Essential Role of GATA3 for the Maintenance of Type 2 Helper T (Th2) Cytokine Production and Chromatin Remodeling at the Th2 Cytokine Gene Loci*

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GATA3 expression is essential for type-2 helper T (Th2) cell differentiation. GATA3-mediated chromatin remodeling at the Th2 cytokine gene loci, including Th2-specific long range histone hyperacetylation of the interleukin (IL)-13/IL-4 gene loci, occurs in developing Th2 cells. However, little is known about the role of GATA3, if any, in the maintenance of established remodeled chromatin at the Th2 cytokine gene loci. Here, we established a Cre/loxP-based site-specific recombination system in cultured CD4 T cells using a unique adenovirus-mediated gene transfer technique. This system allowed us to investigate the effect of loss of GATA3 expression in in vitro differentiated Th2 cells. After ablation of GATA3, we detected reduced production of all Th2 cytokines, increased DNA methylation at the IL-4 gene locus, and decreased histone hyperacetylation at the IL-5 gene locus but not significantly so at the IL-13/IL-4 gene loci. Thus, GATA3 plays important roles in the maintenance of the Th2 phenotype and continuous chromatin remodeling of the specific Th2 cytokine gene locus through cell division.

After antigenic stimulation, naive CD4 T cells differentiate into two distinct helper T cell (Th)1 subsets, Th1 and Th2 cells.

(1). Th1 cells produce IFN-γ to control cell-mediated immunity against intracellular pathogens. Th2 cells produce IL-4, IL-5, and IL-13, and are involved in humoral immunity and allergic reactions (2–4). The outcome of Th cell differentiation depends on the cytokine environment (5, 6). IL-4-mediated STAT6 activation is important for inducing efficient Th2 cell generation (7, 8), although IL-4/STAT6-independent Th2 responses have also been reported in various experimental systems (9–13).

Recent studies have identified several transcription factors that control Th2 cell differentiation (8, 14, 15). Among them, GATA3 appears to be a master transcription factor for Th2 cell differentiation. GATA3 is selectively expressed in Th2 cells and its ectopic expression induces Th2 cell differentiation even in the absence of STAT6 (16–19). Also, GATA3-dependent autoactivation (13, 19) and an instructive role of GATA3 for Th2 cell differentiation (20) were reported.

Changes in the chromatin structure of the Th2 cytokine (IL-4/IL-5/IL-13) gene loci occur during Th2 cell differentiation (14, 21). Th2 cell differentiation induced by ectopic expression of GATA3 results in DNA demethylation (21) and the induction of DNase I-hypersensitive sites in the IL-4 gene locus (19, 22). Recently, we and others demonstrated that histone hyperacetylation of the Th2 cytokine gene loci occurs in developing Th2 cells in a Th2-specific and STAT6-dependent manner (23–25). We demonstrated an essential role for GATA3 in Th2-specific histone hyperacetylation (23). We generated a precise map of the Th2-specific histone hyperacetylation within the type 2 cytokine gene loci, and identified a 71-bp conserved GATA3 response element (CGRE) 1.6 kbp upstream of the IL-13 locus exon 1 (23). The conserved GATA3 response element (CGRE) may play a crucial role for GATA3-mediated targeting and downstream spreading of core histone hyperacetylation within the IL-13 and IL-4 gene loci in developing Th2 cells. However, it is still unclear whether continuous expression of GATA3 is required for the maintenance of the established chromatin remodeling at the Th2 cytokine gene loci.

In the present study, we investigated the role for GATA3 in the maintenance of Th2 cytokine production and the remodeled chromatin using a newly established in vitro site-specific recombination system. The loss of GATA3 expression resulted in decreased Th2 cytokine production, reduction of histone hyperacetylation at the IL-5 gene locus, and increased DNA methylation at the IL-4 gene locus. Thus, GATA3 plays important roles in the maintenance of the Th2 phenotype and continuous chromatin remodeling of the specific Th2 cytokine gene loci.
sorter (Miltenyi Biotec), yielding purity of CD4 cells were purified using magnetic beads and an Auto-MACS cell differentiation cultures were done as described (23, 29). Splenic adenovirus receptor (CAR) Tg mice) has been previously described (27). Role of GATA3 in the Maintenance of Th2 Phenotype

EXPERIMENTAL PROCEDURES

Mice—C57BL/6 mice were purchased from SLC (Shizuoka, Japan). STAT6-deficient mice were kindly provided by Dr. Shizuo Akira (Osaka University, Osaka, Japan). Transgenic mice expressing coxsackie/adeno virus receptor under the control of an lck proximal promoter (cox sackie/adeno virus receptor (CAR) Tg mice) has been previously described (27). All mice used in this study were maintained under specific pathogen-free conditions and were used at 4–6 weeks of age. Animal care was in accordance with the guidelines of Chiba University.

