The Cell Type-Specific Expression of the Murine IL-13 Gene Is Regulated by GATA-3

Hiroko Kishikawa, Jenny Sun, Andrew Choi, Shi-Chuen Miaw and I-Cheng Ho

*J Immunol* 2001; 167:4414-4420; doi: 10.4049/jimmunol.167.8.4414

http://www.jimmunol.org/content/167/8/4414

References

This article cites 29 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/167/8/4414.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
The Cell Type-Specific Expression of the Murine IL-13 Gene Is Regulated by GATA-3

Hiroko Kishikawa,* Jenny Sun,† Andrew Choi,* Shi-Chuen Miaw,* and I-Cheng Ho2a†

IL-13, a Th2 cell-specific cytokine, is a major effector molecule mediating several pathological features of allergic asthma. However, the transcriptional regulation of the IL-13 gene remains unclear. Here we demonstrate, by using intracellular cytokine staining, that IL-13 is not always coexpressed with other Th2 cytokines in normal Th cells on a single cell basis. In addition, we identified and cloned a minimal inducible and cell type-specific promoter of the murine IL-13 gene. The cell type specificity of the minimal IL-13 promoter is mediated by a functionally critical GATA-3 site that binds endogenous GATA-3 proteins, whereas the induction by PMA/ionomycin is mediated by distinct cis-acting elements. Furthermore, by expressing GATA-3 in wild-type and c-maf transgenic Th1 cells, we demonstrate that the expression of IL-13 is regulated by a mechanism distinct from that regulating the expression of IL-4, and that the expression of Th1 and Th2 cytokine genes does not have to be mutually exclusive in effector Th cells. The Journal of Immunology, 2001, 167: 4414–4420.

Type 2 CD4+ helper T (Th2) cells play critical roles in the pathogenesis of allergic asthma, and cytokines produced by Th2 cells mediate several pathological features of allergic asthma (1, 2). For example, IL-4 and IL-13 enhance the production of IgE and trigger the airway remodeling process (3, 4), and IL-5 is a potent chemoattractant for eosinophils (5).

Recent studies suggest that IL-13 might play a more important role than IL-4 or IL-5 as an effector molecule in the pathogenesis of allergic asthma. Adoptive transfer of Ag-specific Th2 cells derived from IL-4-deficient mice still induced airway hyperresponsiveness (AHR) and overproduction of mucus (6, 7). Blockade of IL-13, even after the development of allergic asthma in an animal model, ablated AHR and mucus overproduction (8, 9). In contrast, IL-4 blockade prevented the development of allergic asthma only when applied during the phase of sensitization (10). Furthermore, administration of rIL-13 to airways induced AHR, influx of eosinophils, mucus production, and an increase in serum total IgE (8, 9).

Although the roles of IL-13 in the pathogenesis of allergic asthma have been well studied, very little is known about the transcriptional regulation of the IL-13 gene. The expression of most of the Th2 cytokine genes, such as IL-4 and IL-5, is regulated at the level of transcription and is induced by stimulation with anti-CD3 or PMA/ionomycin (iono). Sequence analysis revealed that the sequences immediately upstream of the first exon of the IL-13 gene are highly conserved through evolution and contain potential binding sites for NF-AT, GATA, and AP-1 (11); however, the functional significance of these binding sites has not been investigated. The IL-13 gene maps to the same chromosomal locus as the IL-4 and IL-5 genes, and the expression of these Th2 cytokine genes is directly related to the chromosomal accessibility of this locus. Indeed, several Th2 cell-specific DNaseI hypersensitivity sites (DHSs) have been identified along the IL-13/IL-4/IL-5 locus (12). Three of the DHSs are located in the IL-13 gene; however, their roles in regulating the expression of the IL-13 gene remain elusive.

Recently, it was shown that the intergenic region between IL-4 and IL-13 functions as a coordinate regulator of IL-4, IL-5, and IL-13 (13). Despite close chromosomal linkage of the Th2 cytokine genes, several pieces of evidence suggest that there is significant heterogeneity of cytokine gene expression by individual Th cells or clones (14–16). However, the IL-13 gene was not examined in these studies, and it remains unclear whether IL-13 is always coexpressed with IL-4 or IL-5 in normal Th2 cells on a single cell basis.

