A mechanism underlying STAT4-mediated up-regulation of IFN-γ induction in TCR-triggered T cells

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Abstract

IL-12 promotes Th1 development/IFN-γ expression by activating STAT4. However, it is still unclear how STAT4 elicits IFN-γ promoter activation. Here, we investigated the mechanism by which IL-12-activated STAT4 functions for IFN-γ induction in TCR-triggered T cells. TCR stimulation induced high levels of IFN-γ production depending on co-stimulation with IL-12. IL-12 stimulation greatly enhanced the promoter-binding activity of c-Jun/AP-1, a critical transcription factor for IFN-γ gene expression in wild-type T cells, but not in STAT4-deficient (STAT4−/−) T cells. Comparable amounts of c-Jun were induced by TCR stimulation in both wild-type and STAT4−/− T cells irrespective of IL-12 co-stimulation. However, c-Jun bound to STAT4 in IL-12-co-stimulated wild-type T cells. c-Jun forming a complex with STAT4 efficiently interacted with the AP-1-related sequence of the IFN-γ promoter. Such an enhanced c-Jun binding did not occur in STAT4−/− T cells. These results show that STAT4 contributes to enhancing IFN-γ expression by up-regulating the binding of TCR signal-induced AP-1 to the relevant promoter sequence.

Introduction

Upon antigen sensitization, naïve CD4 T cells differentiate into two groups of polarized effector cells, Th1 and Th2. These two subsets of cells are defined by the profile of cytokines they produce (1) and this profile correlates with functional differences. Namely, Th1 cells are characterized by the acquisition of the capacity to produce IFN-γ capable of mediating anti-inflammatory/infectious immune responses, whereas Th2 cells secrete mainly IL-4 and function to induce humoral immune responses (2,3).

Considerable effort has been made to investigate the factors that determine differentiation of Th cells (4,5). Among these, the composition of the cytokines in the environment at the time of initial exposure to antigen is of crucial importance to the outcome of Th differentiation (6). Ligation of the IL-4 receptor by IL-4 produced in the milieu drives a naïve Th0 cell down a Th2 differentiation through STAT6 activation, while IL-12 promotes IFN-γ production and Th1 development via signaling pathways that lead to STAT4 activation (7–9). Recent studies have shown that T-bet is critical for IFN-γ expression and Th1 lineage commitment (10). Nevertheless, it is still possible that the IL-12/STAT4 signaling pathway is required to sustain high levels of IFN-γ expression and ensure Th1 development. Indeed, STAT4 functions for IFN-γ expression in TCR/IL-12 (8,9) and IL-12/IL-18 stimulation (11,12). However, it remains unclear how STAT4 functions for IFN-γ gene expression, particularly in the former. This confusion could result from the fact that no apparent STAT4-binding sequence exists on the IFN-γ promoter. In the present study, we investigated how STAT4 acts as a transcription factor to enhance IFN-γ expression in TCR-triggered T cells. The results show that TCR-triggered T cells produce large amounts of IFN-γ depending on the presence of IL-12. AP-1 was previously shown to be induced by TCR stimulation and to function as a potent transcription factor for IFN-γ gene expression (13). Our results demonstrated that the enhanced binding of AP-1 to the IFN-γ promoter sequence was achieved by its interaction with IL-12-activated STAT4. STAT4−/− T cells generated comparable amounts of AP-1 to those in wild-type T cells, but failed to up-regulate the AP-1 binding following IL-12 co-stimulation. These results provide a
Mechanism for STAT4-mediated IFN-γ expression

**Methods**

**Mice**

BALB/c mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). STAT4-deficient (STAT4−/−) BALB/c mice (BALB/c-Stat4 tm1Gru) (8) were obtained from Jackson Laboratories (Bar Harbor, ME) and bred in our laboratory.

**Reagents**

Mouse rIL-12 was provided by Genetics Institute (Cambridge, MA). Anti-CD3 (145-2C11) (14), anti-CD28 (Pv-1) (15), anti-IL-12 (16) and anti-I-A^b^ (34-5-3S) (17) mAb were purified from culture supernatants or ascitic fluids of respective hybridomas. Anti-c-Jun (H-79/sc-1694 and N/sc-45x), anti-c-Fos (4-10G/sc-413) and anti-STAT4 (C-20/sc-486 and L-18/sc-485x) antibodies as well as normal rabbit Ig were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phosphotyrosine mAb (4G10) and anti-phosphoserine-c-Jun (Ser 63) antibodies were from Upstate Biotechnology (Lake placid, NY) and New England Biolabs (Beverly, MA) respectively.

