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Transcription factor YY1 is essential for regulation of the Th2 cytokine locus and for Th2 cell differentiation

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The Th2 locus control region (LCR) has been shown to be important in efficient and coordinated cytokine gene regulation during Th2 cell differentiation. However, the molecular mechanism for this is poorly understood. To study the molecular mechanism of the Th2 LCR, we searched for proteins binding to it. We discovered that transcription factor YY1 bound to the LCR and the entire Th2 cytokine locus in a Th2-specific manner. Retroviral overexpression of YY1 induced Th2 cytokine expression. CD4-specific knockdown of YY1 in mice caused marked reduction in Th2 cytokine expression, repressed chromatin remodeling, decreased intrachromosomal interactions, and resistance in an animal model of asthma. YY1 physically associated with GATA-binding protein-3 (GATA3) and is required for GATA3 binding to the locus. YY1 bound to the regulatory elements in the locus before GATA3 binding. Thus, YY1 cooperates with GATA3 and is required for regulation of the Th2 cytokine locus and Th2 cell differentiation.

Th2 cytokine genes *il4*, *il13,* and *il5* are clustered in chromosome 5 in human and chromosome 11 in mouse. The expression of the Th2 cytokine genes are coordinately regulated during Th2 cell differentiation. Several different regulatory elements have been shown to play an important role in Th2 cytokine gene expression, including promoters of Th2 cytokine genes, enhancers [conserved noncoding sequence 1 (CNS1)/hypersensitive site 1-3 (HSS1-3), hypersensitive site V (HSV)/CNS2, and intronic enhancer (IE)/HSII], a silencer (HSIV), and a locus control region (LCR) (1, 2). The functions of these regulatory elements in Th2 cytokine expression in Th2 cells in vivo have been thoroughly investigated in transgenic mice and knockout mice that have targeted deletion in the regulatory elements (3–10). Among these regulatory elements, Th2 LCR has been shown to coordinately regulate Th2 cytokine genes, to induce chromatin remodeling, and to be required for chromosomal interactions (1, 2). The Th2 LCR is located in the introns of the *Rad50* gene and is composed of four DNase I hypersensitive sites, including RHS4, RHS5, RHS6, and RHS7 (11, 12). In previous studies, we have shown that the Th2 LCR is essential for Th2 cytokine expression and chromosome remodeling in the Th2 cytokine locus and in the pathogenesis of allergic asthma (7, 11, 13).

The entire Th2 cytokine locus undergoes chromatin remodeling during Th2 cell differentiation (1, 2, 14). DNase I hypersensitive sites, which reflect accessibility of chromatin, are developed at the specific regulatory regions during Th2 cell differentiation (15, 16). Histone 3 lysine 9 (H3K9)-acetylation and histone 3 lysine 4 (H3K4)-methylation increase at specific regulatory regions in the Th2 cytokine locus during Th2 cell differentiation (17–20). Th2 cytokine locus also undergoes DNA demethylation during Th2 cell differentiation (11, 21–26). Treatment with drugs that cause chromatin modification such as 5-azacytidine or Trichostatin A induces Th2 cytokine expression upon T-cell receptor (TCR) stimulation without IL-4 (27). In addition, deficiency in chromatin-modifying enzymes in CD4 T cells causes aberrant cytokine expression (28–30). These results suggest that chromatin modifications are critically important for Th2 cytokine expression and Th2 differentiation. GATA3, the key transcription factor for Th2 differentiation (31), can induce chromatin remodeling at the Th2 cytokine locus without IL-4 signaling (32–34), suggesting that it plays a major role in the chromatin remodeling process. However, how GATA3 induces chromatin remodeling during Th2 cell differentiation is poorly understood.

The Th2 cytokine locus also engages in chromosomal interactions by either intra- or interchromosomal association (35–38). The Th2 LCR interacts with the promoters of Th2 cytokine genes and enhancers through long-range intrachromosomal interactions (35). Ectopic expression of *gata3* with the treatment of phorbol myristate acetate (PMA) + ionomycin is sufficient to induce some of intrachromosomal interactions in fibroblast cells (35), suggesting that GATA3 plays an important role in this process. Intrachromosomal interactions in the Th2 cytokine locus are greatly reduced in RHS7-deficient cells (7), suggesting that RHS7 is critical in chromosomal interactions. The Shigematsu group has shown that SATB1-binding sites present in the Th2 cytokine locus undergo long-range intrachromosomal interaction in fully developed Th2 cells (D10 cells), and that SATB1 plays a critical role in these interactions (36). The Th2 cytokine locus also interacts with the *IFN-γ* gene locus, which is present in a different chromosome, as a result of interchromosomal interaction (37).

