

Chromatin remodeling by the SWI/SNF-like BAF complex and STAT4 activation synergistically induce IL-12R β 2 expression during human Th1 cell differentiation

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Interleukin-12 (IL-12) is a key cytokine for the development of T helper type 1 (Th1) responses; however, naïve CD4⁺ T cells do not express IL-12R β 2, and are therefore unresponsive to IL-12. We have examined the mechanisms that control Th1-specific expression of the human IL-12R β 2 gene at early time points after T-cell stimulation. We have identified a Th1-specific enhancer element that binds signal transducer and activator of transcription 4 (STAT4) *in vivo* in developing Th1 but not Th2 cells. T-cell receptor (TCR) signaling induced histone hyperacetylation and recruitment of BRG1, the ATPase subunit of the SWI/SNF-like BAF chromatin remodeling complex, to the IL-12R β 2 regulatory regions and was associated with low-level gene transcription at the IL-12R β 2 locus. However, high-level IL-12R β 2 expression required TCR triggering in the presence of IL-12. Our results indicate a synergistic role of TCR-induced chromatin remodeling and cytokine-induced STAT4 activation to direct IL-12R β 2 expression during Th1 cell development.

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Introduction

Protective immunity against intracellular pathogens requires the differentiation of naïve CD4⁺ T cells into T helper type 1 (Th1) cells that secrete interferon- γ (IFN- γ) and activate cell-mediated immune responses. Interleukin-12 (IL-12) is a key cytokine for the development of Th1 responses (Trinchieri, 2003). IL-12 signals by binding to a receptor composed of two subunits, IL-12 receptor β 1 (IL-12R β 1) and IL-12R β 2 (Presky *et al*, 1996). Expression of IL-12R β 2 is rate-limiting for activa-

tion of signal transducer and activator of transcription 4 (STAT4) and IL-12 responsiveness (Rogge *et al*, 1997; Szabo *et al*, 1997). Consistent with the importance of IL-12/STAT4 signaling for the development of Th1 cells *in vitro*, STAT4-deficient mice show increased susceptibility to infection and are resistant to the development of Th1-mediated autoimmune diseases (Watford *et al*, 2004). STAT4 is thought to mediate its biological effects by binding to IL-12 target genes and inducing transcriptional activation (Watford *et al*, 2004) and chromatin remodeling (O'Sullivan *et al*, 2004). Several IL-12 target genes have been identified in recent years (Rogge *et al*, 2000; Watford *et al*, 2004); however, the mechanisms that link IL-12 signaling to gene activation are still poorly characterized.

IL-12R β 2 is not detected on naïve CD4⁺ T cells, but is rapidly induced during Th1 but not Th2 cell differentiation. Cytokine signaling has a prominent role in IL-12R β 2 induction: IL-12 itself strongly upregulates expression of the receptor (Rogge *et al*, 1997). In addition to IL-12, IFN- α/β are potent inducers of IL-12R β 2 in human T cells, possibly through STAT4 activation (Cho *et al*, 1996; Rogge *et al*, 1998; Athie-Morales *et al*, 2004). Furthermore, IFN- γ can efficiently induce IL-12R β 2 expression in mouse T cells, pointing to STAT1 as an important regulator of IL-12R β 2 expression in this system (Szabo *et al*, 1997; Smeltz *et al*, 2002). It is unclear whether IFN- γ /STAT1 signaling is also important for the induction of IL-12R β 2 expression in naïve human CD4⁺ T cells.

The importance of the chromatin structure at cytokine loci during T helper cell differentiation is consistent with the finding that cytokine transcripts are induced more rapidly and at substantially higher levels after stimulation of differentiated effector cells, compared with naïve CD4⁺ T cells (Smale and Fisher, 2002; Ansel *et al*, 2003). Changes in the chromatin structure at cytokine gene loci have been proposed to explain the increased transcriptional competence of effector CD4⁺ T cells. Several studies have demonstrated epigenetic regulation at the IL-4 locus during Th2 differentiation and at the IFN- γ locus during Th1 differentiation (Lee *et al*, 2006). Th2 differentiation is associated with the appearance of specific DNase I hypersensitive sites (HS) at the IL-4 and the closely linked IL-13 genes (Agarwal and Rao, 1998). Th2 lineage commitment also involves hyperacetylation of histones 3 and 4 at the promoter and enhancer of the IL-4 gene (Avni *et al*, 2002; Fields *et al*, 2002). Current understanding of the regulation of the Th2 cytokines IL-4, IL-5, and IL-13 has greatly benefited from the detailed analysis of the interplay of chromatin modifications and transcription factors at the regulatory regions of these genes (Smale and Fisher, 2002; Ansel *et al*, 2003). Chromatin modifications at the IFN- γ locus in Th1 cells (Agarwal and Rao, 1998; Mullen *et al*, 2002; Morinobu *et al*, 2004) indicate that similar mechanisms

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linking cytokine signaling to chromatin remodeling and gene expression may also regulate Th1 differentiation.

The modifications of the chromatin structure that render genes accessible to the transcription machinery require the activity of two major classes of enzyme complexes. Histone-modifying enzymes catalyze covalent modifications of the amino termini of histones, which may alter the interaction of nucleosomes with DNA or serve as docking sites for chromatin-associated proteins. In particular, hyperacetylation of histone 3 and 4 (H3 or H4) as well as trimethylation of lysine residue 4 of histone 3 (H3 K4) is strongly associated with transcriptionally active genes (Ansel *et al*, 2003; Lee *et al*, 2006). Chromatin remodeling complexes use the energy derived from ATP hydrolysis to reposition nucleosomes, creating template structures that are more accessible to DNA binding proteins. Recent results have demonstrated a critical role for the SWI/SNF-like BAF (Brahma-related gene (BRG1)/Brahma (BRM)-associated factor) complex in the CD4/CD8 lineage decision during thymocyte development (Chi *et al*, 2002, 2003). Furthermore, it was shown that stimulation of resting peripheral T cells via the T-cell receptor (TCR) resulted in the rapid and phosphoinositol-dependent recruitment of BRG1, the ATPase subunit of the BAF complex, to chromatin (Zhao *et al*, 1998).

As expression of IL-12Rβ2 is critical for Th1 cell differentiation, we have characterized the regulatory regions of the IL-12Rβ2 gene and analyzed the mechanisms that control Th1-specific expression of this gene. We have cloned the human IL-12Rβ2 gene and analyzed modifications in the chromatin structure at the IL-12Rβ2 locus in cells differentiating along a Th1 or a Th2 pathway. We have identified an HS unique to Th1 lineage-committed cells, which defines an enhancer element that confers IL-12-responsiveness to a reporter gene. We show that STAT4 is bound to this enhancer element *in vivo* in developing Th1 but not Th2 cells.

To identify the molecular bases underlying changes of the chromatin structure during T helper cell differentiation, we have analyzed the recruitment of SWI/SNF-like chromatin remodeling complexes to the IL-12Rβ2 locus. Triggering of the TCR induces recruitment of BRG1 to the enhancer and promoter of the IL-12Rβ2 gene. BRG1 recruitment is associated with histone hyperacetylation and low-level gene transcription at the IL-12Rβ2 locus. TCR triggering in the presence of IL-12 is required for high-level IL-12Rβ2 expression. Our results indicate a synergistic role of TCR-induced chromatin remodeling by BAF complexes and cytokine-induced STAT4 activation in directing IL-12Rβ2 expression during Th1 cell development.

Results

Cytokines induce rapid expression of IL-12Rβ2 transcripts after stimulation of naïve human CD4⁺ T cells

To analyze the signaling pathways that regulate early expression of IL-12Rβ2, we stimulated naïve human CD4⁺ T cells with anti-CD3 and anti-CD28 antibodies in the absence of exogenously added cytokines or in the presence of IL-12, IFN-α, IFN-γ, or IL-4. The most potent cytokine for early induction of IL-12Rβ2 transcripts was IFN-α (Figure 1, upper panel). We observed a five-fold increase in IL-12Rβ2 mRNA levels already 2 h after stimulation and IFN-α remained the most

