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Friend of GATA Is Expressed in Naive Th Cells and Functions As a Repressor of GATA-3-Mediated Th2 Cell Development1

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The commitment of naive T cells to polarized Th cells requires specific changes in their transcription factors. Retrovirally overexpressed GATA-3 has been reported to induce the Th2 cytokine profile in developing Th1 cells. In this study, we examined the role of the N-terminal finger (Nf) of GATA-3 in Th2 cell development. The Nf, as well as the C-terminal finger and the transactivation domain, is critical for the induction of the Th2 phenotype. Using the GATA-3-Nf as a bait, our yeast two-hybrid screening identified friend of GATA (FOG) in the Th2 cell-specific library. Naive T cells express significant levels of FOG mRNA, which was rapidly down-regulated upon commitment to both Th1 and Th2 lineages. In reporter assays, FOG blocked the GATA-3-mediated activation of several cytokine promoters. Finally, retroviral expression of FOG in developing Th2 cells suppressed both IL-4 and IL-5 and allowed for IFN-γ production, which was accompanied by a significant level of T-bet mRNA expression. Serial deletion mutation analysis indicated that the N-terminal region, but not the consensus C-terminal binding protein-binding motif, of FOG is critical for the effects. Our results clearly indicate that 1) FOG is a repressor of GATA-3 in naive T cells and 2) the down-regulation of FOG induces Th2 cell differentiation by releasing GATA-3 from its repression. The Journal of Immunology, 2002, 168: 4538–4545.

Clariﬁcation by which naive Th cells differentiate into effector cells is critical for understanding T cell immune responses. CD4+ Th cells develop into at least two distinct subsets (1). Th1 cells produce IFN-γ and lymphotoksin and mediate delayed-type hypersensitivity responses and protection against intracellular pathogens and viruses. In contrast, Th2 cells produce IL-4, IL-5, and IL-13, provide help to B cells, and are implicated in atopic and allergic manifestations (1). Th2 cell development has been considered to be essentially an IL-4– (2) and Stat6-dependent process (3–5). The IL-4 signal induces expression of GATA-3 and c-Maf in a Stat6-dependent manner (6–10).

GATA-3 is the key transcription factor of Th2 cell differentiation (6–8, 11): transgenic and retroviral expression of GATA-3 has induced the Th2 cytokine profile in Th1 cells (7, 8, 12), while antisense and dominant-negative GATA-3 has reduced the Th2 cytokines in Th2 clones (7, 13). It is supposed that GATA-3 regulates the Th2 cell phenotype at multiple levels. First, GATA-3 strongly transactivates the IL-5 promoter and weakly activates the IL-4 promoter (6, 11, 13–15). Second, it activates the enhancers found within several regions surrounding the IL-4 gene (15). Third, it appears to exert a more global influence by altering the chromatin structure of Th2-specific gene loci (11, 16–18). The early stage of Th2 differentiation is accompanied by changes in the DNAse I hypersensitivity and DNA methylation patterns of the Th2 cytokine cluster loci (16, 19, 20). The changes render the loci more accessible to GATA-3, as proven by the chromatin immunoprecipitation assay (21) and transgenic mice studies (22, 23).

The six vertebrate GATA factors regulate the development of various tissues and are categorized into two subfamilies (GATA-1/2/3 and GATA-4/5/6). The former are expressed primarily in the hematopoietic system: GATA-1 regulates the differentiation of erythroid and megakaryocytic cells, GATA-2 controls the proliferation of hematopoietic progenitors, and GATA-3 controls the development of early T-lymphoid and effector Th2 cells (7, 24, 25). The latter are implicated in development of heart, intestine, and endoderm (26–29).

Of interest is how GATA factors function in transcriptional control. Each GATA factor contains an N-terminal activation domain and two zinc fingers: the C-terminal finger (CF)1 and the N-terminal finger (Nf) (30–32). The CF is essential for binding to the consensus GATA sequence, whereas the NF stabilizes the DNA binding (33). Moreover, the activities of GATA factors are modulated through CF-mediated interaction with several transcription factors (p300/CBP, EKLF, Sp1, PU-1, Pit-1, Nkx2.5, and NF-AT) (29).

