Cutting Edge: The Differential Involvement of the N-Finger of GATA-3 in Chromatin Remodeling and Transactivation During Th2 Development

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*J Immunol* 2002; 169:4103-4107; doi: 10.4049/jimmunol.169.8.4103
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The development of Th subset is accompanied by subset-specific chromatin remodeling of cytokine gene loci. In this study, we show that the C-terminal, but not the N-terminal zinc finger (N-finger) of GATA-3 mediates the association with the IL-4/IL-13 intergenic DNAse I hypersensitive site and the induction of an extended DNase I hypersensitivity on the IL-4/IL-13 locus. Consistently, deletion of the transactivation domains or the C-finger, but not the N-finger, abrogated the induction of IL-4 and IL-13 as well as the down-regulation of IFN-γ. In contrast, the N-finger of GATA-3 was indispensable for the binding to the IL-5 promoter and the induction of IL-5. The selective use of the N-finger may underlie the differential roles of GATA-3 in the induction of IL-4, IL-13, and IL-5. The Journal of Immunology, 2002, 169: 4103–4107.

E lucidation of the mechanism by which naive Th cells acquire their functional cytokine repertoires is critical to understand the immune defense against infection by pathogens (1). It has now become evident that Th1 and Th2 differentiation is associated with changes in the DNase I hypersensitivity, histone acetylation, and methylation status of the cognate cytokine gene loci (2–8). In addition, it has recently been demonstrated that following polarization of Th subsets, transcriptionally nonpermissive cytokine gene loci are targeted to centromeric heterochromatin (9). However, the molecular basis underlying these subset-specific regulations of chromatin structure remains to be clarified.

We have previously identified HSS1 and HSS2, DNase I hypersensitive (DH) sites induced in the IL-4/IL-13 intergenic region during Th2 development (2). Subsequently, Locksley and colleagues (10, 11) demonstrated that the DNA segment containing HSS1 and HSS2, designated conserved noncoding sequence (CNS)-1, is highly conserved across mammals and is crucial for the coordinate induction of clustered Th2 cytokines, IL-4, IL-13, and IL-5. We have demonstrated that ectopic expression of either activated Stat6 or GATA-3 in developing Th1 cells induces an accessible chromatin configuration at CNS-1 as well as at the IL-4 and IL-13 gene loci (12). Intriguingly, ectopic expression of GATA-3 induces chromatin remodeling of the IL-4 locus even in fully differentiated Th1 cells (13). GATA-3 directly associates with CNS-1 (12), and mediates strong enhancement of the IL-4 promoter activity in transgenic mice when the promoter is linked to CNS-1 and the intronic enhancer (14). Rao and colleagues (3, 6) have also identified Th subset-specific DH sites in the IFN-γ, IL-4, and IL-13 gene loci, which persist in resting Th1 and Th2 cells. Additionally, they have identified an inducible enhancer at 3′ of the IL-4 gene by DH site mapping (4). NFAT1 and GATA-3 bind to this enhancer and mediate synergistic activation (4, 7). Stat6 also associates with this enhancer and maintains increased histone acetylation status (7). As for the IFN-γ locus, it has recently been demonstrated that T box expressed in T cells (15), a critical regulator of Th1 development, controls chromatin remodeling (16).

GATA-3 is selectively induced during Th2 development (17, 18). Ectopic expression of GATA-3 induces Th2 differentiation under conditions that otherwise polarize toward Th1 development (17, 19). Moreover, GATA-3 exerts Stat6-independent autoactivation, thereby creating a feedback pathway which stabilizes Th2 commitment (20). GATA-3 possesses N-terminal transactivation domains (TADs) and two zinc fingers; the N- and the C-finger, both of which are highly conserved among GATA family members. The C-finger of GATA proteins is essential for DNA binding, whereas the N-finger has been shown to contribute to the specificity and stability of DNA binding at certain GATA recognition sequences (21, 22).

Although accumulating evidences clearly indicate that GATA-3 is the key factor for Th2 development, little is known with regard to how GATA-3 mediates chromatin remodeling. In this study, we define the structural requirement of GATA-3 in chromatin remodeling of the IL-4/IL-13 locus as well as in the induction of Th2 cytokines. We demonstrate that the N-finger of GATA-3 plays essential roles in transactivation of the IL-5 expression, but not in chromatin remodeling of the IL-4/IL-13 locus, and propose that the selective use of the N-finger may underlie the differential role of GATA-3 in the induction of IL-4, IL-13, and IL-5.

