Positive and Negative Regulation of the IL-27 Receptor during Lymphoid Cell Activation[1]

Alejandro V. Villarino,* Joseph Larkin III,† Christiaan J. M. Saris,§ Andrew J. Caton,† Sophie Lucas,‡ Terence Wong,‡ Frederic J. de Sauvage,‡ and Christopher A. Hunter[2]*

Previous reports have focused on the ability of IL-27 to promote naive T cell responses but the present study reveals that surface expression of WSX-1, the ligand-specific component of the IL-27R, is low on these cells and that highest levels are found on effector and memory CD4$^+$ and CD8$^+$ T cells. Accordingly, during infection with *Toxoplasma gondii*, in vivo T cell activation is associated with enhanced expression of WSX-1, and, in vitro, TCR ligation can induce expression of WSX-1 regardless of the polarizing (Th1/Th2) environment present at the time of priming. However, while these data establish that mitogenic stimulation promotes expression of WSX-1 by T cells, activation of NK cells and NKT cells prompts a reduction in WSX-1 levels during acute toxoplasmosis. Together, with the finding that IL-2 can suppress expression of WSX-1 by activated CD4$^+$ T cells, these studies indicate that surface levels of the IL-27R can be regulated by positive and negative signals associated with lymphoid cell activation. Additionally, since high levels of WSX-1 are evident on resting NK cells, resting NKT cells, effector T cells, regulatory T cells, and memory T cells, the current work demonstrates that IL-27 can influence multiple effector cells of innate and adaptive immunity. ***The Journal of Immunology,*** **2005, 174: 7684–7691.**

Interleukin-27 is a recently described IL-6/IL-12 family cytokine that is composed of a helical protein (IL-27p28) paired to a soluble receptor-like subunit (EBI3) (1). Produced by macrophages, dendritic cells, and epithelial cells in response to a range of inflammatory stimuli (2), IL-27 mediates its biological activities through a high-affinity receptor complex that includes gp130 and WSX-1 (T cell cytokine receptor (TCCR)[3]) (1, 3). As a shared receptor component for several cytokines, including IL-6, IL-11, IL-27, and Oncostatin (4–6), gp130 is expressed by immune and nonimmune cells in virtually all tissues (5, 6). In contrast, WSX-1 is only known to interact with IL-27 (1), and its expression is restricted to immune sites (3, 7–9). Thus, while germline deletion of ubiquitously expressed gp130 results in gross developmental abnormalities (10), WSX-1-deficient (WSX-1$^{-/-}$) mice are viable and fertile (8, 9). Nevertheless, given that WSX-1 transcripts can be detected in a range of immune cell types and that receptor-deficient mice develop aberrant inflammatory responses upon infection with various pathogens (4), it is clear that this receptor has important immunological functions.

Although WSX-1 mRNA is present in several lymphoid lineages (3, 7–9), initial reports have focused on the ability of IL-27 to influence T cell responses (1, 11–14). Naive CD4$^+$ T cells express high levels of WSX-1 mRNA (7–9), and since these transcript levels appear reduced upon differentiation (Th1/Th2) (8), a consensus has emerged that the effects of IL-27 are directed toward naive T cells and that a loss of WSX-1 expression is concurrent to the acquisition of effector T cell functions (15–19). Furthermore, because IL-27 can promote naive T cell differentiation (1, 11–14) and receptor-deficient mice display enhanced susceptibility to intracellular pathogens (8, 9), it has been proposed that expression of WSX-1 by naive CD4$^+$ T cells is required for efficient induction of type I (Th1) immunity (15–19). However, while these studies have revealed that IL-27 can augment nascent T cell responses, subsequent reports imply that this property may be secondary to a more profound inhibitory role directed toward effector T cells (20, 21). During infection with *Toxoplasma gondii*, WSX-1-deficient mice control parasite replication through the generation of appropriately polarized effector T cell responses but these animals also develop a lethal inflammatory disease that is characterized by escalating T cell activation, proliferation, and cytokine production (21). Therefore, since the current work demonstrates that effector and memory T cells express higher surface levels of WSX-1 than naive precursors, together, these findings are inconsistent with the idea that IL-27 acts primarily on naive T cells. Instead, these data suggest that IL-27 limits parasite-induced inflammation by directly influencing effector T cells that express high levels of WSX-1.

The present study indicates that naive T cells display low surface levels of WSX-1 and that receptor expression is greatly enhanced by mitogenic stimulation. However, for resting NK and NKT cells, high levels of WSX-1 are reduced upon activation. Thus, in total, these findings establish that expression of the IL-27R on distinct lymphoid lineages is governed by a series of positive and negative signals associated with cellular activation. Furthermore, since CD4$^+$ T cell activation induces expression of both WSX-1 and IL-2, a cytokine shown to suppress WSX-1 levels on effector T cells, these studies suggest that IL-27 sensitivity may be regulated by sequential cues within a single cell.

Materials and Methods

Animals and T. gondii *infections*

Mice deficient in WSX-1 were generated as described (9) and provided by Dr. C. Saris (Amgen, Thousand Oaks, CA). WSX-1 $^{-/-}$ mice were bred as

*Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, and †Wistar Institute, Philadelphia, PA 19104; ‡Department of Molecular Biology, Genentech, South San Francisco, CA 94080; and §Department of Inflammation Research, Amgen, Inc., Thousand Oaks, CA 91320

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[2] Address correspondence and reprint requests to Dr. Christopher A. Hunter, 3800 Spruce Street (Rosenthal Building), Room 226, Philadelphia, PA 19104. E-mail address: chunter@vet.upenn.edu

[3] Abbreviations used in this paper: TCCR, T cell cytokine receptor; WT, wild type; SA, streptavidin; T_{reg} cell, T regulatory cell.