Recently, a yeast two-hybrid screening identified friend of GATA (FOG) as a GATA-1 NF-interacting molecule in the MEL cell library (34). Subsequently, its homolog FOG-2 was cloned in the embryonic brain (27) and heart (26, 28) libraries. In vitro reporter assays suggested that FOG and FOG-2 enhance or repress the transcriptional activity of GATA factors depending on the cell and the promoter context (26, 28).

FOG is highly expressed in erythroid and megakaryocytic cell lines and in spleen, liver, and testis (34). FOG−/− embryos showed impaired primitive and definitive erythropoiesis, like

1 Abbreviations used in this paper: CF, C-terminal finger; FOG, friend of GATA; CBP, C-terminal binding protein; DBD, DNA-binding domain; Nf, N-terminal finger; TAD, transactivation domain; RV, retrovirus; GFP, green fluorescent protein; NLS, nuclear localization signal.

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GATA-1−/− embryos, but a more extreme megakaryocytic defect (35). In contrast, as FOG-2 is expressed in embryonic and adult heart, brain, and urogenital tissues (26–30), FOG-2−/− embryos are lethal due to the defects in heart morphogenesis (29, 36). These tissues and organs contain 0.1 µg/ml OVA and 3000-rad irradiated spleen cells (APC) as described (9). Cells were harvested with days 7 and 14 or without (days 3, 5, 7, 10, 12, and 14) stimulation by adding 5 µg/ml Con A (Sigma-Aldrich, St. Louis, MO) for 6 h. Poly(A)^+ RNA was prepared from the pooled cells, and cDNA was synthesized using an oligo(dT) primer by Superscript II reverse transcrip-
tase (Life Technologies, Gaithersburg, MD). CD4^+Mel-14 naïve T cells were stimulated weekly with 0.6 µM OVA and 35°C 1 and 2 days after activation and cultured under the Th1- and Th2-
mediated Th cell culture. Transfected cells were stimulated with PMA (50 ng/ml) and ionomycin (1 µM) 24 h after transfection, harvested 48 h after, and assayed for luciferase and β-galactosidase activity using the Luciferase Assay System (Promega) and Galacto-Light β-galactosidase detection kit (Tropix, Bedford, MA). Luciferase activities were normalized to β-galactosidase activity.

Mammalian two-hybrid assay
GATA-3 Nf cDNA (aa 259–309) was cloned into GAL4 DNA-binding domain (GAL4-DBD) encoding plasmid pM (Clontech Laboratories) to generate pM/GATA-3 Nf. The GAL4 activation domain encoding plasmid pVP16 (Clontech Laboratories) was used as the cloning vector for in-frame FOG constructs. All FOG mutant constructs were prepared by inserting the corresponding fragments in pVP16 in frame. 293T cells in 12-well plates were transiently transfected by FuGene 6 (Roche), according to the manu-
ufacturer’s protocol, using 1 µg of plasmid DNAs (0.4 µg GAL4-DBD vector, 0.4 µg GAL4-activation domain vector, 0.1 µg pG5-loc vector (Promega), and 0.1 µg pSV-β-galactosidase control vector). Transfected cells were harvested 48 h later and assayed as above.

Retroviral constructs
Control retroviral plasmid (pMX-IRES-GFP) and GATA-3 expression retroviral plasmids (pMX-GATA3-wt) were described previously (11). The pMX/GATA3-ΔCF, ΔNF, ΔTAD, and V264G were prepared by inserting the corresponding fragments in pME18S vectors into pMX-IRES-GFP (11). FOG retroviral plasmids were prepared by inserting full-length (pMX-FOG) or mutant FOG cDNAs into the multicloning site in the pMX-
IRES-GFP vector.