Although the regulatory elements in the Th2 cytokine locus and the chromatin remodeling are well understood, the underlying molecular mechanism of chromatin remodeling and chromosomal interaction—in particular, which proteins play a role in this process—has not been adequately determined. To examine the molecular mechanism in the function of the Th2 LCR, we searched for proteins binding to RHS7 using a transcription factor-binding database and an EMSA and discovered YY1 as a candidate binding protein. YY1 bound to several regulatory elements in the Th2 cytokine locus, including RHS7. Overexpression of YY1 induced the expression of Th2 cytokine genes. Th2 cells from CD4-sppecific YY1 knockdown (KD) mice showed a reduction in Th2 cytokine expression, inactive chromatin conformation, and intrachromosomal interactions, and resistance in an animal model of asthma. YY1 interacted with GATA3, and YY1 was required for binding of GATA3 to the Th2 cytokine locus. These results strongly suggest that YY1 is a critical transcription factor mediating Th2 cytokine locus regulation in cooperation with GATA3.

Results

YY1 Bound to RHS7 and Other Regulatory Elements in the Th2 Cytokine Locus. To examine the molecular mechanism of the Th2 LCR, we searched for proteins that bind to RHS7. Using a DNA sequence comparison between human and mouse, the Promo program,

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which is based on the TRANSFAC 8.3 database (http://alggen.lsi.upc.es), and an EMSA, we discovered that a transcription factor YY1 can bind to a short region of RHS7, which we named as RHS7b, in a Th2-specific manner (Fig. S1 *A* and *B*). Specific binding of YY1 to RHS7b was confirmed by antibody-supershift and oligonucleotide competition assays (Fig. S1*C*).

To examine whether YY1 binds to other regulatory elements in the Th2 cytokine locus in vivo, we performed a ChIP assay (Fig. 1*A*). Fragmented chromatin from Th1 or Th2 cells was precipitated with an anti-YY1 antibody, and the relative amount of precipitated DNA was measured by quantitative PCR. YY1 bound to several regulatory elements in the Th2 cytokine locus, including the promoters of *IL-4* and *IL-13* genes, RHS7, CNS1/HSS, HSV/CNS2, HSIV, and HSVa in a Th2-specific manner (Fig. 1*A*).

Next, we examined whether YY1 is induced by Th1 or Th2 stimulation. Naive CD4 T cells from C57BL/6 were stimulated under either a Th1 or Th2 polarizing condition. The amount of *yy1* transcript and YY1 protein was analyzed by quantitative RT-PCR and immunoblot analysis, respectively. YY1 was rapidly induced as early as 12 h after TCR stimulation both in Th1 and Th2 cells, but to a greater extent in Th2 cells at both the RNA and protein level (Fig. 1 *B* and *C*).

Ectopic Expression of *yy1*-Induced Th2 Cytokine Expression. To explore the role of YY1 in Th2 cytokine gene expression, we used ectopic expression of *yy1* with MIEG3 retroviral vector. The vector contains an internal ribosomal entry site, so it coexpresses a GFP in the transduced cells. We transduced *yy1* into naive CD4 T cells and differentiated toward Th1 or Th2. The expression of Th1 and Th2 cytokines was measured by intracellular cytokine staining and by quantitative RT-PCR with sorted GFP^+ cells (Fig. S2). Ectopic expression of *yy1* modestly induced all Th2 cytokines, IL-4, IL-5, and IL-13 in Th2 cells. However, YY1 did not affect the expression of *ifng* in Th1 cells. Next, we performed the same retroviral transduction experiment with RHS7-deficient mice to examine whether RHS7 is required for the function of YY1 (Fig. S2). Although the induction of Th2 cytokines by YY1 was not affected, the amount of Th2 cytokines expressed greatly reduced in RHS7 KO Th2 cells compared with that in WT Th2 cells (Fig. S2), suggesting that expression of Th2 cytokines by YY1 requires RHS7.

