSX-1-deficient DCs were more potent than wild-type DCs in the induction of proliferation of and IFN-γ production by responder cell proliferation. When cocultured with purified NK cells, WSX-1-deficient DCs induced higher IFN-γ production and killing activity of NK cells than wild-type DCs. As such, Ag-pulsed WSX-1-deficient DCs induced Th1-biased strong immune responses over wild-type DCs when transferred in vivo. WSX-1-deficient DCs were hyperreactive to LPS stimulation as compared with wild-type DCs by cytokine production. IL-27 suppressed LPS-induced CD80/86 expression and cytokine production by DCs in vitro. Thus, our study demonstrated that IL-27/WSX-1 signaling potently down-regulates APC function and Th1-promoting function of DCs to modulate overall immune responses. The Journal of Immunology, 2007, 179: 6421–6428.

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3 Abbreviations used in this paper: DC, dendritic cell; BM, bone marrow; WT, wild type; KLH, keyhole limpet hemocyanin; LN, lymph node; MPL, mean fluorescence intensity; SOCS3, suppressor of cytokine signaling; BM, bone marrow; KO, knockout.

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Materials and Methods

Animals

WSX-1−deficient (WSX-1−/−) mice were generated as described previously and were backcrossed more than nine times to C57BL/6 mice (continual backcrossing). Mice were housed in microisolator cages and were used
between 8 and 14 wk of age. Age- and sex-matched wild-type (WT) C57BL/6 mice (Seac Yoshitomi) were used as controls. All experiments were approved by the institutional animal research committee of Saga University and conformed to the animal care guidelines of the American Physiologic Society.

**Reagents**

LPS was purchased from Sigma-Aldrich. Anti-mouse IL-6 Ab and anti-mouse IL-10 Ab were purchased from R&D Systems and were used at 1 μg/ml for in vitro blocking of each cytokine. rIL-27 was purchased from R&D Systems or generated in our laboratory as described previously (4). Anti-STAT3, STAT1, and anti-IκB Abs were purchased from Santa Cruz Biotechnology and Abs against p38 and anti-phosphorylated proteins were purchased from Cell Signaling.

**Cell preparation**

Bone marrow (BM)-derived DCs were prepared from BM suspensions from femurs and tibias of mice as described elsewhere (13). Briefly, bone marrow cells were cultured with 10 ng/ml murine GM-CSF (R&D Systems) and 10 ng/ml murine IL-4 (R&D Systems) for 10 days and used as DCs. For preparation of splenic DCs, spleen cells were stained with FITC-conjugated anti-CD11c Ab (BD Pharmingen) followed by anti-FITC magnetic beads (MACS; Miltenyi Biotec). NK cells and CD8+ cells also were purified using PE-anti-NK1.1 Ab (BD Pharmingen) and PE-anti-CD8 Ab, respectively, followed by anti-PE magnetic beads (Miltenyi Biotec). Splenic DCs cultured without any simulation for 24 h were regarded as mature (14).

**LPS stimulation of DCs in vivo and in vitro**

WT or WSX-1-deficient mice were injected i.p. with LPS (200 μg/mouse). After LPS injection, CD11c+ cells in the spleen were analyzed for CD80 or CD86 expressions. For in vitro stimulation of DCs, splenic DCs were prepared and were stimulated with LPS (100 ng/ml) for 48 h. Cells were examined for surface expressions of CD80 or CD86 as above.

**Quantitative real-time PCR and RT-PCR analyses**

Total RNAs were extracted from cells using TRIzol solution (Invitrogen Life Techniques) and reverse-transcribed with a ReverTra-plus-kit (Toyobo). Expression levels of WSX-1, IL-27 EBI-3, IL-27p28, IL-12 p40, Jagged-1, and Delta-4 in DCs were determined relative to that of β-actin using TaqMan-PCR (Qiagen) and an Applied Biosystems PRISM 7700 sequence detection system according to the manufacturer’s instructions. Oligonucleotide primers and probes were designed using a Primer Express program (Applied Biosystems). The relative expression of each mRNA was determined and normalized to the expression of β-actin. For the expression of perforin and granzyme B in NK cells, RNAs were prepared and reverse-transcribed as above and the same amounts of cDNAs normalized to β-actin were amplified for the gene expression by PCR. Similarly, suppressor of cytokine signaling (SOCS) 3 expression was examined in LPS-stimulated DCs. Primer and probe sequences used are described elsewhere (15, 16).

