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Ikaros Is a Regulator of Il10 Expression in CD4+ T Cells

Sarah E. Umetsu and Susan Winandy

IL-10 is a regulatory cytokine critical for controlling inflammatory responses. Here we show that Ikaros, a zinc finger DNA-binding protein, plays an important role in the regulation of Il10 in murine CD4+ T cells. Upon initial stimulation of the TCR, T cells deficient in Ikaros express significantly lower levels of IL-10 compared with wild-type T cells. In addition, under Th2 skewing conditions, which induce IL-10 production by wild-type T cells, Ikaros null T cells are unable to properly differentiate, producing only low levels of IL-10. Expression of a dominant-negative isoform of Ikaros in wild-type Th2 cells represses IL-10 production but does not significantly alter expression levels of the genes encoding the transcription factors GATA-3 and T-bet. Furthermore, expression of Ikaros in Ikaros null T cells restores expression of the Th2 cytokines IL-10 and IL-4 while reducing production of the Th1 cytokine, IFN-γ. Coexpression of Ikaros and GATA-3 further increases IL-10 production, showing that these two factors have an additive effect on activating Il10 expression. Finally, we show that Ikaros binds to conserved regulatory regions of the Il10 gene locus in Th2 cells, supporting a direct role for Ikaros in Il10 expression. Thus, we provide evidence for Ikaros as a regulator of Il10 and Ifng gene expression and suggest a role for Ikaros in directing lineage-specific cytokine gene activation and repression. The Journal of Immunology, 2009, 183: 5518–5525.
addition, Ikaros binds directly to IL10 regulatory regions, suggesting a direct mechanism of regulation. We also demonstrate that Ikaros is required for the regulation of expression of IFN-γ in both Th1 and Th2 cells. Significantly, expression of Ikaros or GATA-3 in Ikaros null Th2 cells, which aberrantly express IFN-γ, is equivalently potent in restoring a normal Th2 cytokine expression profile.

Materials and Methods

Mice
Ikaros null (Ik−/−) mice (BALB/c background) were generated by intercrossing of Ikaros null heterozygotes (Ik−/+). Genotypes were assessed by PCR analysis of tail DNA as described previously (33). All animal procedures were approved by the Northwestern University Animal Care and Use Committee.

ELISA
Supernatants were collected from triplicate wells of activated T cells between 18 and 40 h. IL-2, IFN-γ, and IL-10 were detected by mouse ELISA kits (eBioscience) following guidelines provided by the kit.

Cell purification
For bulk CD4+ T cell isolation, CD4+ T cells were purified from pooled spleens of wild-type or Ikaros null mice using CD4+ T Cell Isolation Kit (Miltenyi Biotec). For naïve T cell isolation, CD4+CD62L+ cells were purified from pooled spleens of Ikaros wild-type and Ikaros null mice using the CD4+CD62L+ T Cell Isolation Kit (Miltenyi Biotec). Purity was 95%.

In vitro T cell differentiation
Purified cells were stimulated with plate-bound anti-CD3 (clone 2C11) and soluble anti-CD28 (clone 37.5.1) in RPMI 1640 medium supplemented with 10% FCS, 50 μM 2-ME, and 500 U of penicillin-streptomycin per milliliter (complete RPMI 1640). For Th1 differentiation, T cells were cultured with IL-12 (1 ng/ml; PeproTech) and anti-IL-4 (5 μg/ml). For Th2 differentiation, T cells were cultured with IL-4 (10 ng/ml; PeproTech) and anti-IFN-γ (5 μg/ml).

Intracellular cytokine staining and flow cytometric analyses
Polarized Th1 or Th2 cells were restimulated with PMA (20 ng/ml) and ionomycin (500 ng/ml) for 5 h in the presence of GolgiPlug (BD Pharmingen). Cells were first stained with fluorochrome-conjugated anti-CD4 or anti-H-2Kk (Miltenyi Biotec) and then fixed and permeabilized with Cytofix/Cytoperm solutions (BD Pharmingen), followed by intracellular staining with fluorochrome-conjugated anti-IFN-γ, anti-IL-4, and/or anti-IL-10.

