WSX-1: A Key Role in Induction of Chronic Intestinal Nematode Infection

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WSX-1: A Key Role in Induction of Chronic Intestinal Nematode Infection


Chronic infection by the gastrointestinal nematode Trichuris muris in susceptible AKR mice, which mount a Th1 response, is associated with IL-27p28 expression in the cecum. In contrast to wild-type mice, mice that lack the WSX-1/IL-27R gene fail to harbor a chronic infection, having significantly lower Th1 responses. The lower level of Ag-specific IFN-γ responses between KO and C57BL/6 wild-type (WT) mice were comparable, suggesting that IL-27/WSX-1 signaling may be less important for maintaining a Th1 response (4). However, two recent studies on Toxoplasma gondii and Trypanosoma cruzi have demonstrated that WSX-1 plays a role in limiting the intensity and duration of T cell activation, as WSX-1 KO mice infected with either parasite develop a lethal T cell-mediated inflammatory disease (8, 9).

Trichuris muris is a gastrointestinal nematode very closely related to the human counterpart Trichuris trichiura. The majority of inbred mouse strains expel T. muris from its large intestinal niche and make a dominant Th2 response (10–12). However, a few strains of mouse fail to expel T. muris, harbor a chronic infection, and generate an associated Th1 response (13–15). It has been previously observed that there is a predisposition to particular infection levels of T. trichiura and T. muris in humans and mice, respectively. Experimental manipulation of T. muris infection levels allows us to mimic low-level chronic infection (16), which is the norm in human populations.

What is abundantly clear is the failure to expel the parasite is not a failure to mount an immune response per se. Although a number of factors have been shown to play a role in the Th1/Th2 “decision making”, it is arguably the cytokine environment at the time of Ag presentation that is one of the most important factors (17). A number of cytokines, genes, and transcription factors have been shown to be important in driving a type 1 response (18–20), however, the precise mechanisms that underlie initiation of this response in favor of a type 2 response remain unclear.

This paper is the first study to examine the IL-27/WSX-1 interaction in a chronic gastrointestinal nematode infection, showing a dramatic defect in the ability of WSX-1 KO mice to generate a type 1 response. This defect could not be compensated for by the addition of exogenous IL-12. Moreover, this study demonstrates an up-regulation of the genes involved with IL-27/WSX-1 signaling and subsequent activation of the IL-12 signaling cascade in chronic T. muris infection.

Materials and Methods

Mice

WSX-1 KO were generated as described previously (4) and backcrossed onto a C57BL/6 background >10 times. They were bred and maintained in the Biological Services Unit at University of Manchester (Manchester,
infections were associated with low-level infections were 25% of T. muris eggs and high-level infections were ~100 T. muris eggs.

Parasite 

Infection and maintenance of T. muris was conducted as described by Wakelin (21). Low-level infections were associated with 50% of T. muris eggs and high-level infections were 25% of T. muris eggs.

Parasite Ag

Adult worms were cultured in RPMI 1640 (Invitrogen, Paisley, U.K.) and were infected when 6 x 10^6 cells/ml, and 100 g/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). MLNC were stimulated with 50 µg/ml T. muris for 4 h ES Ag was collected after 4 h and overnight culture. The ES was prepared as in Ref. 16.

In vitro cytokine analysis

Mesenteric lymph node cell (MLNC) suspensions from infected or control mice were prepared as described previously (9). Briefly, 5 x 10^6 cells/ml were resuspended in RPMI 1640, 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). MLNC were stimulated with 50 µg/ml T. muris for 4 h ES Ag in 24-well plates (Costar, Cambridge, MA). Supernatants were harvested at 24 h.

Supernatants were analyzed for cytokine content by sandwich ELISA as previously described (10). The Abs used were 11B11 and 24G2 for IL-4, TR-FK-5 and TR-FK-4 for IL-5, 229.4 (E. Schmitt, Johannes Gutenberg University of Mainz, Mainz, Germany) and 2C12 for IL-9, R46A2 and XM1G12 for IFN-γ, and C15.6 and C17.8 for IL-12p40 (all from BD PharMingen, San Diego, CA). IL-13 was measured using a kit (R&D Systems, Minneapolis, MN).

Ab ELISAs

Levels of parasite-specific IgG1 and IgG2a Ab were determined by ELISA and conducted as described in Ref. 22. Briefly, Immulon IV ELISA plates (Dynal Biotech, Oslo, Norway) were coated with T. muris ES overnight Ag at 5 µg/ml. Serum was diluted from 1/20 to 1/2560, and parasite-specific IgG1 was detected with biotinylated anti-mouse IgG1 (Serotec, Oxford, U.K.) and biotinylated anti-mouse IgG2a (BD PharMingen).