**Flow cytometry**

For flow cytometric analyses of surface molecules on DCs, cells were treated with Fc Block (BD Biosciences) followed by staining with FITC-conjugated anti-CD11c Ab plus biotin-anti-CD80 Ab or biotin-anti-CD86 Ab (both from BD Pharmingen) and streptavidin-PE staining and analyzed using a FACScalibur (BD Biosciences) and CellQuest software (BD Biosciences). As negative staining controls, cells were treated likewise except isotype-matched Ig was used instead of anti-CD80/86 Abs.

**Allogeneic MLR assay**

Allogeneic MLR experiments were performed by culturing CD4+ T cells from BALB/c (H-2b) mice as responder with splenic or BM-derived DCs from WT or WSX-1-deficient mice (H-2b) as stimulator. Briefly, 2 × 10^6 of CD4+ T cells were cocultured with DCs at 300–30,000/200 μl/well for 96 h in either the presence or absence of LPS (100 ng/ml; Sigma-Aldrich). During the last 18 h of culture, cell proliferation was measured by uptake of [3H]thymidine. IFN-γ production in the supernatants was measured by an ELISA development kit (R&D Systems).

**In vivo transfer of Ag-pulsed DCs**

Splenic DCs were prepared and incubated at 2 × 10^6/ml with 50 μg/ml keyhole limpet hemocyanin (KLH; Sigma-Aldrich) for 18 h. DCs were washed three times, resuspended in PBS, and then administered into the hind footpad of WT mice (1 × 10^6 cell/40 μl of PBS per mouse). The draining lymph nodes (LN) were removed 5 days after transfer and lymphocytes were cultured at 1 × 10^5 cells/200 μl well with or without KLH (20 μg/ml) for 96 h. Ag-specific proliferation and IFN-γ production were measured as above. KLH-specific IgG2b and IgG1 in the sera of mice on day 5 after transfer were detected as described elsewhere (8).

**Parasite infection and vaccination with DC**

BM-derived DCs (5 × 10^6/ml) were cocultured with Leishmania major (MHOM/SU/73-5-ASKH) promastigotes lysates (2.5 × 10^6/ml at ratio of 10:1 for 18 h, washed with PBS three times, and injected i.v. (5 × 10^6 DC/40 μl PBS per mouse). Control mice were treated with PBS. One week later, mice were infected intradermally with 1 × 10^6 promastigotes of L. major at the footpad. The course of infection was monitored daily by measuring the increase in thickness of the infected footpad over uninfected footpad as described elsewhere (3). Draining LN cells were removed (day 2 after infection for WSX-1-deficient DC-transferred mice or 4 wk after infection for control and WT DC-transferred mice) and examined for proliferation and IFN-γ production as described elsewhere (3). Parasite numbers in the footpads were measured as follows. Footpads were removed from mice, minced, and incubated in RPMI 1640 medium at 25°C. After 3 days of incubation, parasite number in the culture medium was counted microscopically.

**NK cells assay**

Purified NK1.1+ NK cells (5 × 10^6/200 μl per well) were cocultured with WT or WSX-1-deficient DC11c+ splenic DCs in the presence of LPS (100 ng/ml) at indicated mixing ratios for 24 h. Cytokine production in the supernatants was measured by ELISA. For cytotoxicity assay, NK cells were cocultured with WT or WSX-1-deficient DCs at 10:1 ratio for 24 h and were measured for killing activity against [3H]-labeled YAC-1 cells as target. The percentage of specific lysis was calculated as follows: percent lysis = [(cpm experimental well − cpm spontaneous release)/(cpm maximum release − cpm spontaneous release)] × 100. Expression of perforin and granzyme B was also examined by real-time PCR analyses.

**Western blotting**

Splenic DCs (1 × 10^6/ml) were prepared and stimulated with LPS (100 ng/ml) in either the presence or absence of rIL-27 (10 ng/ml; R&D Systems). Immunoblotting to detect phosphorylated STAT3, STAT1, p38, and IκB was performed as described previously (17).

**Determination of cytokine production by DCs**

Splenic DCs were prepared and stimulated with LPS (100 ng/ml) in the presence of IL-27 (10 ng/ml), anti-IL-6, or anti-IL-10 Ab for 24 h. Culture supernatants were examined for IL-12p40, TNF-α, or IL-6 by ELISA (Quantikine HS ELISA Kit; R&D Systems) according to the manufacturer’s direction.

