Th2 LCR is essential for regulation of Th2 cytokine genes and for pathogenesis of allergic asthma

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Previous studies have shown that Th2 cytokine genes on mouse chromosome 11 are coordinately regulated by the Th2 locus control region (LCR). To examine the in vivo function of Th2 LCR, we generated CD4-specific Th2 LCR-deficient (cLCR KO) mice using Cre-LoxP recombination. The number of CD4 T cells in the cLCR KO mouse was comparable to that in wild-type mice. The expression of Th2 cytokines was dramatically reduced in in vitro-stimulated naïve CD4 T cells. Deletion of the LCR led to a loss of general histone H3 acetylation and histone H3-K4 methylation, and demethylation of DNA in the Th2 cytokine locus. Upon ovalbumin challenge in the mouse model of allergic asthma, cLCR KO mice exhibited marked reduction in the recruitment of eosinophils and lymphocytes in the bronchoalveolar lavage fluid, serum IgE level, lung airway inflammation, mucus production in the airway walls, and airway hyperresponsiveness. These results directly demonstrate that the Th2 LCR is critically important in the regulation of Th2 cytokine genes, in chromatin remodeling of the Th2 cytokine locus, and in the pathogenesis of allergic asthma.

chromatin remodeling | differentiation | locus control region

CD4 T cells play an essential role in the activation and regulation of a variety of immune responses. Effector CD4 T cells are composed of several different subsets including Th1, Th2, and Th17 cells (1–5). The expression of subset-specific cytokines is critical for the differentiation and function of T helper cells (6, 7). The Th2 cytokine genes, *il4*, *il5*, and *il13*, are clustered on human chromosome 5 and mouse chromosome 11. The Th2 cytokine locus undergoes structural changes in its chromatin upon Th2 cell differentiation to accommodate the high level expression of Th2 cytokine genes. The changes include acquisition of DNase I hypersensitivity (8, 9), restriction enzyme accessibility (10), histone acetylation (11–16), histone methylation (17), and DNA demethylation (8, 13, 18, 19). Chromatin remodeling and enhancement of transcription of a gene are regulated by *trans*-acting factors that bind to specific DNA regulatory elements. Many laboratories including ours have shown that the Th2 cytokine locus is regulated by a number of regulatory elements including enhancers [CNS-1/HSS (9, 20–22), CNS-2/HSV (20, 23), IE/HSII (20)], a silencer (HSIV) (20, 24), and a locus control region (LCR) (25).

We have shown previously that the expression of Th2 cytokines is coordinately regulated by the Th2 LCR that is located in the 3′ region of the *rad50* gene (25). The Th2 LCR is composed of four DNase I-hypersensitive sites, namely RHS4, RHS5, RHS6, and RHS7 (13, 26). We have shown that deletion of RHS7 causes marked reduction of Th2 cytokine genes under Th0 conditions and partial reduction under Th2 conditions (27), suggesting that RHS7 is important for Th2 cytokine expression. The Th2 LCR interacts with the promoters of Th2 cytokine genes through intrachromosomal associations (28). Deletion of RHS7 disrupts these (27), suggesting that RHS7 is critical for these interactions.

Although deletion of RHS7 has shown a substantial reduction of Th2 cytokine expression in Th0 cells in our previous study (27), their effects are partial in Th2 cells, suggesting that other sequences also play a critical role in the regulation of Th2 cytokine genes. The role of the entire LCR in the regulation of Th2 cytokine genes has not been addressed by deletion mutagenesis. In addition, the physiological consequence of the LCR deletion in relation to Th2-related diseases has not been documented. To examine the function of the entire Th2 LCR in vivo, we generated CD4-specific conditional Th2 LCR-deficient mice and demonstrated that the Th2 LCR is essential for Th2 cytokine expression, chromatin remodeling of the Th2 cytokine locus, and pathogenesis of allergic asthma.

Results

Generation of Mice with CD4-Specific Deletion of Th2 LCR. To examine in vivo functions of the Th2 LCR, we generated mice that have CD4-specific deletion in the Th2 LCR as described in *SI Text* and Fig. S1.

Th2 LCR Is Essential for Th2 Cytokine Gene Expression. To examine the function of Th2 LCR in the regulation of Th2 cytokine genes, we isolated naïve CD4 T cells from the spleens of wild-type and cLCR KO mice, stimulated them in vitro under Th0, Th1 or Th2 conditions, and measured Th2 cytokine expression at the protein level by ELISA and at the transcript level by quantitative reverse transcription-PCR (RT-PCR) (Fig. 1). The expression of all three Th2 cytokines (IL-4, IL-13 and IL-5) was dramatically reduced in LCR($^{-/-}$) CD4 T cells compared to wild-type cells under Th0 and Th2 conditions both at the protein and transcript level (Fig. 1). This result demonstrates that the Th2 LCR is essential for the expression of Th2 cytokine genes. It also suggests that the regulation occurs mainly at the transcriptional level. This result is particularly interesting because the magnitude of the reduction of Th2 cytokines in Th2 cells is much greater in cLCR KO mice compared to RHS7 KO mice in which expression of IL-4 and IL-13 was only partially reduced and that of IL-5 was not changed (27). In addition, we measured the frequency of cells expressing IL-4 and IFN-γ by intracellular staining and found that cells expressing IL-4 were almost completely eliminated in Th0 and Th2 cells from cLCR KO mice (Fig. S2). These results demonstrate that the Th2 LCR is essential for the complete regulation of all three Th2 cytokine genes.

