WSX-1 over-expression in CD4⁺ T cells leads to hyperproliferation and cytokine hyperproduction in response to TCR stimulation

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Abstract

WSX-1 is a component of the IL-27R. Analyses of WSX-1 knockout (WSX-1⁻/⁻) mice have shown that IL-27/WSX-1 signaling is essential for the proper development of T₄₁ responses and that WSX-1 can suppress cellular activation and pro-inflammatory cytokine production. We have generated transgenic mouse lines over-expressing the WSX-1 gene under the control of the T cell-specific CD2 promoter (WSX-1 Tg mice). Unexpectedly, like activated CD4⁺ T cells from WSX-1⁻/⁻ mice, activated CD4⁺ T cells from WSX-1 Tg mice showed increased proliferation, augmented IL-2 production and up-regulated surface expression of activation markers. IL-27-mediated tyrosine phosphorylation of STAT1 was also enhanced in WSX-1 Tg CD4⁺ T cells, but STAT3 activation was normal. Exogenous IL-27 supported the proliferation of wild-type CD4⁺ T cells but suppressed that of WSX-1 Tg cells. WSX-1 over-expression increased IFN-γ production in T₄₁-polarized CD4⁺ T cells, but also promoted T₄₂ cytokine production under T₄₁-polarizing conditions. Importantly, WSX-1 over-expression failed to suppress T₄₂ cytokine production under T₄₂-polarizing conditions. Cytokine hyperproduction was also observed in vivo in WSX-1 Tg mice injected with Con A. Our data suggest that WSX-1 plays a pivotal role in regulating T cell responsiveness to TCR stimulation and that the correct balance of STAT1/STAT3 activation downstream of IL-27R engagement is crucial for the physiological function of IL-27.

Introduction

WSX-1 is a class I cytokine receptor chain with homology to the IL-12R chains and the gp130 subunit of the IL-6R (1, 2). WSX-1 expression is prominent in lymphoid tissues such as spleen, thymus and lymph nodes and is highest in CD4⁺ T cells. IL-27, a newly identified IL-12-related heterodimeric cytokine, was recently reported to bind to WSX-1, and WSX-1 was found to associate with gp130 to compose the fully functional IL-27R complex (3, 4). We and others have reported that IL-27/WSX-1 signaling is important for normal T₄₁ differentiation of naive CD4⁺ T cells and that IFN-γ production is impaired in WSX-1⁻/⁻ mice (2, 5). We and others have reported that IL-27/WSX-1 signaling is important for normal T₄₁ differentiation of naive CD4⁺ T cells and that IFN-γ production is impaired in WSX-1⁻/⁻ mice (2, 5). The importance of IL-27/WSX-1 signaling to T₄₁ differentiation lies in its induction of STAT1 phosphorylation, which in turn leads to increased expression of the T₄₁-specific transcription factor T-bet. Increased T-bet results in up-regulated IL-12Rβ2 expression and the receipt of IL-12 signaling that drives T₄₁ differentiation (6, 7). Accordingly, WSX-1⁻/⁻ mice show impaired IFN-γ production and are thus susceptible to intracellular pathogens, such as Leishmania major and Listeria monocytogenes, that are best combattted by T₄₁ responses (2, 5). However, WSX-1 appears to be more than just a simple promoter of T₄₁ responses. We have recently shown that WSX-1 also acts to attenuate inflammatory responses induced by certain protozoan infections, in that lymphocytes from infected WSX-1⁻/⁻ mice are hyperactivated and over-produce pro-inflammatory cytokines such as IFN-γ, IL-4, tumor necrosis factor (TNF)-α and IL-6 (8, 9). Although the
precise molecular mechanism underlying this suppressive role of WSX-1 is not clear, STAT3 activation downstream of IL-27R engagement has been implicated (8, 10). These observations suggest that WSX-1 may be a pivot upon which the regulation of Tα1 responses turn. WSX-1 first induces Tα1 differentiation and then acts to negatively regulate cellular activation and cytokine production as the immune response requires termination.

