# Lack of Interferon γ Receptor β Chain and the Prevention of Interferon γ Signaling in $T_H1$ Cells

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The ability of interferon γ (IFN-γ) to inhibit the proliferation of type 2 T helper cells ($T_H2$), but not that of type 1 ($T_H1$) cells, suggests that helper cell subsets might differ in their activation of the IFN-γ signaling pathway. The IFN-γ–inducible signal transducing factor (STF-IFNγ) was activated in murine $T_H2$ but not in $T_H1$ cell clones, because in the latter the second chain of the IFN-γ receptor (accessory factor 1 or IFN-γRβ) was absent. Thus, $T_H1$ cells use receptor modification to prevent the activation of STF-IFNγ and achieve an IFN-γ–resistant state.

Molecular distinctions between the $T_H1$ and $T_H2$ subsets of $CD4^+$ T helper cells (*1*) remain poorly defined. Interleukin-4 (IL-4) and IFN-γ differentially regulate the proliferation and differentiation of T helper cell subsets (*2*). For instance, IFN-γ inhibits the proliferation of $T_H2$ but not $T_H1$ cells (*3*). To determine the molecular basis of this dichotomy in cytokine responsiveness, we examined whether different signal transducing factors (STFs) are activated in response to IFN-γ (STF-IFNγ, IFN-γ–activated factor, or the Stat1 homodimer) (*4*) and IL-4 (STF-IL4) (*5*, *6*) in T helper subsets. Binding of IFN-γ to its receptor leads to the activation, by tyrosine phosphorylation, of two receptor-associated tyrosine kinases, Janus kinase–1 (Jak1) and Jak2. These kinases are required for the rapid phosphorylation of Stat1 (p91), a latent cytoplasmic factor belonging to the STAT (signal transducers and activators of transcription) family of proteins. The phosphorylated Stat1 protein homodimerizes to form STF-IFNγ which translocates to the nucleus and binds to specific DNA elements (IFN-γ–activated sites) responsible for IFN-γ–stimulated transcription (*7*). Upon stimulation of cells with IL-4, a different set of kinases, Jak1 and Jak3, is phosphorylated (*8*). These kinases are thought to activate a distinct member of the STAT family (termed IL-4 Stat or Stat6), which homodimerizes to form STF-IL4 (*9*).

To ascertain whether the $T_H1$ and $T_H2$ subsets differ in cytokine signaling, we stimulated established murine $T_H1$ clones (D1.1 and HDK1 cells) (*10*, *11*) and $T_H2$ clones (CDC25, CDC35, and D10.G4.1 cells) (*12*) with IL-4, IFN-γ, or IL-4 plus IFN-γ. We then examined the extracts by electrophoretic mobility shift assay (EMSA) for the presence of STF-IFNγ and STF-IL4 DNA binding activity (Fig. 1). Stimulation with IL-4 led to the appearance of STF-IL4 in both $T_H1$ and $T_H2$ cells. Because $T_H2$ clones secrete IL-4, STF-IL4 was present constitutively in some of these cells. When stimulated with IFN-γ, however, the responses of the two types of clones differed. IFN-γ led to the induction of STF-IFNγ DNA binding activity in the $T_H2$ clones, but not in the $T_H1$ clones. $T_H1$ cells were also cultured with increasing concentrations of IFN-γ, but again, STF-IFNγ was not induced (*13*). Culturing T cells with both cytokines did not affect the appearance of either STF (*5*).

Because $T_H1$ clones produce IFN-γ upon antigen stimulation (*14*), the inability to detect STF-IFNγ in $T_H1$ clones might be a result of prolonged exposure to IFN-γ. Blocking antibodies to IFN-γ (*11*) and to IFN-γ receptor (antibody GR-20) (*15*) were added separately to the culture medium of a $T_H1$ (D1.1 cells) and a $T_H2$ (D10.G4.1 cells) clone at the time of antigen stimulation and kept in the medium until the cells were harvested to assay for IFN-γ stimulation. This treatment did not induce STF-IFNγ in D1.1 cells upon exposure to IFN-γ (*13*). Prolonged exposure of D10.G4.1 ($T_H2$) cells to IFN-γ did not affect the ability of this $T_H2$ clone to activate STF-IFNγ upon reexposure to IFN-γ (*13*).