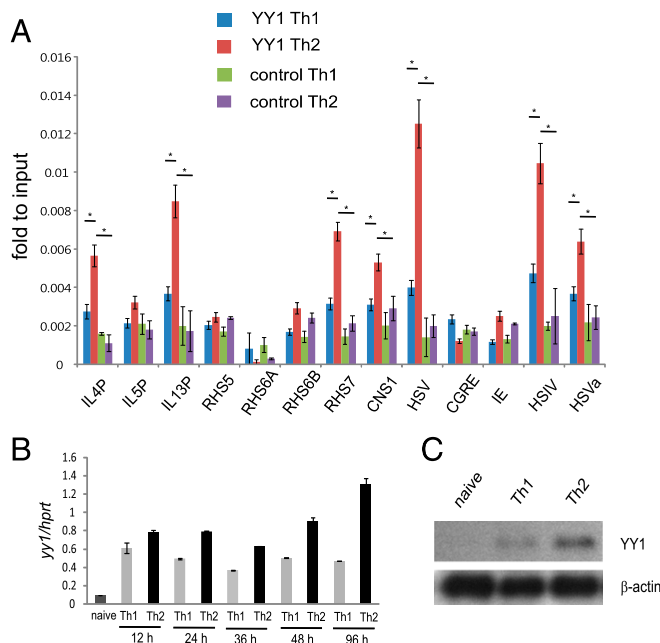

Fig. 1. YY1 binding to Th2 cytokine locus and its expression. (*A*) YY1 binding to the Th2 cytokine locus in a Th2-specific manner. YY1 binding to regulatory regions at the Th2 cytokine locus in Th1 and Th2 cells was measured by ChIP using an anti-YY1 antibody or control IgG. Data are shown as percentages of input DNA. Error bar shows SD ($n = 3$). Data are representative of two independent experiments with similar results. (*B* and *C*) Th2-specific expression of YY1. Expression of YY1 was measured by quantitative RT-PCR at various time points after stimulation (*B*) or by immunoblotting at day 4 (*C*) in Th1 and Th2 cells. Error bar shows SD ($n = 3$). Statistical difference between YY1 Th1 and YY1 Th2 or between YY1 Th2 and control Th2 was analyzed by Student *t* test. $*P < 0.05$.

YY1 KD Reduced Th2 Cytokine Expression. To investigate the function of YY1 in physiological conditions, we used conditional YY1 KD mice (39). This mouse contains pSico-YY1 as a transgene, in which split U6 promoters can be joined together by removing intervening CMV-EGFP by Cre/loxP recombination (39). pSico-YY1 transgenic mice were crossed with CD4-Cre transgenic mice to generate pSico-YY1(CD4-Cre) mice (YY1 KD mice) that have YY1 KD in CD4 T cells. We first examined the expression of YY1 in CD4 T cells from pSico-YY1(CD4-Cre) mice. The amount of YY1 protein was almost completely reduced in CD4 T cells from pSico-YY1(CD4-Cre) mice compared with those from pSico-YY1 control mice (Fig. 2*A*). We also examined whether conditional YY1 KD in CD4 T cells has any developmental defect. The ratio of CD4 single positive to CD8 single positive T cells in the thymus was normal in pSico-YY1(CD4-Cre) mice (Fig. S3*A*). However, the ratios of CD4 to CD8 cells and of T to B cells were reduced by half compared with those of control pSico-YY1 mice in the spleen (Fig. S3 *B* and *C*). The ratio of naive to effector/memory CD4 T cells was no different in pSico-YY1(CD4-Cre) mice from that in pSico-YY1 mice (Fig. S3*D*). The proliferation of splenic naive CD4 T cells from pSico-YY1(CD4-Cre) mice was comparable to that in control mice (Fig. S3*E*). These results suggest that, although there is a partial reduction in the ratio of CD4 T-cell population in the periphery of the pSiro-YY1(CD4-Cre) mice, peripheral CD4 T cells from these mice do not have notable functional defects in activation and proliferation. Therefore, we determined to use these mice for further experiments.

To investigate the functional role of YY1 in Th2 cell differentiation, we examined the Th1/Th2 cytokine expression in pSico-YY1(CD4-Cre) mice. Naive CD4 T cells were isolated from the spleen of pSico-YY1(CD4-Cre) and control pSico-YY1 mice and differentiated into a Th1 or Th2 polarizing condition. The expression of cytokine genes was measured at the transcriptional level by quantitative RT-PCR. Interestingly, the expression of *il4* was found to be greatly reduced in pSico-YY1(CD4-Cre) cells compared with control cells, and that of *il5* and *il13* was also reduced by half compared with those in control cells (Fig. 2*B*). However, the expression of *ifng* was no different between pSico-YY1(CD4-Cre) cells and control cells. The same result was obtained at the protein level, measured by ELISA (Fig. 2*C*). We also measured the expression of subset-specific transcription factors T-bet, GATA3, and STAT6 in these cells. The expression of these factors was no different between YY1 KD and control cells at the protein and transcript levels (Fig. 2 *A* and *B*), suggesting that the reduction of Th2 cytokines in YY1 KD Th2 cells is not due to altered expression of these transcription factors.

YY1 KD Reduced Chromatin Remodeling in the Th2 Cytokine Locus. To investigate whether YY1 plays a role in chromatin remodeling in the Th2 cytokine locus, we measured H3K4-methylation and H3-acetylation states, which are activation markers, using ChIP. Both H3K4-methylation and H3-acetylation were reduced in many regulatory elements in the Th2 cytokine locus, including the promoters of the Th2 cytokine genes, enhancers, and a locus control region in YY1 KD Th2 cells, compared with control cells (Fig. S4 *A* and *B*). We also measured DNA methylation at the *il4* promoter and RHS7 in Th2 cells from WT and YY1 KD mice. DNA methylation at sites between –501 and –274 from the *il4* transcriptional start site and the entire region of RHS7 substantially increased in YY1 KD Th2 cells compared with those in