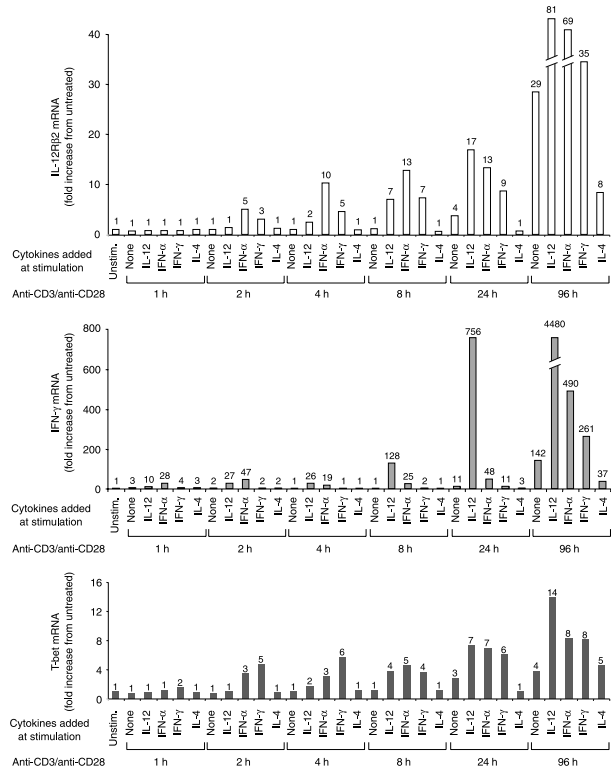


Figure 1 Cytokines rapidly induce IL-12Rβ2, IFN-γ, and T-bet transcripts following stimulation of naïve human CD4⁺ T cells. Naïve CD4⁺ T cells were purified from cord blood and stimulated with anti-CD3/CD28 antibodies in the presence of the indicated cytokines. Cells were harvested at the indicated times and the mRNA levels of IL-12Rβ2, IFN-γ, and T-bet were determined by kinetic real-time PCR. The fold increase of IL-12Rβ2 (upper panel), IFN-γ (middle panel), and T-bet transcripts (lower panel) when comparing stimulated T cells to naïve CD4⁺ T cells is shown.

powerful inducer for the first 8 h. IFN-γ induced IL-12Rβ2 with a similar kinetics but with lower efficiency. Consistent with the finding that naïve T cells do not express IL-12 receptors, IL-12 was found to efficiently induce IL-12Rβ2 only 8 h after stimulation. At later time points, however, IL-12 became the most potent cytokine to induce expression of IL-12Rβ2. Cytokine stimulation in the absence of TCR triggering was inefficient in inducing IL-12Rβ2 (Figure 6C and data not shown).

Similar to IL-12Rβ2, IFN-γ transcripts were very rapidly induced by IFN-α (Figure 1, middle panel). However, at later time points (24 h), IL-12 is a much more potent inducer of IFN-γ transcripts than IFN-α (16-fold) or IFN-γ (70-fold). The kinetics of T-bet mRNA expression in these samples was similar to the one observed for IL-12Rβ2 and IFN-γ (Figure 1, lower panel).

These data suggested a critical role for cytokine receptor signaling in the induction of IL-12Rβ2 during early Th1 cell differentiation. To define how signals mediated by IL-12, IFN-α, and IFN-γ are integrated with TCR signaling to induce IL-12Rβ2 expression, we decided to clone and analyze the regulatory regions of the IL-12Rβ2 gene.

Molecular cloning of the human IL-12Rβ2 gene

We have characterized a genomic DNA contig of 16.5 kb encompassing the 5'-region of the IL-12Rβ2 gene. The

human IL-12Rβ2 gene has a first untranslated exon of 604 bp, followed by a 12.4 kb intron, and a second exon (112 bp) that contains the start codon of the gene. The third exon (288 bp) follows after a 1.2 kb second intron (Supplementary Figure 1A). An mRNA start site was mapped in the vicinity of the 5'-end of the longest cDNA clone (data not shown).

The IL-12Rβ2 core promoter is active in lymphoid and non-lymphoid cells

Analysis of the genomic sequence in the vicinity of the mRNA start site revealed seven potential binding sites for the transcription factor SP1 (5'-GGGCGG-3') (Supplementary Figure 1B). Reporter gene assays with a 0.7 kb DNA fragment encompassing all SP1 elements and the mRNA start site showed that this fragment displays promoter activity in both Jurkat and COS7 cells (Figure 2A), indicating that this region does not confer Th1-cell-specific IL-12Rβ2 expression, but represents a 'core promoter'. This finding is consistent with a previous preliminary characterization of the IL-12Rβ2 gene (van Rietschoten *et al*, 2001).

Reorganization of the chromatin structure at the human IL-12Rβ2 locus during T helper cell differentiation

To identify *cis*-elements controlling Th1-specific expression of IL-12Rβ2, we analyzed the pattern of DNase I HS in cord

blood cells developing along a Th1 or a Th2 pathway. HS were mapped within a 9 kb DNA fragment spanning 5 kb upstream and 4 kb downstream of the mRNA start site 6 days after stimulation, a time at which maximal IL-12Rβ2 expression was observed (Rogge *et al*, 1999). We observed two strong HS in the 5'-upstream region of the IL-12Rβ2 gene in Th1 cells (Figure 2B, right panel, lanes 7–10 and Figure 2B, lower panel). These HS map 3.2 kb (HS1) and immediately upstream of the mRNA start site (HS2), respectively (see Figure 2B, lower panel). HS2 was also detected in Th2 cells (Figure 2B, left panel, lanes 2–5), and is located within the 'core promoter' of the IL-12Rβ2 gene. The strong Th1-specific HS1 suggested the presence of an enhancer element active in differentiating Th1 cells.

We asked whether the appearance of specific HS at the IL-12Rβ2 locus during T helper cell differentiation is associated with histone modifications at these sites. We analyzed H4 acetylation and H3 K4 trimethylation in naïve CD4⁺ as well as in Th1 and Th2 cells (5 days after stimulation) using chromatin immunoprecipitation (ChIP) assays. Formaldehyde-fixed chromatin fragments were precipitated with anti-acetyl H4, anti-trimethyl H3 K4, or control antibodies and the enrichment of specific DNA sequences was determined by amplification of DNA fragments spanning HS1 or HS2. We observed hyperacetylation (Figure 3A) and H3 K4 trimethylation (Figure 3B) at HS1 and HS2 in Th1 cells, but not in naïve CD4⁺ and Th2 cells, consistent with the absence of IL-12Rβ2 transcripts in these cells (Figure 3C). These findings provide

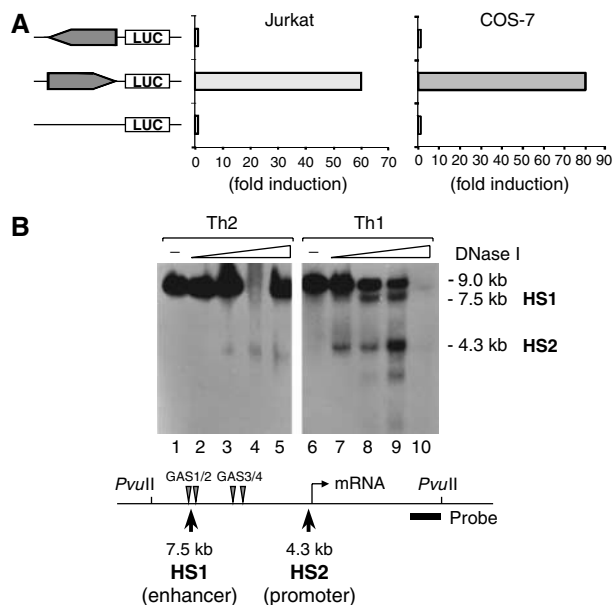


Figure 2 (A) The IL-12Rβ2 promoter is active in lymphoid and non-lymphoid cells. A 740 bp fragment (−173 to +572) of the IL-12Rβ2 gene was cloned in both directions upstream of the luciferase gene. These constructs were transiently transfected into Jurkat cells (left panel) or COS7 cells (right panel) and luciferase activity in cell lysates was measured 24 h after transfection. Transfection efficiency was normalized by measuring β-galactosidase activity of a CMV-βgal vector cotransfected in the cells. Results are representative of five independent experiments. (B) Mapping of DNase I-hypersensitive sites (HS) in the IL-12Rβ2 gene in Th1 and Th2 cells. Nuclei were purified from Th1 (right panel) or Th2 (left panel) cells 6 days after priming and treated with increasing amounts of DNase I (lanes 2–5 and 7–10). Genomic DNA was digested with PvuII and Southern blot analysis performed with a 0.5 kb PvuII–BamHI genomic fragment as a probe. The lower panel indicates the positions of HS with respect to the PvuII restriction site, the GAS elements, the mRNA start site, and the probe used for Southern blot analysis.