To further investigate the physiological roles of WSX-1, we generated lines of WSX-1 transgenic (WSX-1 Tg) mice in which WSX-1 was over-expressed in T cells under the control of the human CD2 promoter and enhancer. To our surprise, CD4+ T cells from WSX-1 Tg mice were hyperactivated in response to TCR stimulation and exhibited elevated cytokine production, enhanced expression of activation markers and increased proliferation both in vitro and in vivo. Paradoxically, these observations are strikingly similar to those observed for WSX-1−/− mice. Although IL-27 stimulation induced a higher level of STAT1 tyrosine phosphorylation in WSX-1 Tg CD4+ T cells than in wild-type (WT) controls, STAT3 tyrosine phosphorylation in WSX-1 Tg CD4+ T cells was equivalent to that in WT CD4+ T cells. Our results demonstrate that IL-27/WSX-1 regulates the extent of TCR-dependent activation in CD4+ T cells and suggest that proper balance of STAT1/STAT3 activation downstream of IL-27R engagement is needed for the Tα1-promoting and -suppressive roles of IL-27/WSX-1.

Methods

Generation of WSX-1 Tg mice

A full-length cDNA-encoding murine WSX-1 was amplified by reverse transcription–PCR using total RNA extracted from naive T cells. The cDNA was subcloned into the human CD2 promoter/enhancer cassette (11) and the resulting plasmid was injected into fertilized oocytes (C57BL/6 × CBA/J) that were implanted into the pseudopregnant C57BL/6 × CBA/J F1 mice. Offspring were examined for integration of the transgene (Tg) by PCR amplification of genomic DNA using the following primers: 5′-AGC AAT TCT GGG CAA CCT TA and 5′-TCC TTG. Mice with Tg integration were backcrossed into the BALB/c background at least four times (continual crossing) and were confirmed to have the H-2d/d haplotype by crossed into the BALB/c background at least four times (continual crossing) and then acts to negatively regulate cellular activation and cytokine production as the immune response requires termination.

To prepare recombinant IL-27, we used a conventional calcium phosphate method to transfect HEK293 cells with murine EBI-3 cDNA in pCMV-3 × FLAG (Sigma–Aldrich, St Louis, MO, USA) plus murine p28 cDNA in pcDNA4.0-myc (Invitrogen, Carlsbad, CA, USA). Culture supernatants containing recombinant murine IL-27 were recovered after 3 days of culture. The presence of biological IL-27 activity in a supernatant was confirmed by its ability to induce STAT1 phosphorylation in CD4+ T cells, as described previously (7).

Cell culture

CD4+ T cells were purified from spleens of either WT, WSX-1 Tg or WSX-1−/− mice using magnetic bead sorting (MACS; Miltenyi Biotec, GmbH, Germany). Purified T cells were cultured in RPMI 1640 medium (GIBCO BRL of Invitrogen) supplemented with 10% FCS and antibiotics. The purity of the resulting CD4 T population was >95%.

Western blotting

Purified splenic CD4+ T cells were lysed in lysis buffer [50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1 mM vanadate, 1 mM NaF, 1 mM dithiothreitol, 0.1 mM (p-amidinophenyl)-methanesulfonyl fluoride hydrochloride] and centrifuged at 12 000 × g for 10 min. The lysates were fractionated by SDS/PAGE and immunoblotted with the antibodies described above using standard procedures.