**Results**

**Augmented expression of WSX-1 in DCs after activation**

WSX-1 is expressed in most immune cells, such as T cells, B cells, NK cells, and macrophages. Although IL-27 is mainly produced by DCs, the precise activation/maturation status of IL-27-producing DCs was not known. To examine the expression of WSX-1/IL-27 in DCs, we first assessed the expression of both subunits of IL-27, p28, and EBI-3 in an activation-dependent manner. However, the expression of WSX-1 was also low in immature DCs but increased after LPS stimulation (Fig. 1B). Because the DCs did not proliferate in this experimental condition (data not shown), these data demonstrated that activated DCs expressed WSX-1 as well as both subunits of IL-27 in an activation-dependent manner.
Prolonged expression of CD80 and CD86 on WSX-1-deficient DCs after in vivo stimulation with LPS

To examine the effect of IL-27/WSX-1 on DC function, we took advantage of WSX-1 knockout (KO) mice (3) and analyzed the expression of some cell surface proteins. The expression of CD80 and CD86, both costimulatory molecules, was slightly lower in WSX-1-deficient DCs than in WT DCs (Fig. 2A). The expression levels of MHC class I molecules (H-2Kd) and the percentages of CD4+ or CD8+ cells within DCs, however, were comparable between the two groups of mice (data not shown), suggesting that there were no differences in the cellular composition between WSX-1-deficient DCs and wide-type DCs. Upon i.p. injection of LPS into mice, the expression of CD80 and CD86 in DCs of both groups began to augment at 6 h after stimulation (data not shown) and at 24 h, ~60% of DCs were positive for CD80 and CD86 expression. At this time point, there was no difference in the percentages of CD80- and CD86-positive cells between the two groups of mice (Fig. 2A). Also, there were no significant differences in the mean fluorescence intensity (MFI) for the respective molecules between the two groups of mice (Fig. 2B). Although the percentages of CD80- and CD86-positive DCs gradually decreased thereafter by 72 h after stimulation, WSX-1-deficient DCs still expressed high levels of CD80 and CD86 at 48 h after stimulation (Fig. 2A). The expression levels were still higher in WSX-1-deficient DCs than in WT DCs at 72 h, albeit showing a decrease at 48 h. MFI for both CD80 and CD86 was also higher in WSX-1-deficient DCs than in WT DCs at 48 h and later (Fig. 2B). The prolonged expression of CD80 and CD86 on WSX-1-deficient DCs indicated that DCs without IL-27/WSX-1 stimulation remained in activated status longer than WT DCs after stimulation. Percentages of viable DCs after in vitro stimulation with LPS were almost equivalent between WT and WSX-1-deficient DCs (data not shown).

In vitro suppression of CD80/86 expression by IL-27

The prolonged expression of CD80/86 by WSX-1-deficient DCs observed above could be secondary but not primary events induced by cytokines produced by in vivo LPS stimulation. To address the direct effect of WSX-1-deficiency on DCs and, more practically, to address the direct suppressive effect of IL-27 on DCs, spleen-derived DCs from WT or WSX-1-deficient mice were stimulated with LPS in vitro. As shown in Fig. 3, WSX-1-deficient spleen-derived DCs showed activated phenotypes even before LPS stimulation in terms of CD80 expression. Although less evident than in vivo observation, there was a little but reproducible increase in the percentage of WSX-1-deficient CD80-positive cells as compared with that of WT cells. Presumably, in vitro stimulation of DCs with LPS (1–100 ng/ml used in our experiments) was strong enough to induce full induction of CD80 expression even in WT cells. As expected, however, addition of rIL-27 (10 ng/ml) significantly reduced CD80 expression by WT but not WSX-1-deficient DCs. IL-27 also suppressed the expression of CD86 by WT but not WSX-1-deficient DCs. Addition of anti-IL-6 Ab to the stimulation culture did not affect the CD80/86 expression by both WT and WSX-1-deficient DCs. Thus, it was unlikely that the higher expression of CD80/86 by WSX-1-deficient DCs is secondary to IL-6 production by stimulated DCs. Addition of IL-10 Ab did not affect the CD80/86 expression either (data not shown). Taken together, IL-27 had an inhibitory effect on LPS-stimulated DC function and, in the absence of its receptor, WSX-1, DCs showed higher sensitivity to LPS stimulation by CD80/86 expression both in vivo and in vitro, although the effect observed in vivo may be partially due to secondary cytokines produced after LPS stimulation.
**AUGMENTATION OF DC FUNCTION BY IL-27R DEFICIENCY**