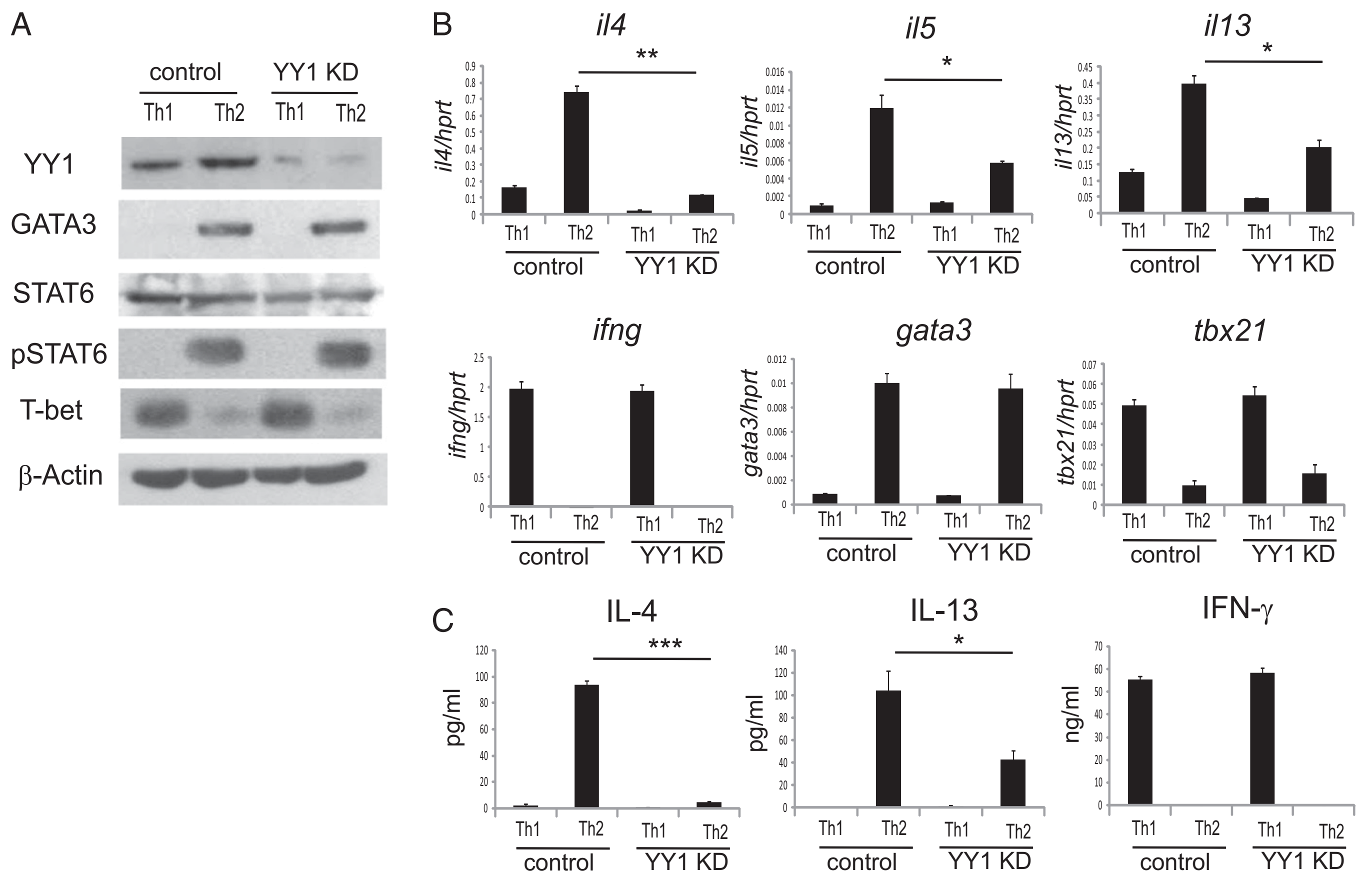

Fig. 2. Expression of transcriptions factors and cytokines in YY1 KD cells. (*A*) Expression of YY1 and other transcription factors in YY1 KD cells. Cell extracts were prepared from in vitro–differentiated Th2 cells from control (pSico-YY1) or YY1 KD [pSico-YY1 (CD4-Cre)] mice. Protein expression was measured by immunoblot analysis with specific antibodies for the transcriptions factors or β-actin as an internal control. The effect of YY1 KD on Th1-/Th2-specific gene expression. Naive CD4 T cells were isolated from control (pSico-YY1) or YY1 KD [pSico-YY1(CD4-Cre)] mice, and differentiated under a Th1 or Th2 polarizing condition. (*B*) Total RNA was isolated from the cells, and gene expression was measured by quantitative RT-PCR. *y* axis shows the relative expression amount of each gene to the internal control *hprt* (encoding HPRT). Data are representative of three independent experiments with similar results. (*C*) Cytokines secreted into the culture media were measured by ELISA. Statistical difference between control Th2 and YY1KD Th2 was analyzed by Student *t* test. $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are representative of three independent experiments with similar results.

control Th2 cells (Fig. S4*C*). Taken together, these results suggest that YY1 is required for permissive chromatin state for gene activation in the Th2 cytokine locus.