**Preparation of T cell populations**

Mouse lymph node cells were depleted of B cells and Ia^+^ antigen-presenting cells by immunomagnetic negative selection as previously described (18). The CD4 and CD8 T cell subsets were positively selected from purified T cell populations using superparamagnetic microbeads conjugated to anti-CD4 or anti-CD8 mAb (Miltenyi Biotec, Sunnyvale, CA).

**Stimulation of T cells with anti-CD3 plus anti-CD28 mAb**

Anti-CD3 (0.5 or 5 μg/ml) and anti-CD28 (2 μg/ml) were co-immobilized to 24-well culture plates (25820; Corning Glass Works, Corning, NY) in a volume of 0.5 ml. After 3 h, solutions were discarded and plates were washed with PBS twice. Purified T cells were cultured in the presence or absence of 1000 pg/ml rIL-12 in 2 ml of RPMI 1640 medium supplemented with 10% FCS and 2-mercaptoethanol at 1.5 × 10^6^ cells/well for various times in a CO_2_ incubator.

**Measurement of IFN-γ concentrations**

IFN-γ concentrations were measured by mouse IFN-γ ELISA (12).

**Preparation of total cell lysates/nucleus extracts and immunoprecipitation/immunoblotting**

Total cell lysates were prepared by lysing cells in cell lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, pH 8.0, 0.2 mM sodium orthovanadate, 0.2 mM PMSF and 0.5% NP-40). Nuclear extracts were prepared as follows. After washing with PBS, cells were resuspended in cell lysis buffer (20 mM HEPES–NaOH, pH 7.9, 20 mM NaF, 1 mM Na_3_VO_4_, 1 mM EDTA and 0.1 mM EGTA) supplemented with 0.2% NP-40, 1 mM dithiothreitol, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 0.1 mM Pefabloc (Roche, Mannheim, Germany). The nuclei were pelleted and then extracted with vigorous agitation at 4°C in the above buffer without NP-40, but containing 0.42 M NaCl, 20% glycerol and protease inhibitors as described above. Immunoprecipitation and immunoblotting were performed as previously described (12).

**Electrophoretic mobility shift assay (EMSA)**

EMSA was performed as previously described (12). The AP-1 consensus oligonucleotide probe (5′-CGCTTGATGACTCA-GCCGGAA-3′) was purchased from Santa Cruz Biotechnology. The oligonucleotide probe corresponding to the AP-1-binding sequence present in the mouse IFN-γ promoter (5′-GCGGGGTCTGTCTGTCAGA-3′) was prepared in our laboratory.

**Oligonucleotide DNA precipitation**

The procedure was essentially the same as that previously described (12). Nuclear extracts were incubated with agarose beads coupled to an AP-1 consensus oligonucleotide (TGACTCA) (Santa Cruz Biotechnology). The binding reaction was performed for 45 min at 4°C in a binding buffer. The agarose beads were washed 5 times with binding buffer. The bound proteins were released with SDS loading buffer, separated by 10% SDS–PAGE, transferred to PVDF membrane and visualized with the relevant antibodies.

**RT-PCR**

The following oligonucleotides were used: IL-12Rβ1 sense primer 5′-CAAGCAGAAGAACCACACA-3′, IL-12Rβ1 anti-sense primer 5′-CAGAGACGCGAATATGATG-3′, IL-12Rβ2 sense primer 5′-AAATTCATTGACGCTGCTCTCA-3′, IL-12Rβ2 anti-sense primer 5′-ATCAGGGGCTGACTCTTCTCA-3′, β-actin sense primer 5′-AGAAAGATCTGATGCGTCTGACG-3′ and β-actin anti-sense primer 5′-CTTCTCGATCCTTCTGCA-GCAATGCC-3′. Cycle parameters were: annealing 1 min at 60°C (IL-12Rβ1 and β2) or 55°C (β-actin), elongation 1 min at 72°C and denaturation 0.5 min at 94°C. Resulting PCR products were separated in 1% agarose gel and visualized by SYBR Green staining. Sequences of the IL-12Rβ1, IL-12Rβ2 and β-actin (for standardization) were amplified out of the genomic DNA of wild-type and STAT4−/− mice.
each cDNA batch with 28, 28 and 20 amplification cycles, respectively.