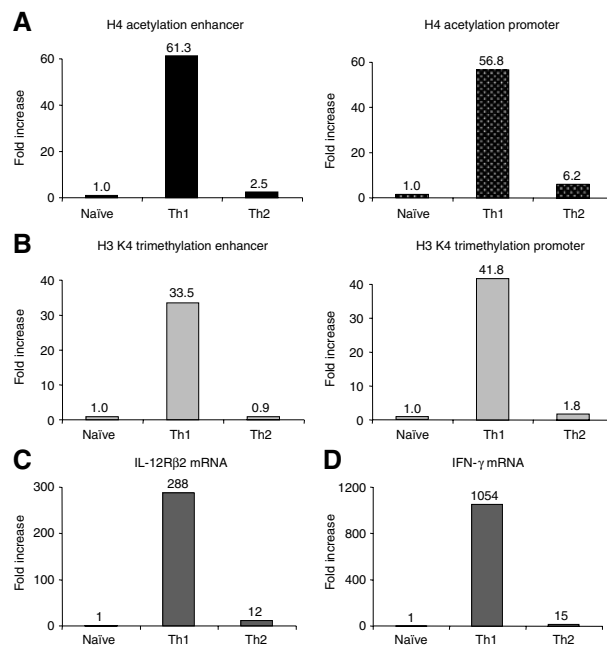


Figure 3 Chromatin remodeling at the IL-12Rβ2 enhancer and promoter is restricted to the Th1 subset. Histone 4 (H4) acetylation (A) and histone 3 lysine 4 (H3 K4) trimethylation (B) were analyzed in naïve CD4⁺ T cells and in Th1 and Th2 cells at day 5 after stimulation. Quantification of the specific enrichment of IL-12Rβ2 enhancer or promoter fragments following ChIP with anti-acetyl H4, anti-trimethyl H3 K4 was performed by Taqman real-time PCR. The mRNA levels of IL-12Rβ2 (C) and IFN-γ (D) were determined in the same samples by real-time PCR with Taqman probes and calculated relative to the expression of the target gene in naïve T cells.

further evidence that HS1 and HS2 define regulatory regions of the IL-12Rβ2 gene.

Characterization of GAS elements present in the IL-12Rβ2 regulatory region

Analysis of the DNA sequence corresponding to HS1 revealed the presence of two GAS (IFN-gamma-activated sequence) elements (GAS1 and GAS2; Figure 2B). Two additional GAS elements were identified approximately 2 kb upstream of the mRNA start site (GAS3 and GAS4; Figure 2B). To test whether these GAS elements bound STAT proteins, we incubated nuclear extracts from IL-12-treated and untreated Th1 cells with biotinylated oligos corresponding to these GAS elements. As a control, we used an oligonucleotide encompassing the GAS element from the human IRF-1 gene, which is a target sequence for both STAT4 and STAT1 (Coccia *et al*, 1999; Galon *et al*, 1999). STAT4 bound *in vitro* to the GAS elements (Figure 4A, upper panel). These elements were not recognized by STAT1, as shown by incubation with nuclear extracts from IFN-γ-treated and untreated Th2 cells, whereas STAT1 bound to the GAS element derived from the IRF-1 gene promoter (Figure 4A, lower panel). Nuclear extracts from Th2 cells were used in this experiment, as downregulation of the IFN-γ receptor beta-chain during Th1 cell differentiation results in very limited responsiveness of Th1 cells to IFN-γ (Bach *et al*, 1995; Groux *et al*, 1997; and L Rogge, unpublished). To analyze whether the GAS elements were capable of directing IL-12 or IFN-γ-inducible gene expression, we generated a reporter gene construct containing these elements (beta2-Luc) and introduced it into Jurkat T cells, together with expression vectors encoding both IL-12 receptor subunits and STAT4. As a control, we used a reporter gene construct containing the STAT1/STAT4 binding site from the IRF-1 gene promoter (IRF1-Luc) (Coccia *et al*, 1999). The IL-12Rβ2 GAS elements confer IL-12 responsiveness to the beta2-Luc construct in IL-12-treated, transfected Jurkat cells (Figure 4B). Consistent with our previous results (Coccia *et al*, 1999), the IRF1-Luc construct was also induced to a similar level following IL-12 treatment. IFN-γ treatment induced a much stronger response with the IRF1-Luc construct than with the beta2-Luc construct (Figure 4B).

We next analyzed whether these GAS elements induce Th1 lineage-specific expression of a reporter gene when introduced into naïve CD4⁺ T cells. We observed increased promoter activity in T cells that were stimulated for 24 h in Th1-inducing conditions, compared with unstimulated cells or cells stimulated in Th2 conditions (Figure 4C). These results demonstrate that the IL-12Rβ2 GAS elements can activate transcription in Th1 but not Th2 cells.

STAT4 is bound to the IL-12Rβ2 gene enhancer in differentiating Th1 cells *in vivo*

To determine whether STAT4 is bound to the IL-12Rβ2 regulatory regions in the early phases of Th1 cell differentiation *in vivo*, we performed ChIP assays with CD4⁺ T cells that had been stimulated for 20 h in Th1 or Th2 conditions, using anti-STAT4 or control antibodies, followed by amplification of a DNA fragment spanning GAS1 and GAS2 (IL12Rb2 enhancer), or GAS3 and GAS4 (IL12Rb2-2 kb). Our results show that STAT4 is bound to the enhancer already 20 h after stimulation in Th1-inducing conditions (Figure 5A). As

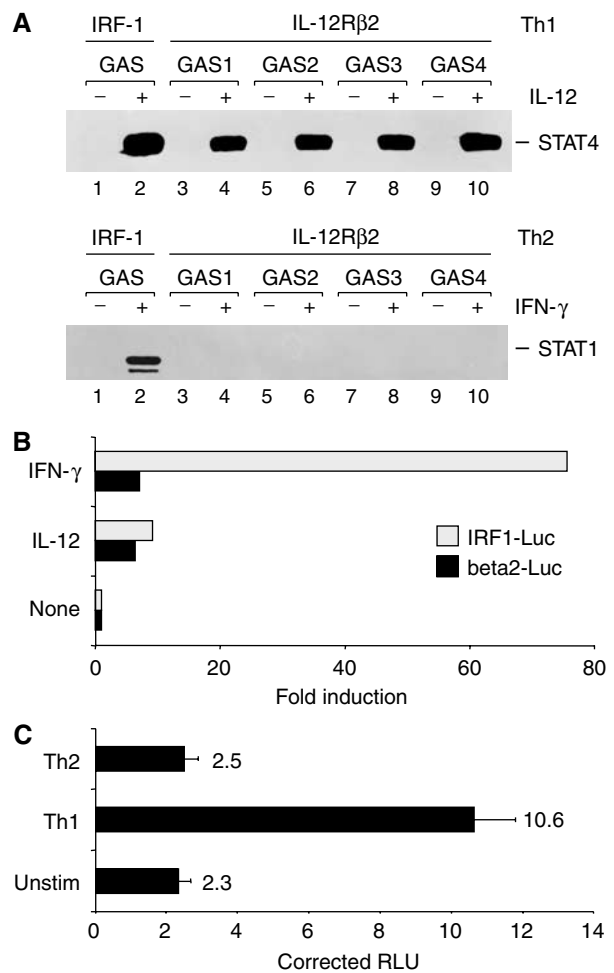


Figure 4 (A) STAT4 binds to GAS elements in the IL-12Rβ2 regulatory region. Biotinylated oligonucleotide probes encompassing the four GAS elements from the IL-12Rβ2 gene (lanes 3–10) or the GAS element present in the human IRF-1 promoter (lanes 1 and 2) were incubated with nuclear extracts from IL-12-treated or untreated Th1 cells (upper panel), or IFN-γ-treated or untreated Th2 cells (lower panel). Proteins bound to the probes were precipitated with streptavidin-agarose, separated on SDS gels, and transferred to PVDF membranes. The membranes were probed with antibodies to STAT4 (upper panel) or STAT1 (lower panel). (B) IL-12Rβ2 GAS elements mediate IL-12-induced transcriptional activation. The beta2-Luc construct (black bars) containing all four IL-12Rβ2 GAS elements was compared with the IRF1-Luc construct (gray bars) for responsiveness to IL-12- and IFN-γ-mediated transcriptional activation. Jurkat cells were transiently cotransfected as described in Supplementary data section to reconstitute IL-12 signaling plus the reporter construct. Cells were left untreated or stimulated for 20 h with IL-12 or IFN-γ. Luciferase activity was measured 24 h after transfection. Results were normalized by measuring the activity of a cotransfected CMV-βgal vector. Transfections were performed in triplicate and the experiment is representative of three. (C) IL-12Rβ2 GAS elements mediate Th1-specific expression of a reporter gene. Naïve CD4⁺ T cells were transfected with the beta2-Luc construct containing the four GAS elements from the IL-12Rβ2 gene. Cells were left unstimulated or stimulated in Th1 or Th2 conditions. Luciferase activity was measured 24 h after stimulation and normalized as above. The bars represent mean and s.d. from one representative experiment out of two.

expected, we observed strong binding of STAT4 to the IFN-γ promoter in Th1 cells. In contrast, we did not observe specific amplification of the DNA fragment encompassing GAS3 and GAS4, indicating that these two GAS elements are not occu-