IL-12 treatment

One microgram of murine rIL-12 in 0.2 ml of PBS was given i.p. every second day from day 0 to 14 p.i. Controls received PBS.

Intracellular cytokine staining

MLNC were restimulated for 24 h at 37°C with ES for IFN-γ and IL-4 staining. PMA (50 ng/ml) and ionomycin (500 ng/ml) was added for the last 3 h of culture for IL-4 staining only. Brefeldin A (1 µg/ml; Sigma-Aldrich, St. Louis, MO) was added for the final 1.5 h of culture. After washing, cells were stained for surface markers (CD4-FITC (RM4-5) or PE (BD Biosciences, Mountain View, CA).

CFSE labeling

Five micromolars CFSE were added for 15 min to MLNC at 5 x 10^6 cells/ml, and 100 µl of FCS/ml cells were added to quench the reaction. Cells were cultured for 96 h under Th0-, Th1-, or Th2-polarizing conditions. IFN-γ CD4 T cells were identified and, using CFSE fluorescence intensity, further subdivided into populations that had undergone 0–7 divisions.

In vitro polarizing conditions

Plate-bound anti-CD3e (2C11) at 3 µg/ml and soluble anti-CD28 (CD28.2; BD PharMingen) at 10 µg/ml were added to all cultures. Th1 polarization was induced by addition of 5 µg/ml rIL-12 and 20 µg/ml anti-murine IL-4 mAb (11B11). Th2 polarization was induced by addition of 50 µg/ml murine rIL-4 and 50 µg/ml anti-IFN-γ mAb (XM1G12). Cocultures were harvested after 24, 48, and 72 h for cytokine analysis and after 96 h to assess proliferating IFN-γ-positive cells by FACS analysis.

RT-PCR

The cecal tip and the MLN were removed from both WSX-1 KO and WT mice at day 21 p.i. and snap frozen in 1 ml of TRIzol. After homogenization, mRNA was prepared and assessed for quality by spectrophotometry at 260 nm and visualization of 18s and 28s ribosomal bands on a 2% agarose gel. Two microliters of mRNA was reverse transcribed using Improm II and Oligo(dT) (Promega, Madison, WI). cDNA (0.4 µg) was used in a 20-µl PCR. The primer sequences were as follows: WSX-1, 5'-AACCTGGAGATATGTTAGGCTAGCT, 5'-CTCTCGTGATGGAAGTGTTGCCAGA; p28, 5'-TGTACCTGAGAGACCTTGCTTCCT, 5'-TTCCTCTCTCTCCTCTCTCTCCT; EB13, 5'-ACACTCCTCTCTGTAGGGTGCTACATAGAAGATATGTTAGGCTAGCT, 5'-ACAGAAGATATGGAAGTGTTGCCAGA; IL-12Rα1, 5'-TCTCCGAAACTCGCCCATCGC; IL-12Rα2, 5'-TCCACATTACTGCCCATACAGA; p28, T-bet, CAGGGTTGG; STAT-1, 5'-AGAAAGATCATCCCA, 5'-ATGGGTTGG; IFN-γ, 5'-AGAAATTCACCTATGAGCCCGACC, 5'-AGAAATTCACCTATGAGCCCGACC; EBI3, 5'-CCCTTGATGTAAGGTTGCCCAGA; IL-12Rβ2, 5'-CCCTACATTACTGCCCATACAGA; CAGGTGTTCGAGACCTGTGACCT.

Statistical analysis

Statistical analysis was conducted using the Mann-Whitney U test. A value of p < 0.05 was considered to be significant.

Results

IL-27/WSX-1 signaling is associated with chronic T. muris infection and WSX-1 KO mice are resistant to infection, making reduced Th1 responses

WSX-1 was first described as a cytokine receptor important for driving type 1 responses and IFN-γ production. To examine whether signaling through WSX-1 is important in the Th1-associated chronic T. muris infection, gene expression at the initial site of Ag encounter was examined. Fig. 1a shows the cecal expression of genes involved in IL-27/WSX-1 signaling at day 21 p.i. from both susceptible (AKR) and resistant (BALB/c) mice. T. muris is intimately associated with the cecal epithelium. EB13 and WSX-1 are both expressed in AKR and BALB/c mice, whereas p28, T-bet, and IL-12Rβ2 are absent and STAT-1 is expressed at a reduced level in resistant BALB/c mice.