Results

Requirement of IL-12 and STAT4 for TCR-stimulated IFN-γ production

Wild-type and STAT4−/− T cells were stimulated with a low (0.5 μg/ml) or high (5.0 μg/ml) dose of immobilized anti-CD3 plus anti-CD28 mAb in the presence or absence of rIL-12. IFN-γ production by wild-type T cells stimulated with a low dose of anti-CD3 was markedly enhanced by co-stimulation with IL-12, while such an IL-12-mediated up-regulation did not occur in STAT4±/± T cells (Fig. 1A). Unlike stimulation with a low dose of anti-CD3, wild-type T cells stimulated with a high dose of anti-CD3 produced relatively large amounts of IFN-γ even in the absence of IL-12 (Fig. 1B, left). Under this condition, IFN-γ production by STAT4−/− T cells was weak irrespective of IL-12 co-stimulation (Fig. 1B, right). By positively selecting CD4+ and CD8+ T cells, we examined their responsiveness to TCR/IL-12 stimulation (Fig. 2). The patterns of IFN-γ production by either the CD4+ or CD8+ T cell subset were similar to those observed for unfraccionated T cells in terms of IL-12-mediated enhancement only in wild-type T cells.

IL-12R, particularly an IL-12Rβ2 subunit, is induced following stimulation of resting T cells with anti-CD3 plus anti-CD28 (18–20). To exclude the possibility that the failure of IL-12 to up-regulate IFN-γ production in STAT4−/− T cells is primarily due to their inability to induce a functional IL-12R complex, the induction of IL-12Rβ1 and β2 was compared between wild-type and STAT4−/− T cells stimulated with anti-CD3/anti-CD28 in the presence or absence of IL-12. As shown in Fig. 3, there was no substantial difference between wild-type and STAT4−/− T cells in the levels of IL-12Rβ1 and β2 mRNA.

IL-12-independent STAT4 phosphorylation in T cells stimulated with a high dose of anti-CD3/anti-CD28

Figure 4(A) shows that stimulation of wild-type T cells with a high dose of anti-CD3 alone or together with anti-CD28 induced moderate levels of IFN-γ production even without IL-12 co-stimulation. Because this was not the case for STAT4−/− T cells, it is possible that this differential IL-12-independent IFN-γ production is also generated depending on STAT4 signaling. To determine this, we examined STAT4 phosphorylation in wild-type T cells stimulated with anti-CD3/anti-CD28 without IL-12 co-stimulation. Unlike IL-12-stimulated STAT4 activation (19,20), STAT4 phosphorylation was not observed shortly after stimulation with anti-CD3 plus anti-CD28 (data not shown). However, STAT4 was phosphorylated 12 h after the stimulation and the levels were slightly increased up to 36 h after the stimulation (Fig. 4B). Figure 4(C) shows that STAT4 phosphorylation is inducible irrespective of CD28 co-stimulation or is only slightly detected depending on CD28 co-stimulation in T cells stimulated with a high or low dose of anti-CD3 respectively. Because significant amounts of IFN-γ are produced by STAT4−/− T cells (Fig. 4A), STAT4 is not necessarily an absolute requirement for IFN-γ production. However, enhanced levels of IFN-γ expression by TCR-triggered T cells associates with the involvement of STAT4 function.

STAT4 is required to up-regulate the binding of AP-1 to the IFN-γ promoter sequence

AP-1 is induced by TCR stimulation and by forming complexes with other transcription factors; this transcription factor interacts with the NF-AT/AP-1 site (21) and CD28-responsive element (22) of the IL-2 gene promoter. An earlier study (13) showed that the AP-1-binding site exists on the IFN-γ gene promoter and that the binding activity of TCR signal-induced AP-1 is enhanced by co-stimulation with IL-12. We examined AP-1-binding activity generated in TCR/IL-12-stimulated

Fig. 2. The up-regulating effect of IL-12 on IFN-γ production is expressed on both CD4 and CD8 T cell subsets. CD4+ and CD8+ T cells from wild-type and STAT4−/− mice were positively selected from purified T cell populations. Cells were stimulated with anti-CD3 (A: 0.5 μg/ml or B: 5.0 μg/ml) together with anti-CD28 in the presence or absence of IL-12.