Flow Cytometry Staining and Flow Cytometry Analysis—In general, one million cells were stained with antibodies as indicated according to a standard method (28). Anti-CD4-fluorescein isothiocyanate (RM4-1; BD Pharmingen) and anti-CD8-PE (53-6.7–2; PE–) were purchased from BD Pharmingen. For detecting hCAR, bintylated anti-CAR antibody (RmBc) (27) and Cy5-conjugated avidin were used. For intracellular staining, aliphycocyanin-conjugated anti-IFN-γ antibody (OXMG1.2; BD Pharmingen), anti-IL-5 antibody (TRFK5; BD Pharmingen), and PE-conjugated anti-IL-4 antibody (11B11; BD Pharmingen) were used (29, 30). Flow cytometry analysis was performed on FACScalibur (BD Biosciences) and results were analyzed with CELLQUEST software (BD Biosciences).

In Vitro T Cell Differentiation Culture—Purification and in vitro Th cell differentiation cultures were done as described (23, 29). Splenic CD4 cells were purified using magnetic beads and an Auto-MACS sorter (Miltenyi Biotec). Splegic CD4 cells were stimulated for 2 days with immobilized anti-TCR mAb (H57-597; BD Pharmingen) and anti-CD28 mAb (37.51; BD Pharmingen) in the presence of IL-2 (25 units/ml), IL-12 (100 units/ml), and anti-IL-4 mAb (11B11, 25% culture supernatant). For Th2 cell differentiation, cells were stimulated with immobilized anti-TCR mAb and anti-CD28 mAb for 2 days in the presence of IL-2 (25 units/ml), IL-4 (100 units/ml), and anti-IFN-γ mAb (R4–6A2, 25% culture supernatant). Cells were then transferred to new wells and cultured for another 3 days in the presence of only the cytokines present in the initial culture. In some experiments, two or three cycles of the anti-TCR plus anti-CD28 stimulation were used.

Virus Infections, Injection, and Strategy for Deletion of GATA3 Transgene—The retroviral vector pMX-IRES-EGFP and a Plat-E packaging cell line were kindly provided by Dr. Toshio Kitamura (University of Tokyo, Tokyo, Japan). Retrovirus vectors containing a loxP-flanked GATA3/IRES-EGFP cassette (pMX-loxP-GATA3-IRES-EGFP-loxP) were generated using the original pMX-IRES-GFP vector (31). The method for the preparation of virus supernatant was described previously (31). An adenovirus vector containing a Cre recombinase expression cassette (Ad-Cre) was kindly provided by Izumi Saito (University of Tokyo, Tokyo, Japan) (Fig. 1B).

To investigate the effect of loss of GATA3 expression in differentiated Th2 cells, we established a site-specific recombination system in CD4 T cells cultured in vitro. The strategy of introduction and deletion of the GATA3 transgene is illustrated in Fig. 1C. First, naive CD4 T cells were stimulated under Th1-skewed conditions, and infected with retrovirus vectors containing a loxP-flanked GATA3/IRES-EGFP cassette. Three days later, GFP-positive retrovirus-infected cells were sorted with a FACSVantage (BD Biosciences) flow cytometer and restimulated under the same Th1-skewed conditions of initial stimulation for a further 5 days. After another cycle of 5-day re-stimulation culture under Th1-skewed conditions, the cells were infected with Ad-Cre to delete the GATA3/IRES-EGFP transgene by expressing NLS-tagged Cre recombinase. The preparation of adenovirus supernatant was done as described previously (31). Entry of adenovirus involves high-affinity binding of the viral fiber capsid protein to a cellular receptor, CAR. We used CAR Tg mouse T cells to avoid the limited expression of CAR on T cells (27). In Figs. 4 and 5, a more strict protocol was used. The outline of the protocol is shown in Fig. 4A. Four days after infection of retrovirus vectors containing a loxP-flanked GATA3/IRES-EGFP, cells were stimulated with immortalized anti-TCR and anti-CD28 for 4 h, stained with anti-IL-4 PE detection mAbs using IL-4 Secretion Assay kit (number 130-090-515; Miltenyi Biotec), and GFP-IL-4+ cells were sorted with purity >98%. The sorted cells were cultured for 6 days in the presence of cytokines (IL-2 and IL-12), and then another stimulation with anti-TCR and anti-CD28 was performed. Two days later, cells were infected with Ad-Cre. Four days after Ad-Cre infection, GFP-+ cells were sorted to exclude the small numbers of GFP (+) GATA3 expressing cells remaining in the culture. After T cell expansion by anti-TCR stimulation, analysis was done on day 25. To exclude the effect of endogenously induced GATA3 molecules, we used naive STAT6-deficient CD4 T cells and Th1-skewed culture conditions containing anti-IL-4 mAb throughout the 25-day cultivation.