**Th1-inducing properties of WSX-1-deficient DCs**

To further examine the impact of WSX-1 deficiency on DC function, we examined the expression of cytokines and some Notch ligands. Although the production of IL-12p70 was very low in WT and WSX-1-deficient DCs, WSX-1-deficient DCs produced significantly higher IL-12p70 before and after in vitro LPS stimulation (Fig. 4A). Production of p40 subunit of IL-12 was also higher by WSX-1-deficient DCs than by WT DCs irrespective of LPS stimulation. Quantitative RT-PCR analyses also confirmed higher expression of IL-12p40 in WSX-1-deficient DCs than in WT DCs (Fig. 4B). The expression of EBI-3, a subunit of IL-27, was also higher in WSX-1-deficient DCs than in WT DCs. There was no significant difference in the expression of p28 of IL-27 between WT and WSX-1-deficient DCs (data not shown). Interaction of Notch with its ligands, such as Jagged and Delta, affects the differentiation of CD4+ T cells (16). The expression of Delta-4, a Th1-inducing Notch ligand, was drastically induced and was much higher in LPS-stimulated WSX-1-deficient DCs than in WT DCs. Interestingly, the expression of Jagged-1, a Th2-inducing Notch ligand, was also induced by LPS stimulation in WT DCs and was higher in WT DCs than in WSX-1-deficient DCs. Expression of TLR4, the receptor for LPS, was not affected by WSX-1 deficiency (data not shown). These data indicated that WSX-1-deficient DCs were more potent in the induction of Th1 responses than WT DCs.

To substantiate the possible augmentation of Ag-presenting function as well as the possible augmentation of Th1-inducing function of WSX-1-deficient DCs, an allogeneic MLR assay was performed where responder CD4+ T cells from BALB/c mice (H-2b) were mixed with either WT DCs or WSX-1-deficient DCs (H-2b) as stimulator and cytokine production and responder proliferation was measured. When splenic DCs were used as stimulator, WSX-1-deficient DCs induced higher proliferation of responder cells than WT DCs in the presence or absence of LPS (Fig. 5A). IFN-γ production was also higher in responder cells plus WSX-1-deficient DCs than in responder plus WT DCs. BM-derived DCs also showed similar results, albeit less in degree in that both IFN-γ production and responder cell proliferation were higher when WSX-1-deficient DCs were used as stimulator (Fig. 5B). There was no significant difference in IL-2 production between cultures with WT DCs and WSX-1-deficient DCs (data not shown). Production of IL-4 was below detectable levels in all culture conditions (data not show). These results demonstrated that WSX-1-deficient DCs were more potent as Th1-inducing APCs than WT DCs.

**Augmented APC functions of WSX-1-deficient DCs in vivo**

Next, we analyzed the APC function of WSX-1-deficient DCs in vivo by adoptive transfer of Ag-pulsed DCs into WT mice. BM-derived DCs were prepared either from WT or WSX-1-deficient mice, pulsed with KLH, and then transferred into footpads of the syngeneic C57BL/6 mice. First, there was no difference in the number of lymphocytes in the draining LN between the two groups of mice after cell transfer (data not shown). Although CD4+ T cells from WSX-1-deficient DC-transferred mice proliferated reproducibly (but not significantly) higher than the cells from WT DC-transferred mice in response to KLH stimulation, these CD4+ T cells from WSX-1-deficient DC-transferred mice produced more IFN-γ than cells from WT DC-transferred mice (Fig. 6A). IL-4 production was below detectable levels in this experimental condition. These data demonstrated that WSX-1-deficient DCs were more potent as Th1-inducing APCs in vivo than WT DCs. Consistent with this, mice transferred with WSX-1-deficient DCs contained higher levels of KLH-specific IgG2a, a subtype dependent on Th1-type immune responses, than WT DC-transferred mice (Fig. 6B). The levels of KLH-specific IgG1, a subtype dependent mainly on Th2-type responses, were similar between the two groups of mice. All of these data strongly suggested that WSX-1-deficient DCs initiate higher levels of Th1-biased immune responses than WT DCs in vivo.
Protection against *L. major* infection bestowed by WSX-1-deficient DC transfer

