



Published in final edited form as:

J Allergy Clin Immunol. 2008 July ; 122(1): 195–201.e5. doi:10.1016/j.jaci.2008.03.012.

Yin-Yang 1 regulates effector cytokine gene expression and T_H2 immune responses

Jia Guo, MD^a, Xin Lin, PhD^a, Marc A. Williams, PhD^a, Qutayba Hamid, MD, PhD^b, Steve N. Georas, MD^a

^aDivision of Pulmonary and Critical Care Medicine, University of Rochester Medical Center,

^bMeakins Christie Laboratories, McGill University, Montreal.

Abstract

Background: The transcription factor Yin-Yang 1 (YY-1) binds to the promoter regions of several T-cell cytokine genes, but the expression and contribution of this factor to cytokine gene expression and T-cell activation *in vivo* is not clear. Objective: We sought to better define the role of YY-1 in T-cell gene regulation and allergic immune responses.

Methods: We studied cytokine gene expression in T lymphocytes isolated from wild-type mice and heterozygous littermates bearing 1 targeted *yy-1* allele (*yy-1*^{+/-} mice). T cells were stimulated with anti-T-cell receptor (anti-TCR) plus CD28 antibodies or with peptide antigen plus antigen-presenting cells by using newly generated *yy-1*^{+/-} TCR transgenic mice. We also studied ovalbumin-driven allergic immune responses in a mouse model of asthma and YY-1 expression in lung tissue from human asthmatic subjects.

Results: CD4⁺ T cells from *yy-1*^{+/-} mice secreted significantly less IL-4 and IFN- γ compared with wild-type littermates after TCR-dependent activation, whereas IL-2 production was not significantly affected. Both airway inflammation and recall splenocyte IL-4 production were inhibited in *yy-1*^{+/-} mice, as was antigen-driven T-cell proliferation. YY-1 expression was higher in airway biopsy specimens from asthmatic compared with control subjects.

Conclusion: These data indicate that YY-1 regulates T-cell cytokine gene expression and allergic immune responses in a gene dose-dependent manner. (*J Allergy Clin Immunol* 2008;122:195–201.)

Keywords

T lymphocyte; cytokine gene regulation; transcription factors; allergic inflammation; asthma

Yin-Yang 1 (YY-1) is an ubiquitously expressed zinc-finger DNA-binding transcription factor that influences the expression of a wide variety of cellular and viral genes.¹ YY-1 is a versatile factor that can either initiate, activate, or repress transcription, dependent on the promoter context,^{1,2} and was recently implicated in genomic imprinting and in

Reprint requests: Steve N. Georas, MD, 601 Elmwood Ave, Box 692, Rochester, NY 14642. Steve_Georas@urmc.rochester.edu.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Clinical implications: YY-1 can contribute to allergic immune responses by regulating T-cell expansion and cytokine gene expression.

mitochondrial function.^{3,4} YY-1 interacts with a diverse array of other transcription factors and chromatin remodeling complexes that influence its transacting ability.^{1,2,5-7} The observation that YY-1 is a negative regulator of p53 helped explain why genetic deletion or knockdown of YY-1 in tumor cells resulted in growth arrest, apoptosis, or both.⁸ Recent studies using conditional deletion in mice discovered a critical role for YY-1 in the differentiation of oligodendrocytes and in B-cell development.^{9,10}

Although YY-1 is widely expressed in cells of the immune system, the function of this factor in immune responses is only beginning to be understood. Conditional deletion of YY-1 early in B-cell development impairs VDJ recombination and immunoglobulin locus contraction, resulting in profoundly reduced numbers of mature B cells in the periphery.¹⁰ YY-1 might play a role in the pathogenesis of allergic diseases because single nucleotide polymorphisms in several asthma- and allergy-associated genes affect YY-1 binding.¹¹⁻¹⁴ Several T-cell cytokine gene-promoter regions contain consensus YY-1 binding sites, including IL-4,¹⁵ IFN- γ ,^{16,17} and IL-5,^{18,19} but most research to date has been performed by using cell lines, and it remains unclear whether or how YY-1 regulates the activation of primary T cells or immune responses *in vivo*. Cameron et al¹⁴ recently showed that YY-1 binds to a polymorphic site in the IL-13 promoter and positively regulates IL-13 expression. Using electrophoretic mobility shift assays, we showed that the IL-4 promoter contains 4 YY-1 binding sites and that overexpressed YY-1 enhanced promoter activity and IL-4 gene expression in both Jurkat and primary T cells independently of nuclear factor of activated T cells (NFAT), a key regulator of T-cell cytokines.¹⁵ The function of YY-1 in IFN- γ gene expression remains confusing. Ye et al¹⁷ discovered 2 YY-1 binding sites in the IFN- γ promoter that appeared to act independently to repress basal promoter activity in Jurkat cells. Using stably integrated IFN- γ promoter reporter constructs in transgenic mice, Soutto et al²⁰ confirmed that the -110 to -225 region of the IFN- γ promoter, which contains the binding sites for YY-1 and other factors, repressed IFN- γ promoter activity *in vivo*. In contrast, Sweetser et al²¹ used site-directed mutagenesis and concluded that YY-1 interacted with adjacently bound NFAT to enhance IFN- γ expression in mitogen-stimulated mouse splenocytes. Thus the precise role that YY-1 plays in regulating CD4⁺ cytokine expression requires further study.