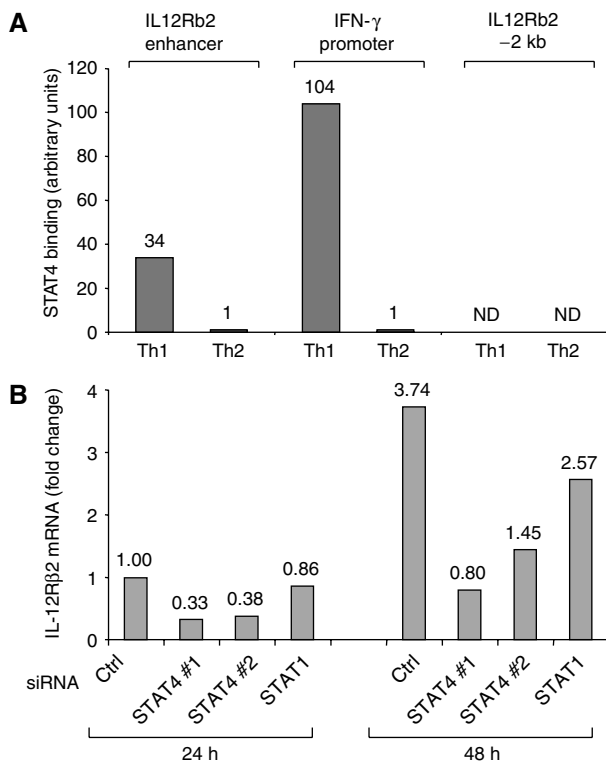


Figure 5 (A) STAT4 is bound to the enhancer of the IL-12Rβ2 gene *in vivo*. Naïve CD4⁺ T cells were stimulated for 20 h with anti-CD3/CD28 in Th1 or Th2 conditions. ChIP assays were performed with anti-STAT4 antibodies or rabbit IgG as control (see Materials and methods). The enrichment of IL-12Rβ2 enhancer sequences in the precipitate was measured by real-time PCR using Taqman probes. Shown is binding of STAT4 to the IL-12Rβ2 enhancer and the IFN-γ promoter in Th1 relative to Th2 cells. No STAT4 binding to a fragment spanning GAS elements 3 and 4 (a sequence element 2 kb upstream of the IL-12Rβ2 mRNA start site) was detected (ND, amplification equal to background). (B) Knock-down of STAT4 decreases IL-12Rβ2 expression. Naïve CD4⁺ T cells were transfected with a control siRNA oligo (CTRL), siRNA oligos targeting STAT4 (STAT4 #1 and STAT4 #2), or an oligo targeting STAT1. Cells were stimulated with anti-CD3/CD28 in the presence of IL-12 and harvested after 24 or 48 h. Transcripts encoding IL-12Rβ2, STAT4, and STAT1 were measured by real-time PCR using Taqman probes. Shown are the expression levels of IL-12Rβ2 mRNA relative to the control at 24 h after stimulation. STAT4 and STAT1 mRNAs were inhibited 60–65 and 75–85%, respectively (not shown). Similar results were observed in five experiments.

pied by STAT4 in the early phase of Th1 cell differentiation. Comparable amplification efficiency of the primers used was tested on genomic DNA (Supplementary Figure 2). These findings are consistent with the fact that GAS1/GAS2 but not GAS3/GAS4 are located within HS1.

To analyze whether STAT4 is required for IL-12Rβ2 expression during Th1 differentiation, we knocked down STAT4 expression using small interfering RNA (siRNA) oligos. Transfection of naïve CD4⁺ T cells with two different siRNAs targeting STAT4 resulted in strongly reduced IL-12Rβ2 mRNA levels in cells stimulated in the presence of IL-12 (Figure 5B). In contrast, transfection of a control siRNA or an siRNA targeting STAT1 did not substantially affect IL-12Rβ2 expression (Figure 5B).

Taken together, our data suggest a fundamental role of STAT4 in the induction of IL-12Rβ2 gene expression during the early phases of Th1 cell differentiation.

Synergy of cytokine and TCR signaling in inducing chromatin modifications and gene transcription of the IL-12Rβ2 gene

To analyze the role of cytokine and TCR signaling in the induction of epigenetic modifications within the IL-12Rβ2 enhancer and promoter, we analyzed H3 acetylation at these loci, early after T-cell stimulation.

First, we asked whether IFN-α treatment in the absence of TCR stimulation is sufficient to induce chromatin remodeling of the IL-12Rβ2 locus. Although IFN-α target genes are very efficiently induced by IFN-α in naïve T cells (Dondi *et al*, 2003), we observed only minor changes in the acetylation status of the IL-12Rβ2 regulatory regions (Figure 6A and B) and a very modest increase of transcripts encoding IL-12Rβ2, IFN-γ, and T-bet (Figure 6C–E) in IFN-α-treated naïve T cells. Stimulation with anti-CD3/CD28 alone increased H3 acetylation at the IL-12Rβ2 enhancer (Figure 6A) or promoter (Figure 6B). However, IL-12Rβ2, IFN-γ, and T-bet transcripts were only weakly induced in response to TCR/CD28 ligation (Figure 6C–E). A further increase in H3 acetylation was found in cells stimulated simultaneously with anti-CD3/CD28 and IFN-α or IL-12. In particular, we observed a striking synergistic effect of TCR and cytokine receptor signaling on the expression of IL-12Rβ2, IFN-γ, and T-bet in the same samples (Figure 6C–E).

T-cell stimulation induces rapid recruitment of the SWI/SNF-like BAF chromatin remodeling complex to the IL-12Rβ2 locus

The molecules that induce chromatin remodeling during T helper cell differentiation at cytokine or cytokine receptor loci are not known. To explore the recruitment of SWI/SNF-like BAF complexes to the enhancer and promoter of the IL-12Rβ2 gene, we performed ChIP assays with antibodies against BRG1. We found that TCR/CD28 stimulation alone induced recruitment of BRG1 to the enhancer and promoter of the IL-12Rβ2 gene (Figure 7A), but not to the GAPDH promoter used as a control (Supplementary Figure 3). BRG1 recruitment occurred in parallel with increased histone 3 acetylation (Figure 7B).

To investigate whether recruitment of the BAF chromatin remodeling complex is implicated in IL-12Rβ2 expression, we targeted BRG1 by RNAi in naïve CD4⁺ T cells. As shown in Figure 7C, knock-down of BRG1 resulted in diminished IL-12Rβ2 expression both in cells stimulated with anti-CD3/CD28 alone and in cells stimulated in the presence of IL-12. This effect was specific to BRG1, as targeting of SNF2h, the ATPase of the ISWI chromatin remodeling complex, did not result in reduced IL-12Rβ2 gene expression. As expected, knock-down of STAT4 did not affect IL-12Rβ2 expression in cells stimulated in the absence of IL-12; however, it resulted in decreased IL-12Rβ2 mRNA levels in cells stimulated in the presence of IL-12. These data indicate an important role for the BAF complex in the remodeling of the chromatin structure at the IL-12Rβ2 locus following TCR stimulation of naïve CD4⁺ T cells.

We then asked whether BRG1 and STAT4 recruitment promoted histone hyperacetylation at the IL-12Rβ2 regulatory regions. BRG1 downregulation caused a strong reduction of H4 acetylation at the IL-12Rβ2 enhancer and promoter (Figure 8A) in cells stimulated with anti-CD3/CD28 alone. Under the same conditions, downregulation of STAT4 did not affect H4 acetylation, as expected (Figure 8A). However, a