To show the functional consequence of the differential activation of Stat1 in $T_H2$ versus $T_H1$ clones, we examined the IFN-γ–mediated induction of the gene encoding interferon response factor–1 (IRF-1), whose transcriptional activation is regulated by STF-IFNγ (*16*). IFN-γ led to the induction of IRF-1 mRNA in a $T_H2$ (D10.G4.1) clone but not in a $T_H1$ (D1.1) clone (Fig. 2). Thus, the lack of STF-IFNγ activation in $T_H1$ cells leads to differential gene expression in $T_H1$ versus $T_H2$ cells upon exposure to IFN-γ.

We analyzed the components of the IFN-γ signal transduction pathway in $T_H1$ and $T_H2$ cells. Immunoprecipitations with

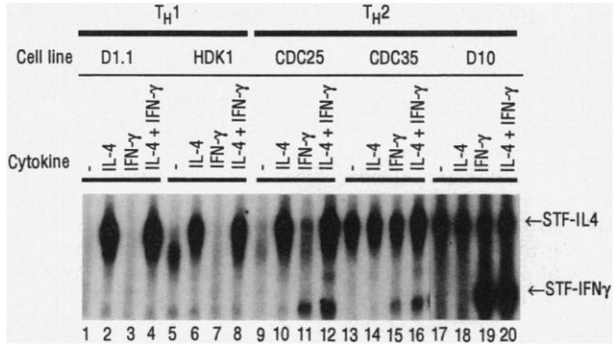


**Fig. 1.** Differential activation of the IFN-γ–inducible signal transducing factor in established murine $T_H1$ and $T_H2$ clones. Whole-cell extracts were prepared and examined by EMSA as previously described (*16*), with a βCASGAS probe to which both STF-IFNγ and STF-IL4 bind (*5*). Samples included extracts from two $T_H1$ clones, D1.1 (lanes 1 to 4) and HDK1 (lanes 5 to 8), and three $T_H2$ clones, CDC25 (lanes 9 to 12), CDC35 (lanes 13 to 16), and D10.G4.1 (D10) (lanes 17 to 20). Clones were either unstimulated (−) or stimulated for 15 min with IL-4 (400 U/ml) (lanes 2, 6, 10, 14, and 18), IFN-γ (66 U/ml) (lanes 3, 7, 11, 15, and 19), or both IL-4 (400 U/ml) and IFN-γ (66 U/ml) (lanes 4, 8, 12, 16, and 20). Derivation and antigen stimulation of the $T_H1$ and $T_H2$ clones have been previously described (*10*–*12*).

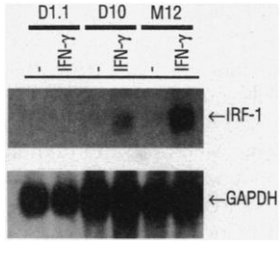


**Fig. 2.** Activation of an IFN-γ (Stat1)–inducible gene, *IRF-1*, in $T_H2$ but not $T_H1$ clones in response to IFN-γ stimulation. Cells from D1.1, D10.G4.1 (D10), or a B cell line M12 were either unstimulated (−) or stimulated with IFN-γ (66 U/ml) for 18 hours. Total RNA was extracted by lithium chloride, and 10 μg of RNA was assayed by Northern (RNA) blotting with standard protocols. The blot was probed with either an IRF-1 complementary DNA (cDNA) (upper panel) or a glyceraldehydephosphate dehydrogenase (GAPDH) cDNA (lower panel) labeled by random hexamer priming.