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homozygotes in a specific-pathogen free environment at the University of Pennsylvania. Wild-type (WT) C57BL/6 mice were purchased from The Jackson Laboratory. For infections, the ME49 strain of *T. gondii* was maintained in mice (Swiss Webster and CBA/CaJ; The Jackson Laboratory) and tissue cysts prepared as described (21). At 5–8 wk of age, groups of three to five mice were infected with 20 cysts i.p. All experiments were conducted following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

Flow cytometry

For ex vivo experiments, spleens were isolated from uninfected or *T. gondii*-challenged mice, dissociated into a single-cell suspension, and depleted of erythrocytes using 0.86% (w/v) ammonium chloride (Sigma-Aldrich). Cells were then washed and immediately stained for surface protein expression using the following Abs: anti-CD3, anti-NK1.1, anti-CD28, anti-CD40L, anti-CD4, anti-CD8, anti-CD25, anti-CD28, anti-CD62L, anti-CD44, and anti-CD45RB (eBioscience). For detection of WSX-1 (TCCR), mAbs were generated as described (22) and provided by Dr. F. de Sauvage (Genentech, South San Francisco, CA). Briefly, recombinant polyhistidine-tagged (HIS8) murine TCCR was expressed in CHO cells (Genentech) and, with Ribi adjuvant (Ribi Immunochem Research), used to hyperimmunize four female Lewis rats (Charles River Laboratories). From these rats, B cells were isolated and expanded before anti-TCCR Ab titers were assayed by ELISA. Those showing anti-TCCR binding activity were fused with rat myeloma cells (YB2/0; American Type Culture Collection) and, after 10–14 days in culture, supernatants were harvested and screened for Ab production. Limiting dilution was then performed to identify clones with the highest anti-TCCR binding activity, and four were chosen for further expansion. Supernatants were harvested from each hybridoma, purified by affinity chromatography (fast protein liquid chromatography; Pharmacia), sterile filtered (0.2-μm pore size; Nalgene), and stored at 4°C in PBS. Purified mAb (clone 2918) was tested for specificity by using 293 cells transfected with TCCR or empty vectors. For flow cytometry experiments, mAb was biotinylated and used in combination with fluorochrome-conjugated streptavidin (SA). Similar distribution and intensity of surface TCCR/WSX-1 staining was observed for SA-PE, SA-PerCP, and SA-allophycocyanin (eBioscience).

For in vitro $CD4^+$ T cell differentiation, splenocytes were isolated from uninfected mice as above and depleted of $CD8^+$ and $NK1.1^+$ cells by magnetic bead separation (Polysciences). Cells were then labeled with CFSE (5 μg/ml; Sigma-Aldrich) and stimulated with soluble anti-CD3 Ab (1 μg/ml) and soluble anti-CD28 Ab (1 μg/ml). For Th1- and Th2-polarizing conditions, cultures were supplemented with either rIL-12 (5 ng/ml) or IL-4 (50 ng/ml), respectively (eBioscience). Where noted, cells were arrested in G_1 phase by adding L-mimosine (300 μM; Sigma-Aldrich) before activation. In some experiments, murine IL-2 was blocked with neutralizing Abs (5 μg/ml; eBioscience) and, where indicated, increasing doses (1, 10, 50, 100 U/ml) of recombinant human IL-2 (Chiron) were added. After 48 or 72 h in culture, surface staining for WSX-1 and CD4 was performed as above.

To assay production of IFN-γ, $CD4^+$ T cells were stimulated as above (nonpolarizing) and, where noted, recombinant murine IL-27 was added (200 ng/ml; Genentech) (14). After 48 h in culture, cells were pulsed with PMA (50 ng/ml; Sigma-Aldrich) and ionomycin (500 ng/ml; Sigma-Aldrich) for 4 h, treated with brefeldin A (10 μg/ml; Sigma-Aldrich) for 2 h, and then stained for intracellular IFN-γ in combination with surface CD4 (eBioscience).

RT-PCR

To assay gene transcription, mRNA was isolated from cells using standard procedures and converted to cDNA as described (21). PCR (34 cycles: 95°C, 30 s; 60°C, 30 s; 72°C, 60 s) was then used to quantify message levels and β-actin expression was used as an internal control to assure equal loading of every reaction. Primers were as follows: WSX-1, 5′-CAA GAAGAGGTCCCGTGCTG, 5′-TTGAGCCCAGTCCACCACAT; gp130, 5′-CGTGGGAAAGGAGATGGTTGTG, 5′-AGGGTTGTCAGGAAGGC TAAG; Foxp3, 5′-CAGCTGCCTACAGTGCCCCTAG, 5′-CATTTGC CAGCAGTGGGTCTAAG.

Results

Expression of the IL-27R is suppressed after activation of NK cells and NKT cells

Acute infection with *T. gondii* induces a robust NK cell response that peaks during the first week of infection (23). Thus, to explore a role for IL-27 in this parasite-induced inflammation, surface levels of WSX-1 were monitored on NK cells before and after challenge with *T. gondii* (day 5). Previous studies indicate that resting NK1.1-positive cells express WSX-1 mRNA (8) and, accordingly, high levels of WSX-1 are evident on 44% of NK cells ($NK1.1^+CD3^-$) from uninfected animals (Fig. 1*A*). During acute toxoplasmosis, there is a dramatic reduction in the percentage of WSX-1^{high} NK cells (15%, Fig. 1*A*) and, to determine whether this decrease is prompted by parasite-induced NK cell activation, expression of WSX-1 was compared between resting ($NK1.1^{high}$) and activated ($NK1.1^{low}$) NK cells (24). Before infection, most splenic NK cells maintain a resting phenotype and display high levels of WSX-1 (52% WSX-$1^{high}NK1.1^{high}$, Fig. 1*A*). In contrast, during infection, most NK cells become activated and their expression of WSX-1 is reduced (10% WSX-$1^{high}NK1.1^{low}$, Fig. 1*A*). Given that surface levels of WSX-1 remain high on resting NK cells from *T. gondii*-challenged animals (33% WSX-1^{high}NK1. 1^{high} vs 10% WSX-$1^{high}NK1.1^{low}$), it is apparent that expression of the IL-27R is suppressed only after these cells become activated. Consistent with these findings, expression of WSX-1 mRNA is reduced after NK cells are stimulated by virally challenged macrophages (25).

Like NK cells, NKT cells ($NK1.1^+CD3^+$) are activated during acute toxoplasmosis and contribute to the protective innate immune response through rapid cytokine production (26). Similar to NK cells, WSX-1 is highly expressed by most NKT cells from uninfected mice (62% WSX-1^{high}), and after infection, there is a significant reduction in the percentage of WSX-1^{high} NKT cells (37%, Fig. 1*B*). Because there is a shift in the phenotype of splenic NKT cells from mostly resting ($CD3^{high}$) in uninfected animals to mostly activated ($CD3^{low}$) in *T. gondii*-challenged mice (27), it is