RAD50 is a protein involved in DNA double-strand-break repair (29, 30). Because CD4 T cells from cLCR KO mice also have a deletion of the terminal exons of the *rad50* gene, these

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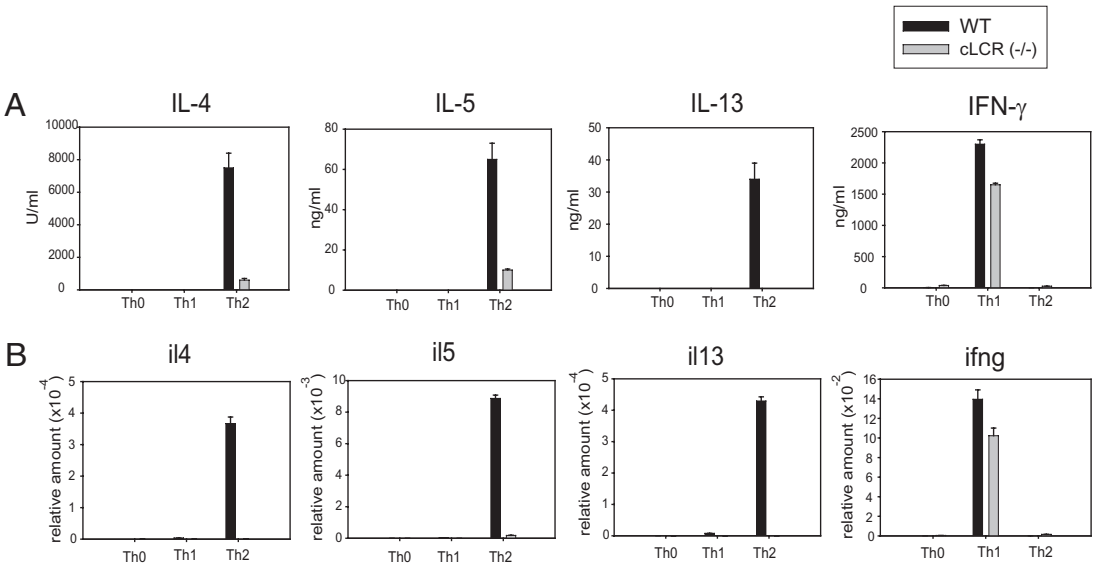

Fig. 1. Expression of cytokines in CD4-conditional LCR($^{-/-}$) mice. Naïve CD4 T cells from wild-type and conditional LCR KO mice were stimulated with Th0, Th1, or Th2 conditions for 5 days. Cells were restimulated with a plate-bound anti-CD3 antibody for 16 h. Cytokines produced in supernatants were measured by ELISA (*A*) or transcripts of cytokines were measured by quantitative RT-PCR using total RNA isolated from the cells.

cells do not produce RAD50 and are presumably defective in DNA double-strand-break repair. Although conventional *rad50* KO mice are embryonic lethal (31), CD4 T cells in cLCR KO mice survived. The reason why CD4 T cells from cLCR KO survive is not known at this time, but it is possible that these cells may have some mechanisms that compensate for the loss of RAD50. In vitro-stimulated CD4 T cells from cLCR KO mice were bigger in size than those from wild-type mice and showed polyploidy. We were thus concerned that the reduction of Th2 cytokine expression might be due to the decrease of naïve or effector/memory cells from cLCR KO mice because of genomic instability rather than a direct effect of the loss of the LCR. Therefore, we carefully examined whether the CD4 T cells from cLCR KO mice have functional defect in differentiation. cLCR KO mice did not show significant defects in the differentiation of CD4 T cells either in vitro and in vivo (*SI Text* and Fig. S3–S5). We therefore concluded that these mice are a suitable model for studying the function of the Th2 LCR.

Th2 LCR Is Essential for Chromatin Structural Changes in the Th2 Cytokine Locus. To investigate whether the Th2 LCR is essential for the opening of chromatin in the Th2 cytokine locus, we examined the histone H3 acetylation and histone H3-K4 methylation status of the Th2 cytokine promoters and regulatory sequences in the cells from wild-type and cLCR KO mice. Both histone H3 acetylation and histone H3-K4 methylation in several regions of the Th2 cytokine locus, including the promoters of *il4*, *il13*, and *il5* genes and the CNS-1/HSS, CNS-2/HSV, HSVa, HSIV, and IE/HSII, were greatly reduced. The highest reduction was in the promoters of Th2 cytokine genes in in vitro-polarized Th2 cells from cLCR KO mice as compared to those from wild-type mice (Fig. 2). However, the β-globin gene locus, which does not play any role in the regulation of Th2 cytokine genes, did not exhibit any changes in its histone H3 acetylation and histone H3-K4 methylation status (Fig. 2), indicating that such changes are specific to the promoters and the regulatory elements in the Th2 cytokine locus and occur specifically in Th2 cells.