RV infection
A retrovirus (RV) packaging cell line, PLAT-E (39), was transfected with retroviral plasmids using FuGene 6 (Roche). Splenic naïve T cells from DO11.10 mice were prepared and activated weekly as previously described (9). Cells were spin-infected with RV supernatants for 4 h at 3000 rpm at 35°C 1 and 2 days after activation and cultured under the Th1- and Th2-
mediated Th cell culture. Transfected cells were harvested on day 7 with a purity of >98% by FACScan (BD Bio-
sciences, Mountain View, CA).

Cytokine ELISA
T cells were stimulated with PMA (50 ng/ml) and ionomycin (1 µM) for 48 h, and supernatants were measured as described (9).

Quantification of mRNA expression
The expression profiles of the GATA and FOG family genes were detected by real-time quantitative PCR (ABI PRISM 7700 Sequence Detection System; PE Applied Biosystems, Foster City, CA). Tissue RNAs were ex-
tracted from tissues of 8-wk-old BALB/c mice using STAT60 (Tel-Test, Friendswood, TX). CD4^+CD62L^− naïve DO11.10 splenic T cells were stimulated weekly with OVA and APC as described above, or with plate-
bound anti-CD3 (10 µg/ml) and anti-CD28 (1 µg/ml). The cells were cul-
tured under Th1- and Th2-priming conditions as described above, except that 10 µg/ml anti-IFN-γ (XMG1.2) was added in place of the anti-IL-12 Ab. The cells were activated with 5 µg/ml Con A on day 14. RNAs were extracted from cultured T cells using an RNeasy kit (Qiagen, Valencia, CA). CD3a cDNAs were synthesized from total RNA using an oligo(dT) primer and random hexamers using Superscript II reverse transcriptase (Life Tech-
nologies). cDNA from 100 ng of total RNA was used per PCR. Real-time
PCR was performed with the PCR SYBR Green sequence detection system (PE Applied Biosystems). The sequences of the primers are as follows: retrovirally induced GATA-3, 5′-CCCTTCCACAGATGTCACC-3′ and 5′-GGATATTACAGCGGCCG-3′; endogenous GATA-3, 5′-GCCATGGTTAGAAGAGCCACG-3′ and 5′-TTGGGAGCTTCACACAC-3′; and ubiq-
tin, 5′-GCGAGTGCTGTTGAAAGCCT-3′ (a second set of primers was also used: 5′-TGGGAGCTCACACAGGTG-3′ and 5′-CAGGTAAGCTTCCGAGTGCTG-3′); FOG-2, 5′-GAACCTGCAAGCCCATTTGA-3′ and 5′-GGTGCATCTGTTGGAAGTCT-3′ (a second set of primers was also used: 5′-GGTGCATCTGTTGGAAGTCT-3′ and 5′-CAGGTAAGCTTCCGAGTGCTG-3′); FO-3 gates were constructed as below. A NotI/XhoI FOG fragment corresponding to aa 817–996 was obtained from a clone (pACT-FOG) in the T cell lymphoma library. An EcoRI/KpnI fragment corresponding to aa 1–264 was PCR-amplified from day 15 embryo Mar-
athon cDNA library using GC2-PCR kit (Clontech Laboratories); missense mutations were corrected by PCR-based mutagenesis. A KpnI/NcoI fragment (aa 265–816) was obtained from a clone in the Th2 cell library (pG7D-
FOG-402). The three fragments were cloned into BamHI/XhoI sites of pME18S to generate pME-FOG.

Reporter and expression plasmid construct
pGL3-MIC-4C (−766) construct was made by inserting the KpnI/HindIII fragment from pHL-4 (−766)Luc (37) (−766 to +63 of the transcription start site) into the pGL3-basic luciferase vector (Promega, Madison, WI). The pGL3-MIC-5 (1.2 kb) and pGL3-MIFN-γ (300 bp) constructs were prepared by inserting a HindIII/XhoI fragment of pGL2-MIC-5 (1.2 kb) (from −1174 to −33 bp upstream of the translation start site) (14) and a PCR-cloned fragment from pMCITK-IFN-γ (−300 to −5 bp) (38), into the pGL3-basic luciferase vector (Promega), respectively.

