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Stat4 Regulates Multiple Components of IFN-γ-Inducing Signaling Pathways

Victoria A. Lawless,* Shangming Zhang, * Osman N. Ozes,* † Heather A. Bruns,* India Oldham,* Timothy Hoey, ‡ Michael J. Grusby, § and Mark H. Kaplan 2 *

Stat4 is activated in response to IL-12. Most functions of IL-12, including the induction of IFN-γ, are compromised in the absence of Stat4. Since the precise role of Stat4 in IFN-γ induction has not been established, experiments were conducted to examine Stat4 activation of IFN-γ and other genes required for cytokine-induced expression of IFN-γ. We first examined IL-12 signaling components. Basal expression of IL-12Rβ1 and IL-12Rβ2 is decreased in Stat4-deficient cells compared with that in control cells. However, IL-12 was still capable of inducing equivalent phosphorylation of Jak2 and Tyk2 in wild-type and Stat4-deficient activated T cells. We have further determined that other cytokine signaling pathways that induce IFN-γ production are defective in the absence of Stat4. IL-18 induces minimal IFN-γ production from Stat4-deficient activated T cells compared with control cells. This is due to defective IL-18 signaling, which results from the lack of IL-12-induced, and Stat4-dependent, expression of the IL-18R. Following IL-12 pretreatment to induce IL-18R, wild-type, but not Stat4-deficient, activated T cells demonstrated IL-18-induced NF-κB DNA-binding activity. In addition, IL-12-pretreated Stat4-deficient activated T cells have minimal IFN-γ production followed by stimulation with IL-18 alone or in combination with IL-12 compared with control cells. Thus, Stat4 activation by IL-12 is required for the function of multiple cytokine pathways that result in induction of IFN-γ. The Journal of Immunology, 2000, 165: 6803–6808.

Interleukin-12 is a pleiotropic cytokine produced by macrophages and dendritic cells (1). The IL-12R is composed of two noncovalently linked receptor chains, IL-12Rβ1 and IL-12Rβ2, expressed on NK and activated T and B cells. These receptor subunits cooperate in IL-12 binding and signaling, with each chain sharing homology with β-chains of the gp130 family of receptors (2, 3). Both receptor chains associate with members of the Janus kinase family of tyrosine kinases, the IL-12Rβ1 chain interacts with Tyk2, and the IL-12Rβ2 chain interacts with Jak2 (4). The IL-12Rβ2 chain is tyrosine phosphorylated and is responsible for recruitment and activation of Stat4 to a specific docking site (5–7). Stat4 is required for the biological functions of IL-12, including its ability to induce IFN-γ production, proliferation of activated T cells, increased cytotoxicity, and development of Th1 cells (8, 9). The precise role Stat4 plays in these responses is still unclear.

Stat4 can interact directly with DNA sequences in the IFN-γ promoter to increase gene transcription (10, 11), providing at least one mechanism for IL-12-induced IFN-γ expression. However, Stat4 may contribute to IFN-γ induction through other distinct mechanisms as well. IL-12 can synergize with IL-18 (also called IFN-γ-inducing factor) to induce IFN-γ. IL-18 was cloned based on its ability to induce the production of IFN-γ from T cells (12). IL-18 is secreted by macrophages and dendritic cells and is important for Th1 and NK function in vivo (13). IL-18 signals follow interaction of cytokine with a specific receptor composed of at least two separate chains, a cytokine-binding IL-18R (originally identified as an IL-1R-related protein) and an accessory protein-like chain, AcPL (14–17). IL-18 stimulation activates the DNA-binding activity of both NF-κB and AP-1 using signaling proteins that include MyD88, TNFR-associated factor (TRAF) 6, IL-1R-associated kinase (IRAK), and c-Jun N-terminal kinase (11, 18–22). The IL-18R chain is differentially expressed on subsets of Th cells (23, 24), potentially as a result of IL-12 stimulation (25, 26). IL-1, which shares many signaling components with IL-18, has also been shown to enhance Th1 development and IFN-γ induction (27, 28). The function of these additional IFN-γ-inducing pathways in the absence of IL-12 signaling has not been carefully examined.

Using previously generated Stat4-deficient mice that specifically lack responses to IL-12, we have now determined that Stat4 contributes to the regulation of IFN-γ production on several levels. In this report, we demonstrate that expression of IL-12Rβ1, IL-12Rβ2, MyD88, and IL-18R is IL-12 inducible in a Stat4-dependent manner. These results suggest that in addition to direct effects on the IFN-γ gene, Stat4 regulates cytokine receptors and signaling pathways required for the induction of IFN-γ.