We also examined the DNA methylation status of the *il4* promoter and CNS-1 regions. As was previously demonstrated (13, 32), wild-type Th2 cells showed decreased DNA methylation in the *il4* promoter and CNS-1 regions compared to Th1 cells. However, Th2 cells from cLCR KO mice showed a dramatic increase in DNA methylation in these regions, in particular at the −343, −274, and −249 sites from the transcription start site of the *il4* gene, in the *il4* promoter, and from the −8473 to −8384 sites from the transcription start site of the *il4* gene within the CNS-1 region compared to wild-type Th2 cells (Fig. 3), suggesting that the Th2 LCR controls the DNA methylation status in these regions.

Collectively, these results indicate that Th2 LCR is essential for structural changes of chromatin in the entire Th2 cytokine locus.

Th2 LCR Is Essential for the Pathogenesis of Allergic Asthma. It is well known that Th2 cytokines play a central role in the pathogenesis of allergic asthma (33–35). To understand the role of the Th2 LCR in the pathogenesis of allergic asthma, we used cLCR KO mice in a mouse model of allergic asthma. We injected ovalbumin (OVA) into wild-type and cLCR KO mice intraperitoneally at day 0 and day 5, and subsequently challenged the mice with aerosolized OVA at days 12, 13, 14, 15, and 16.

One day after the last aerosol challenge, we collected bronchoalveolar lavage (BAL) fluid and counted the number of total cells and the key cell types using differential cell counting (Fig. 4*A*). As is well established, the OVA challenge increased the total cell number in the BAL fluid in wild-type mice. The majority of the cells in the BAL fluid in the wild-type mice were eosinophils, and a smaller but significant number of lymphocytes were also detected. However, in the cLCR KO mice, the numbers of eosinophils and lymphocytes were completely abrogated to the levels found in the PBS-control mice (Fig. 4*A*).

We next examined airway inflammation by staining lung sections with hematoxylin and eosin. The cLCR KO mice showed a marked reduction of infiltrating cells in the peribronchiolar and perivascular regions of the lung compared to the wild-type mice (Fig. 4 *B* and *C*). Mucus production detected by perchloric acid staining was also dramatically reduced in cLCR KO mice compared to that observed in wild-type mice (Fig. 4 *D* and *E*).

Airway hyperresponsiveness is a functional hallmark of asthma. We therefore measured it in wild-type and cLCR KO mice. Sensitized and OVA-challenged (or PBS-challenged) mice were anesthetized and mechanically ventilated in the presence of increasing doses of inhaled methacholine. In contrast to wild-type