PCR Analysis—The levels of EGFP transgene were assessed by semi-quantitative PCR with a specific primer pairs: forward, GTGAACGCT-CAGATCGG-3’ and reverse, 5’-TTACCTTGAGCAGCTGTC. ELISA for the Measurement of Cytokine Concentration—Cells were stimulated with immobilized anti-TCR (3 μg/ml) in 48-well flat bottom plates (2.5 × 105 cells/well) for 24 h at 37 °C. The production of IL-4, IL-5, IFN-γ, and IL-2 was assessed by ELISA as described previously (30). The production of IL-13 was evaluated with a mouse IL-13 ELISA kit (R & D Systems) according to the manufacturer’s protocol.

RESULTS Efficient Adenovirus-mediated Transgene Introduction into CAR Tg CD4 T Cells in Vitro—The aim of this study was to determine the role of GATA3, if any, in the maintenance of the established Th2 phenotype and Th2-type chromatin remodeling. To investigate the effect of loss of GATA3 expression in differentiated Th2 cells, we established a Cre/loxP-mediated site-specific recombination system in T cells. The system constitutes (i) retrovirus-mediated introduction of a loxP-flanked GATA3 transgene for Th2 cell differentiation from naive CD4 T cells, and (ii) subsequent adenovirus-mediated Cre expression to delete the loxP-flanked GATA3 transgene (Fig. 1). Thus, we first evaluated the feasibility of adenovirus-mediated gene transfer in T cells. Naive CD4 T cells express limited amounts of CAR and are known to be resistant to adenovirus infection. To increase the efficiency of adenovirus infection in T cells, we used CAR Tg mice expressing CAR on T cells under the control of the proximal promoter of lck and a CD2 enhancer, in which the majority of CD4 and CD8 T cells in the spleen showed high level cell surface expression of CAR (27). Freshly prepared CD4 T cells from CAR Tg mice were infected with Ad-EGFP. Two days after infection, the majority of CAR Tg CD4 T cells expressed substantial levels of GFP compared with that of non-Tg B6 CD4 T cells (Fig. 2A). A time course of the GFP expression after Ad-EGFP infection was assessed in CAR CD4 T cells cultured under Th1- or Th2-skewed conditions (Fig. 2B). The expression of GFP peaked on day 3 in either Th1 or Th2-skewed culture conditions. The high-level expression was maintained for at least 4 days after infection. Thus, adenovirus-mediated gene transfer was efficient when using CD4 T cells from CAR Tg mice.

Deletion of a loxP-flanked EGFP Transgene by Ad-Cre Infection in Cultured CD4 T Cells—The efficiency of Cre-mediated DNA recombination was next assessed in CAR Tg CD4 T cells using EGFP as an indicator. CAR Tg CD4 T cells were stimulated with anti-TCR mAb plus anti-CD28 mAb and infected with a retrovirus containing a loxP-flanked EGFP cassette (pMX-loxP-EGFP-loxP). GFP-expressing infected cells were sorted, restimulated for 3 days, and then infected with either 1 or 3 × 105 IFU of Ad-Cre as described under “Experimental Procedures.” Fig. 3A shows a representative genomic DNA PCR result assessing the amount of EGFP transgene DNA left in

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Role of GATA3 in the Maintenance of Th2 Phenotype