Materials and Methods

Mice

The generation of Stat4-deficient mice was previously described (8). Mice were backcrossed 10 generations to the BALB/c background or eight generations to the C57BL/6 background. Mice were housed and bred in the Indiana University Laboratory Animal Research Center. Control BALB/c

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3 Abbreviations used in this paper: AcPL, accessory protein-like chain; TRAF, TNFR-associated factor; IRAK, IL-1R-associated kinase.

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and C57BL/6 mice were purchased form Harlan Bioproducts (Indianapolis, IN). Mice were used between 6 and 12 wk of age.

**Abs and cytokines**

Anti-CD3 (145-2C11), anti-IL-4 (11B11), and anti-INF-γ (R4/6A2) were produced and purified in our laboratory. IL-12 was purchased from Genzyme (Cambridge, MA). IL-18 was purchased from PeproTech (Rocky Hill, NJ) or produced as recombinant protein in *Escherichia coli* using the T7 system. IL-18 was expressed with a C-terminal histidine tag and purified by nickel affinity chromatography. IL-1α was provided by M. Harrington (Department of Biochemistry, Indiana University School of Medicine).

**Immunoprecipitations and phosphotyrosine analysis**

Activated T cells were washed in medium and stimulated for 20 min in the absence or the presence of 2 ng/ml IL-12. Cells were immediately washed twice in cold serum-free RPMI 1640 and lysed in cold lysis buffer containing protease and phosphatase inhibitors. Protein concentrations of the extracts were determined, and Jak2 and Tyk2 were immunoprecipitated with purified rabbit polyclonal Abs (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoprecipitates were separated by 7.5% SDS-PAGE and transferred to a nitrocellulose membrane. Immunoblotting was performed with a mouse monoclonal anti-phosphotyrosine Ab (Santa Cruz Biotechnology) and ECL detection kit (Amersham, Piscataway, NJ). Blots were stripped and reprobed with the precipitating Abs to demonstrate equal protein loading. Immunoblots of total cellular extracts were performed using polyclonal Abs to p65 and c-Rel (Santa Cruz Biotechnology).

**IFN-γ assay**

Total splenocytes were activated with 2 μg/ml plate-bound anti-CD3, for 48 h at 2 × 10^6 cells/ml. Cells from mice with a BALB/c background were also incubated with 10 μg/ml anti-IL-4 (11B11). Following activation, cells were washed, washed, and washed at 10^6/ml, and left unstimulated or stimulated with 1 ng/ml IL-12, doses of IL-18 as indicated, or both for 48 h. In some experiments activated cells were pretreated with 2 ng/ml IL-12 for 18 h before additional stimulation as described above. Cell-free supernatants were collected, and IFN-γ levels were determined by ELISA (PharMingen, San Diego, CA).

**Proliferation assay**

Splenocytes were activated as described above, washed, and plated in a microtiter plate at 10^5/ml. Cells were incubated in the presence or the absence of IL-12 and the concentrations of IL-18 indicated. Cells were pulsed with 1 μCi of [³H]thymidine/well for the last 18 h of a 48-h incubation.

**Gel shift analysis**

CD4^+^ cells were purified from 48-h anti-CD3-activated splenocytes by magnetically removing B220^+^ (PharMingen), CDS^+^ (2.43), Fcy receptor^+^ (2.4G2), and MHC class II^+^ cells using a mixture of mAbs and goat anti-CD3 magnetic beads (PerSeptive Biosystems, Framingham, MA). Cells were then treated for 18 h with 2 ng/ml IL-12, washed, and plated at 5 × 10^6/ml in the presence or the absence of 50 ng/ml IL-18 for 1 h. Whole cell extracts were made by lysing cells in 5 mM HEPES, 100 mM KCl, 0.125 M EDTA, 0.025 EGTA, 0.25 mM DTT, 0.25 mM MgCl₂, 5% glycerol, 0.25% Nonidet P-40, and protease inhibitors. Extract protein concentrations were determined, and equal amounts of protein were used for each binding reaction. Gel shifts used oligonucleotides specific for AP-1 (11) and NF-κB (Promega, Madison, WI).