Infection was next conducted in WSX-1 KO and WT C57BL/6 mice. Fig. 1b demonstrates infection data from WSX-1 KO and WT C57BL/6 mice at day 19 (day 18–21 p.i. is the time of optimal cytokine production) and day 35 p.i. (used to confirm resistance and susceptibility as the parasite is sexually mature). Both WSX-1 KO mice and C57BL/6 mice were infected with a low-level infection, which has previously been shown to change the response phenotype of resistant mice (23). It is clear that at both time points worms were obtained from WT C57BL/6 mice, whereas WSX-1 KO mice had expelled their parasites.

Fig. 1c demonstrates Ag-specific Ab production at day 35 p.i. from both WSX-1 KO and WT C57BL/6 mice. It can be seen that WSX-1 KO mice have significantly lower levels of parasite-specific IgG2a (p < 0.05) (which is dependent on IFN-γ (24)) in comparison to infected WT mice. WSX-1 KO mice had similar levels of parasite-specific IgG1 when compared with infected WT mice.

Ag-specific cytokine production is shown in Fig. 1d, where it can be seen that WSX-1 KO mice make significantly reduced levels of type-1-associated cytokines (IFN-γ and IL-12p40; p < 0.05) and significantly enhanced levels of type 2 cytokines (IL-4 and IL-13; p < 0.05). Similar data were obtained for IL-9, although there was no difference between infected KO and WT mice for IL-5 (data not shown). Infection of WSX-1 KO mice supports a role for signaling through IL-27/WSX-1 in chronic T. muris infection, as KO mice expelled their worms and failed to make type 1 responses associated with chronic infection.
Significantly higher proportion of IFN-γ productions \((\text{p} < 0.05)\). Moreover, a significant increase in IL-4-positive CD4\(^+\) cells within CD4\(^+\) T cells in WSX-1 KO mice compared with WT mice \((\text{p} < 0.05)\).

FIGURE 1. The IL-27/WSX-1 signaling pathway is associated with chronic infection and WSX-1 KO mice are resistant to chronic *T. muris* infection, making reduced Th1 responses. *a*, RT-PCR of genes associated with the IL-27/WSX-1 signaling pathway in the cecum at day 21 p.i. using three mice per group. WSX-1 KO \(\square\) and C57BL/6 mice \(\blacksquare\) \((n = 4)\) were infected with a low-level *T. muris* infection. *b*, Mean worm burden at day 19 and 35 p.i. *c*, The relative levels of parasite-specific IgG1 (G1) and IgG2a (G2a) present in a 1/80 dilution of serum taken from mice at day 35 p.i. *d*, i–iv, Ag-specific cytokine production at day 19 p.i. when MLNC were restimulated in vitro for 24 h with 50 \(\mu\)g/ml parasite Ag. *i*, Significantly different \((\text{p} < 0.05)\) from WT mice; KO, WSX-1 KO mice.

**Ag-driven reduction in IFN-γ-positive CD4\(^+\) T cells**

In an attempt to elucidate whether the lowered IFN-γ production was of CD4 or CD8 cell origin, intracellular IFN-γ staining was conducted on both CD4 and CD8 cells. Fig. 2 shows ex vivo cells from infected WSX-1 KO and WT mice at day 19 p.i. It can be seen that the lack of WSX-1 on the T cell causes a significant reduction in Ag-specific IFN-γ-positive cells within the CD4\(^+\) T cell compartment (Fig. 2a; \(\text{p} < 0.05\)), with no significant difference observed in CD8\(^+\) T cells between KO and WT mice (Fig. 2b). IL-4-positive CD8\(^+\) cells were undetectable but there was a significant increase in IL-4-positive CD4\(^+\) T cells in WSX-1 KO mice compared with WT mice \((\text{p} < 0.05)\).

Paradoxically, Fig. 3 shows that significantly more IFN-γ is produced by naive cells from WSX-1 KO mice when stimulated polyclonally in vitro under Th0-, Th1-, or Th2-polarizing conditions (ai–ci). Moreover, a significantly higher proportion of IFN-γ-positive CD4\(^+\) T cells, which proliferated faster, were seen from WSX-1 KO mice again under all polarizing conditions \((\text{p} < 0.05)\; aii–cii\). This agrees with data from others that show that after polyclonal stimulation these mice have the ability to produce IFN-γ in vitro (2).