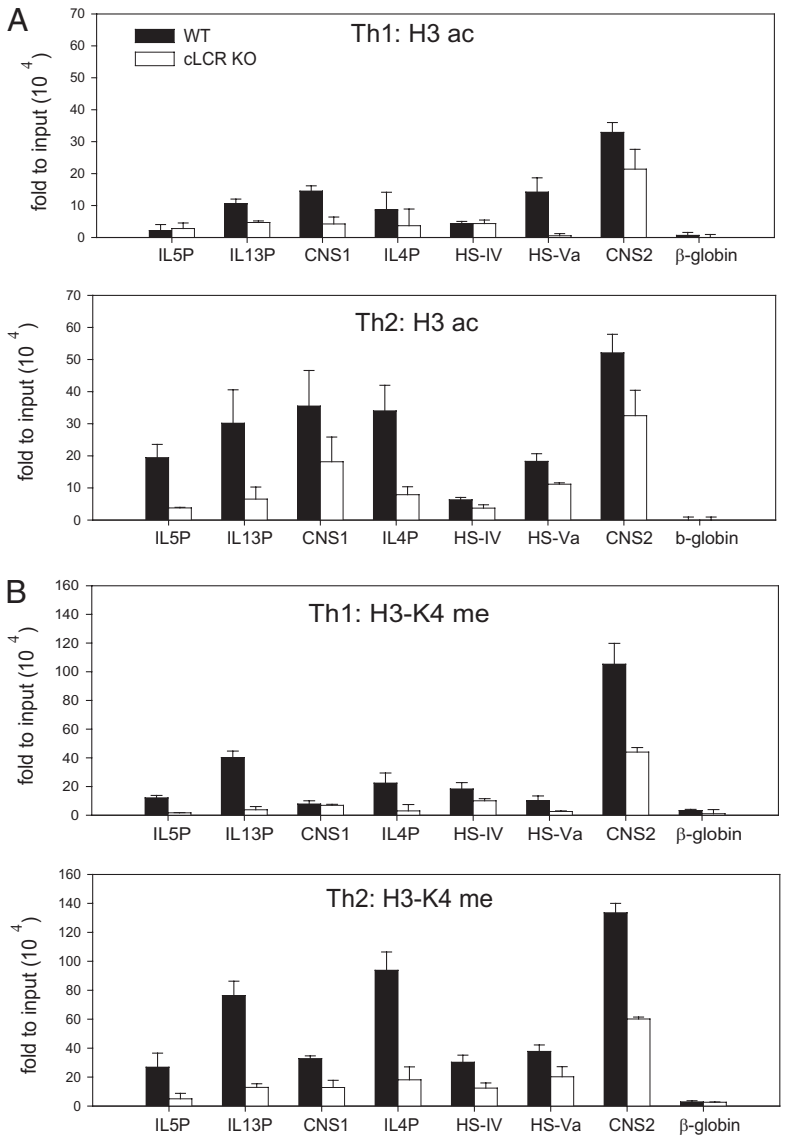

Fig. 2. Histone H3 acetylation and histone H3-K4 methylation in the Th2 cytokine locus. Naïve CD4 T cells from wild-type or cLCR KO mice were in vitro-stimulated into either Th1 (*A*) or Th2 (*B*) cells, and histone H3 acetylation and histone H3-K4 methylation at several regulatory regions in the Th2 cytokine locus in the cells were examined by chromatin immunoprecipitation using anti-acetyl-H3 and anti-methyl-H3-K4 antibodies. Relative amount of precipitated DNA compared to input DNA was measured by quantitative RT-PCR.

mice, cLCR KO mice showed almost complete protection from methacholine-induced increases in mean lung resistance (Fig. 5*A*). We also measured OVA-specific serum IgE level by ELISA, and it was found to be completely absent in the OVA-challenged cLCR KO mice but was present in wild-type mice (Fig. 5*B*). Taken together, these results indicate that the Th2 LCR is essential for the pathogenesis of allergic asthma in an animal model.

Th2 LCR Is Essential for the Expression of Th2 Cytokines and Chemokines in the Lung. To examine whether the resistance to asthma pathogenesis in cLCR KO mice is due to the lack of Th2 cytokine expression in the lung, we measured the expression of Th2 cytokines in the lungs by quantitative RT-PCR analysis. The expression of all three Th2 cytokines, IL-4, -5, and -13, was greatly reduced in the lungs of cLCR KO mice compared to those of wild-type mice, suggesting that the resistance to asthma pathogenesis is directly related to the lack of Th2 cytokine expression in the lung (Fig. S6*A*).

The expression of eotaxin-1, eotaxin-2, and TARC was greatly reduced in OVA-challenged cLCR KO mice compared to that in OVA-challenged wild-type mice (Fig. S6*B*), suggesting that the deficiency of the Th2 LCR reduced the expression of these chemokines, most likely because of the lack of Th2 cytokines in the lung.

Discussion

The Th2 LCR has been suggested to play an important role in the regulation of Th2 cytokine genes. The Th2 locus contains numerous regulatory elements; however, their relative importance has not been fully examined. In this study, we investigated the role of the LCR in the regulation of Th2 cytokine genes using targeted deletion of the entire LCR. We demonstrated that the Th2 LCR is essential for the regulation of Th2 cytokine genes, chromatin remodeling of the Th2 cytokine locus, and the pathogenesis of an animal model of allergic asthma.

Our study showed that deletion of the complete Th2 LCR has dramatic effects on the regulation of Th2 cytokine genes. The