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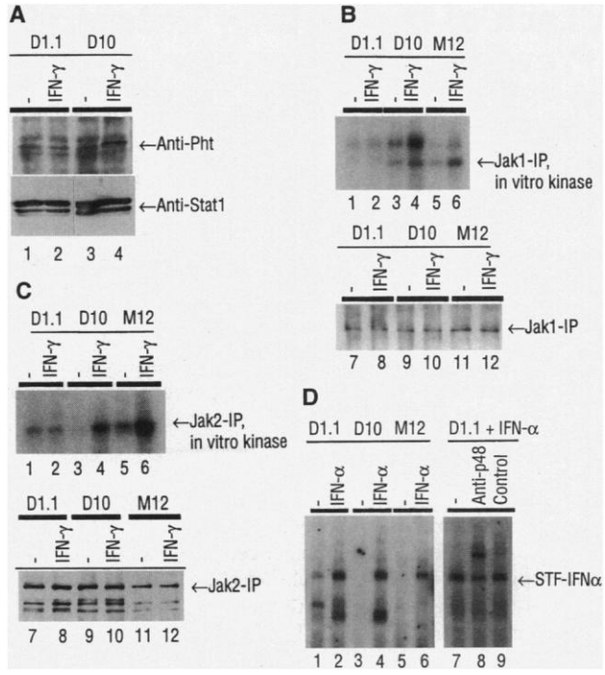


**Fig. 3.** Activation of Stat1, Jak1, and Jak2 in a $T_H2$ but not in a $T_H1$ clone in response to IFN-γ. (**A**) Whole-cell extracts from D1.1 (lanes 1 and 2) or D10 cells (lanes 3 and 4), which were either unstimulated (−) or stimulated with IFN-γ (66 U/ml) for 15 min, were immunoprecipitated with an antiserum to Stat1 (ab1) (*22*) and fractionated on a 7% SDS-polyacrylamide gel before immunoblotting with an antibody to phosphotyrosine (anti-Pht) (antibody 4G10, Upstate Biotechnology) (upper panel). Bands were detected by enhanced chemiluminescence (ECL, Amersham). The blot was then stripped and reprobed with a different antiserum to Stat1 [which recognizes both Stat1α and Stat1β (anti-Stat1)] (ab3) (*22*) (lower panel). (**B**) Extracts from cells of the $T_H1$ clone D1.1 (lanes 1 and 2), the $T_H2$ clone D10.G4.1 (D10) (lanes 3 and 4), or M12 (as a positive control; lanes 5 and 6) were either unstimulated (−) or stimulated with IFN-γ (66 U/ml) for 15 min. The extracts were then immunoprecipitated (IP) with a Jak1 antiserum (*23*) followed by an in vitro kinase assay (lanes 1 to 6) (*24*). An aliquot of this Jak1 immunoprecipitation was immunoblotted with Jak1 antiserum for quantitation (lanes 7 to 12). (**C**) Extracts were prepared as described in (B), immunoprecipitated by Jak2 antiserum (Upstate Biotechnology), and assayed by in vitro kinase reaction (lanes 1 to 6) (*24*). An aliquot of the Jak2 immunoprecipitation was immunoblotted with Jak2 antiserum for quantitation (lanes 7 to 12). (**D**) Activation of the IFN-α–inducible signal transducing factor in $T_H1$ cells. Whole-cell extracts from cells of the $T_H1$ clone D1.1 (lanes 1 and 2) or the $T_H2$ clone D10.G4.1 (D10) (lanes 3 and 4), which were either unstimulated (lanes 1 and 3) or stimulated (lanes 2 and 4) for 20 min with IFN-α (2000 U/ml), were analyzed by EMSA with a probe from the interferon-stimulated response element of ISG15 (O15) (*25*). Extracts from untreated (lane 5) or IFN-α–treated (lane 6) cells of a murine B cell line (M12) were used as a positive control for the induction of STF-IFNα (interferon-stimulated gene factor–3). The identity of STF-IFNα was confirmed by incubation of IFN-α–treated extracts from D1.1 (lane 7) with either an antiserum directed against one of the STF-IFNα components, p48 (*26*) (lane 8), or with a control antiserum to IRF-1 (lane 9) at a 1:20 dilution for 30 min at 4°C after a standard EMSA reaction with the O15 probe for 20 min at room temperature.