Efficient Depletion of Retrovirus-induced GATA3 Expression by Ad-Cre Infection in in Vitro Differentiated Th2 Cells—To investigate the role of GATA3 in the maintenance of the Th2 phenotype, CD4 T cells from STAT6-deficient CAR Tg mice cultured under Th1-skewed conditions were infected with retroviral vectors containing a lox-P-flanked GATA3-IRES-EGFP cassette (pMx-loxP-GATA3-IRES-EGFP-loxP). In the STAT6-deficient T cells cultured under Th1-skewed conditions, endogenous GATA3 induction was minimum. The outline of the protocol is shown in Fig. 4A. Four days after infection of the retrovirus vector, the cells were stimulated with immobilized anti-TCR and anti-CD28 mAbs, stained with anti-IL-4-PE detection mAbs, and GFP “IL-4” cells were sorted. Representative GFP/IL-4 profiles are shown in Fig. 4B. The sorted cells were cultured for 6 days in the presence of cytokines (IL-2 and IL-12), and another cycle of stimulation with anti-TCR and anti-CD28 was performed on day 12. Two days later, the cells were infected with Ad-Cre (3 × 10⁸ IFU). Four days after Ad-Cre infection, GFP⁺ cells were sorted to enrich for GATA3 transgene-depleted cells. Th1-skewed conditions were used throughout the 25-day culture. The cultured cells were harvested, and the expression levels of EGFP and GATA3 were assessed to confirm that GATA3 protein is depleted (Fig. 4, C and D). As can be seen, the levels of GFP fluorescence were reduced (Fig. 4C), and the expression levels of GATA3 protein were decreased dramatically (about 10-fold) in the cells infected with Ad-Cre (Fig. 4D). Without Ad-Cre infection, the expression of GATA3 protein was not changed during the last 7-day cultivation (data not shown).

The mRNA levels of several transcriptional regulators (GATA3, e-Maf, JunB, and T-bet) in the Th2 cells after ablation of GATA3 were assessed (Fig. 4E). As expected, the mRNA levels of GATA3 were ~1/10 of those of LacZ-infected control cells. In contrast, essentially no significant change in e-Maf or JunB expression was detected in the Ad-Cre-infected T cells. The expression of T-bet was reduced by the expression of GATA3, and restored by the depletion of GATA3 transgene.

Expression of GATA3 Is Required for Th2 Cytokine Production in in Vitro Differentiated Th2 Cells—Cytokine production profiles of the cells prepared in Fig. 4 were assessed by cyto­plasmic staining. As can be seen in Fig. 5A, middle panels, more than 40% (39.3 ± 3.5%) of the cells infected with pMx-loxP-GATA3-IRES-EGFP-loxP were IL-4 producing cells, and more than 30% (19.2 ± 18.2%) were IL-5 producing cells. Marginal numbers of IFN-γ producing IL-4 non-producing cells were detected (7.0%). The percentages of IL-4 producing cells were decreased to about 25% (23.1 ± 3.8%) after Ad-Cre infection, and those of IL-5 were about 16% (8.1 ± 7.9%) (compare the percentages depicted in the middle and right panels in IFN-γ/IL-4 and IL-5/IL-4 profiles). A significant number of IFN-γ producing cells was noted, suggesting that some of the cells become IFN-γ producing cells after deletion of the GATA3 transgene. These results suggest that GATA3 expression is important for the maintenance of Th2 cytokine production.

Next, the levels of Th2 cytokines produced in the culture supernatant were determined by ELISA. Little in the way of Th2 cytokines (IL-4, IL-13, and IL-5) were detected in supernatants from non-infected cells cultured under Th1-skewed conditions. In contrast, GATA3-transduced cells produced large amounts of Th2 cytokines and decreased amounts of Th1 cytokines (IFN-γ and IL-2) as previously reported (16–19). As
expected, the production of Th2 cytokines (IL-4, IL-13, and IL-5) was significantly decreased by Ad-Cre infection (Fig. 5B, bottom). IFN-γ production was moderately restored. These results suggest that the continuous expression of GATA3 is important for the production of IL-4, IL-5, and IL-13 in the in vitro differentiated Th2 cells.