*L. major* is intracellular protozoa and that the clearance of the parasites exclusively dependent on proper Th1 responses and IFN-γ production has been reported (18). Given the augmented APC function of WSX-1-deficient DCs with Th1-inducing features, we then examined the effect of the adoptive transfer of DCs pulsed with *L. major* lysates. Since the untreated WT mice with an H-2b haplotype were a resistant strain, a slight but significant swelling of the footpad, up to 1 mm, was observed on day 13 after infection (Fig. 6C). In mice transferred with WT DCs pulsed with *L. major*, lysates showed footpad swelling on day 15 after infection, whose peak was lower than in untreated mice. Mice transferred with WSX-1-deficient DCs pulsed with *L. major* lysates, showed no peak swelling of the footpad, demonstrating successful infection by *L. major* Ag-pulsed DCs and infected with *L. major* in their hind footpad as described in Materials and Methods. Footpad swelling was then measured. O, Untreated mice; □, WT DC transferred; ■, WSX-1-deficient (KO) DC transferred; *, *p < 0.01 and **, *p < 0.05 as compared with KO DC-transferred mice. D, On day 14 after infection, parasite numbers in the footpads from control (□), WT DC-transferred (■), or WSX-1-deficient DC-transferred (KO; □, DC, ■, mice. Data shown are mean ± SD (n = 5 per group). **, *p < 0.01. E, On day 2 after infection, CD4+ T cells from the draining LN were stimulated with *L. major* Ag and were examined for IFN-γ and IL-4 production. □, No Ag; ■, with *L. major* Ag; **, *p < 0.01. Experiments were repeated three times with similar results.

Augmented NK cell IFN-γ production by WSX-1-deficient DCs

Recent lines of evidence show that DCs also augment cytotoxic functions and IFN-γ production of NK cells (19). To address whether WSX-1-deficient DCs are also potent in augmenting NK functions, purified WT NK cells were cocultured with either WT or WSX-1-deficient DCs and production of cytokines, expression of cytotoxic molecules, and killing activity were measured. NK cells primed with WSX-1-deficient DCs secreted more IFN-γ than transferred mice, at 4 wk after infection, later than in WSX-1-deficient DC-transferred mice (data not shown). These data demonstrated that WSX-1-deficient DCs elicited stronger Th1-biased immune responses in vivo after transfer than did WT DCs.
did NK cells with WT DCs, especially at high DC:NK ratios (Fig. 7A). Although not significant, NK cells with WSX-1-deficient DCs produced slightly more TNF-α than did NK cells with WT DCs. In RT-PCR analyses, expression of perforin was augmented in NK cells with WSX-1-deficient DCs than with WT DCs, while there was no apparent difference in the expression of granzyme B (Fig. 7B). There was only a small increase in the killing activity against YAC-1 cells by NK cells primed with WSX-1-deficient DCs over those primed with WT DCs (Fig. 7C). Thus, WSX-1 deficiency also resulted in up-regulation of DC function for NK cell priming, especially for IFN-γ production and perforin expression.