Homozygous deletion of the murine *yy-1* gene results in early embryonic lethality caused by peri-implantation defects.²² Heterozygous mice survive and are fertile, although they possess axial skeletal abnormalities consistent with homeotic transformation.²³ Mutant embryos were recently shown to have impaired growth and viability in direct proportion to the genetic complement of YY-1.²⁴ Thus YY-1 can act in a gene dose-dependent manner to regulate gene expression and differentiation.

We wanted to explore the expression and potential role of YY-1 in T_H cytokine gene regulation and allergic immune responses *in vivo*. Using heterozygous *yy-1*^{+/-} mice, we report that compared with that seen in wild-type (WT) littermates, secretion of CD4⁺ cytokines, including IL-4 and IFN- γ , is significantly impaired by partial YY-1 deficiency, especially at later stages of T-cell activation. We generated new strains of T-cell receptor (TCR) transgenic mice bearing 1 targeted YY-1 allele and observed significant inhibition of IL-4 and IFN- γ gene expression after antigen-specific activation. Targeted microarray arrays

revealed that YY-1 regulates other genes in T cells in addition to cytokines. Using a well-established model of allergen-driven T_H2-dependent airway inflammation, we confirmed that deficiency of *yy-1* attenuated allergen-driven IL-4 gene expression and airway inflammation *in vivo*.

METHODS

Mouse lines

Mice genetically deficient in *yy-1* were generated and previously characterized by Donohoe et al.²² Heterozygous mice were maintained on the C57BL6×129 background or backcrossed more than 10 generations to a BALB/c background. OTII.2²⁵ and DO11.10 mice, transgenic for a TCR that recognizes the ovalbumin (OVA) peptide OVA_{323–339}, were obtained from Dr Shaun Huang (Johns Hopkins University) and Taconic Laboratories, respectively. Heterozygous *yy-1*^{+/-} mice were bred with OT-II.2 and DO11.10 mice to generate offspring that were TCR transgenic but heterozygous for *yy-1*. Animals were maintained at the animal facilities of the Johns Hopkins Medical School and University of Rochester Medical Center in compliance with institutional guidelines and used at 6 to 12 weeks of age.

Cell isolation and analysis by means of flow cytometry

For more information, see the Methods section in the Online Repository at www.jacionline.org.

CD4⁺ T-cell isolation and stimulation

CD4⁺ cells were isolated from mouse spleens with a CD4⁺ negative selection kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells (1×10^6 per well in 24-well plates) were stimulated with coated anti-CD3 plus soluble anti-CD28 antibodies (2 µg/mL each; BD PharMingen, Franklin Lakes, NJ) or calcium ionophore A23187 (0.5 µmol/L) plus phorbol ester phorbol 12-myristate 13-acetate (PMA; 40 ng/mL; Calbiochem, San Diego, Calif). WT and *yy-1*^{+/-} heterozygous TCR transgenic CD4⁺ T cells were incubated with antigen-presenting cells (APCs) plus OVA peptide for up to 4 days, and cytokine secretion was analyzed by means of ELISA. APCs were WT C57BL/6 bone marrow-derived dendritic cells, which were obtained by culturing bone marrow in recombinant mouse GM-CSF (25 ng/mL) plus mIL-4 (10 ng/mL; R&D Systems, Minneapolis, Minn) for 7 days. Loosely adherent cells were collected, washed, and loaded with different concentrations of OVA_{323–339} peptide (Peptides International, Louisville, Ky) and then incubated with OTII.2 CD4⁺ T cells at a dendritic/T cell ratio of 1:5 for 48 hours, followed by analysis of cytokine secretion by means of ELISA.

Cytokine ELISA or cytometric bead array

For more information, see the Methods section in the Online Repository.