**Northern blot analysis**

Splenocytes were activated as described above, and Th1 or Th2 cultures were generated as previously described (29). Cells were washed, plated at 5 × 10^6/ml, and incubated in the presence or the absence of IL-12 for 4 h. RNA was isolated using Trizol (Life Technologies, Gaithersburg, MD) and run on a denaturing formaldehyde agarose gel. Northern blots were probed with cDNAs labeled with random decamer reagents (Ambion, Austin, TX). Probes for IRAK and TRAF6 were provided by M. Harrington (Department of Biochemistry, Indiana University School of Medicine), and IL-18R was supplied by J. Sims (Immunex, Seattle, WA). The MyD88 coding sequence probe was generated by PCR using 5'-TGAATTCATGTCTCAGC GAGAGCCCTCGGGATCCGGTCC-3' and 5'-CTCGCTTGGAGGTCAGC-3'. Underlined segments denote restriction enzyme site added to coding sequence. The AcPL probe was generated by PCR using specific primers (5'-ATGCTCTGTTT 5'-TTCCTCGAGTCAGTCAGGCGGCTTTTGAGTTGGC-3'). Synthesis kit (Roche, Indianapolis, IN). All PCR-generated probes were subcloned and sequenced to verify identity.

**Statistics**

Statistical analysis was performed using a two-tailed Student’s *t* test.

**Results**

**Activation of Janus kinases by IL-12 in the absence of Stat4**

To determine the role of Stat4 in IFN-γ induction, we first assessed mRNA expression of IL-12R chains. Wild-type and Stat4-deficient spleen cells were activated for 48 h with 2 μg/ml plate-bound anti-CD3, washed, and incubated for 4 h in the absence or the presence of 2 ng/ml of IL-12. Total RNA was isolated from cells and subjected to Northern blot analysis. Fig. 1 demonstrates that IL-12Rβ1 and IL-12Rβ2 expression is decreased in the absence of Stat4. Since IFN-γ is known to induce and IL-4 is known to decrease, IL-12Rβ2 expression (30), it seemed possible that the reduced level of IL-12Rβ2 expression on Stat4-deficient cells was due to a lack of IFN-γ or an increase in IL-4 produced in the Stat4-deficient cultures. To test this, we then supplemented medium during the activation period with 500 U/ml IFN-γ and 10 μg/ml anti-IL-4. Expression of either IL-12R chain was not affected by supplementation of anti-CD3-stimulated cultures with IFN-γ and neutralizing IL-4 Ab (data not shown). This suggests that the defect in IL-12R expression in the Stat4^−/−^ cells is not due to a lack of IL-12R expression.
to a lack of IFN-γ and agrees with a previous report that anti-CD3 induced IFN-γ is not decreased in Stat4-deficient cultures (9). Fig. 1 also demonstrates that IL-12Rβ1 and IL-12Rβ2 are induced by IL-12 in a short term stimulation in wild-type, but not in Stat4-deficient, cultures. The induction of IL-12R expression is modest (1.3-fold for IL-12Rβ1 and 1.5-fold for IL-12Rβ2), but is abolished in the absence of Stat4 when expression is normalized to TCRα expression (Fig. 1B). These results suggest that Stat4 may also function directly at the IL-12R promoters and demonstrate that Stat4 is required for the normal expression of IL-12R chains following T cell activation.

To further explore IL-12 signaling in the absence of Stat4 and determine whether decreased receptor expression affected activation of other IL-12 signaling molecules, we analyzed activation of Janus kinases by IL-12 in wild-type and Stat4−/− cells. Spleen cells were activated for 48 h with 2 μg/ml plate-bound anti-CD3. Cells were then washed and incubated in the absence or the presence of 2 ng/ml IL-12 for 20 min. Jak2 and Tyk2 were immunoprecipitated and immunoblotted with an anti-phosphotyrosine Ab. Fig. 2 shows that IL-12 is equally capable of stimulating phosphorylation of Jak2 and Tyk2 in wild-type and Stat4-deficient cells.