All Abs were from eBioscience unless otherwise stated. Abs were FITC, PE or allophycocyanin conjugates. Cells were analyzed on a flow cytometer (FACSCanto; BD Biosciences) using FlowJo software (Tree Star).

Protein preparation and immunoblotting
Protein extracts were prepared by whole-cell lysis with 420 mM NaCl lysis buffer (20 mM Tris (pH 7.5), 0.1% BSA, 1 mM EDTA, and 1% Nonidet P-40) supplemented with protease inhibitors. Abs against Ikaros (H-100, sc-13039X, Santa Cruz Biotechnology) were diluted 1/4000 and incubated with the membrane at room temperature for 1 h.

mRNA isolation and quantitative real-time PCR
Total RNA was isolated from the cells using the SV Total RNA Isolation System (Promega). cDNA was generated with a Superscript III Kit (Invitrogen). Quantitative PCR was performed on cDNA using iQ SYBR Green (Bio-Rad) and the BioRad MyiQ Real-Time PCR machine. Results were analyzed using the Pfaffl method. Data are shown as ratios (E(target))−CT,target/ (E(reference))−CT,reference, where E = efficiency of PCR; target = gene of interest; and reference = HPRT. Primers were generated by the Beacon Design program and synthesized by IDT DNA Technologies. Primer sequences are available upon request.

Retroviral transduction
Retroviruses were produced by transfecting retroviral constructs into the Phoenix packaging cells using Lipofectamine reagent (Invitrogen). Viral supernatants harvested at 48 and 72 h posttransfection were used to infect T cell cultures using 1 ml of viral supernatant supplemented with 4 μg/ml polybrene. Plates were centrifuged at 500 × g for 2 h at 32°C. Supernatants were removed, and cells were recultured in complete RPMI 1640. Successfully transduced cells were sorted by their expression of the H-2Kk marker using H-2Kk MicroBeads (Miltenyi Biotec). Purity was assessed using the MACSelect control Ab. Purity was consistently >90%.

Chromatin immunoprecipitation (ChiP)
Chromatin immunoprecipitation (ChiP) was performed using chromatin prepared from Th2 cells. Briefly, 5 × 10^6 cells were used per sample. Cells were cross-linked with 1% formaldehyde for 10 min at room temperature. Cells were washed, lysed, and sonicated (Fisher Scientific Sonic Dismembrator Model 100) to sheath DNA. Samples were precleared with protein G-agarose/salmon sperm beads (16-201; Millipore). Protein-DNA complexes were immunoprecipitated using the ChiP Assay Kit (17-295; Millipore) and Abs against Ikaros (M-20, sc-9859-X; Santa Cruz Biotechnology). Complexes were collected with Protein G agarose/salmon sperm beads and washed. Protein-DNA complexes were eluted off the beads and cross-links were reversed by heating at 65°C overnight. DNA was recovered by phenol-chloroform extraction and precipitated by ethanol. qPCR analyses were performed on immunoprecipitated DNA and normalized to total chromatin input using the Pfaffl method.

Results
Ikaros null T cells have defects in cytokine production upon initial TCR stimulation
Ikaros has recently been shown to be a transcriptional regulator of IL2 expression in anergic T cells and IL4 expression in Th2 cells (2–4). It is not clear, however, if Ikaros is important in regulating cytokines in naïve, undifferentiated cells. Therefore, Ikaros’ role in regulating cytokine expression during initial in vitro TCR stimulation was examined. For these studies, we used a previously established mouse line with a deletion at the C terminus of the Ikaros gene that removes the last translated exon (Ikaros null) (33). In these mice, Ikaros proteins are not detected at the cellular level.