Stimuli, proliferation assay and determination of cytokine production

Purified splenic CD4+ T cells (2.5 × 10^5 per 200 µl per well) from WT or WSX-1 Tg mice were plated in 96-well plates and stimulated for 72 h with plate-bound anti-CD3: mAb (0–10 µg ml^−1; 145–2C11, 1 µg ml^−1, BD Biosciences PharMingen, San Diego, CA, USA) in the presence or absence of IL-27, phorbol 12-myristate 13-acetate (PMA) (1–10 ng ml^−1) plus ionomycin (50 ng ml^−1) or Con A (1–2.5 µg ml^−1) plus irradiated syngeneic antigen-presenting cells (APCs). To assay CD4+ T cell proliferation, [3H]thymidine ([3H]TdR; 1 µCi) was added to each well for the last 6 h of culture. [3H]TdR incorporation was determined by scintillation counting. The stimulation index was defined as: [sample [3H]TdR incorporation (counts per minute)] – (background)/([3H]TdR incorporation without stimulation) – (background).

For the detection of IL-2 and IL-13 production, a microbead-based ELISA system for the detection of mouse cytokines (Multiplex Antibody Bead Kits; Biosource, Camarillo, CA, USA) was used according to manufacturer’s directions. Measurement of cytokine concentration was performed with Luminex 100 (Luminex, Austin, TX, USA).

Flow cytometry

Purified splenic CD4+ T cells were stimulated with anti-CD3: antibody (1 µg ml^−1) for 72 h. Cells were stained with FITC-conjugated anti-CD4 antibody and PE-conjugated anti-CD25 antibody (BD PharMingen), PE-conjugated anti-CD44 antibody (BD PharMingen), peridinin-chlorophyll–protein 5.5-conjugated anti-CD69 antibody (BD PharMingen) or PE-conjugated anti-Fas antibody (BD PharMingen). Cells were analyzed for marker expression using a FACSCalibur (BD Biosciences, Mountain View, CA, USA) and CellQuest software.
IL-27-dependent STAT1 and STAT3 phosphorylation

Purified splenic CD4+ T cells were activated with plate-bound anti-CD3ε antibody for 24 h, washed twice and stimulated by the addition of titrated doses of culture supernatant containing IL-27 as described above, plus anti-IFN-γ-neutralizing antibodies (1 μg ml⁻¹; R&D Systems, Minneapolis, MN, USA). Cell lysates were subjected to western blotting to detect phospho-STAT1 and phospho-STAT3 using the antibodies described above.

In vitro induction of T cell differentiation

In vitro differentiation of CD4+ T cells into Th1 or Th2 subsets was performed as described previously (12). Briefly, purified CD4+ T cells (1.0 × 10⁶) were stimulated with plate-bound anti-CD3ε antibody (1 μg ml⁻¹) plus anti-CD28 antibody (1 μg ml⁻¹) and IL-2 (10 U ml⁻¹) in the presence of either IL-12 (1 ng ml⁻¹) and anti-IL-4 antibody (clone 11B11, BD PharMingen) (500 ng ml⁻¹) for Th1 differentiation or IL-4 (1000 U ml⁻¹) for Th2 differentiation. Cultured cells were transferred into new wells free of anti-CD3ε antibody, cultured for 4 days and supplied with fresh medium. Cells were collected after 7 days and viable cells (0.5 × 10⁶) were re-stimulated with plate-bound anti-CD3ε antibody in the absence of any additional polarizing cytokines. Supernatants were collected 24 h later and analyzed for IFN-γ production in Th1 differentiation cultures or for IL-4 production in Th2 differentiation cultures. Cytokines were measured in duplicate using ELISA as described above. For histopathological examination of organs from WSX-1 Tg A and WSX-1 Tg B mice revealed no apparent abnormalities. The development of lymphoid organs, including CD4+ T cells, was also normal in both lines of WSX-1 Tg mice (data not shown). In the thymus, total numbers of thymocytes and the development of thymocyte sub-populations were unaffected by WSX-1 over-expression (data not shown). The relative and total numbers of lymph node cells and splenocytes were also comparable in WT, WSX-1 Tg A and WSX-1 Tg B mice (data not shown). These results indicate that over-expression of WSX-1 does not affect lymphocyte differentiation or the homeostasis of mature T cell populations in the periphery.