In agreement with the heterogeneity in cytokine expression, previous studies have suggested that each of the Th2 cytokine genes might be regulated by independent mechanisms. For example, it was shown that the sequence-specific binding of GATA-3, a Th2 cell-specific transcription factor, is critical for the expression of the IL-5 gene (17, 18), whereas a high level of IL-4 gene expression requires the presence of c-maf, another functionally critical Th2 cell-specific transcription factor (19, 20). In addition, Th2 cells derived from c-maf-deficient mice continue to produce IL-5 and IL-13 at levels comparable to those of wild-type (WT) Th2 cells (21). Developing Th1 cells derived from GATA-3 transgenic mice expressed several Th2 cytokine genes, but not IL-13. In contrast, overexpression of an anti-sense GATA-3 in a Th2 clone, D10, inhibited the expression of almost all Th2 cytokine genes, including IL-13 (22). In a transgenic mouse model, the expression of IL-13 and other Th2 cytokines was significantly reduced in Th2 cells derived from mice carrying a “dominant negative” GATA-3 transgene. However, the mechanism that leads to this effect remains unclear because the mutant GATA-3 also inhibited the expression of IFN-γ and was as potent as WT GATA-3 in inducing the expression of Th2 cytokine genes in vitro (23, 24). Although...

---

*Department of Immunology and Infectious Diseases, Harvard School of Public Health, and †Department of Medicine, Harvard Medical School, Boston, MA 02115

© 2001 by The American Association of Immunologists

Received for publication May 29, 2001. Accepted for publication August 8, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by a Junior Award from the Sandler Program for Asthma Research (to I.C.H.), a postdoctoral training grant in cancer immunology from the National Institutes of Health (to H.K.), and a Howard Hughes medical student research training fellowship award (to J.S.).

2 Address correspondence and reprint requests to Dr. I-Cheng Ho, Department of Immunology and Infectious Diseases, Harvard School of Public Health, FWH, Room 205, 652 Huntington Avenue, Boston, MA 02115. E-mail address: ihoch@hsph.harvard.edu

3 Abbreviations used in this paper: AHR, airway hyperresponsiveness; CS, consensus sequence; DHS, DNaseI hypersensitivity site; iono, ionomycin; RV, retroviral vector; ds, double-stranded; GFP, green fluorescent protein; mafTG, c-maf transgenic; WT, wild type.
forced expression of WT GATA-3 in developing Th1 cells resulted in the expression of Th2 cytokine genes, including IL-13 (23), it is unclear whether GATA-3 directly transactivates the IL-13 promoter or indirectly induces IL-13 expression by redirecting the differentiation program into the Th2 pathway.

Here we report that the murine IL-13 gene is not always coexpressed with the IL-4 and IL-5 genes in normal Th2 cells on a single cell basis. In addition, we identified a 2-kb genomic fragment, containing the murine IL-13 promoter, which is sufficient to confer cell type specificity and response to PMA/iono stimulation in vitro, and mapped a minimal IL-13 promoter that retains both properties. The cell type specificity of the minimal IL-13 promoter is mediated by a functionally critical cis-acting element that binds endogenous GATA-3 proteins that is located in the proximal region of the IL-13 promoter, whereas the response to PMA/iono is mediated by distinct cis-acting elements that are conserved in sequence between human and mouse. Ectopic expression of GATA-3, but not c-maf, is sufficient to transactivate the endogenous IL-13 promoter in Th1 cells, which concomitantly express high levels of IFN-γ. Furthermore, ectopic expression of both c-maf and GATA-3 in differentiated Th1 cells synergistically transactivates the endogenous IL-4 promoter, but does not have any synergistic effect on the endogenous IL-13 promoter. Taken together, our data suggest that IL-13 is transcriptionally regulated by a mechanism distinct from that regulating the expression of the IL-4 gene.