CD4+ T cells were purified from wild-type and Ikaros null mice and stimulated with increasing concentrations of anti-CD3 and anti-CD28. The culture supernatants were collected, and cytokine levels (IL-2, IFN-γ, and IL-10) were quantified by ELISA (Fig. IA). Ikaros null T cells produced 5- to 40-fold higher levels of IL-2 and 3- to 5-fold higher levels of IFN-γ compared with wild-type T cells under the same conditions. In contrast, Ikaros null T cells expressed significantly reduced levels of IL-10 (Fig. IA).

Expression of cytokine genes was examined in purified naïve CD4+ T cells and from CD4+ T cells following activation for 24 h with anti-CD3 and anti-CD28 (Fig. IB). As seen at the protein level, unstimulated wild-type and Ikaros null T cells both had very low levels of basal cytokine gene expression, suggesting that in the absence of stimulation lack of Ikaros is not sufficient to allow for increased expression of cytokines. Following activation, there was increased expression of IL2, Ifng, and TNF-α and decreased expression of Il4, Il10, and Il13 in Ikaros null T cells compared with wild-type T cells. Thus, Ikaros null T cells exhibited enhanced production of Th1-type cytokines and decreased production of Th2-type cytokines during an early phase of TCR activation.

Our hypothesis is that Ikaros directly regulates the expression of multiple cytokine genes. However, it is possible that the aberrant levels of cytokine production by Ikaros null T cells is a result of either developmental defects or enhanced responses in vivo, which causes the Ikaros null T cells to be in a more activated state than

3 Abbreviations used in this paper: ChiP, chromatin immunoprecipitation; Treg, regulatory T.
Ikaros REGULATES IL10 IN T CELLS

Ikaros null T cells have defects in cytokine production upon initial TCR stimulation. A, CD4+ T cells were purified from the spleen of wild-type (Ik+/+) and Ikaros null (Ik−/−) mice and stimulated with increasing concentrations of anti-CD3 along with 5 μg of anti-CD28 Abs. Supernatants were taken at 18 h and analyzed by ELISA for the presence of IL-2, IFN-γ, and IL-10. Data represent three separate experiments using three pooled spleens per group. ELISAs were performed in duplicate from triplicate culture wells. B, RNA was purified from naive CD4+ T cells and from cells after activation for 24 h with 5 μg of anti-CD3 and 5 μg of anti-CD28. Message levels of cytokines were examined by quantitative real-time PCR. *, (p < 0.05) p values were calculated using paired Student’s t test. Data represent three separate experiments using three pooled spleens per group. C, CD4+ cells from spleens of wild-type (shaded) and Ikaros null mice (dark line) were stained for CD62L, CD69, CD25, and Foxp3 expression and analyzed by flow cytometry. D, CD4+CD62L+ T cells were isolated from the spleens of wild-type (Ik+/+) and Ikaros null (Ik−/−) mice and stimulated with increasing concentrations of anti-CD3 along with 5 μg of anti-CD28 Abs. Supernatants were analyzed by ELISA for the presence of IL-2, IFN-γ, and IL-10. Data represent three separate experiments using three pooled spleens per group. ELISAs were performed in duplicate from triplicate culture wells.

Because of the dramatic decrease in IL-10 expression observed in Ikaros null T cells upon initial stimulation, we next wanted to examine their potential to produce IL-10 under conditions that promote high levels of IL-10 production. Therefore, wild-type and Ikaros null T cells were isolated and cultured under Th2 conditions, because Th2-differentiated cells produce high levels of IL-10 (15). After 5 days, cells were restimulated, stained for intracellular cytokines, and analyzed by flow cytometry. As previously reported, Ikaros null T cells produced significantly increased levels of IL-2 and IFN-γ and decreased levels of IL-10 as compared with wild-type T cells.