Con A-induced hepatitis experiments

Con A-induced hepatitis experiments were performed essentially as described (13) except that higher doses of Con A were used for mice of the BALB/c background. Briefly, Con A (Sigma–Aldrich) was injected intravenously into mice at 25 mg kg⁻¹ in 200 μl PBS. After 9 and 24 h, sera were collected to determine serum chemistry (alanine aminotransferase) and cytokine production. The concentration of IFN-γ in the 24-h post-injection serum sample was measured in duplicate by ELISA as described above. For histopathological examination of mouse livers, livers were removed 24 h after injection and fixed according to standard procedures. Thin sections were stained with hematoxylin and eosin and examined by light microscopy.

Results

Establishment of WSX-1 Tg mouse lines

To analyze the role of WSX-1 specifically in T cells, we generated WSX-1 Tg mouse lines by transfecting fertilized oocytes with a plasmid expressing the full-length murine WSX-1 cDNA under the control of human CD2 promoter and enhancer (Fig. 1A). From among several Tg-positive offspring, we established two independent lines of WSX-1 Tg mice, WSX-1 Tg A and WSX-1 Tg B. Western blot analysis of lysates of MACS-sorted splenic CD4+ T cells from WT, WSX-1 Tg A or WSX-1 Tg B mice showed that WSX-1 expression was strong in WSX-1 Tg B T cells, moderate in WSX-1 Tg A T cells and weak in WT T cells (Fig. 1B).

Both lines of WSX-1 Tg mice were born at the expected Mendelian frequency, appeared normal and thrived. Gross examination of organs from WSX-1 Tg A and WSX-1 Tg B mice revealed no apparent abnormalities. The development of lymphoid organs, including CD4+ T cells, was also normal in both lines of WSX-1 Tg mice (data not shown). In the thymus, total numbers of thymocytes and the development of thymocyte sub-populations were unaffected by WSX-1 over-expression (data not shown). The relative and total numbers of lymph node cells and splenocytes were also comparable in WT, WSX-1 Tg A and WSX-1 Tg B mice (data not shown). These results indicate that over-expression of WSX-1 does not affect lymphocyte differentiation or the homeostasis of mature T cell populations in the periphery.

Hyperactivation of WSX-1 Tg CD4+ T cells in response to TCR stimulation

Because we had previously demonstrated that activated WSX-1+/− CD4+ T cells exhibit hyperproliferation (5), we expected that WSX-1 Tg T cells would be hyporeactive to stimulation. To our surprise, however, the proliferation of WSX-1 Tg CD4+ T cells stimulated by TCR ligation was significantly greater than that of similarly treated WT cells and about equivalent to that of WSX-1+/− CD4+ T cells (Fig. 2A). This hyperproliferation appeared to depend directly on the expression level of WSX-1 since WSX-1 Tg B cells proliferated more vigorously than WSX-1 Tg A cells (Fig. 2B). Similarly, stimulation of CD4+ T cells with either Con A or PMA plus ionomycin resulted in enhanced proliferation of WSX-1 Tg T cells that increased in a dose-dependent manner (Fig. 2C and D and data not shown). In parallel with their hyper-proliferation, TCR-stimulated WSX-1 Tg CD4+ T cells produced...
more IL-2 than WT cells in a manner that reflected the level of WSX-1 over-expression (Fig. 2E).

We next analyzed the effect of WSX-1 over-expression on the induction of various T cell-activation markers in response to TCR stimulation. As shown in Fig. 3(A), although the base levels of CD25, CD44, CD69 and CD95 expression were comparable in WT and WSX-1 TgB CD4+ T cells prior to stimulation, all four markers were up-regulated to a greater degree in WSX-1 TgB T cells compared with WT cells after TCR stimulation. When we examined the expression of three of these activation markers on WSX-1−/− CD4+ T cells, we found that CD44 was substantially up-regulated on stimulated WSX-1−/− T cells compared with the WT (Fig. 3B), but that there was only a marginal increase in CD25 and CD95. These results clearly show that WSX-1 Tg cells become hyperactivated in response to TCR stimulation and that the degree of this hyperreactivity exceeds that exhibited by stimulated WSX-1−/− T cells.