YY1 Is Required for Long-Range Chromosomal Interactions in the Th2 Cytokine Locus. The regulatory elements for Th2 cytokine gene expression have been shown to interact with the promoters of the Th2 cytokine genes through long-range chromosomal interactions (35, 36). To examine the role of YY1 in long-range chromosomal interactions, we measured the interactions of the *il4* promoter with other regulatory elements at the Th2 cytokine locus in Th1 and Th2 cells from YY1 KD and control mice using a chromosome conformation capture assay. Both Th1 and Th2 cells from control mice showed high frequencies of interactions between the *il4* promoter and other promoters and regulatory elements, including the *il5* promoter, *il13* promoter, CNS1, RHS4-5, and RHS7, confirming poised chromosomal interactions in the Th2 cytokine locus (35). Interestingly, these long-range chromosomal interactions were reduced by about half in YY1 KD Th2 cells compared with those in control Th2 cells (Fig. S5). This result suggests that YY1 is required for full function of long-range chromosomal interactions in the Th2 cytokine locus.

GATA3 Binding to Regulatory Elements in Th2 Cytokine Locus Requires YY1. To explore the molecular mechanism of YY1 in Th2 cell differentiation, we examined whether YY1 interacts with the key Th2 transcription factor GATA3. To this end, we transfected the expression vectors for *yy1* and *gata3* into 293T cells and performed a coimmunoprecipitation assay using specific antibodies against YY1 or GATA3. Our results showed that YY1 and GATA-3 were coimmunoprecipitated in these cells (Fig. 3*A*), suggesting physical association of these proteins.

We also examined the interactions of these proteins using immunofluorescence. Cellular localization of YY1 and GATA3 in in vitro–differentiated Th1 or Th2 cells from C57BL/6 mice was examined using fluorescence-labeled anti-YY1 or anti-GATA3 antibodies. YY1 and GATA3 were colocalized in the nucleus of Th2 cells but not in Th1 cells (Fig. 3*B*), suggesting Th2-specific association of these proteins. Th2-specific association of YY1 with GATA3 was further confirmed by an in situ proximity ligation assay that detects interactions of proteins in cells and tissues (40) (Fig. 3*C*).

Next, we examined whether GATA3 binding to the Th2 cytokine locus requires YY1, using ChIP in in vitro–differentiated Th1 or Th2 cells from YY1 KD or control mice. GATA3 binding to many regulatory elements in the Th2 cytokine locus, including IL4P, IL5P, IL13P, RHS5, RHS6, RHS7, HSV/CNS2, CGRE, IE/HSII, HSIV, and HSVa, increased in Th2 cells compared with that in Th1 cells (Fig. 4*A*). By comparing the binding of YY1 (Fig. 1*A*), we found that some sites of the locus such as IL4P, IL5P, IL13P, RHS7, CNS1, HSV/CNS2, HSII/IE, HSIV, and HSVa were bound by both YY1 and GATA3, but that other sites

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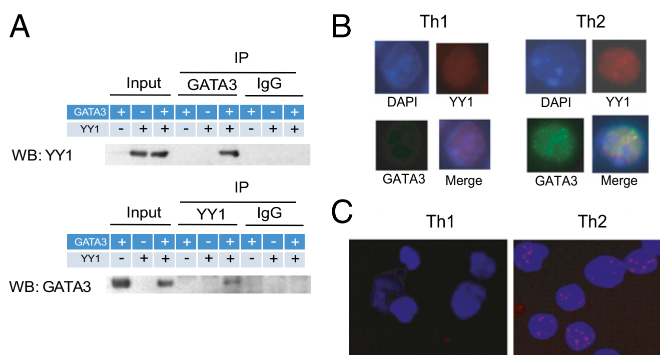

Fig. 3. Interactions between YY1 and GATA3. (*A*) 293T cells were transfected with a *yy1* or *gata3* expression vector. Cell lysates were immunoprecipitated with anti-GATA3, anti-YY1, or a control IgG antibody, and then immunoblotted with anti-YY1 or anti-GATA3 antibody as indicated. (*B*) In vitro–differentiated Th1 or Th2 cells were treated with rabbit anti-YY1 and mouse anti-GATA3 antibodies, and then with Alexa Flour 647–conjugated anti-mouse IgG or Alexa Flour 546–conjugated anti-rabbit IgG. DNA was counterstained with DAPI. Images were recorded with fluorescence microscopy. (*C*) In vitro–differentiated Th1 or Th2 cells were treated with anti-YY1 and anti-GATA3 antibodies, and then in situ proximity ligation assay was performed as described in *SI Materials and Methods*. IN, input cell lysates.

such as RHS5, RHS6, and CGRE were bound by only GATA3. Interestingly, the GATA3 binding to regulatory elements was greatly reduced in YY1 KD Th2 cells (Fig. 4*A*). These results suggest that GATA3 binding to regulatory elements requires YY1. In contrast, YY1 binding to Th2 locus was not much different in WT and GATA3 KO cells (Fig. S6), suggesting that YY1 binding to the locus does not require GATA3.