Ikaros null T cells are unable to produce IL-10 after Th2 differentiation

If Ikaros is required for IL-10 expression, we hypothesized that decreasing Ikaros function in wild-type T cells should reduce their ability to produce IL-10. To test this, Ikaros or a dominant-negative Ikaros isoform (DN-Ik-7) that interferes with Ikaros activity, was expressed in wild-type Th2 cells using a retroviral transduction system, and the effect on IL-10 expression was examined. Ik-1 is the largest and most predominantly expressed Ikaros isoform in T cells and has been shown in ectopic expression and reporter assays to have the highest transcriptional activation and repression activity (6, 7, 11, 35). DN-Ik-7 is an engineered Ikaros isoform that lacks the N-terminal DNA binding domain and therefore is unable to bind DNA but has the ability to dimerize with wild-type Ikaros their wild-type counterparts. Therefore, Ikaros null CD4+ T cells were examined for the expression of T cell markers that are associated with activation state. Naive T cells express high levels of CD62L and do not express CD25 or CD69. Analyses of cell surface marker expression levels by flow cytometry revealed that wild-type and Ikaros null CD4+ T cells express similar levels of CD62L and CD69. Interestingly, a slightly lower percentage of Ikaros null CD4+ T cells express CD25 compared with wild-type T cells. CD25 can also be a marker for regulatory T (Treg) cells. Therefore, this lower percentage of CD25+ T cells could represent a decrease in the numbers of Treg cells in Ikaros null spleens. To determine whether this was the case, intracellular staining for the Treg-associated transcription factor Foxp3 was performed. Wild-type and Ikaros null mice had an equal percentage of CD4+ T cells expressing Foxp3 (Fig. 1C). Therefore, there was not a deficiency in Foxp3+ Treg cells, although Ikaros null Treg cells express lower levels of CD25 compared with their wild-type counterparts. Taken together, these data indicate that Ikaros null T cells are naive and not inappropriately activated in vivo.

However, to ensure that differences in cytokine expression observed in Ikaros null T cells was not due to a preactivated state, naive CD4+CD62L+ T cells were purified from wild-type and Ikaros null mice. Purified T cells were stimulated with increasing concentrations of anti-CD3 and anti-CD28. The culture supernatants were collected at 24 and 48 h, and cytokine levels were quantified by ELISA (Fig. 1D). As seen in the bulk CD4+ sorted cells (Fig. 1A), Ikaros null T cells produced significantly increased levels of IL-2 and IFN-γ and decreased levels of IL-10 as compared with wild-type T cells.

Interfering with Ikaros activity in wild-type Th2 cells decreases IL-10 expression without altering key transcription factor levels

If Ikaros is required for IL-10 expression, we hypothesized that decreasing Ikaros function in wild-type T cells should reduce their ability to produce IL-10. To test this, Ikaros or a dominant-negative Ikaros isoform (DN-Ik-7) that interferes with Ikaros activity, was expressed in wild-type Th2 cells using a retroviral transduction system, and the effect on IL-10 expression was examined. Ik-1 is the largest and most predominantly expressed Ikaros isoform in T cells and has been shown in ectopic expression and reporter assays to have the highest transcriptional activation and repression activity (6, 7, 11, 35). DN-Ik-7 is an engineered Ikaros isoform that lacks the N-terminal DNA binding domain and therefore is unable to bind DNA but has the ability to dimerize with wild-type Ikaros
and inhibit its ability to bind DNA (7). cDNA sequences of Ik-1 and DN-Ik-7 were subcloned into the murine stem cell virus vector (MSCV-ires-H-2Kk), which contains an IRES to allow cotranslation of Ikaros and H-2Kk, a truncated MHC class I protein, from one transcript. Thus, H-2Kk is expressed on the surface and can be used to mark and sort successfully transduced cells.