Augmented IL-27-mediated STAT1 phosphorylation in CD4+ T cells from Tg mice

In a previous report, we demonstrated that stimulation of naive CD4+ T cells with IL-27 resulted in tyrosine phosphorylation of STAT1 and activation of this transducer downstream of WSX-1 (7). STAT3 is also reportedly activated downstream of IL-27R engagement (6, 8), presumably via its gp130 component (4). We, therefore, investigated the activation status of STAT1 and STAT3 in IL-27-stimulated WSX-1 Tg CD4+ T cells. Naive CD4+ T cells from WT or WSX-1 TgB mice were pre-activated with anti-CD3 antibody and stimulated with IL-27.
tyrosine phosphorylation of STAT1 was observed which was more intense in WSX-1 Tg cells than in WT cells (Fig. 4A). Moreover, STAT1 phosphorylation was detectable as early as 5 min after IL-27 stimulation in WSX-1 Tg cells compared with 15 min in WT cells. In striking contrast, no increase in either the level or speed of IL-27-mediated STAT3 phosphorylation was observed in WSX-1 Tg cells compared with WT cells (Fig. 4A). These results indicate that the normal balance of STAT1 and STAT3 activation is impaired in stimulated WSX-1 Tg T cells.

We also examined the effect of IL-27 on the proliferation of WSX-1 Tg T cells since IL-27 reportedly promotes the proliferation of naive WT CD4+ T cells (3). We confirmed that IL-27 does indeed augment CD3-mediated proliferation of WT CD4+ T cells in a dose-dependent manner (Fig. 4B). However, IL-27 stimulation had the exact opposite effect on the proliferation of WSX-1 Tg CD4+ T cells, hampering their proliferation in a dose-dependent manner (Fig. 4B). Taken together, these data suggest that, where WSX-1 is over-expressed, IL-27 stimulation delivers inappropriate signals downstream of the receptor.

Increased cytokine production by WSX-1 Tg CD4+ T cells

We and others have previously reported that WSX-1 plays an important role in the proper development of Th1 responses...
Incorporation of [3H]TdR during the last 6 h of culture was measured for 72 h with plate-bound anti-CD3 antibody (1 ng/ml) plus anti-IFN-γ-neutralizing antibody for the indicated times. Phosphorylation of STAT1 or STAT3 in whole-cell lysates was analyzed by western blotting using anti-PY-STAT1 or anti-PY-STAT3 antibodies, respectively. The filter was stripped and re-probed with anti-STAT1 or anti-STAT3 antibodies as loading controls.

Furthermore, production of IL-2 by TgA (not shown) and IL-2 (10 U/ml) in the presence of (A) IL-12 (1 ng/ml) plus anti-IL-4 antibody (500 ng/ml) to induce Th1 differentiation or (B) IL-4 (1000 U/ml) to induce Th2 differentiation (see Methods). Th0 cells were parallel cultures that received neither IL-12 nor IL-4. After 7 days, cells were re-stimulated with plate-bound anti-CD3 antibody (1 μg/ml) and anti-CD28 antibody (1 μg/ml) for 24 h in the absence of polarizing cytokines. Culture supernatants were analyzed by ELISA for the production of (A) IFN-γ and IL-13 or (B) IL-4 and IL-13. (C) IL-2 production. WT and WSX-1 TgB CD4+ T cells were stimulated as in (A) or (B) to generate T1 or T2 cells, respectively. Tg0 cells received neither IL-12 nor IL-4. IL-2 concentration in culture supernatants was measured 24 h after a secondary stimulation as in (A) and divided by the number of viable cells. *P < 0.05 and **P < 0.01. Data shown are the mean ± SD of triplicate cultures and are representative of three independent experiments.