**Requirement for Stat4 in IFN-γ induction by other cytokines**

Several cytokines, including IL-1 and TNF-α, alone and in combination with IL-12, function to induce IFN-γ (27, 28). The ability of IL-1 and TNF-α to induce IFN-γ production and to synergize with IL-12 is diminished in the absence of Stat4 (data not shown). However, the amounts of IFN-γ induced by these stimuli, even in control activated T cell populations, are small. The ability of IL-18 to induce IFN-γ expression and synergize with IL-12 is more dramatic. To determine whether IL-18 induced IFN-γ expression was similarly dependent on Stat4, we tested the function of IL-18 on wild-type and Stat4-deficient cells. Total splenocytes from wild-type and Stat4-deficient mice were activated with 2 μg/ml plate-bound anti-CD3 for 48 h. Activated cells were then washed and plated for an additional 48 h in the absence or the presence of increasing doses of IL-18. Fig. 3A demonstrates that wild-type cells produce an IL-18 dose-dependent increase in IFN-γ production following IL-18 stimulation. IL-18 function of anti-CD3-activated splenocytes. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. Cells were washed, replated, and incubated for an additional 48 h in the absence or the presence of increasing doses of IL-18 as indicated (A) or in the absence or the presence of 1 ng/ml IL-12 and/or 50 ng/ml IL-18 as indicated (B). IFN-γ levels were determined by ELISA and are expressed as the average ± SD. Asterisks represent significant differences between wild-type and Stat4-deficient cultures (p < 0.05). C, Proliferative responses of wild-type and Stat4-deficient cells. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. Cells were washed, replated, and incubated in the absence or the presence of various doses of IL-12 and IL-18. Data shown are from stimulation with 1 ng/ml IL-12 and/or 50 ng/ml IL-18, as indicated. Cells were pulsed with [3H]thymidine for the last 18 h of a 48-h assay. Asterisks indicate a significant proliferative response over the unstimulated condition (p < 0.05).

**FIGURE 3.** IL-18 function of anti-CD3-activated splenocytes. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. Cells were washed, replated, and incubated for an additional 48 h in the absence or the presence of increasing doses of IL-18 as indicated (A) or in the absence or the presence of 1 ng/ml IL-12 and/or 50 ng/ml IL-18 as indicated (B). IFN-γ levels were determined by ELISA and are expressed as the average ± SD. A, Asterisks indicate significant differences between wild-type and Stat4-deficient cultures (p < 0.05). B, Asterisks represent a significant induction of IFN-γ from the unstimulated condition (p < 0.03). C, Proliferative responses of wild-type and Stat4-deficient cells. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. Cells were washed, replated, and incubated in the absence or the presence of various doses of IL-12 and IL-18. Data shown are from stimulation with 1 ng/ml IL-12 and/or 50 ng/ml IL-18, as indicated. Cells were pulsed with [3H]thymidine for the last 18 h of a 48-h assay. Asterisks indicate a significant proliferative response over the unstimulated condition (p < 0.05).

**FIGURE 2.** Phosphotyrosine analysis of IL-12-activated Janus kinases. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. Cells were washed, replated, and incubated for an additional 20 min in the absence or the presence of 2 ng/ml IL-12. Jak2 and Tyk2 were immunoprecipitated (IP) from total cell extracts and immunoblotted (IB) with anti-phosphotyrosine. Blots were stripped and reprobed with the precipitating Abs to demonstrate equal loading. The arrow in the top panel shows the phospho-Jak2 band to distinguish it from a phosphorylated protein that was nonspecifically precipitated from Stat4-deficient cell extracts.

In contrast, Stat4-deficient cells produced little IFN-γ in the absence or the presence of IL-18. To demonstrate that the synergy with IL-12 was also affected by Stat4 deficiency, cells activated as described above were washed and plated for an additional 48 h in the absence or the presence of 1 ng/ml IL-12, 50 ng/ml IL-18, or both cytokines. IL-12 stimulated a 10- to 20-fold increase in IFN-γ production over that seen in unstimulated wild-type cultures (Fig. 3B). Strikingly, the combination of both cytokines led to a several-hundred-fold increase in secreted IFN-γ levels. There was no significant increase in IFN-γ production following IL-18 stimulation of Stat4-deficient activated T cells. Furthermore, the induction of IFN-γ secretion by a combination of both cytokines was greatly diminished in Stat4-deficient cultures (Fig. 3B). The increase in IFN-γ production in IL-12- and IL-18-treated Stat4-deficient cells may be attributed to synergy between Stat4-independent IL-12 signals, such as the activation of p38 mitogen-activated protein kinase.
The lack of IL-18 responsiveness was observed in Stat4/Stat6 double-deficient cells and Stat4-deficient purified B cells in addition to activated T cells from both the BALB/c and C57BL/6 genetic backgrounds (Fig. 3 and data not shown). Together, these data support a requirement for Stat4 in IL-18 responses.