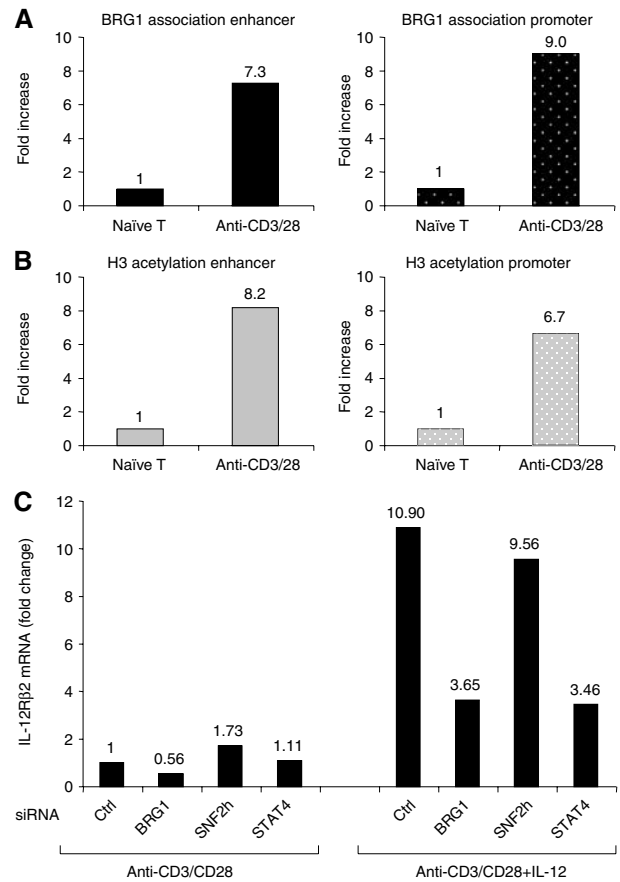
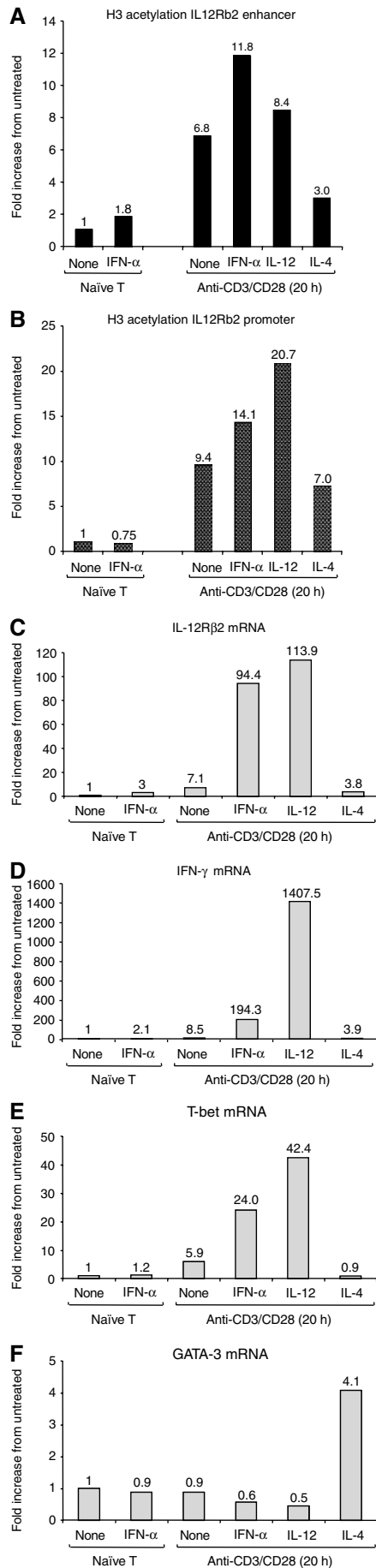


Figure 7 (A) Stimulation of naïve T cells induces recruitment of the BAF chromatin remodeling complex to the IL-12Rβ2 locus. Naïve CD4⁺ T cells were harvested after purification or after stimulation for 20 h with anti-CD3/CD28. (A) ChIP assays were performed with anti-BRG1 or control antibodies, and the enrichment of IL-12Rβ2 enhancer or promoter sequences in the precipitate was measured by real-time PCR. Similar results were obtained using two different anti-BRG1 antibodies. (B) Histone H3 acetylation was analyzed at the IL-12Rβ2 enhancer and promoter in the same samples as described above. (C) Knock-down of BRG1 results in reduced IL-12Rβ2 expression. Naïve CD4⁺ T cells were transfected with a control siRNA oligo (CTRL) or siRNA oligos targeting BRG1, SNF2h, or STAT4. Cells were stimulated with anti-CD3/CD28 in the absence or presence of IL-12 and harvested after 24 h. Transcripts encoding IL-12Rβ2 were determined by real-time PCR. Expression levels of IL-12Rβ2 mRNA are shown relative to the control. BRG1, SNF2h, and STAT4 mRNAs were inhibited 70, 90–95, and 60–65%, respectively (not shown). Similar results were observed in three experiments.

Figure 6 IL-12Rβ2 enhancer and promoter undergo rapid epigenetic changes following T-cell stimulation. Naïve T-cells were left untreated or treated for 20h with IFN-α (1000 U/ml) alone (naïve T). Alternatively, naïve T cells were stimulated with anti-CD3/CD28 antibodies for 20 h in the absence of exogenously added cytokines or in the presence of IFN-α (1000 U/ml), IL-12 (5 ng/ml), or IL-4 (1 ng/ml). Following ChIP of acetylated histone H3, IL-12Rβ2 enhancer fragments (A) or promoter fragments (B) were amplified by Taqman real-time PCR. The mRNA levels of IL-12Rβ2 (C), IFN-γ (D), T-bet (E), and GATA-3 (F) were determined in the same samples by real-time PCR with Taqman probes. Shown are the expression levels, normalized to 18S RNA amplification, in each sample and calculated relative to the expression of the target gene in naïve T cells.

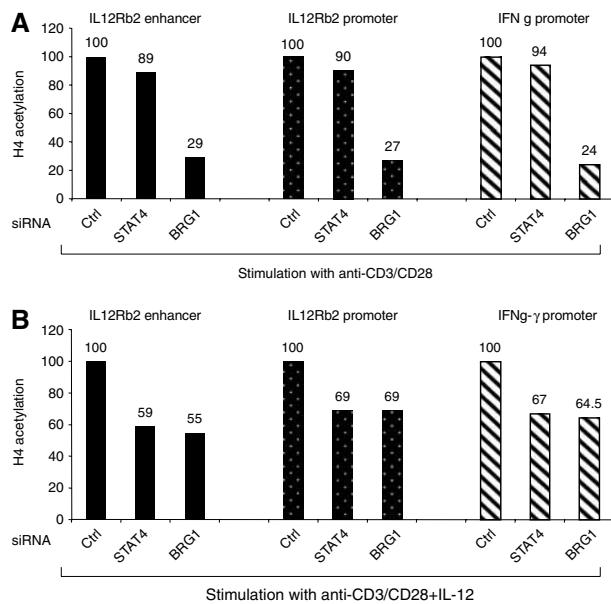


Figure 8 Downregulation of BRG1 and STAT4 inhibits H4 acetylation at the IL-12Rβ2 regulatory regions. Naïve T cells were transfected with the indicated siRNA oligos and stimulated for 24 h with anti-CD3/CD28 antibodies in the absence (A) or presence (B) of IL-12. ChIP assays were performed with anti-acetyl H4 or control antibodies and regions spanning the IL-12Rβ2 enhancer, promoter, or the IFN-γ promoter were amplified by real-time PCR. Values are expressed relative to the control siRNA sample.

reduction in STAT4 levels decreased H4 acetylation of these regions in cells stimulated in the presence of IL-12 (Figure 8B). In these cells, downregulation of BRG1 alone was less efficient in reducing H4 acetylation, consistent with a synergistic role of STAT4 recruitment in promoting chromatin modifications (Figure 8B).

This synergy was further underscored by the study of the kinetics of BRG1 and STAT4 recruitment in the early phases of Th1 development. We could observe BRG1 recruitment already 4 h after culture in Th1 conditions at the IL-12Rβ2 enhancer and promoter (Figure 9A), whereas initial STAT4 recruitment was observed only at the enhancer (Figure 9B). More important STAT4 recruitment to this region was observed at 24 h, consistent with increased IL-12-responsiveness of the developing Th1 cell population.

Together, our data suggest the following model for the sequence of events that lead to the induction of IL-12Rβ2 during the early phases of human Th1 cell differentiation: the inaccessible chromatin structure at the IL-12Rβ2 locus prevents transcription of this gene in naïve CD4⁺ T cells (Figure 10, left panel). TCR signaling is necessary and sufficient to induce the initial opening of the regulatory regions of the IL-12Rβ2 gene. This process is, at least in part, mediated by the BAF complex and is paralleled by increased acetylation of associated histones. The resulting low-level IL-12Rβ2 gene transcription renders the cells responsive to IL-12 (Figure 10, middle panel). STAT4 activation by IL-12 or IFN-α induces binding of this transcription factor to the IL-12Rβ2 enhancer, further remodeling of the locus, and high-level IL-12Rβ2 gene transcription (Figure 10, right panel).

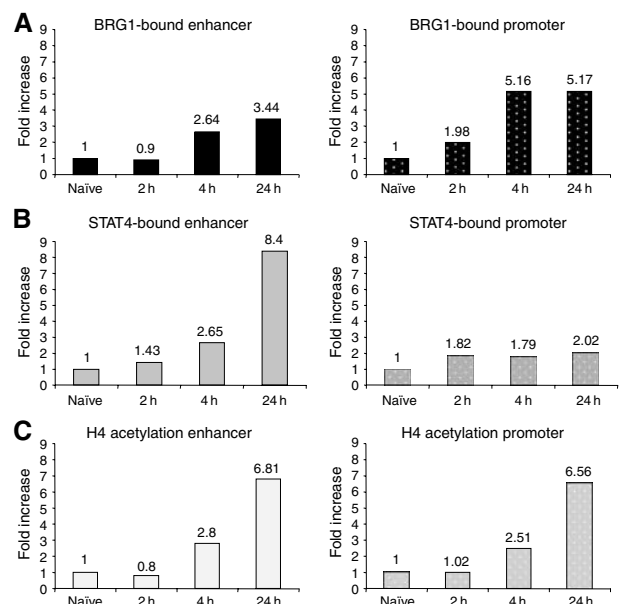


Figure 9 Rapid recruitment of BRG1 and STAT4 to the IL-12Rβ2 regulatory elements during Th1 cell differentiation. Naïve T cells were stimulated with anti-CD3/CD28 antibodies + IL-12 for the indicated times. ChIP assays were performed with anti-BRG1 (A), anti-STAT4 (B), anti-acetyl-H4 (C), or control antibodies. Real-time PCR amplification of the enhancer and the promoter region are represented relative to the unstimulated samples. No specific amplification was observed in samples precipitated with control antibodies (not shown).