Transcriptional reporter assays
Jurkat cells were transfected with 4 µg of plasmid DNAs using XtremeGene Q2 transfection reagent (Roche, Indianapolis, IN) according to the manufacturer’s instructions. All transfections were performed in du-
uplicate and contained 0.1 µg of pSV-β-galactosidase vector (Promega).
Results

GATA-3 induces Th2 cytokines and suppresses IFN-γ production by developing Th1 cells in a zinc fingers- and transactivation domain-dependent manner

To investigate the effects of GATA-3 domains on the induction of Th2 cytokine profiles, we transduced RVs containing GATA-3 and its mutants into developing Th1 cells. Compatible with previous reports (11, 40), wild-type GATA-3 induced the expression of IL-4 and IL-5 and suppressed IFN-γ production (Fig. 1A), whereas deletion of the Cf and the TAD resulted in a complete loss of the Th2 phenotype induction. Interestingly, deletion of the Nf led to a complete loss of IL-5 induction, but both the IL-4 induction and the IFN-γ suppression were partially conserved.

The induction of the Th2 profile was confirmed by quantitative analysis of several Th type-specific genes (Fig. 1B). First, the levels of retrovirally expressed GATA-3 were similar among wild-type and mutant GATA-3-expressing Th1 cells (data of GATA-3-ΔCf and -ΔTAD are not shown). The induction of endogenous GATA-3 was induced by wild-type- but not GATA-3-ΔNf. Moreover, the suppression of T-bet and IL12Rβ2 mRNAs was observed by wild-type GATA-3. In contrast, GATA-3-ΔNf partially suppressed T-bet but not IL12Rβ2 mRNA. These results indicate the critical but differential roles of three domains of GATA-3 for the induction of the Th2 phenotype.

Identification of FOG as a GATA-3-interacting molecule in Th2 cells

To identify proteins that interact with GATA-3, we prepared a yeast two-hybrid library from mixtures of several developmental stages of Th2 cells. We used an N-terminal-truncated GATA-3 (aa 169–422) that encompassed both zinc fingers as a bait to screen the library. More than 800 clones of one million clones demonstrated histidine and adenine auxotrophy, and sequence analysis revealed that 3 of 197 clones encoded FOG (aa 228–819, 443–819, and 505–819). The first one contained the first through the sixth zinc finger domains, and the latter two contained the fifth through the sixth fingers, of which the first, fifth, and sixth fingers are putative interacting domains with GATA factors.

Tissue-restricted and developmentally regulated expression of GATA-3 and FOG family proteins

Next, we examined the tissue- and developmental stage-specific expression of FOG family proteins, including FOG and FOG-2, and GATA-3 using a real-time quantitative RT-PCR (Fig. 2A, left panels). Significantly, higher levels of GATA-3 mRNA were detected in lymph node and thymus, while much lower levels were detected in spleen, liver, and brain stem. FOG mRNA is expressed in many tissues, most prominently in spleen and lymph node and moderately in thymus, kidney, heart, and brain stem. We confirmed the expression of FOG using two different sets of primers (see sequences in Materials and Methods) and obtained similar results (data not shown). In contrast, FOG-2 mRNA is expressed very weakly in thymus and spleen but moderately in brain stem, ovary, and lymph node, and strongly in heart. These data are compatible with previous reports (26–28).

We further compared the expression of FOG and FOG-2 mRNAs in developing Th cells (Fig. 2A, right panels). Naive T cells showed a lower but significant level of GATA-3 mRNA compared with thymic cells. Two weeks of Th1-driving culture with OVA and APC led to the disappearance of GATA-3 and FOG mRNAs. In contrast, 2 wk of Th2-driving culture led to prominent up-regulation of GATA-3 and an almost complete disappearance of FOG mRNA. FOG-2 mRNA was detected very weakly in naive Th cells (<30 × 10−3/ubiquitin) but not in polarized Th1 or Th2 cells. This developmental stage-specific expression was further confirmed by time-course analysis of in vitro cultured Th cells (activated with anti-CD3 and anti-CD28 under Th1/Th2-driving cultures) (Fig. 2B). As controls, the expression of both GATA-3 and T-bet mRNAs was examined (Fig. 2B, middle and lower panels). A slight level of GATA-3 mRNA was expressed in naive T