Materials and Methods

Cell culture and transfection

Murine B cell lines, M12 and I8.81, and EL4 (a murine thymoma cell line) cells were maintained in RPMI 1640 supplemented with 10% FCS. NIH3T3 cells were maintained in DMEM supplemented with 10% FCS. D10 (a Th2 clone) cells were maintained in RPMI 1640 supplemented with 10% FCS and 10% conditioned medium of Con A-stimulated rat splenocytes. D1.1 (a Th1 clone) cells were maintained in RPMI 1640 supplemented with 10% FCS, 5% conditioned medium of Con A-stimulated rat splenocytes, 1% T-STEM (Collaborative Biomedical Products, Bedford, MA), and recombinant human IL-2 at 50 U/ml. Transfection of tumor cell lines was performed by electroporation as described previously (19). For transfection experiments, 10 μg of each murine IL-13 promoter reporter construct, in the backbone of pGL2Basic (Promega, Madison, WI), alone or in combination with 10 μg of a GATA-3 expression vector, pcDNA-GATA3 (a gift from J. Leiden, Abbott Laboratories, Abbott Park, IL), or 10 μg of empty pcDNA-3 vector (Invitrogen, Carlsbad, CA), was used. In addition, 10 ng of a Renilla luciferase reporter vector, pRL-TK (Promega), was added into each transfection as an internal control for transfection efficiency. The transfected cells were left untreated, treated with PMA (10 ng/ml) and iono (1 μg/ml) 1 h pretransfection, or stimulated with plate-bound anti-CD3 (1 μg/ml, for D10 cells). Twenty-four hours after transfection, cell extract was harvested and subjected to luciferase assay by using the Dual Luciferase Reporter System (Promega) according to the manufacturer’s instructions. The firefly luciferase activity thus obtained was normalized against the respective Renilla luciferase activity.

In vitro differentiation of Th cells

CD4+ M14/M14gth naive Th cells were purified from splenocytes and lymph node cells of BALB/c or C57B/6 mice by a Vantage cell sorter (BD Biosciences, Mountain View, CA) and stimulated in vitro with plate-bound anti-CD3 mAb (2C11) and anti-CD28 at 1 μg/ml each in the presence of human IL-2 at 100 U/ml, along with anti-IFN-γ mAb (XMG1.2) at 10 μg/ml and IL-4 (10 ng/ml) (Th2 skewing conditions) or with anti-IL-4 (11B11) at 10 μg/ml and IL-12 (1 ng/ml) (Th1 skewing conditions). Afterward, the stimulated cells were cultured for another 6 days before use. All Abs and cytokines were purchased from BD PharMingen (San Diego, CA).

Retroviral transduction of Th cells

The bicistronic retroviral vector (RV) and the GATA3-expressing retroviral vector, RV-GATA3, were provided by K. Murphy (Washington University School of Medicine, St. Louis, MO). Retroviral transduction of Th cells was described previously (23). Briefly, the packaging Phoenix cells were cotransfected with RVs along with CMV-Env and RSV-Gag-Pol-Lyt-2 vectors (gifts from G. Nolan, Stanford University, Stanford, CA) by using Effectene Transfection Reagent (Qiagen, Valencia, CA). Supernatant from transfected Phoenix cells was used as the source of retrovirus. Naive Th cells were purified from WT C57B/6 or c-maf transgenic (mafTG) mice (20) and differentiated in vitro under Th1 skewing conditions. On the seventh day of in vitro differentiation, the differentiated Th1 cells were re-stimulated with anti-CD3 under Th1 skewing conditions. Twenty-four hours later the restimulated cells were incubated with the virus-containing supernatant at 37°C for 24 h. The infected Th1 cells were washed once, replated in fresh complete medium containing the necessary cytokines, and cultivated for another 6 days before use. We routinely achieve 60% transduction efficiency by using this protocol.