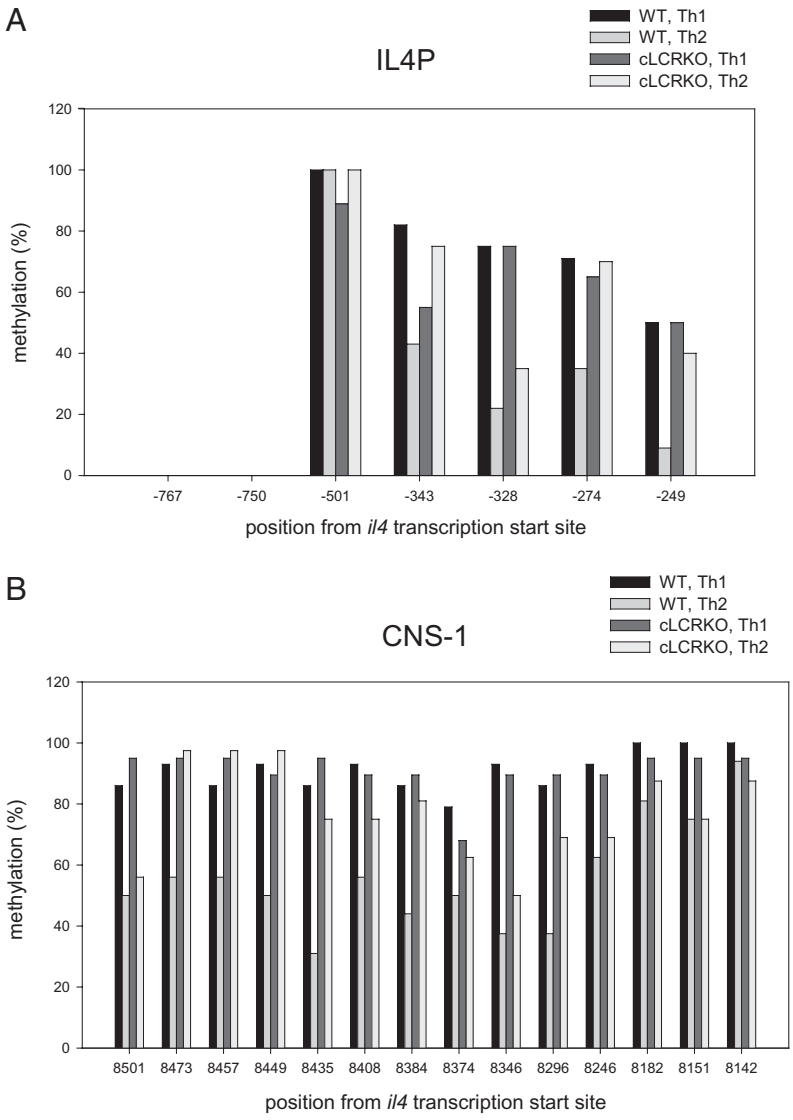

Fig. 3. DNA methylation in the il4 promoter and CNS-1 regions. Naïve CD4 T cells from wild-type or cLCR KO mice were stimulated in vitro into either Th1 (*A*) or Th2 (*B*) cells, and DNA methylation at the *il4* promoter (*A*) and CNS-1 (*B*) regions of the Th2 cytokine locus was examined by bisulfate modification and DNA sequencing (n = 20–30).

expression of all three Th2 cytokines, IL-4, IL-5, and IL-13, was almost completely eliminated under Th2 polarizing conditions, suggesting that the Th2 LCR is essential for the regulation of Th2 cytokine genes. This result contrasted with our previous observations on the effect of deletion of RHS7, which had only a partial effect on IL-4 and IL-13 and no effect on IL-5 under Th2 polarizing conditions (27). Our current study suggests that LCR sequences other than RHS7 also play a critical role or that cooperation of RHS7 with other regulatory elements is required for complete regulation of Th2 cytokine genes. Because Th2 LCR contains four hypersensitive sites, RHS4, RHS5, RHS6, and RHS7, as well as other interstitial sequences, we can speculate that these sites will likely play both redundant and nonredundant roles, and that cooperation of these sites is vital for the regulation of Th2 cytokine genes.

The Th2 LCR may perform complex functions in coordinating Th2 cytokine gene regulation such as tissue-specific enhancement of transcription, chromatin remodeling, and organization of chromatin looping through intra- and interchromosomal interactions (6, 7). Previous studies have shown that RHS7 binds to GATA-3, STAT6, and NFAT (26, 28, 36); that RHS6 binds to STAT6 (26); and that RHS4 binds to SATB1 (37). Studies also indicate that these tissue-specific factors and nuclear matrix organizing factors are essential for the complex functions of the Th2 LCR (26, 28, 37). These factors may in turn recruit other factors such as chromatin-modifying enzymes to the Th2 LCR and other regulatory regions of the Th2 cytokine locus. It has been shown that the ATP-dependent chromatin remodeling factor BRG-1 and Polycomb Group proteins such as YY-1, Mel-18, Ring1A, Ezh2, and Eed are recruited to RHS7, as well as *il4* promoter and several regulatory elements in the Th2 cytokine locus (36–38). These chromatin-modifying factors may induce chromatin remodeling and intra- and interchromosomal interactions. Extensive studies have identified the tissue-specific factors and chromatin-remodeling factors that form multiprotein complexes in the β-globin LCR (39–42). Thus, it is plausible that the Th2 LCR forms a multiprotein complex for coordinated expression of Th2 cytokine genes. Future work will be needed to search for more factors that perform these multiple functions