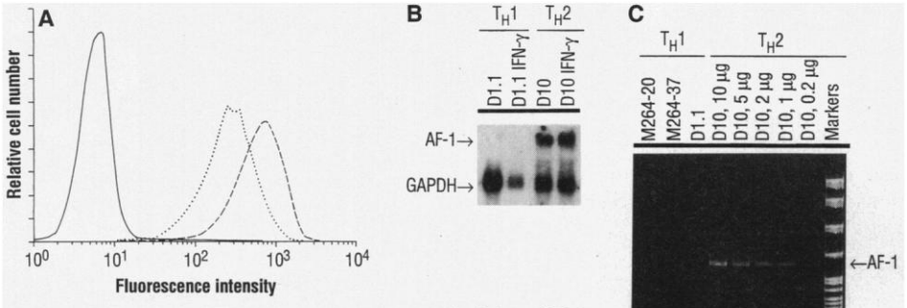


**Fig. 4.** Analysis of the IFN-γ receptor components in $T_H1$ and $T_H2$ clones. (**A**) Cell surface expression of the IFN-γ receptor α chain on representative $T_H1$ and $T_H2$ clones. Cells from the $T_H1$ clone D1.1 (broken line) and the $T_H2$ clone D10.G4.1 (dotted line) were analyzed by FACS with either a rat antibody to mouse IFN-γ receptor (GR-20) (*15*) or an isotype-matched control antibody (solid line) followed by a fluorescein isothiocyanate–conjugated rabbit antibody to rat immunoglobulin G. Clones were analyzed at day 4 after antigen stimulation. (**B**) Total RNA was extracted as described in Fig. 2 and analyzed by Northern blotting with either an AF-1 cDNA (upper panel) or a GAPDH cDNA (lower panel) as a probe. (**C**) RT-PCR analysis of AF-1 mRNA. RT-PCR was performed on three distinct $T_H1$ clones, M264-20 (2 μg), M264-37 (2 μg), and D1.1 (5 μg), as well as on different amounts of D10 (10 μg to 0.2 μg) (*27*). Derivation of M264-20 and M264-37 has been previously described (*28*).

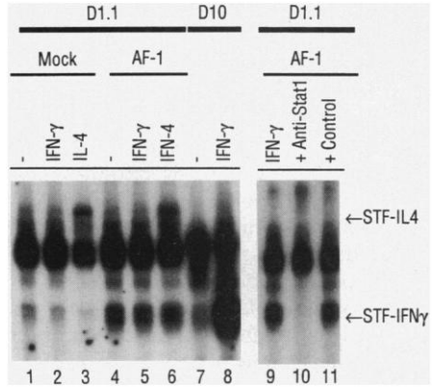


**Fig. 5.** Transient transfection of AF-1 into a murine $T_H1$ clone rescues STF-IFNγ activation in these cells. Cells from a $T_H1$ clone (D1.1), which had either been mock transfected (lanes 1 to 3) or transfected with an AF-1 cDNA plasmid (lanes 4 to 6 and 9 to 11), were either unstimulated (−) or stimulated for 15 min with IFN-γ (66 U/ml) (lanes 2, 5, and 9 to 11) or IL-4 (400 U/ml) (lanes 3 and 6) (*29*). Cells from a $T_H2$ clone (D10), which were either unstimulated (lane 7) or stimulated with IFN-γ (66 U/ml) (lane 8), served as a control. Whole-cell extracts were prepared and examined by EMSA as described in Fig. 1. The identity of STF-IFNγ was confirmed by incubation of IFN-γ–treated extracts from the AF-1–transfected D1.1 with either an antiserum directed against Stat1 (ab1) (lane 10) or with preimmune serum (lane 11) at 1:20 dilution for 30 min at 4°C after a standard EMSA reaction for 20 min at room temperature.