Chromatin immunoprecipitation assays

For more information, see the Methods section in the Online Repository.

Quantitative RT-PCR–targeted array

For more information, see the Methods section in the Online Repository.

OVA sensitization and challenge protocol

Female WT and *yy-I^{+/-}* heterozygous littermate mice 6 to 8 weeks of age on average were sensitized on days 0 and day 7 by means of intraperitoneal injection with 100 μ L of 20 μ g of OVA (Grade V; Sigma, St Louis, Mo) and 5 mg of aluminum hydroxide (Pierce, Cheshire, United Kingdom) in saline. Vehicle control mice were injected intraperitoneally with 100 μ L of saline. On day 14, mice were challenged with intratracheal OVA solution (2.5 μ g in 10 μ L of saline) or saline alone as a control. Mice were killed 24 or 48 hours after OVA challenge for bronchoalveolar lavage (BAL) and isolation of splenocytes. Briefly, after anesthesia with ketamine (6 mg/kg) plus xylazine (0.25 mg/kg), BAL was performed by gently injecting 0.8 mL of PBS into the lung 3 times. BAL fluid cells were placed in pellets and washed, and differential cell counts were prepared by using a cytospin (Shandon, Pittsburgh, Pa) and fixed and stained with Diff-Quik (American Scientific Products, McGraw Park, Ill). Two counts of at least 250 cells were done for each sample in a blinded fashion.

Analysis of cell proliferation

Splenocytes (1×10^6 per well) from WT and *yy-I^{+/-}* OTIL2 mice were labeled with 5, 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) (1 mg/mL) for 10 minutes and incubated for 72 hours with peptide-loaded APCs and analyzed by means of flow cytometry for CFSE dilution as a marker of cell division.

Analysis of YY-1 in lung tissue by means of immunohistochemistry

Human asthmatic and control subjects were recruited from the Québec Respiratory Health Network consisting of the Montreal Chest Institute, Sacré Cœur Hospital, and Laval University. Atopic asthma ($n = 6$) was defined on the basis of clinical history, including intermittent chest tightness, cough, or wheezing according to national guidelines²⁶; positive skin prick test results to 1 or more common aeroallergens; and total serum IgE concentrations of greater than 100 IU/mL. Nonatopic asthmatic subjects ($n = 6$) had a clinical history of perennial symptoms of asthma with no clear allergic trigger, negative skin prick test results with positive histamine reactions, and total serum IgE concentrations of less than 100 IU/mL. Control subjects ($n = 6$) were asymptomatic and nonatopic, defined as a negative skin prick test result with a positive histamine reaction, and also had normal spirometric results. Bronchial biopsy specimens were obtained at segmental divisions from all patients according to American Thoracic Society guidelines.²⁷ Tissues were placed in acetone/methanol, blocked in optimal cutting temperature embedding medium (Sakura Finetechnical, Tokyo, Japan), and then fixed in 4% paraformaldehyde followed by paraffin embedding. Immunohistochemistry was performed on 5-mm sections by using a peroxidase protocol, as previously described,²⁸ and antibodies directed against YY-1 (H-10; Santa Cruz Biotechnology, Santa Cruz, Calif), CD3 (NCL-CD3-PS1; Novocastra, Newcastle Upon Tyne, United Kingdom), or isotype controls. Slides were stained with diaminobenzidine or fast red chromogens (DAKO, Glostrup, Denmark) yielding brown and red colors and counterstained with hematoxylin. Positive signals were quantified with light microscopy at

×20 magnification by using a grid attachment of 0.2 mm². Data were acquired by counting the number of immunoreactive cells and reported as the mean ± SEM of 6 to 8 grids.

RESULTS

Characterization of *yy-1*^{+/-} mice

Because homozygous *yy-1* deficiency is lethal during embryonic development,²² we used heterozygous *yy-1*^{+/-} mice for these studies. To confirm that YY-1 expression was reduced in *yy-1*^{+/-} mice, we analyzed spleen CD4⁺ T cells by means of RT-PCR and Western blotting and observed partial reduction in YY-1 mRNA and protein expression from *yy-1*^{+/-} versus WT littermates (see Fig E1 in the Online Repository at www.jacionline.org). We next studied the size and cellular composition of the thymus, spleen, and lymph nodes in WT and *yy-1*^{+/-} littermates and observed no significant differences in the composition of CD4⁺ and CD8⁺ single- or dual-positive subsets in the thymus (see Fig E1) or of the proportion of B lymphocytes, natural killer cells, and T cells in the spleens and peripheral lymph nodes from WT and *yy-1*^{+/-} mice (data not shown). These data suggest that *yy-1*^{+/-} mice are a valid model to study peripheral immune responses.