Although the most dramatic functions of IL-18 involve IFN-γ induction, IL-18 has also been shown to induce a proliferative response in activated T cells (23, 26). To test whether the lack of IL-18 responsiveness described in the IFN-γ induction assay above was specific for the IFN-γ gene or was a more generalized phenomenon, we examined the proliferative responses to IL-18 or the combination of IL-12 and IL-18 of wild-type and Stat4-deficient activated T cells. Wild-type T cells displayed a significant increase in proliferation following stimulation with IL-18 (Fig. 4C). IL-12 also stimulated an increase in proliferation that was enhanced by the addition of IL-18. Strikingly, Stat4-deficient cultures showed no significant increase in proliferation in response to IL-18 alone or in combination with IL-12 (Fig. 4C). Thus, the inability of Stat4-deficient lymphocytes to respond to IL-18 is not restricted to IFN-γ gene expression.

**IL-18 signaling in the absence of Stat4**

It has already been shown that IL-18 does not activate Stat4 itself (19). To investigate the mechanism of IL-18 unresponsiveness in Stat4-deficient activated T cells, we examined the expression of genes involved in IL-18 signaling. We performed Northern blot analysis using RNA from wild-type and Stat4-deficient T cells activated as described above and incubated for 4 h in the absence or the presence of IL-12. Expressions of IRAK and TRAF6 were equivalent in both wild-type and Stat4-deficient cells, with no apparent induction by IL-12 (Fig. 4A). The IL-18R accessory protein, AcPL, was expressed at equivalent or slightly higher levels in Stat4-deficient cells compared with wild-type cells. There was an induction of MyD88 expression (2-fold) following IL-12 stimulation of wild-type cells, consistent with the identification of a Stat binding site in the MyD88 promoter (32). Importantly, the IL-18R (previously termed IL-1R-related protein) showed Stat4-dependent induction by IL-12 and dramatically lower levels of expression in Stat4-deficient cells, suggesting that it may be a crucial component of Stat4-dependent IL-18 responsiveness.

To further demonstrate the decreased IL-18R expression in Stat4-deficient T cells, we differentiated Stat4+/−/ and Stat4-deficient purified CD4+ T cells to the Th1 phenotype. IL-18R expression is easily detected in Stat4-expressing Th1 cells and is further induced by treatment of cells with IL-12 and IL-18 (Fig. 4B). In contrast, Stat4-deficient T cells had barely detectable expression of IL-18R that was not affected by treatment of the cells with IL-12 and IL-18 (Fig. 4B). As previously described (23, 24), IL-18R was not expressed in Th2 cells (Fig. 4B).

To further demonstrate decreased IL-18 signaling in the absence of Stat4, CD4+ cells from wild-type and Stat4-deficient mice were activated for 48 h with anti-CD3 as described above. Activated T cells were then incubated with IL-12 for 18 h to induce IL-18R expression, washed, and cultured in the presence or the absence of IL-18 for 1 h. Total cellular extracts were then used in an EMSA with probes for the IL-18-activated transcription factor NF-κB. In wild-type cells there was a 3.5-fold increase in the binding activity of NF-κB following stimulation with IL-18 (Fig. 5). In contrast, Stat4-deficient cells had a lower basal level of NF-κB-binding activity. Importantly, IL-18 did not induce NF-κB-binding activity in Stat4-deficient T cells. To confirm equivalent expression of transcription factors present in the NF-κB complex, we performed Western blot analysis for p65 and c-Rel in the extracts described above. There was no appreciable difference in the expression of p65 or c-Rel between wild-type and Stat4-deficient T cells (Fig. 5 and data not shown). This further demonstrates that there is an IL-18 signaling defect that occurs in the absence of Stat4.

To confirm that IL-18 biological responses were still affected following pretreatment with IL-12 as in Fig. 5, we tested these cells for IFN-γ secretion as shown in Fig. 3A. Fig. 6A demonstrates that IL-12-pretreated Stat4-deficient activated T cells produce minimal IFN-γ in response to increasing doses of IL-18, while wild-type cells were sensitized to IL-18 by IL-12 pretreatment and secreted levels of IFN-γ even higher than those shown in Fig. 3A. This sensitivity was also displayed when IL-12-pretreated wild-type cells were stimulated with IL-12 and IL-18 and cells secreted

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**FIGURE 4.** Expression of IL-18 signaling components in the absence of Stat4. A. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. Cells were washed, replated, and incubated for an additional 4 h in the absence or the presence of 1 ng/ml IL-12 as indicated. RNA was isolated and used for Northern blot analysis as described in Materials and Methods. B. RNA was isolated from Stat4+/− Th1, Stat4-deficient Th1, or wild-type Th2 cells left unstimulated or treated as indicated for 24 h.