![FIGURE 1](http://www.jimmunol.org/)

FIGURE 1. Retroviral expression of GATA-3 in developing Th1 cells induces Th2 cytokine patterns in a zinc fingers- and transactivation domain-dependent manner. A, Induction of IL-4 and IL-5 and suppression of IFN-γ by retrovirally expressed GATA-3 in developing Th1 cells. Naive Th cells from DO11.10 spleens were infected with RV vectors containing GATA-3 wild-type and deletion mutants. Cells were sorted 7 days after the activation and activated by PMA and ionomycin. Supernatants were collected after 48 h and assayed by ELISA. Uninfected cells cultured under Th1- and Th2-driving conditions were analyzed as controls. Data bars represent the average of three independent samples, and error bars show the SD. B, GATA-3-mediated induction of endogenous GATA-3 and suppression of T-bet and IL-12Rβ2 mRNA expression. Total RNA was isolated from the above cells and analyzed for expression of retroviral GATA-3, endogenous GATA-3, c-Maf, T-bet, and IL12Rβ2 using the TaqMan quantitative PCR assay. mRNA levels are normalized to ubiquitin mRNA (×10−3/ubiquitin).
cells, rapidly up-regulated during Th2 cell development, but down-regulated during Th1 cell development (6). In contrast, T-bet mRNA was not expressed in naive T cells, but was rapidly up-regulated in Th1 cells but not Th2 cells (41). Interestingly, FOG mRNA was expressed significantly in naive Th cells but down-regulated quickly during the development to both Th1 and Th2 lineages (Fig. 2B, upper panel). FOG-2 mRNA was not detected in either of these polarized Th cells (detection limit, $1 \times 10^{-5}$ ubiquitin) (data not shown). These results demonstrate that FOG and GATA-3 are coexpressed in naive T cells but that the FOG mRNA was rapidly down-regulated during Th cell differentiation.

FOG represses GATA-3-dependent transcription from cytokine gene promoters

FOG family proteins have been reported to function as both co-activators and repressors of GATA-dependent transcription depending on cellular context (34, 42). To study the effects of FOG on GATA-3-dependent transcription in lymphocytes, Jurkat cells were cotransfected with expression plasmids containing GATA-3 and FOG and luciferase reporter plasmids containing mouse IL-5, IL-4, and IFN-γ promoters (Fig. 1). Consistent with previous reports (11, 13, 15), GATA-3 alone transactivated the IL-5 and IL-4 promoters.

FIGURE 3. FOG represses the GATA-3-mediated activation of cytokine promoters in Jurkat cells. A, Activation of the IL-5, IL-4, and IFN-γ promoters by GATA-3, and repression by FOG. pGL3-mIL-5-, mIL-4-, or mIFN-γ plasmid (1.3 g) was cotransfected with pSV-β-galactosidase (0.1 g) into Jurkat cells. Expression vectors for GATA-3 (pME-GATA-3-wt, 1.3 g) and FOG (pME-FOG, 1.3 g; or 0.3 or 0.65 g for the IL-5 promoter), or equivalent amounts of empty pME18S vector, were added as indicated (a total of 4 g of plasmids). After 24 h, cells were stimulated with PMA and ionomycin, and after 48 h luciferase and β-galactosidase activities were measured. The basal promoter activity was arbitrarily assigned as a value of 1. Representative results from three independent experiments, each performed in duplicate, are shown. B, FOG represses the IL-5 promoter through the NF of GATA-3. The parallel experiments for IL-5 promoter activation were done using expression vectors for GATA-3 (pME-GATA-3-wt, -ΔCF, -ΔNF, -ΔTAD, or -V264G) and FOG (pME-FOG).
promoters by 5- and 3-fold, respectively (Fig. 3A). In contrast, forced expression of FOG had little effect on the basal levels of IL-5 promoter activation but significantly repressed the transactivation by GATA-3 in a dose-dependent manner (Fig. 3A, left panel). FOG also repressed GATA3-mediated activation of IL-4 and IFN-γ promoters. Next we examined the effect of interaction between GATA-3 and FOG on the transactivation (Fig. 3B). Interestingly, expression of FOG repressed the transactivation of the IL-5 promoter by GATA-3-wild-type and -ΔTAD to the basal level of activation but did not repress the transactivation by GATA-3-ΔNf and -V264G, which are supposed not to interact with FOG. GATA-3-ΔCf showed no transactivation. These results demonstrated that FOG specifically represses GATA3-mediated transcription of several cytokine promoters through interaction with the N-finger of GATA-3.