Discussion

It is debated which cytokines and signaling pathways play the most important role in the induction of IL-12Rβ2 expression during the early phases of Th1 development. We and others have previously demonstrated an important role for TCR signaling and STAT4 activation in the induction of IL-12Rβ2 gene expression in human T cells (Rogge *et al*, 1997; Athie-Morales *et al*, 2004). In contrast, IFN-γ was demonstrated to induce IL-12Rβ2 in mouse T cells, indicating a role for STAT1 in the regulation of IL-12Rβ2 expression in this system (Szabo *et al*, 1997; Rogge *et al*, 1998; Smeltz *et al*, 2002). Our present data demonstrate that IFN-α is the most potent inducer of IL-12Rβ2 in the first hours after T-cell activation. IFN-α-induced IL-12Rβ2 transcription is not mediated indirectly via the induction of IFN-γ, because the effect is also observed in the presence of neutralizing anti-IFN-γ antibodies (Rogge *et al*, 1997; data not shown). IL-12 signaling is much more efficient at inducing both IFN-γ and IL-12Rβ2 at later times after T-cell stimulation. The mechanism of this observation has yet to be determined; however, a previous report has correlated the more sustained activation of STAT4 by IL-12, when compared with IFN-α, with the higher efficiency of IL-12 in inducing IFN-γ transcription (Athie-Morales *et al*, 2004).

To understand the regulation of the IL-12Rβ2 gene at the molecular level, we have cloned and analyzed the regulatory regions of the human IL-12Rβ2 gene. We have identified a Th1-specific enhancer element that is recognized by STAT4, and that can activate a reporter gene in an IL-12-dependent manner. Consistently, our finding that STAT4 is bound to the enhancer element *in vivo* in developing human Th1 but not in Th2 cells supports the fundamental role of STAT4 in IL-12Rβ2 expression.

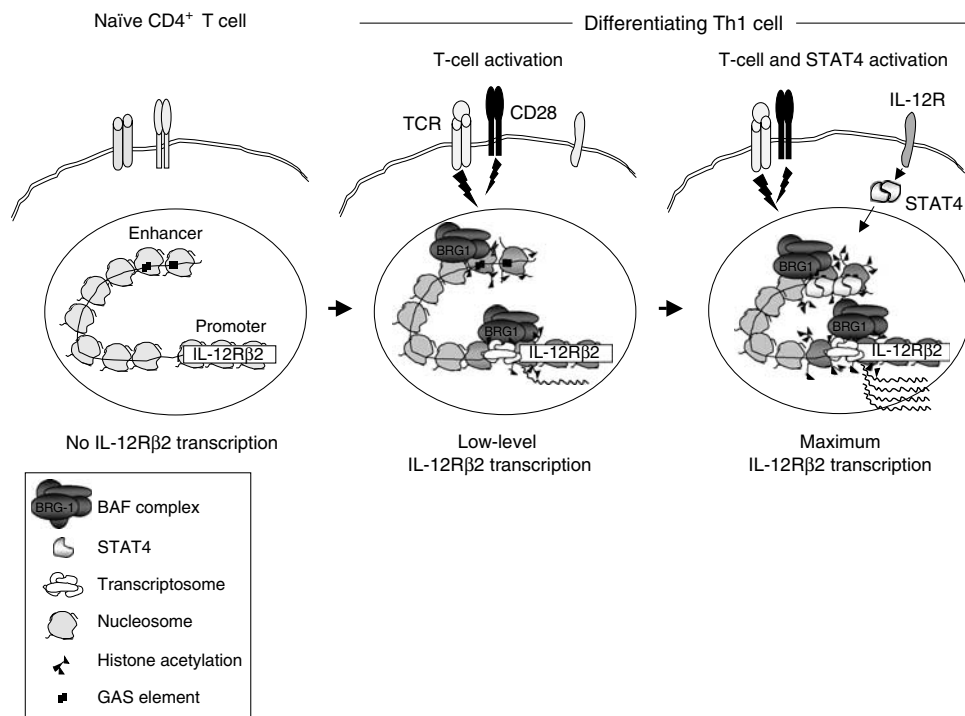


Figure 10 Induction of IL-12Rβ2 expression during Th1 development: a model. A color version of this figure is available at The EMBO Journal Online (Supplementary Figure 5).

Previous studies performed with mouse T cells have shown that IFN- γ /STAT1 signaling is of critical importance for the induction of T-bet (Lighvani *et al*, 2001; Afkarian *et al*, 2002), and it has been proposed that T-bet, in turn, activates IL-12Rβ2 gene expression (O’Shea and Paul, 2002; Berenson *et al*, 2004). Inspection of the enhancer and promoter of the human IL-12Rβ2 gene did not reveal consensus binding sites for T-bet (Supplementary Figure 1B). In addition, retrovirus-mediated expression of T-bet in human Th2 cells strongly induced IFN- γ transcripts; however, we observed only a minor upregulation of IL-12Rβ2 mRNA and protein (data not shown). This finding is consistent with recent data from the Strober laboratory demonstrating that retrovirus-mediated T-bet expression in CD4⁺ T cells from wild-type or T-bet-deficient mice did not result in IL-12Rβ2 expression, and that Th1 cells from T-bet^{-/-} mice expressed normal levels of IL-12Rβ2 (Usui *et al*, 2006). At present, we cannot exclude a role for the IFN- γ /STAT1/T-bet pathway in IL-12Rβ2 gene regulation; however, our data argue in favor of the direct activation of the IL-12Rβ2 gene by a combination of STAT4 and TCR signaling in the early phase of Th1 development.

TCR/CD28 signaling induced histone acetylation at the enhancer and promoter of the IL-12Rβ2 gene, which was further increased in cells stimulated in the presence of IL-12. A similar pattern of histone acetylation has recently been observed at the loci of the IL-4 and IFN- γ genes during T helper cell differentiation. Stimulation of naïve CD4⁺ T cell via the TCR was sufficient to induce detectable levels of histone acetylation at the regulatory regions of these genes; however, the presence of IL-4 or IL-12 was required for high-level and sustained histone acetylation of the IL-4 and IFN- γ loci, respectively (Avni *et al*, 2002; Morinobu *et al*, 2004). In contrast, histone acetylation of the IL-12Rβ2 regulatory

regions was lower in cells stimulated in the presence of IL-4 than in cells stimulated in the absence of cytokines. This is consistent with an active inhibition of IL-12Rβ2 expression by IL-4 in the early phases of Th2 cell differentiation, as shown previously (Rogge *et al*, 1997; Szabo *et al*, 1997; Ouyang *et al*, 1998; Usui *et al*, 2003). Our observation that signaling through the IFN- α receptor alone is not sufficient to induce H3 acetylation provides further evidence for the crucial role of TCR signaling to initiate chromatin remodeling at the IL-12Rβ2 locus. We found that BRG1, the ATPase subunit of the SWI/SNF-like BAF chromatin remodeling complex, is recruited to the enhancer and promoter of the IL-12Rβ2 gene following TCR/CD28 stimulation of naïve human T cells. These data suggest that the BAF complex plays an important role in the initial opening of the IL-12Rβ2 locus in the early phases of Th1 cell differentiation. The mechanism that leads to the recruitment of BRG1 to the IL-12Rβ2 locus is currently not known; however, previous work has shown that the rapid recruitment of the SWI/SNF-like BAF remodeling complex to chromatin following T-cell stimulation is mediated by phosphoinositols (Zhao *et al*, 1998). The notion that nuclear inositol signaling may play an important role in relaying signals from the cell surface to chromatin via the BAF complex has recently been supported by the finding that the activities of the yeast SWI/SNF and INO80 chromatin remodeling complexes are also regulated by inositol signaling (Shen *et al*, 2003; Steger *et al*, 2003). In addition to phosphoinositol signaling, recruitment of BRG1 to chromatin appears to be regulated through a variety of protein–protein interactions. Of particular interest is the targeting of BRG1 to the promoter of the interferon-induced IFITM3 gene via a direct interaction with the ubiquitous transcription factor SP1 (Liu *et al*, 2002). The presence of multiple SP1 binding sites

in the IL-12R β 2 'core' promoter may provide an explanation for the recruitment of BRG1 to this region and for the rapid chromatin remodeling of this region. The importance of protein-protein interactions to recruit BRG1 to specific promoters has also been analyzed in the context of IFN-responsive genes, which have been shown to be upregulated by BRG1 (Liu *et al*, 2002). In this case, BRG1 is targeted to the promoter via a direct interaction with the amino-terminal domain of STAT2 (Huang *et al*, 2002). Given the conservation of this domain among STAT proteins, it is possible that a similar interaction with STAT4 facilitates recruitment of BRG1 to the IL-12R β 2 enhancer.