Our findings in WSX-1 Tg T cell cultures were reminiscent of the hyperproduction of various pro-inflammatory cytokines by WSX-1 Tg lymphocytes (8, 9, 15). To determine the in vivo relevance of our results, we examined pro-inflammatory cytokine production using the Con A-induced hepatitis model (15). We have already shown using this system that WSX-1 Tg
NKT cells and WSX-1−/− CD4+ T cells produce devastating quantities of pro-inflammatory cytokines that cause lethal liver damage in the mutant mice (15). As predicted from the unexpectedly similar phenotypes of WSX-1 Tg and WSX-1−/− mice, WSX-1 Tg mice were highly susceptible to Con A-induced hepatitis and exhibited massive liver necrosis (Fig. 6A and B). Over-production of IFN-γ upon Con A injection of WSX-1 Tg mice was also observed (Fig. 6C), although to a lesser extent than in WSX-1−/− mice (15). Elevation of other cytokines such as IL-4 and TNF-α was not detected, presumably due to the well-documented strain specificity of Con A sensitivity (13).

In summary, this study describes the surprising finding that over-expression of WSX-1 has much the same effect as an absence of WSX-1. Inappropriate WSX-1 signaling, as might result from over-expression of this receptor chain, appears to generate an imbalance in STAT1 and STAT3 activation and impairs the suppressive effects of WSX-1 on cytokine production. Our results strongly point to WSX-1 as a key modulator of Th responses to activating stimuli.

**Discussion**

In this study, we generated lines of Tg mice that over-express WSX-1 in T cells. The characterization of these mutant animals revealed a paradox that may shed light on the true physiological function of WSX-1. Although the development of lymphoid organs in WSX-1 Tg mice was normal, WSX-1 Tg CD4+ T cells were hyperreactive to TCR stimulation in terms of increased proliferation, IL-2 production and activation-marker expression (Figs 2 and 3). Upon IL-27 stimulation, STAT1 phosphorylation was augmented in WSX-1 Tg CD4+ T cells, while STAT3 phosphorylation remained comparable to that in WT cells (Fig. 4). Over-expression of WSX-1 induced over-production of IFN-γ but CD4+ T cells cultured under Th1-polarizing conditions, but also over-production of IL-4 by CD4+ T cells cultured under Th2-polarizing conditions (Fig. 5). This hyperproduction of both Th1 and Th2 cytokines was also observed in vivo (Fig. 6). All of these results were quite unexpected, given the functions for WSX-1 established from the study of WSX-1−/− mice. Characterization of the knockout animals suggested that WSX-1 is both critical for T h1 development and important for the regulation of cellular proliferation and pro-inflammatory cytokine production. We had presumed we would find that WSX-1 Tg cells were less reactive to TCR stimulation than the WT and more biased towards Th1 differentiation. Instead, the phenotypes of WSX-1 Tg CD4+ T cells were strikingly similar to those of WSX-1−/− CD4+ T cells. Indeed, the magnitude of hyperreactivity was even more intense in WSX-1 Tg cells than in WSX-1−/− cells. Furthermore, although WSX-1 signaling has been reported to suppress Th2 cytokine production (16), we found that WSX-1 over-expression failed do so in our system.

Many cytokines exert their biological effects by binding to specific class I receptors and triggering the delivery of downstream signals via specific sets of JAK and STAT molecules. IL-27R is just such a receptor. We and others have shown that...
STAT1 is activated and bound to the cytoplasmic portion of WSX-1 while both STAT1 and STAT3 is activated downstream of IL-27 binding to the complete IL-27R complex (WSX-1 plus gp130) (4, 6–8). While STAT1 is important for Th1 development and reciprocal suppression of Th2 development downstream of stimulation of IL-27R or the IFN-γ receptor (6, 7, 17, 18), STAT1 activation has also been implicated in cell growth arrest and/or apoptosis (19–21). Thus, the lymphocyte hyperproliferation and failed Th1 development observed in WSX-1−/− cells could plausibly be explained by the lack of STAT1 signaling downstream of WSX-1. However, WSX-1 Tg cells also show lymphocyte hyperproliferation, and STAT1 phosphorylation is augmented in these cells in response to IL-27 stimulation. Moreover, WSX-1 over-expression is associated with hyperproliferation and IL-4 over-production in T cells cultured under Th1-polarizing conditions. These phenomena appear on the surface to be inconsistent with the hitherto described functions of STAT1.