CD4+ T cells were isolated from wild-type mice, stimulated, and differentiated under Th2-polarizing conditions. After 5 days, cells were restimulated and transduced with retroviruses containing the negative control vector that encodes no Ikaros protein (MSCV), Ikaros (Ik-1), or the dominant-negative Ikaros isoform (DN-Ik-7). Cells were rested for 2 days and then restimulated, stained for intracellular cytokines, and analyzed by flow cytometry. To confirm the presence of Ik-1 and DN-Ik-7, protein extracts were made from the H-2Kk expressing cells and analyzed by Western blot (Fig. 3A). Endogenously expressed Ikaros is observed in all three lanes. Levels of transduced Ik-1, which runs at a slightly higher m.w. than endogenous Ikaros due to the presence of a flag-epitope tag, and DN-Ik-7, which runs ~10 kDa lower, are expressed at roughly equivalent levels to those of the endogenous Ikaros protein (Fig. 3A).

In the presence of DN-Ik-7, the percentage of IL-10 producing T cells was significantly reduced (Fig. 3B). This was consistent over multiple experiments (Fig. 3C). IL-10 expression was not significantly affected by overexpression of Ik-1 (data not shown), possibly due to the fact that endogenous Ikaros levels are already high and thus overexpression does not have an observable effect. Cells expressing DN-Ik-7 had decreased levels of Il10 message compared with cells expressing MSCV, suggesting that Ikaros is functioning at the level of transcription to affect IL-10 expression (Fig. 3D).

Ikaros null T cells differentiated under Th2 conditions express lower levels of Gata3 and higher levels of Tbet compared with wild-type Th2 cells (4). This suggests that Ikaros could indirectly control Il10 expression through the regulation of Gata3 or Tbet expression. To eliminate this possibility, sorted MSCV- and DN-Ik-7-transduced cells were analyzed for the expression of the gene encoding the Th1 transcription factor T-bet and the gene encoding the Th2 factor GATA-3 (Fig. 3E). There was no significant difference in the expression of these factors between cells transduced with control MSCV or DN-Ik-7. Thus, Ikaros appears to regulate IL-10 expression, without altering expression levels of Gata3 or Tbet, suggesting a more direct mechanism.

Expression of Ikaros or GATA-3 in Ikaros null T cells restores IL-10 expression

If Ikaros is needed for IL-10 expression in Th2 cells, reintroduction of Ikaros into Ikaros null T cells should restore IL-10 expression. To test this, Ikaros null T cells were cultured under Th2 conditions for 24 h and then transduced with the negative control vector MSCV or Ik-1. In cells transduced with Ik-1, the percentage of cells expressing IL-10 was significantly increased compared with cells transduced with MSCV, suggesting that Ikaros could at least partially restore the defect in IL-10 expression (Fig. 4A). In addition, in cells transduced with Ik-1, the percentage of cells expressing IFN-γ was decreased, and the percentage of cells expressing IL-4 was increased compared with cells transduced with MSCV (Fig. 4A). Because normal Th2 cytokine expression was largely restored solely by restoring Ikaros expression, it further supports our hypothesis that the defect in cytokine expression seen in Ikaros null T cells in not due to a defect in thymic development but to a requirement for Ikaros in peripheral T cell function.

Because Ikaros null Th2-polarized cells have lower levels of Gata3 expression compared with their wild-type counterparts (4),
it was examined if restoring wild-type levels of GATA-3 in Ikaros null T cells could also increase IL-10 production. Similar to the previous experiment, Ikaros null T cells were cultured under Th2 conditions for 24 h and then transduced with a retroviral vector containing \( \text{Gata3} \) cDNA (MSCV-Gata3-IRES-GFP) (36). Transduction of GATA-3 in these cells resulted in an increase in the percentage of cells producing IL-10 as well as IL-4 and a decrease in the percentage of cells producing IFN-\( \gamma \) (Fig. 4A). Notably, the effect of GATA-3 on increasing IL-10 and IL-4 was not as strong as with Ik-1, but GATA-3 appeared to be more effective in suppression of IFN-\( \gamma \).