Retroviral gene transduction of FOG into primary T cells results in decreased Th2 cytokine production and induced IFN-γ production.

The experiments described above argue for the repression by FOG of the GATA-3-mediated transcription. The fact that FOG is expressed in naive T cells but down-regulated during Th cell development raises the possibility that the regulation of FOG level is critical in the processes leading to Th2 cell development. To test this, a retroviral transfer of FOG into naive T cells was performed.

We generated a RV vector expressing both FOG and GFP (RV-FOG) and used a retroviral vector containing GFP alone (RV-GFP) as a control. The CD4+CD62L+ T cells from DO11.10 TCRβ-transgenic mouse spleens were activated with OVA and APC under Th2-priming culture and infected with RV 18 and 42 h after primary activation. After 7 days, GFP-positive cells were isolated by FACS and stimulated with OVA and APC for one more week under Th2-priming culture. On day 14, the cells were stimulated with PMA/ionomycin for measurement of cytokine production.

Fig. 4 shows that FOG significantly suppressed the production of IL-4 (78 vs 317 and 316 ng/ml in RV-GFP-infected and uninfected Th2 cells) and IL-5 (95 vs 205 and 222 ng/ml in RV-GFP-infected and uninfected Th2 cells) (Fig. 4A). In contrast, FOG allowed for the production of IFN-γ despite the Th2-priming culture condition (449 vs 38 and 107 ng/ml in RV-GFP-infected and uninfected Th2 cells). The production of IL-10 was only slightly reduced by FOG. Similar phenotypic changes were observed on day 7 (data not shown).

The Th type-specific gene expression was examined on the same samples by quantitative RT-PCR (Fig. 4B). Both RV-GFP- and RV-FOG-infected Th2 cells expressed similar levels of GATA-3 mRNA to uninfected Th2 cells. In contrast, T-bet mRNA was slightly induced in RV-FOG- but not RV-GFP-infected Th2 cells. IL12Rβ2 mRNA was not detected in either of the RV-infected Th2 cells.

The N-terminal region of FOG is critical for the repression of GATA-3 in Th2 cells.

To more precisely identify the domains of FOG required for GATA-3-binding and transcriptional repression, we constructed a series of N- and C-terminal truncations of FOG (Fig. 5A). The binding of FOG to GATA factors is reported to be mediated by the first, the sixth, the ninth, and, weakly, the fifth fingers of FOG and N-finger of GATA factors (43). The putative repression domains were reported to be the consensus C-terminal binding protein (CtBP)-binding (Pro-Ile-Asp-Leu-Ser: PIDLS) motif between the sixth and seventh fingers (44) or the N-terminal domain (45). The nuclear localization signal (NLS) was supposed to be around the 3′ side of the PIDLS motif (Fig. 5A). FOG-Δ3′ mutant lacks the C-terminal regions (aa 762–995) harboring the seventh to ninth fingers, the PIDLS motif, and the NLS. FOG-Δ3′B lacks the three fingers but contains the PIDLS and the NLS. FOG-Δ5′ lacks the N-terminal putative repression domain (aa 1–48). FOG-Δ(S'/S′') lacks both the N-terminal (aa 1–48) and the C-terminal (aa 817–