Fig. 3. STAT4−/− T cells can express IL-12Rβ1 and β2 mRNA following anti-CD3/anti-CD28 stimulation. Purified wild-type and STAT4−/− T cells were stimulated for 12 h with 0.5 μg/ml anti-CD3 plus anti-CD28 in the presence or absence of IL-12. Total RNA was isolated and examined in RT-PCR for the expression of IL-12Rβ1 and β2 mRNA.
While AP-1-binding activity was detectable in T cells stimulated with a low dose (0.5 μg/ml) of anti-CD3 plus anti-CD28, the binding of AP-1 was markedly enhanced by IL-12 co-stimulation. Using wild-type and STAT4−/− T cells, we examined whether STAT4 is involved in IL-12-mediated up-regulation of AP-1 binding. As shown in Fig. 6, concurrent stimulation with IL-12 again up-regulated AP-1-binding activity in wild-type T cells. However, such an up-regulation was not induced in STAT4−/− T cells. This was the case in EMSA using oligonucleotide probes for either the consensus AP-1-binding sequence (Fig. 6, upper panel) or the AP-1-binding sequence present in the mouse IFN-γ promoter (Fig. 6, lower panel). Thus, STAT4 is required to up-regulate the binding of TCR signal-induced AP-1 to the relevant promoter sequence.

**STAT4 deficiency does not affect the induction of c-Jun/AP-1 by TCR stimulation**

To determine whether STAT4 deficiency influences the induction of AP-1, we examined the amounts of c-Jun as a major component of AP-1. Total cell lysates and nuclear extracts were prepared from wild-type and STAT4−/− T cells stimulated with anti-CD3/anti-CD28 in the presence or absence of IL-12. Total cell lysates were immunoblotted with anti-c-Jun antibody and then rebotted with anti-STAT4 antibody (lower panels) and then rebotted with anti-STAT4 antibody (lower panels) (B and C).

**Fig. 4.** STAT4 is phosphorylated during stimulation with high doses of anti-CD3 together with anti-CD28 in the absence of IL-12. (A and C) Purified T cells from wild-type and STAT4−/− mice were stimulated with anti-CD3 (0.5 or 5 μg/ml) alone or together with anti-CD28 in the presence of 10 μg/ml anti-IL-12 mAb for 24 h. (B) Purified wild-type T cells were stimulated with anti-CD3 (5 μg/ml) plus anti-CD28 in the presence of 10 μg/ml anti-IL-12 mAb for the indicated times. Culture supernatants were assayed for IFN-γ concentrations by ELISA (A). Total cell lysates were immunoprecipitated with anti-STAT4 antibody, immunoblotted with anti-phosphotyrosine mAb (upper panels) and then rebotted with anti-STAT4 antibody (lower panels) (B and C).

**Fig. 5.** IL-12 stimulation is required for enhanced induction of AP-1-binding activity in TCR-triggered T cells. Purified T cells were stimulated with anti-CD3 (0.5 μg/ml) plus anti-CD28 (2 μg/ml) in the presence or absence of 1000 pg/ml IL-12 for the indicated times. Nuclear extracts were examined in EMSA for binding to an oligonucleotide probe corresponding to a consensus AP-1-binding sequence.

**Fig. 6.** STAT4−/− T cells fail to up-regulate AP-1-binding activity induced by anti-CD3/anti-CD28 stimulation in the presence of IL-12. Purified wild-type and STAT4−/− T cells were stimulated with anti-CD3 (0.5 μg/ml) plus anti-CD28 in the presence or absence of IL-12. Nuclear extracts were examined in EMSA for their binding to an oligonucleotide probe corresponding to the consensus AP-1-binding sequence (upper panel) or the AP-1-binding sequence present in the mouse IFN-γ promoter (lower panel).
also show that no essential difference in cellular and nuclear amounts of another AP-1 component, c-Fos, was seen between wild-type and STAT4±/± T cells. Thus, while STAT4 ±/± T cells exhibit a markedly reduced level of AP-1-binding activity (Fig. 6), these T cells can induce comparable amounts of c-Jun/AP-1 to those in wild-type T cells.

**STAT4 interacts with c-Jun to form a complex**

Our recent study (12) demonstrated that IL-12 and IL-18 activate STAT4 and c-Jun/AP-1 respectively, and that the binding activity of c-Jun/AP-1 is up-regulated through its interaction with STAT4, providing a mechanism of IL-12/IL-18 synergy for IFN-γ promoter activation. Therefore, we examined whether such a complex is also formed following TCR/IL-12 stimulation (Fig. 7C). Nuclear extracts from TCR or TCR/IL-12-stimulated wild-type and STAT4±/± T cells were allowed to interact with agarose beads coupled to the AP-1-binding oligonucleotide sequence. The bound proteins were then analyzed for c-Jun and STAT4 by immunoblotting using relevant antibodies (Fig. 8). A much larger amount of c-Jun bound to the AP-1 sequence in the nuclear extracts from IL-12-co-stimulated than IL-12-non-co-stimulated wild-type T cells. Serine phosphorylation of c-Jun was found in the former group, which is consistent with the observation that c-Jun interacts with STAT4 (Fig. 7C). In the deficiency of STAT4, the binding of c-Jun to the AP-1 sequence was not enhanced in the TCR/IL-12-co-stimulated group. Taken together, these results indicate that STAT4 interacts with c-Jun and thereby facilitates c-Jun binding to the AP-1 sequence.