Materials and Methods

Cytokines and Abs

Mouse rIL-2, rIL-4, and rIL-12, and anti-mouse IL-4 Ab (11B11) were purchased from R&D Systems (Minneapolis, MN).
Retroviral transduction and DNase I hypersensitivity analysis

Retroviral constructs pMXI-enhanced green fluorescent protein, -GATA-3-enhanced green fluorescent protein, -ΔTAD, -ΔNf263–287, -ΔCF, and -KRR have been described (13). Retroviral constructs pMXI-GATA-3-ΔNf263–287 and -V264G, which contain a 25-aa deletion (aa 263–287) and an aa substitution of glycine for valine at aa 264, respectively, were generated by PCR mutagenesis. The integrity of each mutation was confirmed by DNA sequencing. Naive Th cells were purified as previously described (12), and were infected with retrovirus-containing supernatants in the presence of 0.5 μg/ml polybrene (Sigma-Aldrich, St. Louis, MO) on days 1 and 2 after primary antigenic stimulation under Th1-polarizing conditions. Green fluorescent protein cells were sorted on day 7 and were allowed to develop for another 2 wk with weekly antigenic stimulation. DNase I hypersensitivity of the IL-4/IL-13 locus was analyzed as previously described (12).

Flow cytometric analysis of intracellular cytokine synthesis

Cells were stimulated with PMA (50 ng/ml) plus ionomycin (1 μM) for 4 h. Monensin (2 μM) was added 2 h before harvest. Cells were subsequently fixed, permeabilized with 0.5% saponin, and were stained with mAbs specific for IFN-γ, IL-4, IL-5, or IL-13.

EMSA

COS7 cells were transiently transfected with various GATA-3 expression constructs. Nuclear extracts were prepared after 48 h and were analyzed for DNA binding activity to the oligonucleotide probes encompassing HSS2 (12), IL-5 promoter (23), and TCRα enhancer (24).

Results

To address the relative contributions of the domains of GATA-3 in chromatin remodeling of the IL-4/IL-13 locus, a series of mutant constructs of GATA-3 were introduced into developing Th1 cells and were examined whether they could induce chromatin remodeling of the IL-4/IL-13 locus (Fig. 1). ΔTAD and ΔNf mutants lack portions of the TAD and the N-finger, respectively, and ΔCF mutant is defective in the entire C-finger. KRR mutant, in which aa residues KRR located at 304–306 are altered to AAA, has been shown to have dominant negative effects and to attenuate asthma pathogenesis in vivo (25, 26). As we and others have previously demonstrated (12, 20), retroviral transduction of wild-type GATA-3 resulted in the induction of DNase I hypersensitivity over the entire IL-4/IL-13 locus. ΔCF mutant induced DH site I of the IL-13 locus, but failed to induce any other Th2-specific DH sites. ΔTAD mutant induced an accessible chromatin conformation at the IL-13 locus, but the IL-4 locus as well as CNS-1 was left inaccessible. Interestingly, deletion of the N-finger or introduction of KRR mutation did not affect the ability of GATA-3 to remodel the entire IL-4/IL-13 locus. These results indicate that the C-finger, but not the N-finger of GATA-3, is essential for chromatin remodeling of the IL-4/IL-13 locus.

Next, we examined the cytokine-inducing profile of each GATA-3 mutant (Fig. 2). As expected, wild-type GATA-3 induced the production of IL-4, IL-13, and IL-5 (85, 38, and 15% of cells stained positive). In contrast, barely detectable amounts of these Th2 cytokines were induced by ΔCf and ΔTAD mutants. Deletion of the N-finger, either partially (ΔNf280–287) or entirely (ΔNf263–287), reduced IL-13-producing cells (8 and 12%, respectively), while it only modestly affected the induction of IL-4 (63 and 71% of...
ΔNf280–287 and ΔNf263–287 mutant-introduced cells produced IL-4. Remarkably, the induction of IL-5 was almost completely abolished by the deletion of the N-finger (only 3% or less produced IL-5). V264G mutant, which has a single amino acid substitution within the N-finger, whose corresponding residue in GATA-1 is essential for mediating contact with friend of GATA-1 (27), showed Th2 cytokine-inducing capability almost the same as that of wild-type GATA-3. KRR mutation diminished IL-4- and IL-5-producing cells (59 and 8%), in line with the previous report (26). These results indicate that the TAD and the C-finger of GATA-3 are essential for the induction of IL-4 and IL-13, while the N-finger, along with the TAD and the C-finger, is indispensable for the induction of IL-5. GATA-3 is also known to suppress the production of IFN-γ (19). This activity was retained in ΔNf, V264G, and KRR mutants, while mutants deficient in either the TAD or the C-finger completely lost the activity, indicating that the suppression of IFN-γ production requires, as is the case for the induction of IL-4, the TAD and the C-finger, but not the N-finger of GATA-3.