To determine whether Ikaros and GATA-3 could work collaboratively to increase IL-10 expression, both factors were transduced into Ikaros null Th2-polarized cells. GATA-3 and Ik-1 were additive in their abilities to increase IL-10 expression, suggesting that they may act to increase IL-10 expression in a nonlinear pathway (Fig. 4B). Alternatively, they may act coordinately, but increased expression of one can partially overcome lack or decreased expression of the other.

In all of these experiments, transduction of Ik-1 was performed at 24 h, which is before Th2 differentiation is complete. To determine whether introduction of Ikaros at a later time point would also result in restoration of IL-10 production, Ikaros null T cells were cultured for 5 days under Th2-polarizing conditions and then transduced with Ik-1. Cells transduced with Ik-1 showed an increase in the percentage of cells producing IL-10 compared with cultures transduced with the negative control vector MSCV (Fig. 4C), providing further support that Ikaros’ role in regulating \( \text{Il10} \) is not through altering expression of these factors.

Interfering with Ikaros activity in wild-type Th1 cells increases IFN-\( \gamma \) expression

To determine whether Ikaros could activate the production of IL-10 in cells that would not normally be expressing IL-10, the effect of overexpression of Ikaros in Th1 cells was examined. Wild-type CD4\(^+\) T cells were stimulated and differentiated under Th1 conditions. After 5 days, cells were restimulated and transduced with negative control vector MSCV or Ik-1. Overexpression of Ik-1 could not drive expression of IL-10 (Fig. 5A) or IL-4 (data not shown) in the Th1 cells.

Ikaros null T cells expressed high levels of IFN-\( \gamma \) upon initial stimulation (Fig. 1) as well as under Th2-polarizing conditions (Fig. 2A) and (4). In addition, introduction of Ik-1 into Ikaros null T cells cultured under Th2-polarizing conditions was able to suppress IFN-\( \gamma \) expression (Fig. 4A). To determine whether interfering with Ikaros activity in wild-type Th1 cells could alter the production of IFN-\( \gamma \), DN-Ik-7 was expressed in differentiated Th1 cells. Cells expressing DN-Ik-7 showed an increased percentage of...
cells expressing IFN-γ compared with cells expressing the negative control vector (Fig. 5B). Thus, in Th1 cells, although overexpression of Ikaros could not drive inappropriate IL-10 expression, interfering with Ikaros activity with DN-Ik-7 could increase IFN-γ production.

**Ikaros binds to the II10 promoter and intronic regulatory regions**

An analysis of II10 sequence alignments across multiple species was performed using the program rVISTA (rvista.dcode.org). Several Ikaros consensus binding sites (GGGAA) were identified within highly conserved regulatory regions of the II10 locus (Fig. 6A). A highly conserved site in the proximal promoter (−901 bp) is located within 40 bp of a known GATA-3 binding site (28) (Fig. 6B). To assess whether Ikaros binds to these sites, ChIP experiments were performed using chromatin prepared from Th2-polarized wild-type T cells, because these cells express high levels of IL-10. Ikaros binding was observed at a consensus binding site located in the proximal promoter (site B), as well as at a site located in intron 4 (site C) (Fig. 6C), providing strong evidence for a direct role of Ikaros in regulating II10 expression.