To gain insight into this process, we measured the kinetics of YY1 and GATA3 binding in developing Th1 and Th2 cells. Naive CD4 T cells were stimulated under a Th1 or Th2 polarizing condition, and the binding of YY1 and GATA3 was measured with ChIP at various time points. YY1 binding to at least some of the regulatory elements, including the promoters of *IL-4* and *IL-5* genes, RHS7, CNS1, HSIV, and HSVa, increased at 12 h in Th2 cells (Fig. 4*B*), whereas GATA3 binding to these elements increased at a later time point (at 24 h). These results suggest that YY1 binding to some regulatory sites precedes GATA3 binding. In contrast, the binding of YY1 to Th2 locus was very low at all times in Th1 cells except RHS7, suggesting that Th2 specificity may be due to differential binding of YY1 to Th2 locus (Fig. 4*B*). Taken together with the data showing defective GATA3 binding in YY1 KD cells, these data suggest that YY1 may recruit GATA3 in those regulatory elements in Th2 cells.

YY1 Is Required but Not Sufficient for Th2 Cytokine Expression. To examine the functional relationship between YY1 and GATA3, we transduced the retroviral *gata3* expression vector into YY1 KD cells and transduced the retroviral *yy1* expression vector into GATA3$^{fl/fl}$ cells together with RV-Cre (conditional GATA3-deficient cells) (Fig. 5). In the conditional GATA3-deficient cells, exons of *GATA3* gene flanked by loxp sites can be deleted by retroviral expression of *Cre* recombinase. As was shown previously (32–34), ectopic expression of *gata3* induced *il4* expression in control Th1 cells (Fig. 5*A*). The expression of *il4*, *il5*, and *il13* in YY1 KD Th2 cells was substantially reduced compared with that in YY1-sufficient control cells. When *gata3* was ectopically expressed in YY1 KD Th1 or Th2 cells, *il4* was induced to a level comparable to control cells (Fig. 5). This result suggests that although YY1 is required for the full expression of Th2 cytokine genes, this necessity can be overcome by overexpression of *gata3*. In contrast, ectopic expression of *yy1* could not induce *il4*, *il5*, and *il13* in either Th1 or Th2 cells from conditional GATA3-deficient mice (Fig. 5), suggesting that, unlike GATA3, YY1 is necessary but not sufficient to regulate Th2 cytokine expression.

YY1 Is Essential for Animal Model of Asthma. To investigate the role of YY1 in Th2 cell function in vivo, we used an animal model of allergic asthma. We sensitized and challenged control or YY1 KD mice with ovalbumin (OVA) and examined airway inflammation in the lung. Eosinophils and lymphocytes were recruited in bronchoalveolar lavage fluid in OVA-challenged control mice, but these cells were greatly reduced in OVA-challenged YY1 KD mice (Fig. S7*A*). Lung-infiltrating inflammatory cells were also greatly reduced in these mice (Fig. S7*B*). In addition, the production of mucus was modestly reduced in these mice (Fig. S7*C*). The amount of serum IgE (Fig. S7*D*) and the expression of Th2 cytokine and chemokine genes (Fig. S8) were also greatly reduced in OVA-challenged YY1 KD mice compared with those in OVA-challenged control mice. These results suggest that YY1 is essential for the pathogenesis of allergic asthma in vivo.