GATA3 Is Required for the Maintenance of Hyperacetylation of Histone H3 in the IL-5 Gene Locus but Not in the IL-13/IL-4 Gene Loci—Finally, we assessed the chromatin remodeling status of the Th2 cytokine gene loci after deletion of the GATA3 transgene. Acetylation status of histone H3 (K9/14) in the nucleosomes associated with the Th2 cytokine gene loci was determined by ChIP assays. The levels of acetylation in in vitro differentiated Th2 cells cultured under Th2-skewed conditions for 5 days are shown for comparison. The relative band intensities (Ac-H3/Input DNA) are shown in Fig. 6B. As we reported previously, ectopic expression of GATA3 induced histone hyperacetylation in the Th2 cytokine gene loci (Fig. 6A, Ac-H3, second column) (23). As shown in Fig. 6, A and B, significantly reduced histone hyperacetylation of the IL-5 promoter region was detected by the Ad-Cre-mediated deletion of the GATA3 transgene. The decrease in the acetylation of IL-4 promoter, IL-13 promoter, Vα enhancer, CNS1, and GATA3 response element was marginal. Acetylation of the IFN-γ promoter was decreased by GATA3 expression, and significantly increased by deletion of the GATA3 transgene (Fig. 6, A and B, bottom).

GATA3 Is Required for the Maintenance of Continuous Demethylation of the IL-4 Intron 2 Region—Demethylation of the IL-4 intron 2 region in developing Th2 and established Th2 cells was reported previously (21). Here, we used a methylation-specific PCR technique to evaluate the methylation status of IL-4 intron 2 (34). After treatment of genomic DNA with bisulfate, unmethylated cytidine is converted to uridine but methylcytidine is preserved as cytidine. In this system, primers that distinguish uridine (thymidine) and cytidine at sites of CpGs were used to evaluate the levels of methylation. We focused on two cytidine residues within the IL-4 intron 2 region, and four patterns (both methylated, M/M; one methylated and one demethylated, M/U or U/M; and both demethylated, U/U) would be detected. In GATA3 non-transduced cells, 50% of the genome contained methylated cytidine at both residues (Fig. 6, C and D, top). In GATA3-transduced cells, only 20% of this region was methylated at both residues, 40% was unmethylated and 40% was hemimethylated (Fig. 6, C and D, middle). When the GATA3 transgene was deleted, 50% of the genome contained methylated cytidine at both residues (Fig. 6, C and D, bottom), suggesting that the methylation pattern was compatible to that of non-GATA3 transduced cells. These results suggest that continuous GATA3 expression is required to maintain the unmethylated status of the Th2 cytokine gene loci in differentiated Th2 cells.

DISCUSSION

In the present study, we established a Cre/LoxP-based site-specific recombination system in cultured CD4 T cells using a unique adenovirus-mediated gene transfer technique. Ectopic
expression of GATA3 induced Th2 cell generation without IL-4 or STAT6 activation. Using these Th2 cells, the role of GATA3 expression in the maintenance of Th2 phenotype was examined by deleting the GATA3 transgene with adenovirus-mediated expression of the Cre protein. The reduction of GATA3 expression in the in vitro differentiated Th2 cells resulted in decreased production of all Th2 cytokines tested (IL-4, IL-13, and IL-5) (Fig. 5), decreased histone hyperacetylation of the IL-5 gene locus (Fig. 6, A and B), and increased methylation of DNA at the IL-4 intron 2 region (Fig. 6, C and D). These results suggest that continuous expression of GATA3 is required for the maintenance of Th2 cytokine production and remodeled open chromatin at the specific Th2 cytokine gene loci.

The production of Th2 cytokines, particularly IL-5 and IL-13, were reported to be highly dependent on the transcriptional activity of GATA3 (16, 35, 36). We reported that expression of GATA3 induced more than a 10-fold increase in IL-5 and IL-13 promoter activities, whereas that of the IL-4 promoter was increased only about 2-fold (23). Therefore, the decreased IL-5 and IL-13 production after deletion of the GATA3 transgene by Cre-induced recombination is explained at least in part by the decreased transcriptional activity of GATA3.