**FIGURE 5.** Induction of NF-κB-binding activity by IL-18 in the absence of Stat4. Splenocytes from wild-type and Stat4-deficient mice were activated with anti-CD3 for 48 h. CD4+ cells were enriched as described in Materials and Methods. Cells were then treated with IL-12 for 18 h, washed, and cultured in the absence or the presence of 50 ng/ml IL-18 for 1 h. Total cellular extracts and EMSA were performed as described in Materials and Methods. Bottom, Immunoblot analysis of p65 was performed on extracts from the top.
NF-κB deficient cells was surprising, and Stat4 may regulate their expression (34). Our results suggest that IL-12R expression requires both IFN-γ and IL-12 signaling to achieve normal levels, since IFN-γ alone did not recover IL-12R expression in the absence of Stat4. Indeed, an IL-12-dependent, IFN-γ-independent, up-regulation of the IL-12Rβ2 chain has been described (35). In this report we further show that IL-12-induced IL-12Rβ1 and IL-12Rβ2 expression is Stat4 dependent. Importantly, Stat4-dependent IL-12R expression does not appear to be strictly required for IL-12 signaling, since IL-12 induces normal levels of Jak2 and Tyk2 phosphorylation in the absence of Stat4 (Fig. 2). Nevertheless, Stat4-induced IL-12R expression may be required to increase IL-12 responsiveness in some biological settings.

In many ways IL-18R expression parallels IL-12Rβ2 expression. Both receptor chains appear as T cells are activated to differentiate. IL-18R and IL-12Rβ2 are expressed on Th1, but not on Th2, cells (23, 24, 30). The obvious functional consequence is that differentiated Th1 cells become more susceptible to IFN-γ-inducing signals. Our report reveals a positive regulatory loop, in that IL-12, via Stat4, can increase expression of its own receptor and the receptor for IL-18. Furthermore, Stat4 is required for IL-18R expression in differentiated Th1 cells. IL-18 is an important regulator of NK and Th1 responses (13, 17). Thus, the defects in Stat4-deficient mice, and potentially in IL-12- and IL-12R-deficient patients (36–38), reflect not only deficiencies in IL-12 signaling, but IL-18 signaling as well.

IL-12-stimulated biological functions require Stat4. However, the role of Stat4 in many of these responses has not been precisely determined. In this report we found that Stat4 regulates several genes that are required for signaling pathways leading to induction of the IFN-γ gene. This is in addition to the direct effects Stat4 has on the IFN-γ gene itself. Thus, in eliciting the effects of IL-12, Stat4 very likely has direct and indirect roles in generating and regulating immune responses. Understanding the role of Stat4 will require further investigation into novel aspects of IL-12 biology and further identification of Stat4 target genes.

Discussion

Stat4 is required for IL-12-induced biological activities, including the induction of IFN-γ production. This observation may be attributed at least in part to Stat4 binding directly to the IFN-γ promoter resulting in increased gene transcription (10, 11). This report further demonstrates that Stat4 is required for IL-12-induced IFN-γ expression. This may occur through several distinct mechanisms. First, the IL-12-induced expression of MyD88 may affect signaling from the IL-18R (Fig. 4). Second, Stat4 regulates IL-12R expression that directly affects cellular responses to IL-18 (Fig. 4). Stat4 may also regulate genes other than those identified here that are involved in IL-18 signaling or the synergy between IL-12 and IL-18. Stat4 regulation of IFN-γ may also include interactions between Stat4- and IL-18-induced transcription factors such as NF-κB or AP-1 at the IFN-γ promoter. Indeed, IL-18-activated NF-κB has been shown to be important in IFN-γ induction (33). Thus, Stat4 may act as a gateway, both directly and indirectly controlling expression of IFN-γ.

The reduced level of expression of both IL-12R chains in Stat4-deficient cells was surprising, and Stat4 may regulate their expression through distinct mechanisms. IL-12Rβ2 expression is up-regulated by anti-CD3 signaling, although the exact signaling requirements are not clear. Jnk2 appears to play an indirect role in anti-CD3-induced IL-12Rβ2 induction. In Jnk2-deficient mice, inefficient up-regulation of the IL-12Rβ2 chain results in decreased Th1 development (34). However, supplementation of Jnk2-deficient cells with IFN-γ recovers Th1 differentiation, suggesting that anti-CD3 induced IFN-γ is required for the induction of IL-12Rβ2 high levels of IFN-γ. No significant increase in IFN-γ secretion was observed in IL-12-pretreated Stat4-deficient cultures following treatment with IL-12 and IL-18.

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Stat4 regulation of cytokine-induced IFN-γ