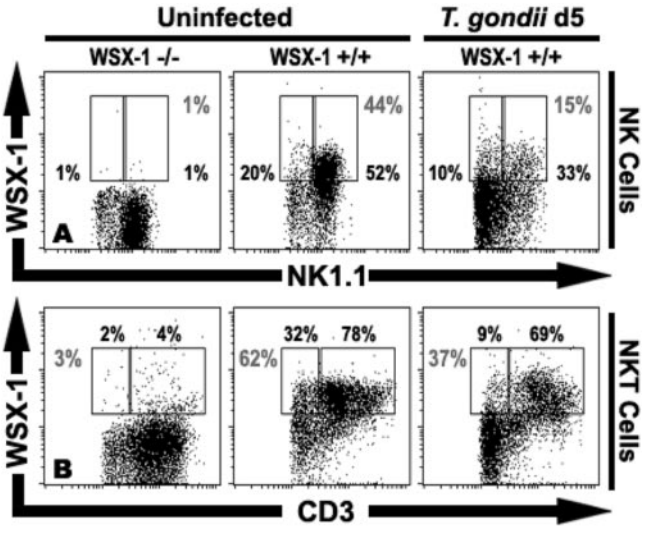

FIGURE 1. Expression of the IL-27R is reduced upon activation of NK cells and NKT cells. Surface levels of WSX-1 were monitored on splenic NK cells and NKT cells before and after 5 days of infection with *T. gondii*. *A*, For NK cells, only $NK1.1^+CD3^-$ events are displayed and the percentage of total WSX-1^{high} cells is presented in gray in the *upper right corner* of each plot. The percentage of activated ($NK1.1^{low}$) or resting ($NK1.1^{high}$) WSX-1^{high} NK cells is noted in black beside the respective NK1.1 gate. *B*, For NKT cells, only $NK1.1^+CD3^+$ events are displayed and the percentage of total WSX-1^{high} cells is presented in gray in the *upper left corner* of each plot. The percentage of activated ($CD3^{low}$) or resting ($CD3^{high}$), WSX-1^{high} NKT cells is denoted in black above the respective CD3 gate. *A* and *B*, WSX-1-deficient mice were used as negative controls for surface receptor staining on NK cells and NKT cells. These data are representative of three to five individual experiments (three mice per group). See Table I for a detailed statistical analysis of WSX-1 expression by NK and NKT cells.

likely that, as with NK cells, the decline in WSX-1 levels is related to parasite-induced activation of NKT cells. Accordingly, most resting NKT cells display high levels of WSX-1 before (78% WSX-1^{high}CD3^{high}) or after (69% WSX-1^{high}CD3^{high}) infection with *T. gondii*, and in both groups, NKT cell activation is associated with decreased expression of this receptor (32%/9% WSX-1^{high}CD3^{low}, Fig. 1*B*). Likewise, before or during infection, surface levels of WSX-1 are low on NKT cells that express CD40L (our unpublished data), a costimulatory molecule that is induced by activation (28).

Expression of the IL-27R is enhanced by activation of CD4$^+$ and CD8$^+$ T cells

While NK and NKT cells contribute to the inflammatory response during acute toxoplasmosis, it is well established that T cells are required for resistance (29). Thus, given that WSX-1 is required to suppress the development of pathogenic T cell responses during acute toxoplasmosis (21), expression of this receptor was monitored on CD4 T cells throughout infection. Consistent with the notion that IL-27 can promote naive T cell responses (1, 11–13), there are low levels of WSX-1 on the surface of CD4$^+$ T cells from uninfected animals (21% WSX-1^{high}, Fig. 2*A*). However, since expression of IL-27R is increased during infection (49% WSX-1^{high}), these data also support the hypothesis that IL-27 can directly suppress effector T cells (Fig. 2*A*). To address whether this infection-induced change is dependent on T cell activation, receptor levels were compared between naive and effector CD4$^+$ T cells. Similar to the intermediate expression of WSX-1 noted on total CD4$^+$ T cells from uninfected mice (21% WSX-1^{high}, Fig. 2*A*), approximately one-fourth of naive CD4$^+$ T cells (CD62L^{high}) express high levels of this receptor in either uninfected (17% WSX-1^{high}) or *T. gondii*-challenged mice (28% WSX-1^{high}, Fig. 2*B*). However, when compared to naive counterparts, effector T cells (CD62L^{low}) from either group display a 2-fold increase in their expression of IL-27R (Fig. 2*B*). Thus, since splenic T cell populations shift from mostly naive to mostly effector during acute toxoplasmosis, the gross increase in WSX-1 levels (49% WSX-1^{high}, Fig. 2*A*) reflects a predominance of WSX-1^{high} effector T cells (*B*).

Like CD4$^+$ T cells, CD8$^+$ T cells promote resistance to *T. gondii* (29, 30), and during infection of WSX-1-deficient animals, both subsets display increased activation, proliferation, and IFN-γ production (21). Therefore, since acute toxoplasmosis promotes expression of WSX-1 by CD4$^+$ T cells (Fig. 2*A*), this receptor was monitored on CD8$^+$ T cells. Although previous reports indicate that CD8$^+$ T cells have reduced WSX-1 mRNA expression when compared with their CD4$^+$ counterparts (7–9), surface levels of this receptor are comparable between CD4$^+$ (21% WSX-1^{high}) and CD8$^+$ (22% WSX-1^{high}) T cells from uninfected mice (Fig. 2, *A* and *C*). Furthermore, as noted for CD4$^+$ T cells, *T. gondii* infection leads to a significant increase in the percentage of CD8$^+$ T cells expressing high levels of WSX-1 (59% WSX-1^{high}, Fig. 2*C*). However, unlike CD4$^+$ T cells, this rise in WSX-1 levels is not strictly linked to changes in the expression of CD62L (Fig. 2*D*).