More importantly, however, we detected decreased histone hyperacetylation at the IL-5 gene locus and increased methylation of IL-4 gene intron 2 following GATA3 ablation (Fig. 6), suggesting that the levels of openness of chromatin at specific Th2 cytokine gene loci were dependent on the expression of GATA3. Interestingly, the levels of acetylation at the IL-13/IL-4 gene loci were not significantly affected by the ablation of the GATA3 protein (Fig. 6). It is possible that small amounts of residual GATA3 are sufficient for the maintenance of acetylation of the IL-13/IL-4 gene loci but not for that of the IL-5 locus. Alternatively, GATA3 independent molecular events that maintain the histone hyperacetylation are operating at the IL-13/IL-4 gene loci in differentiated Th2 cells. Histone H3-K4 methylation and histone H3-K9/14 acetylation appear to be associated with transcriptionally active chromatin (37). Disruption of an H3-K4-specific methyltransferase, MLL containing a SET domain, resulted in reduced histone acetylation (38, 39). Thus, unknown but critical molecular events may control histone H3-K9/14 acetylation as well as histone H3-K4 methylation in in vitro differentiated Th2 cells.

Recently, we have reported that the induction of histone hyperacetylation at the IL-5 gene locus is dependent on STAT6 and GATA3, but the signal requirements are distinct from that for the IL-13/IL-4 gene loci (40). The remodeling process of the IL-5 gene locus is more sensitive to CD28-induced NF-κB activation. It is possible that molecular events governing the maintenance of histone hyperacetylation of the IL-13/IL-4 gene loci and that of IL-5 locus are distinct.
We also demonstrated that GATA3 expression is required for the maintenance of demethylation of the IL-4 intron 2 region (Fig. 6, C and D). It is not clear whether GATA3 is involved directly in the methylation processes of the methyltransferase complex at this time. Recently, Tamaru et al. (42) reported that methylation of lysine 9 of histone H3 is a mark for DNA
methylation in Neurospora crassa (41). Also very recently, a tight correlation between methylation of lysine 9 of histone H3 and DNA methylation was reported in mammalian cells (42). Finally, it has been reported in many systems that there is an inverse correlation between acetylation and methylation of histone H3 lysine 9 in chromatin activation (43–45). Thus, it is likely that GATA3 is required solely for selective targeting of the histone acetyltransferase complex to the Th2 cytokine gene loci, and that this causes the appearance of demethylation indirectly. Whereas Hutchins et al. (46) reported that GATA3 is not required for the induction of DNA demethylation of intron 2 of the IL-4 gene locus, we detected demethylation of one site in the same intron 2 region by ectopic expression of GATA3 (Fig. 6). The reason for this apparent discrepancy is not clear, but it is possible that the PCR detection system we used here, to assess demethylation at specific sites in the IL-4 intron 2 region, may be more sensitive than the Southern blotting method used by Hutchins et al. (46).

In Fig. 4, the levels of residual GATA3 after Cre introduction appeared to be about 10% of control. However, the numbers of IL-4 or IL-5 producing cells and the levels of actual cytokine production were only reduced 2–3-fold (Fig. 5, A and B). These results may suggest that a certain low level of GATA3 protein is enough to maintain Th2 cytokine gene expression in some cells. It is also possible that the Th2 phenotype is already fixed in certain numbers of Th2 cells, in which unknown GATA3-independent mechanisms control the maintenance of the Th2 phenotype.

Murphy and colleagues (13) reported that the expression of GATA3 is controlled by autoactivation. Two distinct promoters control the expression of GATA3 (47). A newly identified promoter is suggested to be responsible for GATA3-dependent GATA3 transcription (GATA3 autoactivation). Thus, we performed Northern blot analysis to assess endogenous GATA3 levels, and could not detect any endogenous GATA3 after deletion of the GATA3 transgene. One possible explanation is that the expression level of GATA3 after in vitro site-specific recombination was too low to activate a GATA3-dependent promoter. In any event, the effect of deletion of the GATA3 transgene could not have been complicated in any way by the expression of endogenous GATA3 protein that might have been induced by so called autoactivation, as there was no endogenous GATA3 expression.

In our Cre/LoxP-based site-specific recombination system, the retrovirus-introduced transgene was deleted from the genome quite efficiently by adenovirus-mediated Cre introduction (Fig. 3, A and B). Thus, this system has proven to be a powerful tool for studying stage-specific roles of GATA3, and may be useful in this regard with various factors that are crucial for T cell activation, differentiation, and function. In summary, we demonstrated an important role for GATA3 in the maintenance of Th2 cytokine production, and remodeled
open chromatin at the specific Th2 cytokine gene loci using a newly established in vitro site-specific recombination system.

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Essential Role of GATA3 for the Maintenance of Type 2 Helper T (Th2) Cytokine Production and Chromatin Remodeling at the Th2 Cytokine Gene Loci

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