We next examined the DNA binding properties of each GATA-3 mutant (Fig. 3). To this end, three GATA-3 binding sequences were used; one in HSS2 (12), another in the IL-5 promoter (23), and a third in the TCRα enhancer (24). Western blot analysis using an anti-GATA-3 Ab showed that mutant GATA-3 proteins of expected sizes accumulated in the nuclei (Fig. 3, upper panel). EMSA revealed that the C-finger, but not the N-finger, of GATA-3 is essential for the binding to HSS2. This is compatible with our findings that the ΔNf mutant is capable of inducing chromatin remodeling of the IL-4/IL-13 locus (Fig. 1b). In contrast, deletion of the N-finger as well as the C-finger severely compromised the binding of GATA-3 to its recognition sequence within the IL-5 promoter, and completely abrogated the binding to the TCRα enhancer. The differences in DNA binding capabilities of the ΔNf mutant suggest that the N-finger of GATA-3 may be selectively required for mediating transactivation, not chromatin remodeling.

**Discussion**
Recent studies have established that GATA-3 is the key factor for Th2 development (17–20). However, little has been demonstrated regarding how GATA-3 mediates Th2-specific chromatin remodeling. In this study, we defined the structural requirement of

**FIGURE 2.** Intracellular cytokine synthesis of Th cells introduced with the GATA-3 mutants. Cells described in the legend to Fig. 1 were analyzed for the production of IFN-γ, IL-4, IL-13, and IL-5. Production of these cytokines in cells infected with control retroviral vector developed under parallel Th1-polarizing conditions and uninfected Th2 cells from the same experiments are shown as controls. Proportions of cells in the quadrants are shown. Proportions of ΔNf263–287 mutant-introduced cells stained positive for each Th2 cytokine are also indicated (c).

**FIGURE 3.** The DNA binding properties of the GATA-3 mutants. Nuclear extracts were analyzed for expression of GATA-3 proteins by immunoblot, and for DNA binding activity to the oligonucleotide probes from HSS2 (12), IL-5 promoter (23), and TCRα enhancer (24).
GATA-3 in chromatin remodeling of the IL-4/IL-13 locus. Our results demonstrate that the induction of an open chromatin conformation at the IL-4 locus and CNS-1 requires the TAD and the C-finger of GATA-3 (Fig. 1b). Concomitantly, ∆TAD and ∆CF mutants showed severely impaired production of Th2 cytokines, in agreement with previous reports using Th1 clone (13) as well as developing Th cells (28, 29) (Fig. 2b). In contrast, the N-finger of GATA-3 is dispensable for chromatin remodeling of the entire IL-4/IL-13 locus (Fig. 1b). Correspondingly, ∆NF mutant induced, albeit decreased amounts, the IL-4 and IL-13 expression, and potentially suppressed the IFN-γ production (Fig. 2, b and c). Although the N-finger-independent IL-4 induction is consistent with previous reports (13, 29), the other report has shown that the N-finger-deleted GATA-3 mutant fails to induce IL-4 (28). Because relatively larger regions (aa 249–308) were removed in the construct used in the latter study, it could be possible that the surrounding regions of the N-finger might be involved in the IL-4 induction. Note that amino acid residues extending from the first to the fourth zinc-coordinating cysteine residues were removed in ∆NF mutant used in the present study (Fig. 2a), whereas additional 35 aa residues were removed in the construct used in the aforementioned study (28). It could be also conceivable that the differences in retroviral infection conditions may account for the discrepancy. In either case, it is important to note that both studies have also demonstrated that the autoactivation capacity of GATA-3 is lost upon deletion of the N-finger (28, 29). Thus, the observed IL-4 induction is likely to be mediated by retrovirally transduced ∆NF mutant protein per se.