Intracellular cytokine staining

The protocol for intracellular cytokine staining was described previously (25). Briefly, Th cells were stimulated with PMA (10 ng/ml) and iono (1 μg/ml) for 2 h, then 2 μM monensin (Sigma, St. Louis, MO) was added for an additional 2 h. Cells were collected, washed, fixed in 4% paraformaldehyde, and permeabilized with saponin buffer (0.1% saponin in PBS). The fixed and permeabilized cells were incubated with PE-, FITC-, or biotin-conjugated anti-cytokine Abs or control Abs for 30 min at room temperature. Abs against murine IL-4, IL-5, and IFN-γ were purchased from BD PharMingen; biotinylated polyclonal Ab against murine IL-13 was purchased from R&D Systems (Minneapolis, MN). The cells incubated with the biotin-conjugated Abs were washed twice, incubated with Tri-color-conjugated streptavidin (Caltag Laboratories, Burlingame, CA) for an additional 20 min at room temperature. The stained cells were washed with saponin buffer twice and resuspended in PBS. The resuspended cells were subjected to flow cytometric analysis on FACS (BD Biosciences) and analyzed with CellQuest software.

EMSA

T4 polynucleotide kinase was used to end label 100 ng of double-stranded (ds) oligonucleotides with [32P]ATP (DuPont-NEN Research Products, Wilmington, DE). The labeled ds-oligonucleotides were isolated and purified on 12% polyacrylamide gels, eluted overnight at 37°C in 1× TE (10 m Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0)), and precipitated in ethanol. Binding assays were performed at room temperature for 20 min using 5 μg of nuclear extract prepared from anti-CD3-stimulated D10 cells, 500 ng of poly(dI-dC), 20,000 cpm of probe in a 15-μl volume of 20 mM HEPES (pH 7.9), 100 mM KCl, 5% glycerol, 1 mM EDTA, and 5 mM DTT. For supershift assay, 1 μl of monoclonal anti-GATA-3 or control Ab (Santa Cruz Biotechnology, Santa Cruz, CA) was added. For cold competition, unlabelled WT or mutant ds-oligonucleotides in 50-fold molar excess were added. The samples were then fractionated in 4% nondenaturing polyacrylamide gels containing 0.5× TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8.0)) at room temperature. The sequences of the WT oligonucleotide correspond to −110 to −30 (see Fig. 3B) of the murine IL-13 gene.

ELISA and RT-PCR analysis of IL-13

Cultured cells at 0.5 × 10⁶ million/ml were stimulated with PMA (10 ng/ml) and iono (1 μM) for 24 h. The supernatants were collected, and ELISA was performed as described previously (19). The capturing and detecting Abs against murine IL-13 were purchased from R&D Systems. For RT-PCR, cultured cells were stimulated as described. Six hours later, total RNA was prepared from stimulated cells. Total RNA (0.5 μg) was used for each RT-PCR by using the ACCESS RT-PCR SYSTEM (Promega) according to the manufacturer’s instruction. The sequences of primers used are IL-13 sense 5′-GACTGAGCTCCTGGCTTCGC-3′, IL-13 antisense 5′-TGGAGCCTCAAGCTGATGCCC-3′, β-actin sense 5′-GGGCCGCCTCAGAGCAACA-3′, and β-actin antisense 5′-CGTCTGCTTTTACGAGGCTG-3′.

Results

Discordant expression of IL-13 and other Th2 cytokines in normal Th2 cells

IL-13 is produced mainly by T, in particular Th2, cells. However, it is unclear whether the IL-13 gene is always coexpressed with other Th2 cytokine genes on a single cell basis. To address this question, we purified naive CD4+ Th cells from WT mice and stimulated them in vitro under Th2 skewing conditions. On day 7,
the differentiated Th2 cells were restimulated with PMA/iono and analyzed by intracellular cytokine staining. As shown in Fig. 1, whereas IL-13 was coexpressed with IL-4 in a majority of Th2 cells (∼50%), a significant number of cells produced either IL-13 or IL-4, but not both. A similar scenario was observed for IL-4 and IL-5 staining. In contrast, nearly all IL-5-producing cells also expressed IL-13, but not vice versa. These results demonstrate that the IL-13 gene is not always coexpressed with other Th2 cytokine genes.