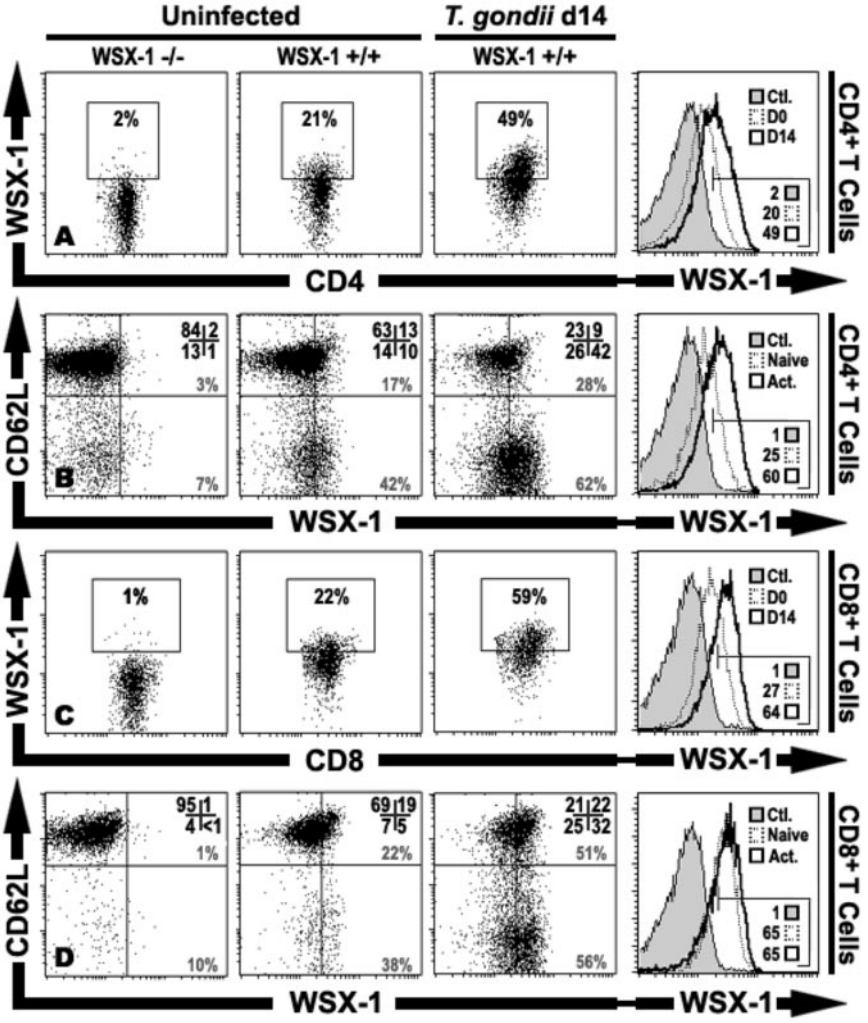

FIGURE 2. Expression of the IL-27R is enhanced upon activation of CD4$^+$ and CD8$^+$ T cells. Surface levels of WSX-1 were monitored on splenic T cells from uninfected or *T. gondii*-challenged mice (day 14 postinfection). *A*, For dot plots and histograms, only CD4$^+$ events are displayed and the percentage of total WSX-1^{high} cells is presented in black within the respective gates. *B*, The percentage of WSX-1^{high} naive (CD62L^{high}) or effector (CD62L^{low}) CD4$^+$ T cells is displayed in gray in the *bottom right corner* of the respective quadrant. The percentage of total CD4$^+$ T cells in each quadrant is presented as an integer in the *upper right*. For histograms, only CD4$^+$ T cells from infected animals are displayed and the percentage of naive (CD62L^{high}) or activated (CD62L^{low}), WSX-1^{high} cells is presented within the appropriate gate. *C*, For dot plots and histograms, only CD8$^+$ events are displayed and the percentage of total WSX-1^{high} cells is presented in black within the respective gates. *D*, The percentage of WSX-1^{high} naive (CD62L^{high}) or effector (CD62L^{low}) CD8$^+$ T cells is displayed in gray in the *bottom right corner* of the respective quadrants. The percentage of total CD8$^+$ T cells in each quadrant is presented as an integer in the *upper right*. For histograms, only CD8$^+$ T cells from infected animals are displayed, and the percentage of naive (CD62L^{high}) or activated (CD62L^{low}), WSX-1^{high} cells is noted within the appropriate gate. *A–D*, WSX-1-deficient mice were used as negative controls for surface receptor staining on CD4$^+$ T cells and CD8$^+$ cells. These data are representative of three to five individual experiments (three mice per group). See Table I for a detailed statistical analysis of WSX-1 expression by CD4$^+$ and CD8$^+$ T cells.

Nevertheless, since these studies establish that *T. gondii* infection promotes expression of WSX-1 by $CD8^+$ T cells (Fig. 2, *C* and *D*), they are consistent with the hypothesis that IL-27 can suppress parasite-induced $CD8^+$ T cell responses (21) and with previous reports demonstrating that it can promote antiviral (31) and antitumor $CD8^+$ T cell responses (32, 33).

Positive and negative regulation of the IL-27R during $CD4^+$ T cell differentiation

Because in vivo activation promotes expression of WSX-1 by $CD4^+$ T cells, surface receptor levels were also monitored during in vitro differentiation. When naive $CD4^+$ T cells are left unstimulated or cultured with anti-CD28 mAb alone, there is little proliferation and relatively few cells (18%) are WSX-1^{high} (data not shown and Fig. 3*A*). However, upon ligation of the TCR/CD3, $CD4^+$ T cells undergo cellular division and display high surface levels of WSX-1 (80% WSX-1^{high}, Fig. 3*C*). Furthermore, despite evidence that WSX-1 mRNA levels are reduced during $CD4^+$ T cell differentiation (Th1/Th2) (8), induction of this receptor is apparent on the surface of $CD4^+$ T cells when activated under nonpolarizing (80% WSX-1^{high}, Fig. 3*C*), Th1-polarizing (74% WSX-1^{high}, Fig. 3*D*), or Th2-polarizing conditions (data not shown). These findings demonstrate that TCR-dependent activation can induce expression of WSX-1 regardless of the cytokine environment present at the time of T cell priming (Fig. 3). Additionally, these studies suggest that TCR ligation is sufficient to induce the high levels of WSX-1 that are apparent on effector Th1 cells during infection with *T. gondii* (Fig. 2).

When cultured with L-mimosine, $CD4^+$ T cells remain in G_1 phase of the cell cycle and do not proliferate (34). However, while arrested cells can become activated and produce IL-2 (our unpublished data) (34), treatment with L-mimosine blocks the ability of TCR ligation to induce WSX-1 (7% WSX-1^{high}, Fig. 3*B*). These data imply that, like the IL-12R (35, 36), at least one round of cellular division is required for optimal expression of the IL-27R by $CD4^+$ T cells (Fig. 3*B*). Still, although 80% of $CD4^+$ T cells express high levels of WSX-1 after cycling for 48 h (Fig. 3*B*), only 66% of these cells remain WSX-1^{high} after 72 h in culture (*E*). To determine whether this decline in receptor levels is related to prolonged cellular division, expression of WSX-1 was compared between $CD4^+$ T cells from successive proliferative generations. Consistent with the hypothesis that WSX-1 is rapidly induced by TCR-dependent activation, $CD4^+$ T cells from the first (76%