One possible explanation to reconcile these conflicting observations is that the disproportionate expression of WSX-1 in WSX-1 Tg mice (relative to its gp130 counterpart) caused it to act as a decoy receptor, binding IL-27 and preventing it from triggering the functional IL-27R. Another possibility is that STAT1 and STAT3 activation must be properly coordinated to mediate the physiological functions of IL-27R, and an imbalance in this coordination abrogates IL-27R function. A balance of STAT signaling is already known to be important for cytokine secretion since STAT3 activation is associated with both pro-inflammatory signaling (IL-6R) and anti-inflammatory signaling (IL-10R) (22). In addition, the pro- or anti-inflammatory properties of STAT3 are influenced by auxiliary regulatory signals (23). We speculate that an abnormality in the balance of STAT1 and STAT3 activation, caused by either WSX-1 deficiency (decreased STAT1 and STAT3 activation) or WSX-1 over-expression (increased STAT1 activation), can lead to T cell hyperactivation and cytokine hyperproduction. In support of this hypothesis, the addition of exogenous IL-27 induced an altered pattern of STAT1 and STAT3 phosphorylation in WSX-1 Tg CD4+ T cells that was associated with an altered effect on proliferation, i.e. the same dose of IL-27 that promoted the proliferation of WT cells inhibited the proliferation of WSX-1 Tg CD4+ T cells (Fig. 4B).

The molecular basis for the hyperproliferation and cytokine hyperproduction in WSX-1 Tg and WSX-1−/− cells is currently unknown. At first glance, the hyperproliferation of WSX-1 Tg cells might be explained in part by their enhanced IL-2 production and CD25 (IL-2Rα) expression (Figs 2E and 3A). CD25 expression is also slightly increased in WSX-1−/− cells in response to TCR stimulation (Fig. 3), and we have previously reported that WSX-1−/− CD4+ T cells over-produce IL-2 (8). CD4+ T cells activated by TCR engagement plus CD28-mediated co-stimulation show downstream activation of JNK (24), which in turn activates transcription factors required for transcription of the IL-2 gene (25, 26). However, we have found that the intensity of JNK activation induced by TCR engagement plus CD28 co-stimulation does not differ in WSX-1 Tg and WT cells (unpublished observation). NF-κB signaling is also important for IL-2 production downstream of TCR stimulation (27). However, serine phosphorylation of IκBα in WSX-1 Tg CD4+ T cells in response to TCR stimulation is comparable to that in WT cells (unpublished observation). Thus, further studies are necessary to elucidate the molecular mechanisms underlying the regulation of cell proliferation and cytokine production by WSX-1.

In conclusion, we have demonstrated that CD4+ T cells with constitutive over-expression of WSX-1 are highly reactive to TCR stimulation and show increased proliferation and cytokine production. Although the precise mechanism underlying WSX-1’s influence on TCR-dependent signaling in CD4+ T cells is not clear, we suggest that IL-27 stimulation normally give rise to an appropriately balanced activation of STAT1 and STAT3 and that this balance is crucial for the physiological function of IL-27/IL-27R. Molecular dissection of the mechanisms underlying WSX-1-mediated promotion of Th1 differentiation and suppression of cellular activation may eventually give rise to opportunities for therapeutic intervention, particularly in cases of infectious or autoimmune disease where inappropriate cell activation and/or cytokine production are factors.

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Abbreviations
APC antigen-presenting cells
[^3H]Tdr[^3H]thymidine
PMA phorbol 12-myristate 13-acetate
PY phosphotyrosine
Tg transgene or transgenic
WT wild type

References