**Exogenous IL-12 fails to drive chronic infection in WSX-1 KO mice**

As WSX-1 was initially shown to be important for early T cell responses, it was thought that IL-12 may be able to compensate for the lack of WSX-1. Fig. 4a shows worm burden at day 35 p.i. from WSX-1 KO mice and C57BL/6 WT mice treated with either rIL-12 or PBS as a control. IL-12-treated WSX-1 KO mice failed to support a patent chronic infection. To ensure that the IL-12 treatment was able to modulate an effective Th2 response toward a Th1 response (as WT mice given a low-level infection already make a type 1 response), a high-level infection (Th2 inducing) was given to WT mice and then treated with IL-12. Fig. 4b shows that IL-12 treatment does indeed drive a high-level infection to chronicity in WT mice \((\text{p} < 0.05)\). Intriguingly, Fig. 4c shows the parasite-specific IgG2a response in infected WSX-1 KO treated with IL-12 was elevated, indicating that IL-12 treatment did alter the Ab isotype response, despite data showing no elevation of the Th1 response \((\text{Fig. 4d})\). To further confirm rIL-12 was unable to affect the T cell response, the prevalence of IFN-γ\(^+\)CD4\(^+\) and CD8\(^+\) cells within the MLN was assessed. The percentage of Ag-stimulated IFN-γ\(^+\)CD4\(^+\) cells \((0.58 \pm 0.16\% \; \text{plus IL-12} \; \text{vs} \; 0.56 \pm 0.05\%; \text{minus IL-12})\) and IFN-γ\(^+\)CD8\(^+\) cells \((0.97 \pm 0.28\% \; \text{plus IL-12} \; \text{vs} \; 0.55 \pm 0.07\%; \text{minus IL-12})\) again showed no significant increase in response to the rIL-12 regime.

A possibility existed that IL-12-treated WSX-1 KO mice were making competent Th1 responses but excessive Th2 responses were eliminating the worms. Fig. 4d demonstrates that this was not the case and that there is no significant difference \((\text{p} > 0.05)\) between the levels of Ag-specific cytokine production seen in WSX-1 KO mice (Th1 or Th2) with IL-12 treatment.

In a further attempt to show WSX-1 KO mice were not displaying exaggerated Th2 responses in our system, a comparison of Ag-specific, in vitro cytokine production between MLNC from WT mice given a high-dose infection and WSX-1 KO mice given
a low-dose infection is shown in Fig. 4e. As both strains expel their worms effectively, it was interesting to note that comparable levels of Th2 cytokines were produced. Although levels of IL-4 are greater in the WSX-1 KO mice compared with WT mice (63.0 U/ml compared with 37.2 U/ml, respectively), the principal difference in the cytokine response is found in the residual IFN-γ levels, with a 13-fold reduction observed in WSX-1 KO animals.

**Down-regulation of the IL-27/WSX-1 signaling pathway in the cecum of infected WSX-1 KO mice**

Gene expression analysis confirmed disrupted IL-27/WSX-1 signaling pathways in vivo in WSX-1 KO compared with WT mice given a low-dose infection. Unsurprisingly, WSX-1 expression was observed in the MLN and the cecum of WT but not WSX-1 KO mice (Fig. 4f). STAT-1 and EBI3 had less intense bands in the cecum of infected WSX-1 KO compared with C57BL/6 mice, and both p28 and T-bet were absent in the WSX-1 KO cecal samples. All these genes were expressed in the MLN, in the case of EBI3 and STAT-1 at similar levels, and to a lower level in the case of p28 and T-bet. IL-12Rβ2 expression was observed only in the MLN of infected WT mice.

**Discussion**

We have shown that the genes involved in the IL-27/WSX-1 interaction and subsequent initiation of the IL-12 cytokine signaling cascade are present in a strain of mouse that is susceptible to *T. muris*, e.g., AKR, whereas in contrast, genes within this pathway are either absent or expressed to a lower extent in mice that expel their worms and are resistant to infection (Fig. 1a). In WSX-1 KO mice, in which clearly this pathway is interrupted at the initial stage, then there is a failure to establish chronic infection, and a subsequent down-regulation in type 1 responses. Ag-specific cytokine production (Fig. 1d) shows that in comparison to infected C57BL/6 WT mice, infected WSX-1 KO mice had reduced levels of both IFN-γ and IL-12p40 and raised levels of IL-4 and IL-13. Raised levels of type 2 cytokines have been observed in TCCR KO mice immunized with keyhole limpet hemocyanin (and, interestingly in this case, IL-5 levels were also unaffected) (3). This demonstrates that WSX-1 is important for progression to chronic *T. muris* infection and associated Th1 responses.