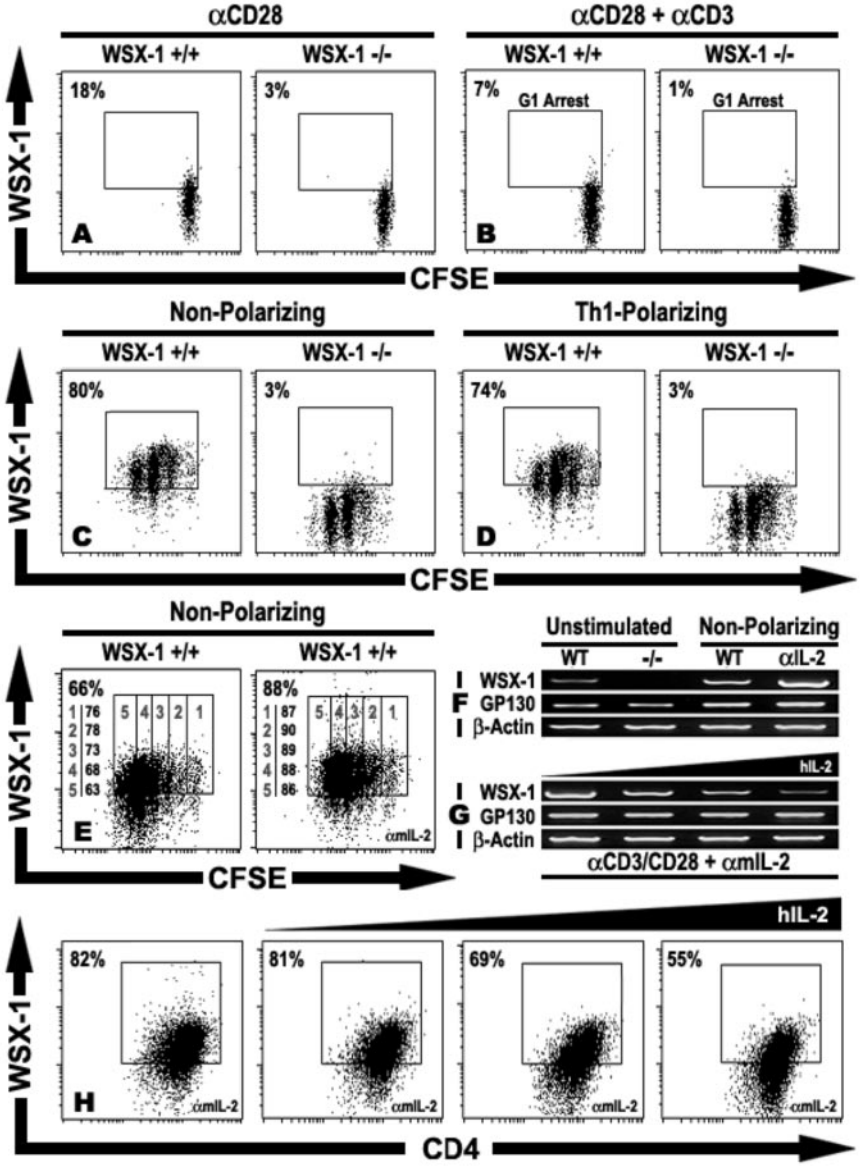

FIGURE 3. Positive and negative regulation of the IL-27R during in vitro $CD4^+$ T cell differentiation. $CD4^+$ T cells from uninfected WT and WSX-1-deficient mice were labeled with CFSE, cultured in vitro and expression of WSX-1 was assayed. *A*, $CD4^+$ T cells were stimulated with anti-CD28 mAb for 48 h and the percentage of WSX-1^{high} cells is displayed in the *upper left* of each plot. *B*, $CD4^+$ T cells were treated with L-mimosine and stimulated with anti-CD28 mAb and anti-CD3 mAb (αCD3/28) for 48 h. *C*, $CD4^+$ T cells were activated under nonpolarizing conditions (αCD3/28) for 48 h and the percentage of WSX-1^{high} cells is displayed in the *upper left* of each plot. *D*, $CD4^+$ T cells were activated under Th1-polarizing conditions (anti-CD3/28 plus rIL-12) for 48 h and the percentage of WSX-1^{high} cells is displayed in the *upper left* of each plot. *E*, $CD4^+$ T cells were cultured (anti-CD28/anti-CD3) for 72 h and, where noted, anti-murine IL-2 mAb was added (+αmIL-2). The percentage of total WSX-1^{high} cells is displayed in the *upper left* of each plot and, additionally, the percentage of WSX-1^{high} cells in each proliferative generation is presented as a numbered integer on the *left margin*. *F*, $CD4^+$ T cells were left unstimulated or cultured under nonpolarizing conditions and, where noted, anti-murine IL-2 mAb was added (+αmIL-2). After 48 h, mRNA was isolated and RT-PCR was performed to detect WSX-1, gp130, and β-actin. *G*, $CD4^+$ T cells were activated in the presence of anti-murine-IL-2 mAb (αCD28/αCD3 + αmIL-2), and cultures were supplemented with increasing doses (*left* to *right*: 1, 10, 50, and 100 U/ml) of recombinant human IL-2. After 48 h, mRNA was isolated and RT-PCR performed to detect WSX-1, gp130, and β-actin. *H*, $CD4^+$ T cells were cultured as in *G*, and after 72 h, surface levels of WSX-1 were assayed. The percentage of WSX-$1^{high}CD4^+$ T cells is displayed in the *upper left* of each plot. *A–H*, For all flow cytometry and RT-PCR, WSX-1-deficient mice were used as negative controls. These data are representative of three to five individual experiments.

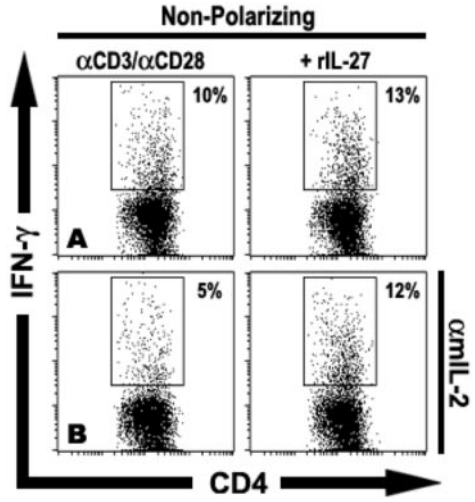

FIGURE 4. Enhanced IL-27 responses in the absence of IL-2. WT $CD4^+$ T cells were cultured (αCD28/αCD3) with (*B*) or without (*A*) anti-IL-2 mAb and, where noted, recombinant murine IL-27 was added. After 72 h, cells were pulsed with PMA and ionomycin for 4 h before staining for surface CD4 and intracellular IFN-γ (*A* and *B*). Gates indicate specific IFN-γ staining compared with control mAb and the percentage of IFN-γ^+CD4^+ cells is displayed in the *upper right* of each plot. These data are representative of three to five individual experiments.