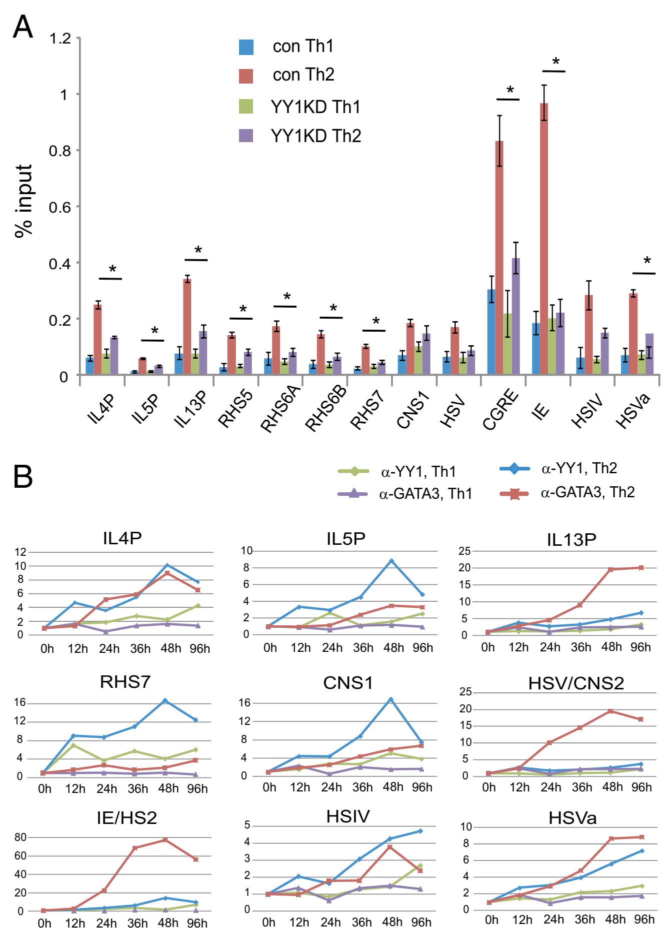

Fig. 4. YY1 recruits GATA3 to Th2 cytokine locus. (*A*) Reduced GATA3 binding to the Th2 cytokine locus in YY1 KD Th2 cells. Naive CD4 T cells were isolated from control (pSico-YY1) or YY1 KD [pSico-YY1(CD4-Cre)] mice, and differentiated under a Th1 or Th2 polarizing condition. GATA3 binding to regulatory elements in the Th2 cytokine locus was measured by ChIP using an anti-GATA3 antibody. Statistical difference was analyzed by Student t test. $n = 3$. $*P < 0.05$. Data are representative of three independent experiments with similar results. (*B*) YY1 binding to the Th2 cytokine locus precedes GATA3 binding. The kinetics of YY1 and GATA3 binding to the Th2 cytokine locus at various time points after Th2 stimulation. y axis shows relative binding compared with the initial binding at time 0 h (naive CD4 T cells). Data are representative of three independent experiments with similar results.

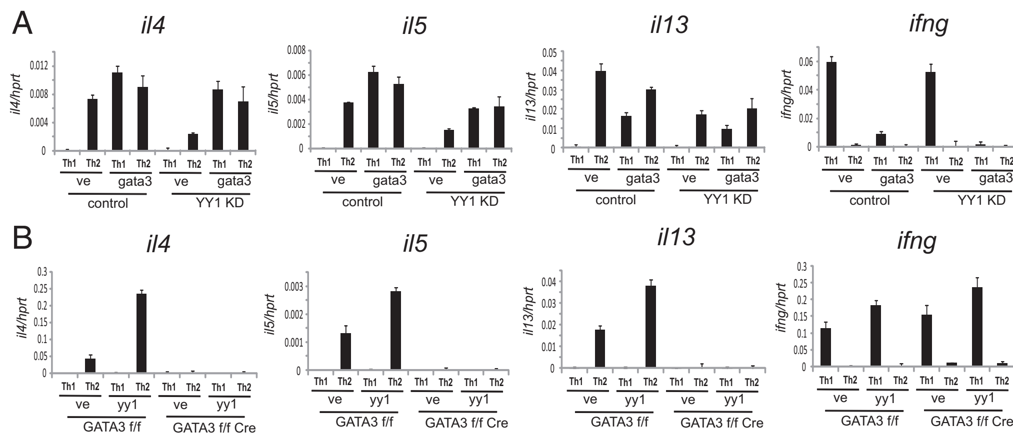

Fig. 5. YY1 is required but not sufficient for Th2 cytokine expression. Naive CD4 T cells from control or YY1 KD mice were transduced with control or *gata3* retroviral vector (*A*), or naive CD4 T cells from GATA3$^{fl/fl}$ mice were transduced with control, yy1, or yy1+Cre retroviral vectors (*B*). Then, cells were induced to differentiate into Th1 or Th2 cells. Total RNA was isolated from the cells, and gene expression was measured by quantitative RT-PCR. Data were normalized by the amount of *hprt*. Data are representative of three independent experiments with similar results.

Discussion

In this study, we examined the role of Th2 LCR-binding transcription factor YY1 in Th2 cell differentiation. Reduction of YY1 resulted in the decreased expression of Th2 cytokine genes, repressed chromatin conformation, decreased intrachromosomal interactions, and resistance in an animal model of asthma. YY1 physically associated with GATA3 and bound to promoters of Th2 cytokine genes before GATA3. These results strongly suggest that YY1 is a critical transcription factor for differentiation of Th2 cells.

Our results showed that YY1 is necessary for Th2 cytokine expression, chromatin remodeling, and intrachromosomal interactions in the Th2 cytokine locus. YY1 is a transcription factor that acts either as an activator or repressor depending on the promoter context (41–43). YY1 interacts with a number of factors including basal and tissue-specific transcription factors, coactivators, and corepressors (41–43). In addition, YY1 has pleiotropic effects on many different cellular processes such as cell growth and differentiation, apoptosis, development, and tumorigenesis (41–43). Likewise, YY1 seems to exert several different functions in Th2 cell differentiation. The first function of YY1 in Th2 differentiation is that it may be a necessary factor in chromatin modification. Our data showed that YY1 KD resulted in more repressed chromatin states with lower levels of H3K4-me1 or 3 and higher levels of DNA methylation. YY1 is well known to interact with several chromatin modification factors such as histone acetyltransferases, histone deacetylases, histone methyltransferase, and chromatin remodeling factor INO80 (41-45). YY1 also has been shown to function in the formation of heterochromatin and double-strand breaks (46). Thus, it is possible that YY1 interacts with some of these factors to modify or remodel chromatin status in the Th2 cytokine locus; it will be interesting to elucidate the detailed mechanism of chromatin modification and the role of YY1 in this process.