Enhanced responses of WSX-1-deficient DCs to LPS stimulation

Finally, we examined the functions of WSX-1-deficient DCs (Fig. 8). TNF-α production by LPS-stimulated WSX-1-deficient DCs was significantly higher than that by WT DCs (Fig. 8A). Although IL-12p40 production was also slightly augmented in WSX-1-deficient DCs, there was no difference in IL-6 production between WT and KO DCs (data not shown). Addition of rIL-27 suppressed both TNF-α and IL-12 production by WT DCs but not WSX-1-deficient DCs, demonstrating the suppressive effect of IL-27 on cytokine production by DCs (Fig. 8A). Then we examined the signal pathways in LPS-stimulated DCs, including phosphorylation of STAT1/3, IκB, and p38 (20) (Fig. 8, B and C). Phosphorylation of STAT3 was augmented in WSX-1-deficient DCs over that of WT DCs upon LPS stimulation (Fig. 8B). Similarly, phosphorylation of p38 and IκB, both phosphorylated downstream of LPS signaling through TNFR-associated factor 6, was augmented in WSX-1-deficient DCs over that of WT DCs. Although simultaneous IL-27 stimulation augmented the STAT3 phosphorylation
in WT DCs, it had no effect in WSX-1-deficient DCs. STAT1 phosphorylation was faintly detected only in WT DCs, which was augmented by IL-27 stimulation. Because the faint STAT1 activation was not detected in WSX-1-deficient DCs, this was presumably induced by endogenously produced IL-27. Time course experiments also detected augmented STAT3 phosphorylation in WSX-1-deficient DCs. By 60 min after LPS stimulation, STAT3 phosphorylation was detectable in WSX-1-deficient DCs when it was not detectable in WT DCs (Fig. 8C). STAT3 is known to be activated downstream of IL-6R and IL-10R. Addition of anti-IL-6 Ab showed no effects on TNF-α and IL-12 production both by WT and KO DCs (Fig. 8A). Addition of anti-IL-10 Ab slightly augmented TNF-α production by WT DCs, up to almost similar levels to those by KO DCs. However, there was no significant difference in IL-10 produced in the supernatants, 1785.5 ± 72.9 and 1963.3 ± 70.3 ng/ml by WT and WSX-1-deficient DCs, respectively. Anti-IL-10 Ab addition showed no apparent effect on IL-12 production. The expression of suppressor of cytokine signaling (SOCS) 3, induced downstream of STAT3 activation, was drastically augmented in WSX-1-deficient DCs over that of WT DCs upon LPS stimulation (Fig. 8D). These data in toto demonstrated that IL-27 showed direct suppressive effects and also that WSX-1-deficient DCs were hyperreactive to LPS stimulation by cytokine production, including TNF-α and IL-12, and also by downstream signaling, including STAT3, p38, and IκB phosphorylation. Thus, it was indicated that IL-27/WSX-1 plays an inhibitory role in the LPS activation of DCs and the lack of this inhibition resulted in the augmentation of overactivation and cytokine production of DCs.

**Discussion**

Our results illustrated the augmented functions of WSX-1 (IL-27Rα)-deficient DCs as APCs. Since the immunosuppressive roles of IL-27/WSX-1 have been proved mainly in T cells and macrophages (10, 11, 21–23), this is the first demonstration that IL-27R signaling also suppresses the function of DCs. The data also show a possibility that WSX-1-deficient DCs with augmented Th1-inducing function be used as an adjuvant for vaccination against infection and cancer.

As shown in Fig. 1, although the expression of IL-27 (p28 plus EBI-3) was observed even in mature DCs before activation, the expression of WSX-1 in DCs was only observed in LPS-stimulated DCs but not in immature or mature DCs. Therefore, while being required for initial Th1 development, IL-27/WSX-1 has a suppressive role on activated DCs as a negative feedback system to attenuate the excess of immune responses, just like IL-27/WSX-1 is suppressive on activated (but not naive) T cells (24). As has been reported previously (6, 7), IL-27/WSX-1 suppresses possibly lethal immunopathology induced by cytokine secretion and, without the suppressive effect, the mice died from lethal inflammation. Similarly, in *Mycobacterium tuberculosis* infection, inflammatory cytokines, including TNF-α, were overproduced in WSX-1-deficient mice (21). Although these inflammatory cytokines such as TNF-α and IFN-γ are beneficial for bacterial clearance, WSX-1-deficient mice died from cachexia induced by the cytokines (21). Because lack of EBI-3 (a subunit of IL-27) resulted in enhanced neutrophil migration and oxidative burst during experimental pneumonia concomitant with high bacterial clearance (25), the high oxidative burst in the absence of IL-27/WSX-1 signaling may induce tissue inflammation while being beneficial for bacterial clearance.

Recently, two groups independently reported the inhibition of IL-2 production by IL-27 (22, 23). For the distinct functions of IL-27, Villarino et al. (26) proposed an intriguing model in their report. In their model, because naive T cells express barely detectable levels of WSX-1 (26), they received little inhibitory signal of IL-27 through WSX-1 and the naive T cells with their IL-2 production began to proliferate at activation phase. During the polarizing phase, activated and committed T cells now expressed WSX-1 and subsequently IL-12Rβ2 for IFN-γ production (with relatively little IL-2 production, presumably to avoid excess proliferation). Our current findings are in line with this model; at the initial T cell activation phase, expression of WSX-1 (and IL-27) by DCs was relatively low and the DCs were capable of delivering stimulatory signals through CD80/86 to T cells. At later phases, DCs expressed substantial levels of WSX-1 and, by receiving an inhibitory signal through IL-27/WSX-1, DCs were less stimulatory to attenuate excessive T cell proliferation. IL-27/WSX-1 thus seems to warrant effective (but not excessive) proliferation and properly controlled differentiation of Th1 cells by regulating cytokine production by T cells and also APC function of DCs.