Identification and cloning of an inducible cell type-specific murine IL-13 promoter

The murine IL-13/IL-4 locus contains several Th2 cell-specific DHSs (12). Three of the DHSs (A, B, and C) fall within the IL-13 gene (Fig. 2A). To examine the function of these DHSs in dictating the expression of the IL-13 gene, we first isolated a ∼2-kb genomic fragment, corresponding to −1990 to +48 of the murine IL-13 gene and containing DHS-A and DHS-B, by PCR amplification. The 2-kb IL-13 promoter was cloned into the pGL2Basic vector in a 5′ to 3′ orientation. The resulting reporter construct was used to transfect a variety of cell lines and Th clones. As shown in Fig. 2B, the activity of the 2-kb IL-13 promoter was induced in EL4 (a murine thymoma line) and D10 (a Th2 clone) by stimulation with PMA/iono, but not in M12 (a murine B lymphoma line), 18.81 (a human B cell line), D1.1 (a murine Th1 clone), and NIH3T3 cells. More importantly, the PMA/iono-induced activity of the 2-kb IL-13 promoter correlated well with the expression of the IL-13 gene as examined by RT-PCR and ELISA (Fig. 2C). Somewhat unexpectedly, addition of the second intron, containing DHS-C, of the IL-13 gene into the 2-kb IL-13 promoter reporter construct, downstream to the transcriptional unit, did not further enhance the promoter activity (data not shown). Taken together, these results demonstrate that the 2-kb IL-13 promoter, containing only DHS-A and DHS-B, is sufficient to confer the expression pattern and kinetics of the murine IL-13 gene in vitro.

The cell type specificity and induction by PMA/iono of the IL-13 promoter are mediated by distinct cis-acting elements

To map functionally important cis-acting elements within the 2-kb IL-13 promoter, we compared the sequences of the 2-kb promoter between murine and human IL-13 genes and identified two regions, henceforth called consensus sequence (CS)1 and 2 (Fig. 3).

FIGURE 1. Intracellular cytokine staining of Th2 cells. Purified naive CD4+ Th cells were stimulated in vitro under the Th2 skewing conditions as described in Materials and Methods. On day 7, the Th2 cells were restimulated with PMA/iono. The production of Th2 cytokines was then examined with intracellular cytokine staining by using indicated anti-cytokine Abs.

FIGURE 2. A, Schematic diagram of the murine IL-13 gene. The numbered filled and open boxes represent translated and untranslated exons, respectively. A, B, and C, Locations of Th2 cell-specific DHSs. This diagram is adapted from a previous report by Agawan et al. (12). B, Luciferase assays of the murine IL-13 promoter. The 2-kb IL-13 promoter (A) in the backbone of the pGL2Basic reporter vector was used to transfect indicated cell lines and Th clones, which were subsequently stimulated with PMA/iono before harvest for luciferase assays. Data represent the fold increase in luciferase activity over that obtained from empty pGL2Basic vector in unstimulated cells, arbitrarily set at 1. C, ELISA (top panel) and RT-PCR (middle and bottom panels) analysis of the expression of IL-13 and β-actin in indicated cell lines and Th clones.

FIGURE 3. The sequences of human and mouse CS-1 (A) and CS-2 (B) of the IL-13 gene. The numbers indicate positions relative to the transcriptional start site, which is designated as +1. The potential GATA binding sites in CS2 are boxed. The dashed lines in the human IL-13 gene represent nucleotides identical to those of the murine IL-13 gene at corresponding positions.
Each of the CSs is at least 80 bp in length and is >85% similar in sequence between mouse and human. Interestingly, even the locations, relative to the transcriptional start site, of the CSs are also maintained through evolution, and correspond well to those of DHS-A and DHS-B, respectively. To determine the function of each CS, we generated a series of deletion mutants of the 2-kb IL-13 promoter reporter, which were used to transfect EL4 and M12 cells. EL4 and M12 cells were chosen because they are easily transfectable and each represents an IL-13 producer and an IL-13 nonproducer, respectively. Although deletion of CS1 (−1535 and −579 promoter constructs in Fig. 4) resulted in an ~50% decrease in the PMA/iono-induced activity of the IL-13 promoter, the −579 deletion mutant could still be induced by PMA/iono and retained the cell type specificity. Deletion of the intervening sequences between CS1 and CS2 did not result in any further loss of promoter activity. Interestingly, a smaller fragment, containing only the 3’ half of CS2 (−109 promoter construct), did not respond to PMA/iono but was still sufficient to retain cell type-specific activity. Similar results were obtained from D10 cells that were stimulated with anti-CD3. In contrast, all the deletion mutants of the IL-13 promoter remained inactive in M12 cells. Taken together, these results demonstrated that the −254 to +48 fragment contained the minimal murine IL-13 promoter and that the cell type specific induction by PMA/iono of the murine IL-13 promoter are mediated by distinct cis-acting elements.