The important feature of the N-finger of GATA-3 revealed in this study is its differential requirement in the induction of each Th2 cytokine (Fig. 2, b and c). Remarkably, its deletion severely compromised the binding to the IL-5 promoter and to the TCRα enhancer, but not to HSS2 (Fig. 3). GATA-3 has been shown to strongly transactivate the IL-5 promoter and the TCRα enhancer by directly interacting with its critical regulatory elements, while it has little effect on the proximal IL-4 promoter (13, 24, 30, 31). Thus, the DNA binding specificity of ∆NF mutant correlates with its capability to induce DNase I hypersensitivity and individual Th2 cytokine productions. It is tempting to speculate that the DNA binding properties of the N-finger-deficient derivative may help distinguish the GATA elements involved in chromatin remodeling from those regulating transactivation.

Our results also indicate that GATA-3 can induce chromatin remodeling of the IL-13 locus in the absence of its TAD (Fig. 1b). In this regard, it is interesting to note that the isolated zinc finger DNA-binding domain of erythroid Krüppel-like factor has been shown to interact with the subunits of SWI/SNF complex and mediate chromatin remodeling of the β-globin promoter (32). Intriguingly, SWI/SNF subunits have also been shown to interact with the zinc finger domains of GATA-1 (32). Thus, it is conceivable that GATA-3 interacts with the components of a chromatin remodeling complex through its zinc finger domains. Because ∆TAD mutant failed to induce DH sites at the IL-4 locus and CNS-1 (Fig. 1b), additional proteins which cooperate with the TAD of GATA-3 may be required for chromatin remodeling of the IL-4 locus and CNS-1.

Our study also disclosed, for the first time, the structural requirement of GATA-3 in the IL-13 expression. Deletion of the TAD or the C-finger abrogated the induction of IL-13, while deletion of the N-finger caused a partial decrease (Fig. 2, b and c). Of note, despite the normal induction of DH sites at the IL-13 locus by ∆TAD mutant (Fig. 1b), the production of IL-13 was severely impared by deletion of the TAD (Fig. 2b). Therefore, it seems likely that the induction of IL-13 requires, in addition to chromatin remodeling, GATA-3-dependent transactivation, which is mediated through the TAD and the N-finger. In agreement with this notion, it has recently been demonstrated that GATA-3 directly transactivates the IL-13 promoter (33). It is also possible that the failure of ∆TAD mutant in the induction of chromatin remodeling at CNS-1 (Fig. 1b) leads to severely compromised IL-13 production (Fig. 2b), since recent in vivo evidences presented by Locksley and colleagues (10, 11) unequivocally indicate that CNS-1 is critical for the expression of IL-13.

Taken together, our results demonstrate that the regulatory roles of GATA-3 in development and effector functions of Th2 cells are organized into overlapping yet distinct structural domains. Of these, the selective use of the N-finger might be of particular interest; it is dispensable for the binding to HSS2 and chromatin remodeling of the IL-4/IL-13 locus, and plays minor roles in the induction of IL-4 as well as the suppression of IFN-γ production. In contrast, the N-finger of GATA-3 is required for optimal induction of IL-13 and plays essential roles in the binding to the IL-5 promoter and the induction of IL-5. Based on these observations, we propose that the induction of IL-4 requires GATA-3-mediated chromatin remodeling during Th2 development, which is attained in the N-finger-independent manner, followed by transactivation in effector Th2 cells which is mediated mainly by factors other than GATA-3, like c-maf and NFAT. Optimal induction of IL-13 requires GATA-3 in both the N-finger-independent and -dependent steps; chromatin remodeling during Th2 development and transactivation in effector Th2 cells. In contrast, the induction of IL-5 absolutely depends on GATA-3-mediated transactivation in effector Th2 cells, which is accomplished through the N-finger-dependent binding to the IL-5 promoter.

Acknowledgments

We are grateful to Drs. H. J. Lee and N. Arai for useful discussions and Dr. J. M. Kim for constructive comments throughout the study.

References