FIGURE 4. Retroviral gene transduction of FOG into naive T cells suppresses Th2 cytokines and induces IFN-γ production in developing Th2 cells. A, Retroviral transduction of primary CD4+CD62L+ T cells with control RV-GFP and RV-FOG. FACS-purified naive T cells from DO11.10 TCRβ-transgenic mouse spleens were infected with the indicated RVs 18 and 42 h after primary activation by OVA and APC and cultured in the Th2-priming condition. Cells were sorted on day 7 for GFP expression, activated with OVA and APC, and cultured in the same condition for one more week. The cells were stimulated on day 14 with PMA and ionomycin, and cytokine production was measured after 48 h. Control uninfected cells were cultured in Th1- and Th2-priming conditions. Empty indicates RV-GFP-infected cells, and FOG indicates RV-FOG-infected cells. B, Expression of GATA-3, T-bet, and IL12Rβ2 mRNAs in RV-infected Th2 cells. Quantitative PCR of Th cell markers was performed as in Fig. 1B.
FIGURE 5. The N-terminal region of FOG is critical for the suppression of Th2 cytokines and the induction of IFN-γ in developing Th2 cells. A. Structure of FOG mutants. The FOG truncation mutants are shown schematically below the full-length FOG protein. Numbers shown to the right indicate the amino acids of FOG included in each mutant. *+, The putative GATA-interacting zinc finger; &, the CtBp-binding site and NLS. B. The 3′- and 5′-truncated mutants of FOG maintain the ability to interact with GATA-3. The GAL4-(adenovirus major late promoter)-luciferase plasmid pGL5-luc was cotransfected into 293T cells with pM-GATA-3-Nf and pVP16 plasmids encoding FOG fusion proteins. The mean fold activation relative to that of pM-GATA-3-Nf and pVP16 is shown. Representative results from three experiments, each in duplicate, are shown. C. Retroviral transduction of primary CD4+CD62L+ T cells with RV-FOG mutants. Naive DO11.10 transgenic naive T cells were stimulated with OVA and APC and infected with RV-GFP, RV-FOG, or RV-FOG mutants. On day 7, cells were sorted for GFP expression and cultured, and cytokine production was measured as in Fig. 4. Representative results from three independent experiments are shown.

The requirement of functional domains is important for understanding the mechanism of GATA-3 functions. For GATA-1, TAD is essential for the transactivation in nonhematopoietic cells but is dispensable for the megakaryocytic conversion of mouse myeloid cell line 416B (46) and terminal maturation of erythroid cells (47, 48). In contrast, although the Nf is dispensable for transactivation, it is critical for the erythroid and megakaryocytic maturation (46–48). Our experiment with GATA-3 mutants indicated that the Nf is critical for the induction of endogenous GATA-3 and IL-5 but partially dispensable for the induction of IL-4 and suppression of IFN-γ. This is partially in contrast to a previous report (40) showing that Nf-deleted GATA-3 mutants failed to induce IL-4 in developing Th1 cells, probably due to the differences in retroviral expression levels and culture conditions. These results indicate the functional significance of the Nf of GATA-3.

Using the Nf-containing region as a bait, we identified FOG in the Th2 cell yeast two-hybrid library. Our quantitative PCR analysis revealed that FOG is expressed in a broad range of tissues, including lymphoid tissues such as thymus, spleen, and lymph nodes, while the expression of FOG-2 is restricted mainly to heart, brain, and ovary (Fig. 2A) (26–28). Although FOG mRNA expression was detected in several erythroid, megakaryocytic, and progenitor cells (34), its expression in lymphoid cells was not examined in detail. We found that naive Th cells express significant levels of FOG mRNA and that its expression is down-regulated during development to both Th1 and Th2 cells, while FOG-2 is not expressed significantly in Th cells (Fig. 2). The expression of FOG protein in naive Th cells and its down-regulation was reported in a recent paper (49).

It has been shown previously that FOG and GATA-1 can synergistically transactivate the NF-E2 p45 promoter (34). However, the effects of FOG proteins depend on both cell types and promoter contexts. Thus, other GATA-dependent promoters are strongly repressed by FOG (44), and the anti-MHC promoter is activated in
assays revealed that FOG represses the GATA-3-mediated transactivation of all the cytokine promoters, including IFN-γ.