**Discussion**

While not all IFN-γ production appears to depend on STAT4 (23,24), IL-12 stimulation and STAT4 activation contribute to the development of IFN-γ-producing Tγ1 cells. However, the specific transcriptional target of STAT4 involved in IFN-γ gene expression after TCR triggering remains unclear. The present study showed that T cells triggered with low magnitudes of TCR stimuli produce large amounts of IFN-γ depending on IL-12 stimulation and STAT4 involvement. While TCR stimulation leads to the generation of a potent transcription factor, AP-1, for IFN-γ promoter activation, IL-12 and STAT4 were required to induce high levels of AP-1 binding to the relevant IFN-γ promoter sequence. The results further demonstrated that
STAT4 and c-Jun/AP-1 form a complex, and that c-Jun interacting with STAT4 binds much more efficiently to the AP-1 sequence than c-Jun alone. These results provide a mechanistic explanation of how STAT4 activated during TCR/IL-12 concurrent stimulation promotes IFN-γ gene expression.

Recent studies have demonstrated that T-bet is a potent transactivator of the IFN-γ gene and functions as the master regulator of Tₜ,1 lineage commitment (10). Because IFN-γ is a potent inducer of T bet expression in TCR-triggered T cells (25,26), T-bet and IFN-γ expression forms a positive feedback loop. As TCR-triggered T cells can express IFN-γ in the absence of STAT4 signaling, STAT4 is not necessarily essential for T-bet induction (26,27). While IFN-γ and T-bet expression occur independently of IL-12/STAT4 signaling, IFN-γ-stimulated T-bet expression was recently shown to induce IL-12Rβ2 expression that is a requirement of Tₜ,1 development (26). Here, we observed that detectable, albeit small, amounts of IFN-γ were produced by both wild-type and STAT4⁻/⁻ T cells stimulated with anti-CD3 (0.5 µg/ml) plus anti-CD28 without IL-12 stimulation. Consistent with this, comparable levels of IL-12Rβ1 and β2 mRNA expression were induced in both types of T cells after anti-CD3/anti-CD28 stimulation. However, IFN-γ production was greatly up-regulated only in wild-type T cells when they were co-stimulated with IL-12 and such an up-regulation was not observed in STAT4⁻/⁻ T cells. Thus, STAT4 is required to enhance/prolong IFN-γ expression in developing T cells. The following scenario (28) may be feasible. TCR triggering induces IFN-γ production required for T-bet expression. T-bet induction does not necessarily require IL-12/STAT4 signaling. T-bet functions to induce IL-12R expression. Stimulation of these developing T cells with IL-12 markedly augments Tₜ,1 development and IFN-γ production in a STAT4-dependent way.

TCR signaling is regarded as the typical physiologic stimulus for IFN-γ induction. However, IL-12 and IL-18 induce IFN-γ production in a TCR-independent manner (29,30), and exhibit a striking synergy when they act in a combination (11,12,31). Two different mechanisms of synergy have been reported. First, IL-12 up-regulates IL-18R expression (11,32). Second, IL-12 and IL-18 activate different transcription factors: the former activates STAT4, and the latter NF-kB (31) and AP-1 (13). Our recent study (12) showed that STAT4 contributes to IFN-γ promoter activation by interacting with c-Jun/AP-1 and up-regulating its binding to the AP-1-relevant sequence. AP-1 is a more potent transcription factor for IFN-γ promoter activation than STAT4 (13). However, the induction of high AP-1 binding required the interaction of AP-1 with STAT4 (12). Additionally, our study (12) gave the following observations. (i) There is no apparent STAT4-binding sequence on the mouse IFN-γ promoter. In fact, STAT4 did not bind to the promoter fragment (positions −436 to +113) for which the IL-12/IL-18 transcriptional synergy was observed (12). This represented a new mechanism of synergistic promoter activation in which a transcription factor acts without directly binding to the promoter sequence. (ii) Unlike AP-1 binding, the binding activity of NF-kB was not up-regulated in the presence of STAT4 (12). Thus, IL-12 signals functioned to selectively enhance AP-1 binding.