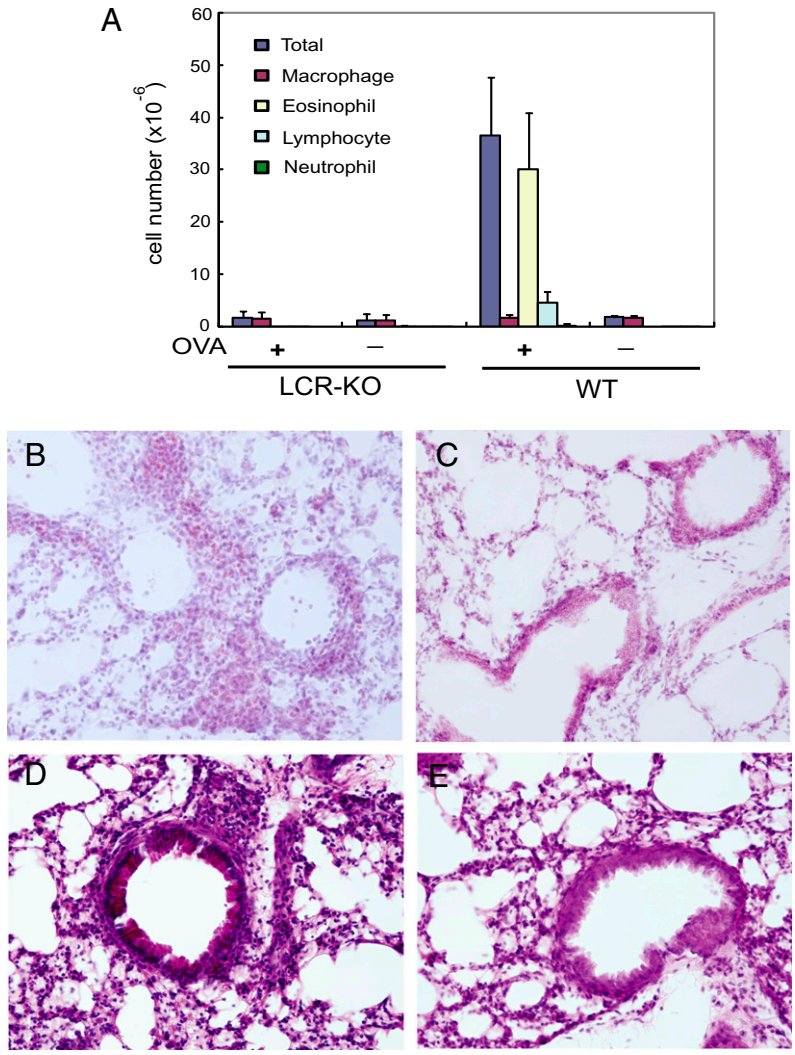

Fig. 4. Differential cell count (*A*), airway inflammation (*B* and *C*), and mucus secretion (*D* and *E*). Wild-type or cLCR KO mice were sensitized and challenged with OVA or PBS as described in *Materials and Methods*. (*A*) Differential cell counting of the BAL fluids was done by staining with Diff-Quik solution. (*B* and *C*) Paraffin-embedded lung sections were stained with hematoxylin and eosin. (*B*) Wild-type mice challenged with OVA. (*C*) The cLCR KO mice were challenged with OVA. (*D* and *E*) Paraffin-embedded lung sections were stained with PAS for mucus staining. (*D*) Wild-type mice were challenged with OVA. (*E*) The cLCR KO mice were challenged with OVA.

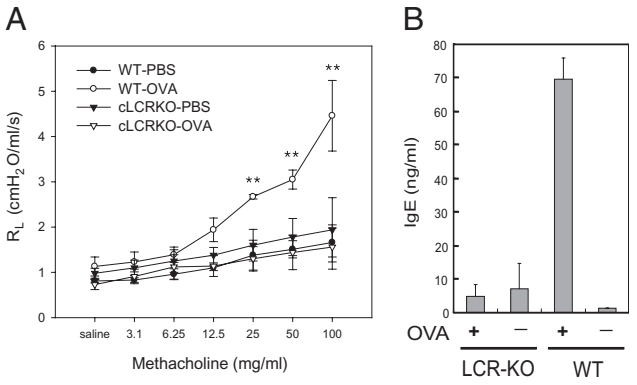

Fig. 5. Airway resistance (*A*) and serum IgE (*B*). Wild-type or cLCR KO mice were sensitized and challenged with OVA or PBS as described in *Materials and Methods*. (*A*) Airway hyperresponsiveness was measure as average lung resistance (R_L) in anesthetized ventilated mice. Data are mean ± standard deviation of six mice. **, $P < 0.01$ compared with wild-type mice. (*B*) Serum IgE amount was measured by ELISA.

for better understanding of the molecular mechanisms of the Th2 LCR.

Excessive Th2 responses to allergens are thought to play a major role in asthma pathogenesis, especially during the early stages of disease (33–35). Our study clearly showed that deletion of the Th2 LCR in CD4 T cells caused resistance to allergic asthma, indicating that the Th2 LCR is essential for the pathogenesis of the disease. The resistance is most likely due to lack of the expression of Th2 cytokines and chemokines, lack of the recruitment of eosinophil and lymphocytes in the lung, and the resultant lack of airway inflammation. This result suggests that regulation of the function of the Th2 LCR has the potential to be used as a therapeutic strategy for allergic diseases such as allergy, allergic asthma, and atopic dermatitis. Clinical trials to use blocking antibodies against IL-4 or IL-5 alone for treatment of allergic asthma have not been effective (43), partly because of functional redundancy of Th2 cytokines. Because deletion of the Th2 LCR in our study caused almost complete reduction of all three Th2 cytokines and resistance to pathogenesis of allergic asthma, therapies directed at regulating the function of the Th2 LCR may be an effective way to get around this redundancy

problem. Further work is necessary to elucidate the detailed molecular mechanisms employed by the Th2 LCR and its contribution to the pathogenesis of allergic asthma.