Although Th1-promoting mechanisms through IL-27/WSX-1 have been clearly shown (4, 27), the suppressive mechanisms have not been fully understood. Although IL-2 suppression by IL-27 has been reported (22, 23), we have no evidence that IL-2 suppression explains the suppression of APC function in DCs in the current study. Because both STAT1 and STAT3 are activated downstream of the WSX-1-gp130 receptor complex upon binding of IL-27 (24, 28), one or both of these transcription factors may be responsible of the suppression. Actually, STAT1 is responsible for the inhibition of Th17 development by IL-27 (11, 29). Thus, it is possible that the lack of STAT1 activation in response to LPS stimulation in WSX-1-deficient DCs (Fig. 8, B and C) led to insufficient SOCS1 induction and subsequent failure of LPS signaling inhibition, as demonstrated in SOCS1-deficient cells (20, 30). In contrast, we reported that STAT3 was partially involved in IL-27-mediated suppression of cytokine production including IL-17 by T cells (24). Holscher et al. (21) reported similar overactivation of WSX-1-deficient macrophages and direct suppression of macrophage activation by IL-27. Because IL-27 stimulation induced STAT3 phosphorylation in macrophages, the STAT3 activation was assumed responsible for IL-27-mediated suppression, as observed in IL-10 stimulation. However, as shown in Fig. 8, B and C, WSX-1-deficient DCs showed overphosphorylation of STAT3 in response to LPS stimulation. Roles of STAT3 in IL-27/WSX-1-mediated suppression may be distinct in LPS activation of DCs.

Addition of anti-IL-10 Ab increased the production of TNF-α and IL-12 by LPS-stimulated WT DCs (Fig. 8A). Thus, IL-10 produced by stimulated DCs appears to be responsible for IL-27-mediated suppression at least partially. However, since there was no difference in IL-10 production by WT and WSX-1-deficient DCs, other suppressive mechanisms, in addition to IL-10 production, should also be responsible for the IL-27-mediated DC suppression. Ruckerl et al. (31) reported that IL-10, IL-4, and IL-27 modulate macrophage activation in a distinct manner, respectively. Interestingly, these cytokines collaborate in attenuation of macrophage activation by successive up-regulation of the IL-4Rα and then WSX-1 on macrophages. It is reasonable to assume that similar factors exert their suppressive effects in a synergistic manner on DCs.

In addition to their augmented APC function, WSX-1-deficient DCs are characterized by their high Th1-inducing function. The WSX-1-deficient DCs with augmented APC function and Th1-inducing potential may have great advantage in eventual therapeutic usage. As shown in an *L. major* infection assay (Fig. 6, C and D), WSX-1-deficient DCs pulsed with *L. major* lysates bestowed Th1-biased protective immunity. Because WSX-1-deficient mice were in the resistant C57BL/6 background, the footpad swelling in
the control C57BL/6 mice was small. However, the footpad swelling in WSX-1-deficient DC-transferred mice was reproducibly less than control and WT DC-transferred mice (Fig. 6). Of note, IFN-γ production by draining LN cells from WSX-1-deficient DC-transferred mice was remarkably higher than that from control and WT DC-transferred mice early after infection (day 2 after infection). In susceptible backgrounds such as BALB/c, the same effect of WSX-1 deficiency could induce more drastic curative effects over control mice, which should show exacerbated footpad swelling with far less IFN-γ production upon infection. So far, various approaches have been taken into consideration to augment anti-pathogen or antitumor vaccination, such as expression of cytokines or costimulatory molecules by DCs. WSX-1-deficient DCs, or WSX-1-knockdown DCs, may be a promising adjuvant to augment Th1-skewed immunity. In addition to their Th1-promoting function, WSX-1-deficient DCs also effectively activated NK cells through increasing perforin expression and especially through increasing IFN-γ production (Fig. 7). Although NK cells activated with WSX-1-deficient DCs showed only a marginal increase in the killing activity against YAC-1 cells over NK cells activated with WT DCs, the increase in perforin and IFN-γ expression may cause augmented NK activity against other target cells. Thus, WSX-1-deficient DCs may be of potential importance in NK cell-mediated defense against viral infection and tumors. Possible augmentation of APC function of WSX-1-deficient DCs toward CD8+ T cells is currently under investigation.

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Disclosures

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