$WSX\text{-}1^{high}$) and second (78% $WSX\text{-}1^{high}$) proliferative generations display the highest receptor levels (Fig. 3*E*). Thereafter, expression of WSX-1 wanes with each division and, by the fifth generation, only 63% of $CD4^+$ T cells display high levels of WSX-1 (Fig. 3*E*). Given that proliferation is required for optimal induction of WSX-1 (Fig. 3, *A* and *B*) and that continued cellular division appears to prompt a reduction in receptor levels (*E*), these in vitro studies demonstrate that expression of the IL-27R can be regulated by sequential positive and negative cues.

IL-2 can suppress expression of the IL-27R by activated $CD4^+$ T cells

Because effector T cells must divide several times before levels of WSX-1 decline (Fig. 3*E*), it is possible that receptor expression can be maintained if cells are hindered from reaching this proliferative threshold. In turn, since autocrine IL-2 production enhances $CD4^+$ T cell proliferation (37), it is not surprising that depletion of this cytokine increases the percentage of cells that express high levels of WSX-1 after 72 h in culture (88% $WSX\text{-}1^{high}$, Fig. 3*E*). However, although it is clear that T cells proliferate less when IL-2 is neutralized in these cultures, expression of WSX-1 does not decline even in cells that reach the fourth (88% $WSX\text{-}1^{high}$) and fifth (86% $WSX\text{-}1^{high}$) proliferative generations (Fig. 3*E*). Thus, aside from its role in promoting T cell proliferation, it is apparent that IL-2 also inhibits the expression of WSX-1 by activated $CD4^+$ T cells. In accord with this hypothesis, WSX-1 mRNA levels are enhanced when IL-2 is depleted during $CD4^+$ T cell differentiation (Fig. 3*F*). Furthermore, in the absence of endogenous murine IL-2, the addition of recombinant human cytokine leads to a dose-dependent reduction in expression of WSX-1 mRNA and surface protein (Fig. 3, *G* and *H*). Together, these studies demonstrate that, similar to its role in regulating the IL-7R (38), IL-2 can directly suppress expression of the IL-27R.

Given that gp130 is abundant in naive and effector T cells (Fig. 3, *F* and *G*) (39), it is likely that surface levels of WSX-1 limit the availability of heterodimeric IL-27R. Therefore, since IL-2 can selectively inhibit expression of WSX-1 but not that of gp130 (Fig. 3, *F* and *G*), it may suppress the ability of $CD4^+$ T cells to respond to IL-27. Accordingly, previous reports indicate that neutralization

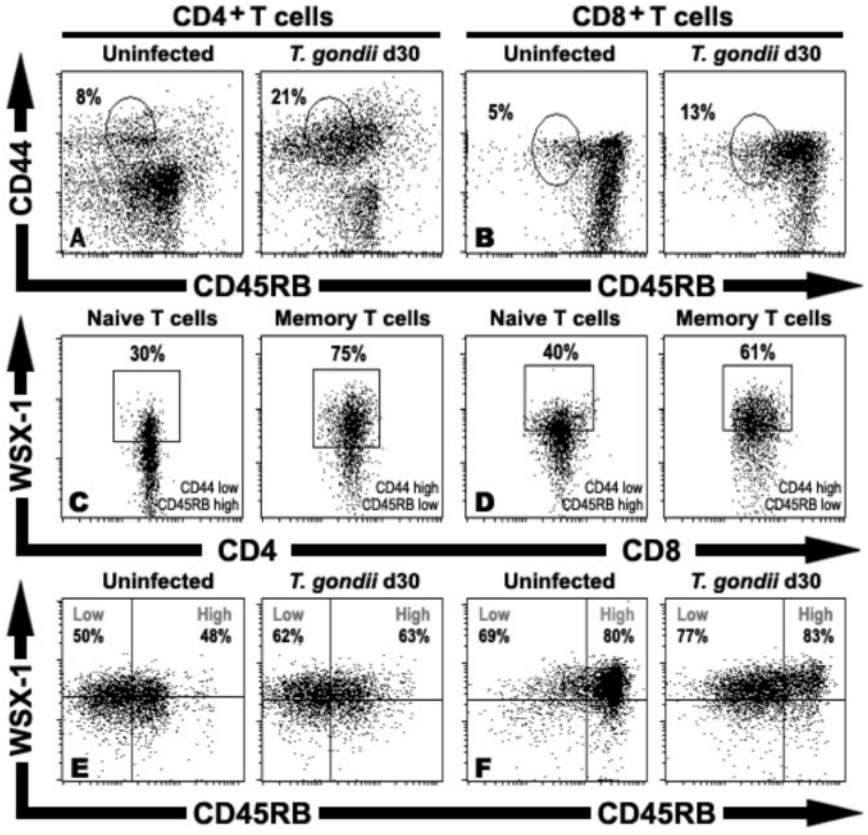

FIGURE 5. Expression of the IL-27R by memory T cells. Surface levels of WSX-1 were monitored on memory T cells from the spleens of uninfected or *T. gondii*-challenged WT mice (day 30 postinfection). *A* and *B*, The percentage of memory ($CD44^{high}CD45RB^{low}$) $CD4^+$ (*A*) or $CD8^+$ (*B*) T cells is displayed in the *upper left* of each plot. *C*, Splenocytes were isolated from chronically infected mice, and the percentage of $WSX\text{-}1^{high}$ naive ($CD44^{low}CD45RB^{high}$) or memory ($CD44^{high}CD45RB^{low}$) $CD4^+$ T cells is noted within the respective gates. *D*, The percentage of $WSX\text{-}1^{high}$ naive and memory $CD8^+$ T cells is displayed within the respective gates. *E* and *F*, $CD44^{high}$ (effector/memory) $CD4^+$ (*E*) or $CD8^+$ (*F*) cells were identified from uninfected or *T. gondii*-challenged animals. The percentage of $WSX\text{-}1^{high}$ effector ($CD45RB^{high}$) or memory ($CD45RB^{low}$) T cells is displayed within the appropriate quadrant. *A–F*, WSX-1-deficient mice were used as negative controls for surface receptor staining on $CD4^+$ T cells and $CD8^+$ cells. These data are representative of three to five individual experiments (three mice per group). See Table I for a detailed statistical analysis of WSX-1 expression by memory $CD4^+$ and $CD8^+$ T cells.

of IL-2 enhances the ability of IL-27 to promote T cell proliferation (1). Likewise, in the presence of IL-2, IL-27 can enhance IFN-γ production only modestly, but when IL-2 is depleted, IL-27 promotes a 2-fold increase in the percentage of IFN-γ-positive $CD4^+$ T cells (5 vs 12%, Fig. 4*B*). Because levels of WSX-1 are enhanced in the absence of IL-2 (Fig. 3, *E–H*), these data are consistent with the hypothesis that IL-27 sensitivity can be modulated by IL-2-dependent changes in receptor expression. However, since IL-2 and IL-27 can each promote T cell proliferation and IFN-γ production, an alternative interpretation of these studies is that the ability of IL-27 to augment T cell responses is magnified when a dominant growth factor (IL-2) is removed. Even so, these two mechanisms are not mutually exclusive, and it is possible that both affect the potency of IL-27 in IL-2-deficient T cell cultures.