**GATA-3 transactivates the minimal IL-13 promoter through a functionally critical GATA site**

To identify and clone trans-acting factors that regulate the cell type-specific expression of the murine IL-13 gene, we performed a yeast one-hybrid screen by using CS2 region as bait. One of the cDNA clones thus obtained encoded the zinc finger domain of GATA-3 (data not shown). In agreement with this data, overexpression of GATA-3 in M12 cells resulted in an ~25-fold increase of the activity of the 2-kb IL-13 promoter (Fig. 5). Deletion analysis revealed that GATA-3-induced transactivation required only the 3’ half of CS2 (Fig. 5, −109 promoter construct). The 3’ half of CS2 contains at least four potential GATA-3 binding sites, located at −105, −95, −82, and −44, respectively (Figs. 3 and 6A). To identify the functionally critical GATA site within the minimal IL-13 promoter, we performed EMSAs by using the 3’ half of CS2 as a probe. As shown in Fig. 6B, the 3’ half of CS2 bound at least two protein complexes (a and b) present in D10 cell extract. The mobility of both complexes was further retarded by anti-GATA-3 Abs, but not by control Abs. In addition, the formation of the complexes was inhibited by an unlabeled WT probe or by an unlabeled probe bearing a mutation in the −105 (Mut1), −82 (Mut3), or −44 (Mut4) GATA sites. In contrast, the formation of the complexes was only negligibly, or not at all, inhibited by an unlabeled probe bearing a mutation in the −95 (Mut2) GATA site. The EMSA results demonstrate that the −95 GATA site is capable of binding endogenous GATA-3 proteins. To further examine the function of the −95 GATA site, we introduced Mut1 and/or Mut2 mutations into the −109 IL-13 promoter reporter construct. The resulting constructs and a GATA-3 expression vector, pcDNA-GATA3, were used to transfect M12 cells. As shown in Fig. 6C, left panel, the Mut2 mutation dramatically attenuated GATA-3-induced transactivation. The same result was obtained with the Mut1 and Mut2 double mutant (Mut1/Mut2). In contrast, no such attenuation was observed with the Mut1 mutant. Furthermore, Mut2 or Mut1/Mut2, but not Mut1, nearly completely abrogated the cell type-specific activity of the −109 IL-13 promoter in EL4 cells (Fig. 6C,

**FIGURE 4.** Luciferase assays of the 2-kb and truncated IL-13 promoters. **Left,** Schematic diagram of the 2-kb and truncated IL-13 promoters. The filled bars represent CSs and are indicated accordingly. The indicated promoter constructs in the backbone of the pGL2Basic vector were used to transfect M12 (left panel), EL4 (left and middle panels), and D10 (left and right panels). Data represent the fold increase in luciferase activity over that obtained from empty pGL2Basic vector cells, which were left untreated or stimulated with PMA/iono (for M12 and EL4) or anti-CD3 (for D10) arbitrarily set at 1. ND, Not done.

**FIGURE 5.** GATA-3 is a potent transactivator of the IL-13 promoter. M12 cells were cotransfected with indicated IL-13 promoter reporter constructs along with pcDNA-GATA3 or empty pcDNA-3 vector and stimulated with PMA/iono as described in Materials and Methods. Data shown are the fold increase in luciferase activity induced by overexpression of GATA-3.
Taken together, these results demonstrate that the −95 GATA site can bind endogenous GATA-3 proteins and is functionally critical for GATA-3-induced transactivation and cell type-specific activity of the minimal IL-13 promoter.