In conclusion, this study established an essential role of the Th2 LCR in the regulation of Th2 cytokines. This study may not only provide a fundamental mechanism for the regulation of the Th2 cytokine locus during cell lineage commitment, but may also help in developing a therapeutic strategy for Th2 cell-mediated diseases such as allergy and asthma.

Materials and Methods

In Vitro CD4 Cell Differentiation. Isolation of splenic CD4 cells, sorting of naïve CD4 T cells, and in vitro differentiation were done as described in ref. 27.

Quantitative RT-PCR for Cytokines and Chemokines. Total RNA was isolated from naïve CD4 and in vitro differentiated Th1 or Th2 cells using TRIzol reagent (Invitrogen). Reverse transcription was performed using SuperScript II RT (Invitrogen). Quantitative PCRs for the *il4*, *il5*, *il13*, *ifng*, *eotaxin1*, *eotaxin2*, *TARC*, and *hprt* cDNA were performed with real-time fluorogenic 5′-nuclease PCR using the 7500 Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions.

Intracellular Cytokine Staining. Naïve CD4 T cells were stimulated with Th0, Th1, or Th2 conditions for 5 days. Cells were then restimulated with 1 μM ionomycin (Sigma) and 10 nM PMA (Sigma) for 5 h. Intracellular staining was performed using the Cytofix/Cytoperm kit (BD) according to the manufacturer's instructions. Flow cytometric analysis was performed with a FACSCalibur (BD).

Chromatin Immunoprecipitation. Th1 or Th2 cells ($1–2 \times 10^6$) were cross-linked with 1% formaldehyde and quenched with 0.125 M glycine. Cells were lysed with a buffer containing 1% SDS and sonicated at the high-power setting for 15 min using a Bioruptor sonicator (Diagenode). Cell extracts were precleared with protein A agarose/salmon sperm DNA (Upstate) and incubated with anti-acetyl H3 (Upstate 06-599), a mixture of anti-trimethyl-H3-K4 and anti-monomethyl H3-K4 (Millipore 07-473 and 07-436, respectively), or rabbit IgG (sc-2027; Santa Cruz Biotechnology) as a negative control. Antibody-bound chromatin was precipitated by Protein A agarose, washed, and eluted with sodium bicarbonate-SDS. The chromatin was reverse cross-linked by incubating at 65 °C for 4 h, followed by protease K treatment. The amount of precipitated DNA was quantified by real-time PCR using the SYBR green method with the primers listed in Table S1.

DNA Methylation Analysis. DNA methylation analysis using bisulfate modification was performed as described in ref. 13.

Procedures for Animal Model of Allergic Asthma. Procedures for sensitization and challenge, total and differential cell counting of BAL fluid, measurement of serum IgE, lung histology, and airway hyperresponsiveness are described in *SI Text*.