Memory $CD4^+$ and $CD8^+$ T cells express the IL-27R

The preceding studies demonstrate that expression of WSX-1 is greater on effector T cells than on naive precursors. Thus, to determine whether the IL-27R is also present at high levels on the surface of memory T cells, expression of WSX-1 was compared with that of T cell memory markers. In uninfected animals, a small fraction (8% $CD4^+$; 5% $CD8^+$) of the T cell repertoire displays a memory phenotype ($CD44^{high}CD45RB^{low}$), but after challenge with *T. gondii*, there is a significant increase in the percentage of memory $CD4^+$ (21%, Fig. 5*A*) and $CD8^+$ T cells (13%, *B*). As with effector T cells, expression of WSX-1 by memory $CD4^+$ (75% WSX-1^{high}) and $CD8^+$ T cells (61% WSX-1^{high}) is much higher than that of naive counterparts (30/40% WSX-$1^{high}CD44^{low}CD45RB^{high}$; Fig. 5, *C* and *D*). Moreover, while their ontogeny may be distinct (40), memory ($CD44^{high}CD45RB^{low}$) and effector T cells ($CD44^{high}CD45RB^{high}$) display comparable surface levels of WSX-1 (Fig. 5, *E* and *F*). Consequently, although rIL-27 does not enhance the proliferation of $CD4^+CD44^{high}CD45RB^{low}$ memory T cells (1), the current work establishes that they do express the IL-27R.

Regulatory $CD4^+$ T cells express the IL-27R

For $CD4^+$ T cells, enhanced expression of WSX-1 relates to changes in activation markers CD62L (Fig. 2) and CD44 (Fig. 5). In contrast, although expression of CD25 can be indicative of recent cellular activation (21), there is no correlation between levels of CD25 and WSX-1 on the surface of effector T cells (52% WSX-$1^{high}CD62L^{low}CD25^{low}$ vs 57% WSX-$1^{high}CD62L^{low}CD25^{high}$; Fig. 6, *E* and *F*). For $CD4^+$ T cells with a naive phenotype ($CD62L^{high}$), expression of CD25 can denote distinct functional subsets and, specifically, constitutive expression of this marker has been used to distinguish T regulatory cells (T_{reg} cells) ($CD62L^{high}CD25^{high}$) (41). Therefore, since there are no previous reports investigating a role for IL-27 in T_{reg} cell biology, expression of WSX-1 was examined on this T cell subset. Similar to effector T cells, T_{reg} cells display increased levels of WSX-1 (47% WSX-1^{high}) when compared to naive Th cells (23% $CD62L^{high}CD25^{low}$; Fig. 6, *C* and *D*). To confirm these findings, naive T cells and T_{reg} cells were isolated from WT mice and mRNA levels assayed for WSX-1, gp130, and Foxp3, a transcription factor restricted to T_{reg} cells (41). In agreement with the surface receptor levels (Fig. 6, *C* and *D*), expression of WSX-1 mRNA is enhanced in T_{reg} cells ($Foxp3^+CD62L^{high}CD25^{high}$) over naive Th cells ($Foxp3^-CD62L^{high}CD25^{low}$, Fig. 6*G*). Because expression of gp130 confirms the presence of the complete IL-27R (Fig. 6*G*), these data suggest that, as with effector and memory T cells IL-27 can influence T_{reg} cell responses. However, since WSX-1-deficient mice have normal numbers of functional T_{reg} cells (Fig. 6, *A* and *B*, and our unpublished data), it is also evident that IL-27 is not required for the development of this subset.

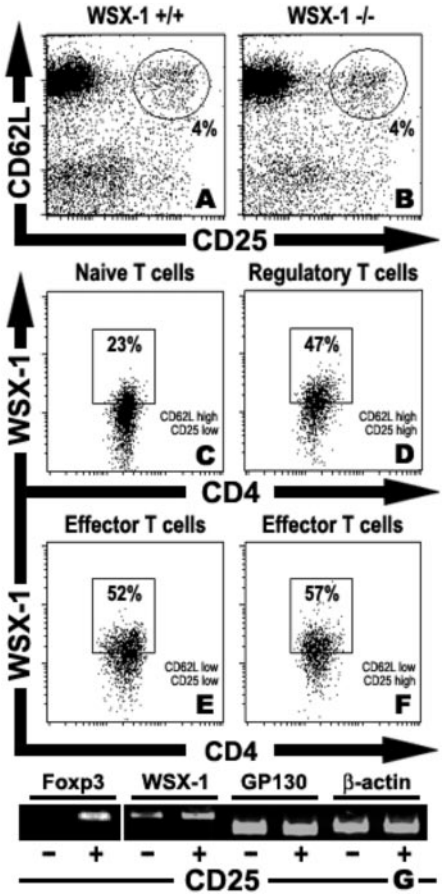

FIGURE 6. Expression of the IL-27R by T_{reg} cells. Surface levels of WSX-1 were monitored on T_{reg} cells from the spleens of uninfected WT and WSX-$1^{-/-}$ mice. *A* and *B*, The percentage of regulatory $CD4^+$ T cells ($CD62L^{high}CD25^{high}$) from WT (*A*) or WSX-$1^{-/-}$ (*B*) mice is displayed beneath the appropriate gates. *C–F*, The percentage of WSX-1^{high} naive ($CD62L^{high}CD25^{low}$) (*C*), regulatory ($CD62L^{high}CD25^{high}$) (*D*), and effector ($CD62L^{low}CD25^{low}$/$CD62L^{low}CD25^{high}$) (*E* and *F*) $CD4^+$ T cells is displayed within the respective gates. *G*, Naive ($CD4^+CD62L^{high}CD25^{low}$) and regulatory ($CD4^+CD62L^{high}CD25^{high}$) $CD4^+$ T cells were FACS sorted from WT mice, mRNA was isolated and RT-PCR was used to detect expression of Foxp3, WSX-1, gp130, and β-actin. *A–G*, For flow cytometry and RT-PCR, WSX-1-deficient mice were used as negative controls. These data are representative of two to three individual experiments. See Table I for a detailed statistical analysis of WSX-1 expression by naive, regulatory, and effector $CD4^+$ T cells.