Ex vivo CD4⁺ T cells from infected WSX-1 KO mice and WT C57BL/6 mice revealed a reduced number of IFN-γ-positive Ag-activated CD4⁺ T cells in WSX-1 KO mice compared with WT mice (Fig. 2). WSX-1 has been shown to be present mainly on CD4⁺ cells and NKT cells, although unlike CD4⁺ T cells, NKT cells have previously been shown not to play an important role during *T. muris* infection (25). However, when naive cells were taken from both WSX-1 KO mice and WT C57BL/6 mice and stimulated in vitro under Th0-, Th1-, or Th2-polarizing conditions, it was shown under all conditions that WSX-1 KO mice produced higher levels of IFN-γ-positive CD4⁺ T cells that proliferated faster than similar cells from WT mice (Fig. 3). Comparison between Figs. 2 and 3 highlights the differences between in vitro and ex vivo studies, and suggests that polyclonal stimulation does not necessarily reflect Ag-driven events, and raises the possibility that as yet undefined costimulatory events subtly influence the outcome of WSX-1/IL-27 interactions in vivo.

Treatment of WSX-1 KO mice with IL-12 failed to reverse their defect in their ability to harbor a chronic infection and low level of IFN-γ and IL-12p40 production. Surprisingly, the administration of IL-12 at a dosage regime that readily promotes susceptibility in WT mice failed to do so in WSX-1 KO mice. This supports the hypothesis that WSX-1 is critical to Th1 responses and consequent susceptibility to infection.

Although recent studies have demonstrated that WSX-1 plays a role in limiting the intensity and duration of T cell activation and WSX-1 KO mice show enhanced Th2 responses rather than a defect in Th1 responses (8, 9), we feel this is not the case in our system. The differences between those studies and ours may reflect that WSX-1 has a dual role both in initiation and regulation of T cell responses. In addition, both CD8 and NK cells, together with CD4 cells, have been shown to be important in resistance to *T. gondii* and *T. cruzi* infections. However, with *T. muris* infection, resistance is exclusively CD4 cell mediated (26).

Comparison of two models of resistance, i.e., WSX-1 KO mice given a low-level infection and WT C57BL/6 mice given a high-level infection, demonstrated comparable levels of type 2 cytokine production. Although IL-4 levels were higher in the KO mice, the principal difference was in the reduced level of IFN-γ production from WSX-1 KO mice. Crucially, IL-13, the key type 2 cytokine responsible for IL-4-independent resistance (27), generated after high-dose infection in WT mice is similar to that seen after a low-dose infection in WSX-1 KO mice. This argues that the inability of IL-12 to change the outcome of infection in WSX-1 KO mice is related to a defect in the immunoregulatory mechanisms operating in vivo, namely the generation of type 1 immunity.

It has recently been demonstrated that the IL-27/WSX-1 interaction leads to phosphorylation of STAT-1, which in turn induces expression of T-bet (5). Recent work has also shown that IL-27 regulates the responsiveness of naive CD4 cells through STAT-1-dependent and -independent mechanisms (28). The present data
show a low level or absence in the gene expression of transcription factors downstream of IL-27/WSX-1 signaling in the cecum of resistant WSX-1 KO mice (Fig. 4f). In addition to explaining why IL-12 failed to rescue the WSX-1 KO (as IL-12Rβ2 was undetectable in these mice), it is likely that down-regulation of this pathway is associated more generally with naturally induced Th2 responses (and not an artifact of knocking out the WSX-1 gene) as a similar pattern of gene expression was observed in resistant BALB/c mice given a high-dose infection (Fig. 1).

It is clear that the IL-27/WSX-1 interaction occurs early on in the immune response and probably there are a number of type-1-promoting transcription factors that may play an important role in chronic infection, e.g., suppressor of cytokine signaling proteins, class II transactivator, friend of GATA-1, and Runx1 (17–19, 29), although their relationship to WSX-1 is as yet undefined. Moreover, recent evidence is emerging demonstrating that Toll-like receptor (TLR) signaling pathways play an important role in determining the outcome of T. muris infection. In that study, it was
shown C3HeJ mice (that cannot signal through TLR4), TLR4 KO mice, or myeloid differentiation factor 88 KO mice are not susceptible to chronic *T. muris* infection, and show depressed type 1 responses compared with susceptible WT mice (30). Therefore, it would be envisaged that IL-27/WSX-1 signaling may play an important part of the bridge between the innate and adaptive immune responses to *T. muris* and, furthermore, commitment to a chronic infection occurs early after initial Ag encounter, with susceptibility not simply being the subsequent down-modulation of a type 2 response. An understanding of the role of IL-27/WSX-1 in the regulation of type-2-mediated pathologies such as allergic inflammation is clearly important; the present data also confirm an important in vivo role for WSX-1/IL-27 in type-1-mediated responses following gastrointestinal nematode infection, and may be involved in type-1-mediated pathological conditions such as inflammatory bowel disease and autoimmunity.

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**References**