an antiserum to Stat1 followed by immunoblotting with an antibody to phosphotyrosine confirmed that IFN-γ induces tyrosine phosphorylation of Stat1 in a $T_H2$ (D10.G4.1) but not in a $T_H1$ (D1.1) clone (Fig. 3A, upper panel). Reprobing the blot with a different Stat1 antiserum revealed that D1.1 and D10.G4.1 cells contain equal amounts of Stat1 (Fig. 3A, lower panel). Subsequently, antisera to the Janus kinases (Jaks) were used to determine if these proteins were present and activated. Equal amounts of Jak1 (Fig. 3B, lower panel) and Jak2 (Fig. 3C, lower panel) proteins were immunoprecipitated from $T_H1$ and $T_H2$ clones. In vitro kinase assays revealed that IFN-γ induced the autophosphorylation activity of Jak1 (Fig. 3B, upper panel) and Jak2 (Fig. 3C, upper panel) in $T_H2$ cells, but not in $T_H1$ cells. However, $T_H1$ cells responded to IFN-α, by the induction of STF-IFNα (Fig. 3D). As expected, appearance of this complex was blocked by an antiserum against p48, a known component of STF-IFNα (Fig. 3D). Because STF-IFNα induction requires the activation of both Jak1 and Stat1 (*17*), these results demonstrate that in $T_H1$ cells, these components can function normally in response to another ligand. Thus, $T_H1$ cells inhibit IFN-γ signal transduction through a ligand-specific mechanism.

The observation that the IFN-γ, but not the IFN-α, pathway was down-regulated in

$T_H1$ cells prompted us to focus our attention on the IFN-γ receptor (IFN-γR). This receptor is composed of two chains, IFN-γRα and the recently cloned accessory factor–1 (AF-1) (also referred to as IFN-γRβ) (*18*). Although IFN-γRα can, by itself, bind IFN-γ with high affinity (*19*), interaction of this chain with AF-1 is required for IFN-γ–mediated signaling, including the activation of STF-IFNγ and the induction of IRF-1 gene expression (*20*). We therefore examined the $T_H1$ and $T_H2$ clones for the expression of these receptor components. Fluorescence-activated cell sorting (FACS) analysis of cell surface expression of IFN-γRα (Fig. 4A) revealed that the clones contain roughly equal amounts of IFN-γRα chain. However, when we examined these clones for the presence of AF-1–encoding mRNA by Northern (RNA) analysis (Fig. 4B), we found that the $T_H2$, but not the $T_H1$, clone expressed the AF-1 mRNA transcript. Reverse transcription of RNA from three different $T_H1$ clones followed by the polymerase chain reaction (RT-PCR) confirmed the absence of AF-1 expression in these cells (Fig. 4C). To test whether reintroduction of AF-1 expression could rescue IFN-γ signaling in $T_H1$ cells, we transiently transfected a complementary DNA (cDNA) encoding AF-1 into a $T_H1$ clone (D1.1). Transfection of AF-1, but not a mock transfection, led to the appearance of STF-IFNγ in $T_H1$ cells (Fig. 5). Detection of this complex by EMSA was blocked by an antiserum against Stat1 (Fig. 5).

Our data indicate that $T_H1$ cells cannot activate the Jak-STAT pathway in response to IFN-γ because the AF-1 component of the IFN-γ receptor is not expressed. Down-regulation of the IFN-γ signaling pathway in $T_H1$ cells may allow the immune system to selectively inhibit the proliferation of $T_H2$ cells, while permitting $T_H1$ cells to escape the antiproliferative effects of the IFN-γ that they secrete. Preliminary data reveal that precursor T helper cells are able to activate STF-IFNγ. If these cells are cultured in the presence of IFN-γ, the resulting T cell population, which is greatly enriched in $T_H1$ cells, does not activate STF-IFNγ in response to IFN-γ restimulation (*21*). Thus, during differentiation into $T_H1$ cells, T cells may lose the capacity to activate STF-IFNγ. This finding is consistent with a model in which modulation of cytokine signaling may play an important role in the acquisition of specific T helper cell phenotypes.