Studies addressing the mechanisms of IFN- β gene expression in response to viral infection have indicated that histone acetyltransferases (HATs) are targeted to the IFN- β promoter before recruitment of the BAF complex (Chi, 2004). In contrast, the BAF complex is recruited to the promoter of the IFN-inducible IFITM1 gene before the action of HATs (Cui *et al*, 2004). A similar kinetics could apply to the control of the IL-12R β 2 gene, as shown by the effect of BRG1 downregulation on H4 acetylation at the IL-12R β 2 enhancer and promoter. The same approach shows that STAT4 recruitment is also important for the complete remodeling of the IL-12R β 2 regulatory regions. It should be noted in this context that several transcriptional activators have been shown to bind directly to both ATP-dependent chromatin remodeling complexes and HATs, indicating a cooperation between the three elements (Narlikar *et al*, 2002). A good example of this is STAT2, which recruits the BAF complex and HATs to IFN-inducible genes by binding BRG1 and p300/Creb-binding protein (CBP), respectively (Bhattacharya *et al*, 1996; Huang *et al*, 2002).

Very recently, Zhang and Boothby (2006) reported that chromatin remodeling at the mouse IFN- γ promoter during early Th1 development is mediated by BRG1. They demonstrated that BRG1 recruitment is dependent on STAT4 and can be inhibited by the immunosuppressive drug cyclosporine A (CsA) (Zhang and Boothby, 2006). Our results demonstrate that BRG1 recruitment and chromatin remodeling at the human IL-12R β 2 locus are initiated by TCR signaling and do not depend on STAT4 activation. A possible explanation for this difference may be that NFAT, which is required for induction of IFN- γ transcription in mouse cells, does not seem to play a role in the early activation of the human IL-12R β 2 gene, which is not inhibited by CsA (S Gasparian, manuscript in preparation).

In conclusion, our data demonstrate that signaling through the TCR is necessary and sufficient to start chromatin remodeling of the IL-12R β 2 locus at an early time point after T-cell stimulation. TCR-induced recruitment of BRG1 to the enhancer and promoter of the IL-12R β 2 gene strongly suggests that the SWI/SNF-like BAF chromatin remodeling complex plays an important role in the initial opening of the locus. Recruitment of the BAF complex is associated with histone acetylation and low-level IL-12R β 2 expression, resulting in IL-12 responsiveness. The presence of IFN- α or IL-12 at the time of TCR stimulation induces binding of STAT4 to the IL-12R β 2 enhancer and increased histone acetylation, and results in a striking synergistic effect on the expression of transcripts encoding IL-12R β 2, as well as IFN- γ and T-bet. However, signaling through the IFN- α / β receptor (which is expressed on naïve T cells) in the absence of TCR stimulation

does not induce significant chromatin remodeling and expression of IL-12R β 2. These data are consistent with a model in which recruitment of the BAF chromatin remodeling complex induced by TCR signaling is an obligate requirement to render the IL-12R β 2 locus susceptible to activation by STAT4.

Materials and methods

Molecular cloning of the human IL-12R β 2 gene

Screening of genomic libraries with a DNA fragment derived from the IL-12R β 2 cDNA spanning the 5'-untranslated region and 240 nucleotides of the coding region (clone B5-10, generously provided by Ueli Gubler) was performed according to standard methods. Analysis of three overlapping phages by restriction mapping, Southern blot, and DNA sequence analysis allowed the assembly of a contig of 16.5 kb that contains part of the human IL-12R β 2 gene.

Purification and stimulation of naïve CD4⁺ T cells

Naïve CD4⁺ T cells were purified as described (Rogge *et al*, 2000). The purity of the CD4⁺ T cells was typically >98%, as determined by flow cytometry. Purified naïve T cells were stimulated with anti-CD3 mAb (1 μ g/ml) (1XE, CLB) and anti-CD28 mAb (1 μ g/ml) (BD Pharmingen) in the presence or absence of IL-12 (2.5 ng/ml), IFN- α (1000 U/ml), IFN- γ (1000 U/ml), or in the presence of IL-4 (1 ng/ml). Th1 and Th2 lines were generated from cord blood leukocytes depleted of CD8⁺ T cells and NK cells as described (Rogge *et al*, 2000).

Quantification of mRNA levels

Total RNA was purified with RNeasy columns (Qiagen). The mRNA levels of IL-12R β 2, IFN- γ T-bet, GATA-3, STAT1, STAT4, BRG1, and SNF2h were determined by real-time PCR with Taqman probes using a protocol provided by the manufacturer (Applied Biosystems). Expression levels were normalized to 18S RNA amplification levels in each sample and calculated relative to the expression of the target gene in unstimulated (naïve) T cells (Figures 3 and 6) or cells transfected with a control siRNA oligo (Figures 5 and 7).

Chromatin immunoprecipitation assays

ChIP assays were performed according to the Upstate Biotechnology protocol using the following antibodies at 4°C overnight: anti-STAT4 (C-20), anti-BRG1 (H88) (Santa Cruz Biotech), anti-BRG1 (SNF2 β /BRG1), anti-acetylated histone 3 and histone 4 (H3 or H4), anti-trimethylated histone 3 lysine 4 (H3 K4) (Upstate Biotechnology), and purified rabbit IgG (control).

Quantification of IL-12R β 2 gene fragments following ChIP was performed by real-time quantitative PCR using Taqman probes as described (Litt *et al*, 2001; Mutskov and Felsenfeld, 2004). For normalization, 1% of 'input' DNA (before IP) from each sample was analyzed in parallel and the amount of IL-12R β 2 DNA in each sample was calculated according to the instructions provided by Applied Biosystem using the equation $2^{-(C_{t\text{sample}} - C_{t\text{input}})}$ (C_t , cycle threshold) (Livak and Schmittgen, 2001). Real-time PCR using the ABI Prism 7300 sequence detection system was carried out in triplicate following a protocol supplied by the manufacturer (Applied Biosystems). Primers and Taqman probes were selected using Applied Biosystems Primer express software. Primer sequences and references for computation are given in the Supplementary data. ChIPs with control antibodies were used to assure that C_t values from samples with specific antibodies resulted from specific IPs (Supplementary Figure 4). On average, signals obtained with anti-STAT4 or anti-BRG1 antibodies were 25–50-fold lower than those observed with anti-acetyl H3/H4 antibodies. This is comparable with results obtained in a study by Bremner and colleagues (Ni *et al*, 2005).

RNA interference

Naïve CD4⁺ T cells from peripheral blood were purified by negative selection using a 'naïve CD4⁺ T cells isolation kit' (Miltenyi Biotec). Naïve CD4⁺ T cells were resuspended in 'human T cell Nucleofector solution' (Amaxa) at a concentration of $30\text{--}40 \times 10^6$ cells/ml and 95 μ l of cell suspension was mixed with 5 μ l of the oligo (1 μ M final concentration). Transfection was performed as described in the protocol from the manufacturer using the program U-14. Immediately after nucleofection, cells were transferred to prewarmed medium in a 24-well plate and incubated overnight (without

stimulation). Cells were stimulated the next morning with anti-CD3/CD28 in the absence or presence of IL-12 as above. Efficiency of the knock-down was determined by real-time PCR using Taqman probes and confirmed by Western blots. We tested eight siRNA duplexes (Qiagen) for inhibition of STAT1, STAT4, and BRG1 expression and four duplexes for SNF2h inhibition. The sequences (sense strand) of the most potent siRNA duplexes are given in Supplementary data, and the knock-down efficiencies (% suppression of the mRNA encoding the target gene) measured 24 h after stimulation are indicated in brackets.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