According to previous studies (12,13), how binding of AP-1 to the relevant IFN-γ promoter sequence is up-regulated is key for enhanced IFN-γ promoter activation. As TCR signaling generates AP-1, the present study aimed to investigate whether the up-regulation of AP-1-binding activity through the STAT4/AP-1 interaction also occurs during TCR/IL-12 co-stimulation. To focus on the role for STAT4, the induction of AP-1-binding activity was compared between wild-type and STAT4⁻/⁻ T cells. High AP-1-binding activity was induced only in wild-type T cells depending on the interaction of c-Jun/AP-1 with STAT4. There was no substantial difference in c-Jun induction between wild-type and STAT4⁻/⁻ T cells as well as between the presence and absence of IL-12 signals. In TCR stimulation alone, low levels of c-Jun/AP-1 binding were observed in EMSA and oligonucleotide DNA precipitation, and the levels were comparable between wild-type and STAT4⁻/⁻ T cells. In TCR/IL-12 co-stimulation, c-Jun/AP-1 induced in wild-type T cells exhibited enhanced binding to the AP-1-binding sequence by interacting with STAT4, whereas c-Jun/AP-1 generated in STAT4⁻/⁻ T cells did not. Thus, these results indicate that STAT4 is critical for up-regulating the binding of AP-1 to the relevant IFN-γ promoter sequence (Fig. 9).

While IFN-γ production is up-regulated by the IL-12/STAT4 signaling pathway, it is also obvious that moderate amounts of IFN-γ are produced without IL-12 stimulation so far as T cells are given high magnitudes of TCR stimuli (5 µg/ml anti-CD3). In this context, our present results showed that stimulation of wild-type T cells with a high dose of anti-CD3 plus anti-CD28 results in detectable, albeit low, levels of STAT4 phosphorylation in the absence of exogenous IL-12. It is unlikely that STAT4 activation was induced by endogenously produced IL-12 because antigen-presenting cells had been depleted of a responding population and, moreover, cultures included sufficient amounts of anti-IL-12 mAb to neutralize endogenous IL-12 that may be produced by residual antigen-presenting cells, if any. Although the mechanism of IL-12-independent STAT4 phosphorylation is unknown, the following postulation may be feasible. A recent study (33) showed that lipid rafts enriched in cytokine receptors and associated signaling molecules provide an efficient place for cytokine signaling. Therefore, condensation of raft-associated cytokine receptors due to potent raft aggregation may lead to the activation of cytokine receptor-associated signaling molecules that usually occurs as a result of ligand binding. Anti-CD3/anti-CD28 co-stimulation induces aggregation of TCR and lipid rafts (34).
Further studies will be required to directly investigate the mechanism of an IL-12-independent STAT4 phosphorylation. Regarding an IL-12-independent mechanism, an earlier study (24) showed that CD4+ T cells require IL-12 and STAT4 activation for IFN-γ induction, whereas CD8+ T cells can produce IFN-γ independently of IL-12. However, IL-12 and STAT4 requirements in IFN-γ induction were compared between CD4+ and CD8+ T cells stimulated with a high dose of anti-CD3 (10 μg/ml) plus anti-CD28 followed by re-stimulation with anti-CD3 (24). In contrast, our results showed that like CD4+ T cells, CD8+ T cells in a primary culture require IL-12 stimulation and STAT4 activation for IFN-γ induction. Our observations also contrast with the differential requirement of T-bet induction for IFN-γ expression by CD4+ versus CD8+ T cells (35).

Although an additional pathway for IL-12/STAT4-independent IFN-γ production exists (23), it is evident that IL-12 stimulation and STAT4 activation is required for IFN-γ gene expression by T cells triggered with low magnitudes of TCR stimuli. Our present results illustrate a role for STAT4 in mounting a high binding activity of AP-1 that is induced by TCR stimulation. These results are consistent with the observation (12) that under synergistic IFN-γ production of IL-12 and IL-18, IL-12-induced STAT4 contributes to IFN-γ expression by up-regulating the binding activity of IL-18-induced AP-1 via the formation of the STAT4-c-Jun/AP-1 complex. The present results could also contribute to a better understanding of STAT4-mediated T-bet,1 differentiation in terms of the acquisition of the capacity to promote IFN-γ-expression in TCR-triggered T cells.

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Abbreviations
EMSA electrophoretic mobility shift assay

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Mechanism for STAT4-mediated IFN-γ expression


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