**Discussion**

Effector function of CD4+ T cells requires selective activation and repression of multiple cytokine genes to generate immune responses specifically suited to particular invading organisms. Regulation of these cytokine genes requires an array of transcription factors that work to bring about stable gene expression programs in differentiated T cells. Recent reports have demonstrated that Ikaros is required for repression of II2 in anergic T cells and activation of II4, II5, and II13 in Th2 cells. In this study, we demonstrate that Ikaros functions as an activator of II10 and a repressor of Ifng in T cells in both the naive and differentiated states. These data suggest that Ikaros is at the center of regulatory networks controlling programs of cytokine expression in T cells, and that its role is independent of T cell differentiation. IL-10 is produced at high levels by Th2 cells but can also be produced by all other T cell subsets under certain conditions, and so it is likely regulated independently of the Th2 cytokine locus (encoding II4, II5, and II13), which is more restricted in its expression. Ikaros null T cells show a deficiency of IL-10 production at both the protein and message level following initial TCR stimulation, demonstrating that Ikaros activity is required for the activation of II10. Ikaros is also important for IL-10 expression after a T cell has undergone Th2 differentiation, as demonstrated by the dramatic decrease in IL-10 expression in Ikaros null Th2 cells as compared with their wild-type counterparts (Fig. 2). In this case, Ikaros null T cells have differentiated in the absence of Ikaros, and therefore, differences in IL-10 expression could be due to abnormalities in their ability to undergo differentiation. However, expression of DN-Ik-7 in differentiated wild-type Th2 cells causes a decrease in the percentage of cells expressing IL-10, providing evidence that this is not the case.

Under Th2-polarizing conditions, Ikaros null T cells showed significant overproduction of IFN-γ and a deficiency in production of IL-10 and IL-4. However, they also express significantly
Because both Ikaros and GATA-3 are expressed in Th2 cells, they are more effective in activating IL-10 expression than Ikaros and GATA-3 are transduced together into Ikaros null Th2 cells, suggesting that Ikaros plays a direct role in regulating Th2 cell differentiation program. When both Ikaros and GATA-3 are expressed in Th2 cells, they are more effective in activating IL-10 expression than is alone (Fig. 4B). Because both Ikaros and GATA-3 are known to bind to the Il10 locus, they may act coordinately. In both the Ikaros promoter and intronic regions, GATA-3 and Ikaros binding sites are in close proximity (Fig. 6). This close proximity of GATA-3 and Ikaros sites has also been observed in regulatory regions of Il4 (1, 4) and Ifng (37). In addition, Ikaros has recently been found to directly interact with GATA-1 in developing erythroid cells (38). This interaction with Ikarost enhances GATA-1 binding to regulatory regions across the β-globin locus (38). Thus, it is possible that Ikaros interacts with GATA-3 in a similar manner. Cooperative protein-protein and also protein-DNA interactions may stabilize an Ikaros-GATA-3 DNA binding complex and enhance the function of GATA-3.

Additionally, we demonstrate that Ikaros functions as a repressor of Ifng, in naïve T cells, as well as Th1 and Th2 cells. Reintroduction of Ikaros into Ikaros null Th2-polarized cells is sufficient to increase IL-10 and IL-4 production and decrease IFN-γ production. It is striking that reintroduction of Ikaros alone can revert the abnormal Th1 cytokine expression pattern of high levels of IFN-γ and reduced IL-4 and IL-10 observed in Ikaros null Th2-polarized cells into a more normal Th2 cytokine expression pattern, increasing IL-4 and IL-10 expression and reducing IFN-γ levels. It is important to restate that this effect is independent of affecting levels of Gata3 and Thet expression (Fig. 4D). Transcription factors can be important early on in T cell differentiation to set up a cytokine gene locus for expression or late in differentiation for acute gene expression, or they may play both roles. In the case of Il10, our data support a model whereby Ikaros controls gene expression acutely and not solely as a factor targeting locus conformation during differentiation. First, expression of DN-Il-7 in fully differentiated wild-type Th2 cells causes a decrease in the percentage of cells expressing IL-10, suggesting that Ikaros activity is still important after differentiation and reprogramming of the gene locus has occurred. In addition, reintroduction of Ikaros was effective in increasing IL-10 production when it was introduced at 24 h poststimulation as well as when it was introduced 5 days postdifferentiation.

Our findings, along with Ikaros’ role in activating the Th2 locus and repressing Ifng, shows Ikaros to be an essential element in the regulation of multiple cytokines critical for T cell effector function.

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Disclosures
The authors have no financial conflict of interest.

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