References

- Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, Murphy TL, Murphy KM (2002) T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4⁺ T cells. *Nat Immunol* **3**: 549–557
- Agarwal S, Rao A (1998) Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation. *Immunity* **9**: 765–775
- Ansel KM, Lee DU, Rao A (2003) An epigenetic view of helper T cell differentiation. *Nat Immunol* **4**: 616–623
- Athie-Morales V, Smits HH, Cantrell DA, Hilken CM (2004) Sustained IL-12 signaling is required for Th1 development. *J Immunol* **172**: 61–69
- Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A (2002) T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* **3**: 643–651
- Bach EA, Szabo SJ, Dighe AS, Ashkenazi A, Aguet M, Murphy KM, Schreiber RD (1995) Ligand-induced autoregulation of IFN-gamma receptor beta chain expression in T helper cell subsets. *Science* **270**: 1215–1218
- Berenson LS, Ota N, Murphy KM (2004) Issues in T-helper 1 development—resolved and unresolved. *Immunol Rev* **202**: 157–174
- Bhattacharya S, Eckner R, Grossman S, Oldread E, Arany Z, D'Andrea A, Livingston DM (1996) Cooperation of Stat2 and p300/CBP in signalling induced by interferon-alpha. *Nature* **383**: 344–347
- Chi T (2004) A BAF-centred view of the immune system. *Nat Rev Immunol* **4**: 965–977
- Chi TH, Wan M, Lee PP, Akashi K, Metzger D, Chambon P, Wilson CB, Crabtree GR (2003) Sequential roles of Brg, the ATPase subunit of BAF chromatin remodeling complexes, in thymocyte development. *Immunity* **19**: 169–182
- Chi TH, Wan M, Zhao K, Taniuchi I, Chen L, Littman DR, Crabtree GR (2002) Reciprocal regulation of CD4/CD8 expression by SWI/SNF-like BAF complexes. *Nature* **418**: 195–199
- Cho SS, Bacon CM, Sudarshan C, Rees RC, Finbloom D, Pine R, O'Shea JJ (1996) Activation of STAT4 by IL-12 and IFN-α. Evidence for the involvement of ligand-induced tyrosine and serine phosphorylation. *J Immunol* **157**: 4781–4789
- Coccia EM, Passini N, Battistini A, Pini C, Sinigaglia F, Rogge L (1999) IL-12 induces expression of interferon regulatory factor-1 via signal transducer and activator of transcription-4 in human T helper type 1 cells. *J Biol Chem* **274**: 6698–6703
- Cui K, Tailor P, Liu H, Chen X, Ozato K, Zhao K (2004) The chromatin-remodeling BAF complex mediates cellular antiviral activities by promoter priming. *Mol Cell Biol* **24**: 4476–4486
- Dondi E, Rogge L, Lutfalla G, Uze G, Pellegrini S (2003) Down-modulation of responses to type I IFN upon T cell activation. *J Immunol* **170**: 749–756
- Fields PE, Kim ST, Flavell RA (2002) Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. *J Immunol* **169**: 647–650
- Galon J, Sudarshan C, Ito S, Finbloom D, O'Shea JJ (1999) IL-12 induces IFN regulating factor-1 (IRF-1) gene expression in human NK and T cells. *J Immunol* **162**: 7256–7262
- Groux H, Sornasse T, Cottrez F, de Vries JE, Coffman RL, Roncarolo MG, Yssel H (1997) Induction of human T helper cell type 1 differentiation results in loss of IFN-gamma receptor beta-chain expression. *J Immunol* **158**: 5627–5631
- Huang M, Qian F, Hu Y, Ang C, Li Z, Wen Z (2002) Chromatin-remodelling factor BRG1 selectively activates a subset of interferon-alpha-inducible genes. *Nat Cell Biol* **4**: 774–781
- Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA (2006) T helper cell differentiation: regulation by *cis* elements and epigenetics. *Immunity* **24**: 369–379
- Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, Nguyen BV, Gadina M, Sher A, Paul WE, O'Shea JJ (2001) T-bet is rapidly induced by interferon-γ in lymphoid and myeloid cells. *Proc Natl Acad Sci USA* **98**: 15137–15142
- Litt MD, Simpson M, Recillas-Targa F, Prioleau MN, Felsenfeld G (2001) Transitions in histone acetylation reveal boundaries of three separately regulated neighboring loci. *EMBO J* **20**: 2224–2235
- Liu H, Kang H, Liu R, Chen X, Zhao K (2002) Maximal induction of a subset of interferon target genes requires the chromatin-remodeling activity of the BAF complex. *Mol Cell Biol* **22**: 6471–6479
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^{−(ΔΔC_T)} method. *Methods* **25**: 402–408
- Morinobu A, Kanno Y, O'Shea JJ (2004) Discrete roles for histone acetylation in human T helper 1 cell-specific gene expression. *J Biol Chem* **279**: 40640–40646
- Mullen AC, Hutchins AS, High FA, Lee HW, Sykes KJ, Chodosh LA, Reiner SL (2002) Hlx is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. *Nat Immunol* **3**: 652–658
- Mutskov V, Felsenfeld G (2004) Silencing of transgene transcription precedes methylation of promoter DNA and histone H3 lysine 9. *EMBO J* **23**: 138–149
- Narlikar GJ, Fan HY, Kingston RE (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**: 475–487
- Ni Z, Karasov E, Yu T, Callaghan SM, Der S, Park DS, Xu Z, Pattenden SG, Bremner R (2005) Apical role for BRG1 in cytokine-induced promoter assembly. *Proc Natl Acad Sci USA* **102**: 14611–14616
- O'Shea JJ, Paul WE (2002) Regulation of T(H)1 differentiation—controlling the controllers. *Nat Immunol* **3**: 506–508
- O'Sullivan A, Chang HC, Yu Q, Kaplan MH (2004) STAT4 is required for interleukin-12-induced chromatin remodeling of the CD25 locus. *J Biol Chem* **279**: 7339–7345
- Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, Murphy KM (1998) Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* **9**: 745–755
- Presky DH, Yang H, Minetti LJ, Chua AO, Nabavi N, Wu C-Y, Gately MK, Gubler U (1996) A functional Interleukin-12 receptor complex is composed of two β type cytokine receptor subunits. *Proc Natl Acad Sci USA* **93**: 14002–14007
- Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U, Sinigaglia F (1997) Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J Exp Med* **185**: 825–831
- Rogge L, Bianchi E, Biffi M, Bono E, Chang SY, Alexander H, Santini C, Ferrari G, Sinigaglia L, Seiler M, Neeb M, Mous J, Sinigaglia F,

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Conflict of interest

The authors have no conflicting financial interests.

- Certa U (2000) Transcript imaging of the development of human T helper cells using oligonucleotide arrays. *Nat Genet* **25**: 96–101
- Rogge L, D'Ambrosio D, Biffi M, Penna G, Minetti LJ, Presky DH, Adorini L, Sinigaglia F (1998) The role of Stat4 in species-specific regulation of Th cell development by type I IFNs. *J Immunol* **161**: 6567–6574
- Rogge L, Papi A, Presky DH, Minetti LJ, Miotto D, Semenzato G, Fabbri LM, Sinigaglia F (1999) Antibodies to the interleukin 12 receptor β 2 chain mark human Th1 but not Th2 cells *in vitro* and *in vivo*. *J Immunol* **162**: 3926–3932
- Shen X, Xiao H, Ranallo R, Wu WH, Wu C (2003) Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science* **299**: 112–114
- Smale ST, Fisher AG (2002) Chromatin structure and gene regulation in the immune system. *Annu Rev Immunol* **20**: 427–462
- Smeltz RB, Chen J, Ehrhardt R, Shevach EM (2002) Role of IFN-gamma in Th1 differentiation: IFN-gamma regulates IL-18R alpha expression by preventing the negative effects of IL-4 and by inducing/maintaining IL-12 receptor beta 2 expression. *J Immunol* **168**: 6165–6172
- Steger DJ, Haswell ES, Miller AL, Wente SR, O'Shea EK (2003) Regulation of chromatin remodeling by inositol polyphosphates. *Science* **299**: 114–116
- Szabo SJ, Dighe AS, Gubler U, Murphy KM (1997) Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J Exp Med* **185**: 817–824
- Trinchieri G (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* **3**: 133–146
- Usui T, Nishikomori R, Kitani A, Strober W (2003) GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet. *Immunity* **18**: 415–428
- Usui T, Preiss JC, Kanno Y, Yao ZJ, Bream JH, O'Shea JJ, Strober W (2006) T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. *J Exp Med* **203**: 755–766
- van Rietschoten JG, Smits HH, van de Wetering D, Westland R, Verweij CL, den Hartog MT, Wierenga EA (2001) Silencer activity of NFATc2 in the interleukin-12 receptor beta 2 proximal promoter in human T helper cells. *J Biol Chem* **276**: 34509–34516
- Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ (2004) Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* **202**: 139–156
- Zhang F, Boothby M (2006) T helper type 1-specific Brg1 recruitment and remodeling of nucleosomes positioned at the IFN- γ promoter are Stat4 dependent. *J Exp Med* **203**: 1493–1505
- Zhao K, Wang W, Rando OJ, Xue Y, Swiderek K, Kuo A, Crabtree GR (1998) Rapid and phosphoinositide-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95**: 625–636