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24. Immunoprecipitates were washed twice in kinase buffer (20 mM Hepes, pH 7.4, 2 mM $MnCl_2$, 10 mM $MgCl_2$, 1 mM dithiothreitol, 150 mM NaCl, 0.1 mM sodium orthovanadate, and 0.4 mM phenylmethylsulfonyl fluoride), then resuspended in 40 μl of kinase buffer and subjected to an in vitro kinase reaction with [γ-$^{32}$P]adenosine triphosphate (ATP) as previously described [O. Colamonici, H. Uyttendaele, P. Domanski, H. Yan, J. Krowleski, *J. Biol. Chem.* **269**, 3518 (1994)].
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27. The cDNA was obtained by reverse transcription with the antisense primer 5′-AAT ACT TGT AGC ATC CAG AA-3′. Half of the cDNA was then subjected to PCR amplification with the above primer and the following sense primer: 5′-GAA CAA ATC GAA GAG TAT CT-3′. All PCR was performed with 25 cycles consisting of 1 min at 94°C, 1 min at 44°C, and 1.5 min at 72°C with a 7-min extension at 72°C for the last cycle. DNA amplification products were analyzed by acrylamide gel electrophoresis.
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29. Transient transfections of D1.1 were carried out by electroporation as previously described [J. Lederer, J. Liou, M. Todd, L. Glimcher, A. Lichtman, *J. Immunol.* **152**, 77 (1994)] except that a Cell-Zap II instrument (Andersen System) and 40-μF capacitance were used. Cells were assayed 48 hours after transfection. The constitutive STF-IFNγ DNA binding activity detected in extracts of AF-1–transfected D1.1 is likely to be due to the presence of endogenous IFN-γ secreted by these cells. Addition of a neutralizing IFN-γ antibody to the culture of the AF-1–transfected D1.1 decreased the amount of STF-IFNγ in untreated cells.


30. We thank C. Cepko for the Jak1 antiserum; D. Levy for the p48 antiserum; S. Pestka for the AF-1 cDNA; K. Calame, N. Braunstein, and L. Chess for critically reading the manuscript; and S. Mauze for technical assistance. Supported by NIH (P.R. and C.S.), the James S. McDonnell Foundation (C.S.), Warner Lambert Grant, the Pew Scholars Program, the Stephen I. Morse Fellowship (A.P.), and the American Academy of Allergy and Immunology (A.P.). DNAX Research Institute is supported by the Schering-Plough Corporation.

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## TECHNICAL COMMENTS

# Seasonal Precipitation Timing and Ice Core Records

Commenting on our work with isotope tracers and the origin of moisture in general circulation model simulations (*1*), Eric J. Steig *et al.* (*2*) suggest that changes in the seasonal distribution of precipitation may provide strong control on isotopic variability in Greenland ice cores. In principle, we agree with the thrust of their comment. In a broad sense, without consideration of specific processes, the seasonality effects discussed by Steig *et al.* and the moisture source effects described in our report are two classes of the same general phenomenon: evaporation, distillation, and transport of isotopes over different temperature regimes. Although the analysis of Steig *et al.* for Greenland precipitation over the last century suggests that seasonal effects are a significant component of interannual isotopic variability, general circulation models (GCMs) represent one of the few means of assessing the importance of this phenomenon for interpreting the isotopic record over glacial cycles. The GCM approach is important for understanding the relationship between $\delta^{18}O$ and temperature because (i) thermodynamic principles and analysis of modern isotopic data suggest that present-day spatial $\delta^{18}O$-temperature correlations cannot be considered an exact surrogate for the temporal relationship between these variables and (ii) geographic isotopic variability—for example, the differences in isotopic values among ice