Discussion

The data presented here indicate that distinct lymphoid cell lineages express varying levels of WSX-1 and that expression of this receptor is profoundly affected during an acute inflammatory response (Table I). Moreover, since the activation of NK cells and NKT cells leads to a reduction in WSX-1 levels while a similar event in T cells promotes expression, these studies demonstrate surface levels of WSX-1 can be regulated by both positive and negative signals (Table I). Accordingly, TCR ligation prompts expression of WSX-1 by $CD4^+$ T cell but also induces the production of IL-2, a cytokine that inhibits expression of this receptor (Fig. 3). In fact, since $CD4^+$ T cells must undergo several rounds of division before IL-2 can suppress receptor levels, this effect is restricted to the committed effector cells that are most likely to be

Table I. *Statistical analysis of WSX-1 expression by lymphoid cells*[a]

	Percentage WSX-1^{high}							
	Uninfected				Infected-*T. gondii*			
	Mean	Dev.	Total (n =)	t test (p =)	Mean	Dev.	Total (n =)	t test (p =)
Innate immunity								
NK cells								
Resting NK1.1^{high}	56.8	±3.5	4		49.0	±7.1	6	
Activated NK1.1^{low}	30.7	±8.5	4	0.05	17.8	±1.5	6	10^{-4}
NKT cells								
Resting CD3^{high}	68.6	±4.1	4		74.0	±5.5	7	
Activated CD3^{low}	42.9	±1.6	4	0.02	37.0	±9.2	7	10^{-5}
Adaptive immunity								
CD4$^+$ T cells								
Naive CD62L^{high}CD25^{low}	22.2	±1.4	5		33.9	±4.5	8	
Regulatory CD62L^{high}CD25^{high}	50.4	±1.1	5	10^{-7}	60.8	±3.5	8	10^{-6}
Activated CD62L^{low}CD25^{low}	45.8	±4.3	5	10^{-3}	51.0	±11.9	8	10^{-3}
Activated CD62L^{low}CD25^{high}	51.4	±8.1	5	0.01	63.9	±2.9	8	10^{-5}
Memory CD44^{high}CD45RB^{low}	49.7	±9.5	4	0.03	48.6	±10.6	6	10^{-3}
CD8$^+$ T cells								
Naive CD62L^{high}	27.4	±0.9	4		51.9	±4.8	6	
Activated CD62L^{low}	42.3	±2.5	4	0.02	56.9	±2.6	6	0.11
Memory CD44^{high}CD45RB^{low}	48.3	±6.4	4	0.02	44.6	±5.3	6	0.12

[a] The mean percentage of WSX-1^{high} NK cells, NKT cells, CD4$^+$ T cells, and CD8$^+$ T cells from either uninfected or *T. gondii*-challenged mice is presented with the SD (Dev.) and number of samples (Total) in each experimental group. Statistical differences (p values) between resting/naive cells and other experimental groups were determined by Student's t test.

WSX-1^{high} (Fig. 3). Together, these data imply that, within a single cell, IL-27 sensitivity may be regulated through sequential induction and suppression of WSX-1. Given that acute infection with *T. gondii* is a potent stimulus for CD4$^+$ T cell proliferation (21) and WSX-1 expression (Table I), it is possible that, in vivo, effector cells that display low levels of WSX-1 (38% WSX-1^{low}CD62L^{low}) may have acquired and subsequently lost the receptor (Fig. 2).

Although activation prompts a reduction in expression of WSX-1 by NK cells (Table I), the appearance of this receptor on resting cells implies that, like IL-12 (15), IL-27 can influence nascent NK cell responses. However, since IL-27 alone does not affect NK cell proliferation, IFN-γ production, or cytotoxicity (14), it is likely that it must synergize with other inflammatory stimuli (i.e., costimulatory molecules, cytokines) to promote cellular effects. Consistent with this hypothesis, IL-27 can induce IFN-γ production by primary human NK cells when combined with IL-2 or IL-12 (1). Still, given that NK1.1-positive cells from WSX-1$^{-/-}$ mice produce more IFN-γ than WT cohorts during infection with *Trypanosoma cruzi* (42), it is also possible that IL-27 can cooperate with anti-inflammatory factors, such as inhibitory receptors or regulatory cytokines, to suppress NK cell responses. Likewise, resting NKT cells are mostly WSX-1^{high} (Table I), and in the absence of this receptor, they produce more IFN-γ and IL-4 than WT counterparts during infection with *T. cruzi* (42) or Con A-induced hepatitis (43). In turn, since the current studies establish that they express high levels of WSX-1 before activation, they support the idea that WSX-1 is required to curb NK and NKT cell hyperactivity during acute inflammatory responses (42, 43).

WSX-1 is only known to interact with IL-27 but gp130 participates in the receptor for several cytokines that influence T cell responses (5, 6). By pairing with IL-6Rα, gp130 mediates signaling for IL-6, a cytokine that, like IL-27, is required for resistance to *T. gondii* (44). However, despite a shared receptor subunit, it is clear that IL-27 and IL-6 have distinct biological functions and that the ligand-specific receptor components (WSX-1/IL-6Rα) are regulated differently. Because naive CD4$^+$ T cells coexpress IL-6Rα and gp130 on their surface (39), while levels of WSX-1 are relatively low, it is likely that IL-6 signaling is favored in these cells. In contrast, surface levels of WSX-1 increase upon activation (Table I), while those of IL-6Rα decline (39). Thus, due to enhanced expression of WSX-1 (Fig. 2) and diminished competition for gp130 (39), effector T cells are more responsive to IL-27 that their naive predecessors. Through sequential expression of ligand-specific receptor components (first IL-6Rα and then WSX-1), two cytokines that operate through gp130 can influence CD4$^+$ T cells during distinct stages of differentiation.

Because the preceding studies demonstrate that key effector cells of innate (NK cells and NKT cells) and adaptive (effector, regulatory, and memory T cells) immunity express high levels of WSX-1 (Table I), they are consistent with the hypothesis that IL-27 can limit pathogen-induced inflammation (20, 21, 42). Therefore since a lack in WSX-1 leads to the development of pathogenic inflammatory responses during infection with *T. gondii* (21) and *T. cruzi* (42), it is likely that a loss of IL-27-mediated immunosuppression contributes to the severe autoimmunity associated with attenuated gp130 signaling in mice (45–47). Given that expression of WSX-1 mRNA has been detected in mast cells (3, 20), B cells (1, 7–9, 48), and monocytes (3), the current findings also build on growing evidence that IL-27 can influence a variety of immune cell types and thereby represents a viable therapeutic target for inflammatory disorders.

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Disclosures

C. A. Hunter and A. V. Villarino are named on a pending patent, "Methods for modulating an inflammatory response."

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