**Forced expression of GATA-3 in developed Th1 cells is sufficient to induce the production of IL-13**

To determine whether GATA-3 is sufficient to induce the expression of endogenous IL-13 in normal Th cells, we infected Th1 cells, generated by in vitro differentiation under Th1 skewing conditions for 7 days, with bicistronic retroviruses expressing both green fluorescent protein (GFP) and GATA-3 (RV-GATA3), or GFP only (RV). It was shown that expression of retroviral GATA-3 in developing, but not fully developed, Th1 cells could reprogram Th cell differentiation into the Th2 pathway and subsequently enhance the production of all Th2 cytokines. To avoid this effect of GATA-3, we chose to use fully developed Th1 cells in this experiment to isolate the effect of GATA-3 on the endogenous IL-13 promoter. The infected Th1 cells were then restimulated with PMA/iono, and the production of IL-13 was examined by intracellular cytokine staining. Previously, it was shown that transduction with RV-GATA3 was insufficient to effectively transactivate the endogenous IL-4 promoter in developed Th1 cells. As controls, no more than 1% of the uninfected (GFP−) or RV-infected (GFP+ of WTTh1/RV) Th1 cells and ~68% of WT Th2 cells (WTTh2/RV) produced IL-4. In contrast, a significant number (40%) of the RV-GATA3-infected Th1 cells, as compared with 72% of WT Th2 cells and 2–2.5% of the uninfected or RV-infected Th1 cells, stained positive for IL-13 (Fig. 7B). These data demonstrate that forced expression of GATA-3 alone efficiently transactivates the endogenous IL-13, but not IL-4, promoter in Th1 cells.

GATA-3 and c-maf synergistically transactivate the endogenous IL-4, but not IL-13, promoter in developed Th1 cells

It was demonstrated that overexpression of c-maf or, to a lesser degree, GATA-3 in tumor cell lines transactivated an IL-4 promoter reporter (17, 19). To determine whether GATA-3 and c-maf have synergistic effects on the endogenous IL-13 and IL-4 promoters in Th1 cells, we also infected mafTG Th1 cells (20), generated by in vitro differentiation under Th1 skewing conditions for 7 days, with RV or RV-GATA3 viruses. This protocol allows us to generate Th1 cells ectopically expressing c-maf alone or c-maf with GATA-3. In agreement with our previous report (20), ectopic expression of c-maf alone resulted in only a small number (~7%) of Th1 cells (mafTGTh1/RV) expressing IL-4 (Fig. 7A); however, ectopic expression of c-maf and GATA-3 synergistically induced endogenous IL-4 production (21% of GFP+mafTGTh1/RV-GATA3).

**FIGURE 6.** A, Sequences of the potential GATA-3 binding sites within CS2. Mut represents the altered sequence of each potential GATA-3 site. B, EMSA analysis of CS2. EMSA was performed by using a radiolabeled 3′ half of the CS2 region (~110 to ~30 of the murine IL-13 promoter) and D10 cell nuclear extract in the presence or absence of indicated Abs or cold competitors. WT and Mut represent unlabeled WT and altered 3′ half of CS2 regions, respectively. NFAT is an unlabeled ds-oligonucleotide containing a consensus NF-AT binding site. a and b, GATA-3 containing protein complexes.

C, Luciferase assays of the WT and mutant IL-13 promoters. The indicated WT ~109 (WT) or mutant promoter reporter constructs were used to transfect M12 (left panel) or EL4 (right panel) cells. M12 cells were also cotransfected with pcDNA-GATA3 or empty pcDNA-3 vector. The transfected cells were stimulated with PMA/iono as described in Materials and Methods. Data in the left panel are the fold increase induced by overexpression of GATA-3. Data in the right panel are the fold increase in PMA/iono-induced activity over that obtained from empty pGL2Basic.
In contrast to its role in enhancing the production of IL-4, ectopic expression of c-maf alone did not transactivate the endogenous IL-13 promoter (5% of mafTGTh1/RV vs 2% of WTTh1/RV) (Fig. 7B). Furthermore, no synergistic effect between c-maf and GATA-3 was observed in the production of IL-13 (43% of GFP+/H11001 mafTGTh1/RV-GATA3 vs 40% of GFP+/WTTh1/RV-GATA3).