We have shown that the expression of FOG was down-regulated during Th cell development of naive T cells. Similar down-regulation of FOG was reported in other GATA-mediated differentiation systems (53, 54). FOG acts as a repressor of GATA-1-mediated induction of eosinophil-specific genes in multipotent hematopoietic precursors and is down-regulated during the eosinophil differentiation (53, 54). Drosophila FOG homolog U-shaped is expressed in hemocyte precursors as a negative regulator of GATA homolog Serpent but down-regulated during crystal cell lineage commitment (42). Moreover, ectopic overexpression of FOG suppressed the erythroid differentiation in Xenopus embryos (55). Therefore, these results, including ours, suggest a general phenomenon that the down-regulation of the FOG family is a prerequisite for GATA-mediated differentiation. Although a previous report (35) suggested that FOG is not required for the control of T lymphocyte development by GATA-3, it is possible that the precise analysis of effector Th cell development was hindered by the embryonic lethality of FOG−/− mice.

Our data indicate that FOG inhibits Th2 cytokines and allows for IFN-γ production in developing Th2 cells (Figs. 4A and 5C). Therefore, it appears that overexpression of FOG inhibits the GATA-3-mediated process of Th2 cell development. The level of GATA-3 mRNA seems not to be influenced by FOG (Fig. 4B). Conversely, a slight but significant increase in T-bet mRNA was observed in RV-FOG-infected Th2 cells, corresponding to the induction of IFN-γ production (Fig. 4). These results, including FOG mRNA expression patterns, suppression of in vitro IL-5 promoter activity, and repression of Th2 cytokines in RV-infected Th2 cells, are compatible with a recent report (49), except that our results show no suppression of endogenous GATA-3 in Th2 cells by RV-FOG.

As shown in Fig. 2B and previous papers (49), naive Th cells express a significant level of FOG and a low level of GATA-3. In contrast, differentiated Th2 cells express a high level of GATA-3 but a much lower level of FOG; differentiated Th1 cells express a high level of T-bet and a much lower level of FOG. Thus, it is supposed that the activity of the low levels of GATA-3 in naive Th cells is repressed by high levels of FOG, while in Th2 cells GATA-3 exerts its full activity in the absence of FOG. Moreover, the different expression patterns of GATA-3 and FOG indicate that not only GATA-3 but also FOG may interact with other partners in distinct cells, such as neuronal and hematopoietic cells, and thus exert different functions (27, 42, 44).

Recently, Ho et al. (56) cloned another GATA-3-interacting protein, repressor of GATA, which is rapidly induced by TCR stimulation and represses both Th1 and Th2 cytokines. In contrast to repressor of FOG, FOG is expressed in naive Th cells and down-regulated during Th cell differentiation, and it represses specifically Th2 cytokines in a GATA-dependent manner. However, the components in the GATA-3-containing complexes remain to be clarified, and GATA-3 might require other NF-interacting coactivators during Th2 development, because GATA-3−/− has decreased Th2 cytokine production despite abolished FOG interaction (Fig. 1).

Although the mechanism of repression by FOG proteins remains unsolved, previous studies suggest the role of a corepressor CtBP (42, 44). Ectopically expressed FOG suppresses crystal cell production in a CtBP-dependent manner (42). In contrast, CtBP binding is not required for the repression of cardiac promoters in NIH3T3 cells (45) or for the Drosophila eye and heart development (42), where the conserved N-terminal regions of FOG and FOG-2 are necessary for the repression. We examined the serial N- and C-terminal deletion mutations for the repression on Th2 phenotype (Fig. 5A). Deletion of aa 1–48 of FOG abolished the repression, while C-terminal deletions did not significantly reduce the repression until all of the GATA-interacting zinc fingers were removed (Fig. 5C). These results indicate that the N-terminal region, but not the CtBP-binding motif, of FOG is critical for the repression of GATA-3-mediated Th2 cytokine gene activation. These findings will be important for development of inhibitors of GATA-3 and FOG by increasing our understanding of the transcriptional complexes in Th2 cell development.

In summary, FOG functions in naive Th cells as a repressor of GATA-3, and the down-regulation of FOG is an essential step for Th2 cell development.

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