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1. Abbas AK, Murphy KM, Sher A (1996) Functional diversity of helper T lymphocytes. *Nature* 383:787–793.
2. Dong C, Flavell RA (2001) Th1 and Th2 cells. *Curr Opin Hematol* 8:47–51.
3. Glimcher LH, Murphy KM (2000) Lineage commitment in the immune system: The T helper lymphocyte grows up. *Genes Dev* 14:1693–1711.
4. Stockinger B, Veldhoen M (2007) Differentiation and function of Th17 T cells. *Curr Opin Immunol* 19:281–286.
5. Weaver CT, Hatton RD, Mangan PR, Harrington LE (2007) IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 25:821–852.
6. Ansel KM, Djuretic IM, Tanasa B, Rao A (2006) Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol* 24:607–656.
7. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA (2006) T helper cell differentiation: Regulation by cis elements and epigenetics. *Immunity* 24:369–379.
8. Agarwal S, Rao A (1998) Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation. *Immunity* 9:765–775.
9. Takemoto N, et al. (1998) Th2-specific DNase I-hypersensitive sites in the murine IL-13 and IL-4 intergenic region. *Int Immunol* 10:1981–1985.
10. Guo L, et al. (2002) In TH2 cells the Il4 gene has a series of accessibility states associated with distinctive probabilities of IL-4 production. *Proc Natl Acad Sci USA* 99: 10623–10628.
11. Avni O, et al. (2002) T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 3:643–651.
12. Fields PE, Kim ST, Flavell RA (2002) Cutting edge: Changes in histone acetylation at the IL-4 and IFN-γ loci accompany Th1/Th2 differentiation. *J Immunol* 169:647–650.
13. Fields PE, Lee GR, Kim ST, Bartsevich VV, Flavell RA (2004) Th2-specific chromatin remodeling and enhancer activity in the Th2 cytokine locus control region. *Immunity* 21:865–876.
14. Kaneko T, et al. (2007) Chromatin remodeling at the Th2 cytokine gene loci in human type 2 helper T cells. *Mol Immunol* 44:2249–2256.
15. Messi M, et al. (2003) Memory and flexibility of cytokine gene expression as separable properties of human T(H)1 and T(H)2 lymphocytes. *Nat Immunol* 4:78–86.
16. Yamashita M, et al. (2002) Identification of a conserved GATA3 response element upstream proximal from the interleukin-13 gene locus. *J Biol Chem* 277:42399–42408.
17. Yamashita M, et al. (2004) Interleukin (IL)-4-independent maintenance of histone modification of the IL-4 gene loci in memory Th2 cells. *J Biol Chem* 279:39454–39464.
18. Kim ST, Fields PE, Flavell RA (2007) Demethylation of a specific hypersensitive site in the Th2 locus control region. *Proc Natl Acad Sci USA* 104:17052–17057.
19. Lee DU, Agarwal S, Rao A (2002) Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. *Immunity* 16:649–660.
20. Lee GR, Fields PE, Flavell RA (2001) Regulation of IL-4 gene expression by distal regulatory elements and GATA-3 at the chromatin level. *Immunity* 14:447–459.
21. Loots GG, et al. (2000) Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. *Science* 288:136–140.
22. Mohrs M, et al. (2001) Deletion of a coordinate regulator of type 2 cytokine expression in mice. *Nat Immunol* 2:842–847.
23. Agarwal S, Avni O, Rao A (2000) Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer in vivo. *Immunity* 12:643–652.
24. Ansel KM, et al. (2004) Deletion of a conserved Il4 silencer impairs T helper type 1-mediated immunity. *Nat Immunol* 5:1251–1259.
25. Lee GR, Fields PE, Griffin TJ, IV, Flavell RA (2003) Regulation of the Th2 cytokine locus by a locus control region. *Immunity* 19:145–153.
26. Lee DU, Rao A (2004) Molecular analysis of a locus control region in the T helper 2 cytokine gene cluster: A target for STAT6 but not GATA3. *Proc Natl Acad Sci USA* 101: 16010–16015.
27. Lee GR, Spilianakis CG, Flavell RA (2005) Hypersensitive site 7 of the TH2 locus control region is essential for expressing TH2 cytokine genes and for long-range intrachromosomal interactions. *Nat Immunol* 6:42–48.
28. Spilianakis CG, Flavell RA (2004) Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nat Immunol* 5:1017–1027.
29. Kinoshita E, van der Linden E, Sanchez H, Wyman C (2009) RAD50, an SMC family member with multiple roles in DNA break repair: How does ATP affect function? *Chromosome Res* 17:277–288.
30. Williams RS, Williams JS, Tainer JA (2007) Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochem Cell Biol* 85:509–520.
31. Luo G, et al. (1999) Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci USA* 96:7376–7381.
32. Rose S, Lichtenheld M, Foote MR, Adkins B (2007) Murine neonatal CD4+ cells are poised for rapid Th2 effector-like function. *J Immunol* 178:2667–2678.
33. Busse WW, Lemanske RF, Jr, (2001) Asthma. *N Engl J Med* 344:350–362.
34. Galli SJ, Tsai M, Piliponsky AM (2008) The development of allergic inflammation. *Nature* 454:445–454.
35. Holgate ST (1999) The epidemic of allergy and asthma. *Nature* 402 (6760, Suppl) B2–B4.
36. Wurster AL, Pazin MJ (2008) BRG1-mediated chromatin remodeling regulates differentiation and gene expression of T helper cells. *Mol Cell Biol* 28:7274–7285.
37. Cai S, Lee CC, Kohwi-Shigematsu T (2006) SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. *Nat Genet* 38:1278–1288.
38. Jacob E, Hod-Dvorai R, Schif-Zuck S, Avni O (2008) Unconventional association of the polycomb group proteins with cytokine genes in differentiated T helper cells. *J Biol Chem* 283:13471–13481.
39. Bank A (2006) Regulation of human fetal hemoglobin: New players, new complexities. *Blood* 107:435–443.
40. Dean A (2006) On a chromosome far, far away: LCRs and gene expression. *Trends Genet* 22:38–45.
41. Li Q, Peterson KR, Fang X, Stamatoyannopoulos G (2002) Locus control regions. *Blood* 100:3077–3086.
42. Mahajan MC, Weissman SM (2006) Multi-protein complexes at the β-globin locus. *Brief Funct Genomic Proteomic* 5:62–65.
43. Barnes PJ (2004) New drugs for asthma. *Nat Rev Drug Discov* 3:831–844.