The second function of YY1 is that it may mediate the chromosomal interactions between the Th2 LCR and the promoters of Th2 cytokine genes in the Th2 cytokine locus. This possibility is supported by a previous report that YY1 is critical to "locus contraction" in the process of IgH locus rearrangement during B-cell differentiation (47). Locus contraction is a phenomenon in which chromosomal regions contract resulting from formation of chromatin loops for long-range gene rearrangement at the Ig gene loci. Locus contraction may be a specific process for Ig or TCR gene loci; however, the underlying mechanism of chromosomal looping may be similar to intrachromosomal interactions at the β-globin and Th2 cytokine loci. Thus, YY1 may have a critical role in the chromosomal looping process, which our data support. It will be interesting to determine how YY1 mediates chromosomal looping. One possibility is that YY1 may interact with the nuclear matrix in the formation of chromosomal loops. YY1 has been shown to associate with a nuclear matrix (48, 49). It has also been shown that intrachromosomal interactions in the Th2 cytokine locus are mediated by SATB1 (36), which is a matrix-binding protein.

The third function of YY1 is that it may recruit GATA3 in the Th2 cytokine locus. Our results showed that YY1 and GATA3 coimmunoprecipitated in cells overexpressing these proteins, and these two proteins were colocalized in Th2 cells, suggesting physical association between them. GATA3 binding to the Th2 cytokine locus is greatly reduced in YY1 KD Th2 cells, suggesting that YY1 is required for the binding. Protein-binding kinetics shows that YY1 bound to the Th2 cytokine locus about 12–24 h before GATA3 binding. These data suggest that YY1 may directly recruit GATA3 to the Th2 cytokine locus after initiation of Th2 differentiation. It is well known that YY1 recruits corepressors and coactivators to DNA (YY1 binding site), and it is one of the suggested mechanisms for YY1 function (41–43). Corepressors and coactivators do not have sequence-specific DNA binding domains, so it is reasonable to speculate that they require other transcription factors to be recruited to DNA. It is not clear why GATA3 requires another protein to be recruited to DNA, because GATA3 contains its own DNA-binding domain. One possibility is that GATA3 may bind to its cognate DNA binding sites, but this binding may be synergistically increased by cooperative binding of YY1. Another possibility is that YY1 may provide a platform for facilitated binding of GATA3. As mentioned previously in the first and second suggested YY1 functions, YY1 may initiate chromatin remodeling by recruiting coactivators to be accessible for GATA3 binding or it may provide a platform in a nuclear matrix for GATA3 binding. Further study will be needed to elucidate the detailed mechanism of protein recruitment in the regulatory elements.

A previous study by Guo et al. has shown that YY1 activates *il4* promoter activity (50), suggesting direct activation of IL4 promoter is another function of YY1. The same group has also shown that YY1 heterozygote mice, which express a low level of YY1, manifest reduced symptoms of experimental allergic asthma (51). Our data using CD4-specific YY1 KD mice are consistent with their result and further specifies the role of CD4 T-cell–driven YY1 in an experimental allergic response.

The binding sites of YY1 before GATA3 were confined to some, but not all, regulatory elements (Fig. 4*A*). The reason for this is not clear. One possibility is that this confined YY1 binding may initiate chromatin remodeling at the whole Th2 locus by sequential recruitment of the GATA3 or chromatin-modifying factors, and this initiation process may not require binding of YY1 to all regulatory elements. It is also not clear why YY1 binds to Th2 locus in a Th2-specific manner. One possible explanation is differential expression of YY1 between Th1 and Th2 cells. Th2 cells express a higher, although modest, amount of YY1 than does Th1 cells after TCR-stimulation (Fig. 1 *B* and *C*). Another possibility, although not

mutually exclusive, is that Th2-specific binding of YY1 to Th2 locus may occur by recognizing cell type–specific features such as Th2-specific factors, signaling molecules, or chromatin status or by cooperating with them, although we do not have direct evidence for that. Cell type–specific binding of YY1 to a given promoter or enhancer has been shown in many other studies (41-43).

In conclusion, our study strongly suggests that YY1 is a necessary and critical factor for Th2 cell differentiation by cooperating with GATA3 and by impacting chromatin modification and chromosomal interactions. This study helps elucidate the fundamental process of cell differentiation of T-helper cells, information that may prove useful in the development of a therapeutic strategy for curing Th2-mediated diseases. Detailed mechanisms of chromatin modification and chromosomal interactions mediated by YY1 await elucidation in further study.

Materials and Methods

We used many molecular and cell biological methods to analyze the function of YY1 in Th2 cell differentiation. We also used WT, YY1 KD, GATA3$^{flox/-}$, CD4-Cre transgenic, and RHS7-deficient mice for the experiment. The full information is listed in the *SI Materials and Methods*.

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