Interestingly, while a substantial number of the RV-GATA3-infected WT or mafTG Th1 cells expressed IL-13, they continued to produce IFN-γ at levels comparable to those of WT Th1 (Fig. 8). A similar result was obtained when we gated on the RV-GATA3-infected mafTG Th1 cells that stained positive for IL-4 (data not shown). This observation suggests that forced expression of GATA-3 and c-maf cannot reverse the Th1 phenotype and that the expression of IFN-γ and Th2 cytokines is not mutually exclusive.

Discussion

To the best of our knowledge, this report is the first detailed analysis of the transcriptional regulation of the murine IL-13 gene, a Th2 cell-specific cytokine. The cell type specificity and induction by PMA/ion of the murine IL-13 promoter were each mediated by distinct cis-acting elements, which are conserved in sequence between human and mouse. The cell type specificity requires only the minimal IL-13 promoter and is dependent on a functionally critical GATA-3 site located in the 3’ half of CS2. In contrast, optimal induction by PMA/Ion requires upstream sequences, including CS1.

Retroviral transduction of GATA-3 into WT or mafTG Th cells is an ideal system for examining the effect of ectopic expression of GATA-3 and/or c-maf on the endogenous promoters of Th2 cytokine genes in differentiated Th1 cells. Recently, it was shown that forced expression of GATA-3 in Th1 cells and a Th1 clone resulted in the remodeling of the IL-13/IL-4 locus as examined by DNaseI hypersensitivity assays (26, 27). This would imply that GATA-3 could induce the production of both cytokines. However, we found that only IL-13, but not IL-4, was produced at a significant level by the GATA-3-expressing Th1 cells. This discrepancy indicates that the expression of the IL-13 and IL-4 genes, while only 10 kb away from each other in the murine genome, is regulated by distinct transcriptional mechanisms. For the IL-13 gene, GATA-3 may function as both a chromatin remodeling factor and a critical cell type-specific transcription factor. In contrast, high level expression of IL-4 appears to require both GATA-3 and c-maf. Ectopic expression of GATA-3 or c-maf alone in Th1 cells has been shown to be insufficient to induce significant levels of IL-4 production (20, 23). Here, we directly demonstrate, for the first time, that GATA-3 and c-maf synergistically transactivate the endogenous IL-4 promoter, even in Th1 cells. Although c-maf is a potent transactivator of the IL-4 gene, it did not play any critical role in transactivating the endogenous IL-13 gene. This is in agreement with a previous report showing the production of IL-13 by c-maf-deficient Th2 cells was not impaired (21).

The differences in transcriptional regulation that we observed between the IL-13 and IL-4 genes provide an attractive explanation for the observations that many Th2 cells express either IL-4 or IL-13, but not both. Heterogeneity in the expression of other Th2 cytokine genes has been previously reported (14–16), but we clearly demonstrate that IL-13 is not always coexpressed with other Th2 cytokines in normal Th2 cells on a single cell basis.
Recently, a report by Kelly et al. (28) showed that approximately half of 23 IL-4-producing Th2 clones also expressed the IL-13 gene as examined by RT-PCR. Our results concur with this observation and further demonstrate that the IL-13 gene can be expressed by non-IL-4-producing Th2 cells. It remains unclear how a Th2 cell chooses to express IL-13, IL-4, and/or IL-5, and whether a Th2 cell can alternate the expression of these cytokine genes. In a Th2 cell, because the IL-13/IL-4/IL-5 locus is easily accessible, the expression of IL-13, IL-4, and/or IL-5 might therefore be determined by the relative levels of c-maf, GATA-3, and, possibly, other Th2 cell-specific transcription factors. This hypothesis requires further examination.

Several groups have reported that GATA-3, in addition to promoting Th2 cell differentiation, can repress the production of the opposing Th1 cytokine, IFN-γ, by various degrees (23, 27). Here, we show that ectopic expression of GATA-3 alone or in combination with c-maf was sufficient to force developed Th1 cells to produce high levels of IL-13 or IL-4 but did not significantly suppress the expression of IFN-γ. This was true even when we examined only the bright GFP-positive population of the RV-GATA3-infected mafTG Th1 cells. The discrepancy still cannot be satisfactorily explained and might be due to differences in the source of Th cells (WT vs TCR transgenic) or the modality of infection (in cell culture or not in cell culture production). J. Exp. Med. 196:1737.