Reduced cytokine expression after polyclonal stimulation in *yy-1*^{+/-} T cells

We next studied how partial YY-1 deficiency affected T-cell production of IL-4 and IFN- γ compared with production of IL-2, which is not known to be regulated by YY-1. Fig 1 shows that IL-4 production was significantly attenuated in anti-CD3/CD28-stimulated *yy-1*^{+/-} CD4⁺ T cells compared with WT control cells, especially at later stages of T-cell activation (Fig 1, *A*; $P < .05$ for *yy-1*^{+/-} vs WT; days 2–4), whereas IL-2 secretion was not significantly affected (Fig 1, *B*). Interestingly, IFN- γ secretion was also reduced, although not to the degree of IL-4 secretion (Fig 1, *C*). The delayed effects of *yy-1* deficiency on IL-4 gene expression could reflect differences in the expression of other YY-1-dependent factors required for maximal IL-4 expression or delayed occupancy of the IL-4 promoter. To distinguish between these possibilities, we analyzed binding of YY-1 to the IL-4 promoter by using chromatin immunoprecipitation (ChIP) assays in T cells activated with anti-CD3 and anti-CD28 antibodies and found that YY-1 bound to the IL-4 promoter with delayed kinetics, peaking after approximately 48 hours of T-cell activation (Fig 1, *D*). Consistent with reduced expression of YY-1 and IL-4 in *yy-1*^{+/-} mice, binding of YY-1 to the IL-4 promoter was barely detectable in *yy-1*^{+/-} T cells. Thus these data are compatible with a model in which YY-1 directly activates IL-4 gene expression in acutely stimulated T cells.

We next analyzed the secretion of 5 cytokines by using a multiplex bead array (see the Methods section) and also analyzed CD4⁺ T cells isolated from WT and *yy-1*^{+/-} mice on the BALB/c background. Similar to the case of C57BL/6×129 *yy-1*^{+/-} mice (Fig 1), the secretion of IL-4 and IFN- γ , but not that of IL-2, was significantly attenuated by using BALB/c *yy-1*^{+/-} CD4⁺ T cells (see Fig E2 in the Online Repository at www.jacionline.org). Interestingly, the secretion of other T_H2 cytokines, including IL-5 and IL-13, was also reduced, indicating that YY-1 deficiency compromises T_H2 cytokine secretion in general (see Fig E2).

Partial YY-1 deficiency affects the expression of other T-cell genes

We next isolated total RNA from WT and *yy-1*^{+/-} CD4⁺ T cells and studied the expression of gene products implicated in T_H differentiation by using a quantitative RT-PCR array. We considered gene products whose expressions differed by 2-fold or greater with a *P* value of .05 to be significantly different: 8 genes met these criteria, all of which were underexpressed in *yy-1*^{+/-} versus WT T cells (see Fig E3 in the Online Repository at www.jacionline.org). Strikingly, the 2 most highly downregulated genes were IL-13 (0.19-fold) and IL-4 (0.25-fold; see Fig E3). Other significantly reduced genes included IFN- γ , the cytokine receptor tumor necrosis factor receptor superfamily, member 8, and the signaling molecules IFN regulatory factor 4, Junb, and suppressor of cytokine signaling 1 (see Fig E3). Thus YY-1 deficiency compromises T-cell activation in a selective manner, with a notable reduction in effector cytokine gene expression.

YY-1 is required for IL-4 and IFN- γ expression in antigen-activated CD4⁺ T cells

To rigorously test the idea that YY-1 is involved in antigen-specific CD4⁺ T-cell responses, we next generated 2 TCR transgenic mouse lines by crossing OTII.2 and DO11.10 TCR transgenic mice, which express a TCR specific for the OVA₃₂₃₋₃₃₉ peptide, with *yy-1*^{+/-} mice. The transgene in OTII.2 mice, which are on the C57BL/6 background, is integrated into the Y chromosome, and we confirmed that only male mice expressed an expanded population of CD4⁺V α 2⁺ lymphocytes (data not shown). In coculture with peptide-loaded APCs, we found the secretion of IL-4 and IFN- γ from OTII.2 x *yy-1*^{+/-} T cells was markedly attenuated when compared with that of WT control cells, whereas IL-2 secretion was not significantly affected (Fig 2). A similar pattern was observed with DO11.10 x *yy-1*^{+/-} TCR transgenic T cells on the BALB/c background (see Fig E2), indicating that partial *yy-1* deficiency attenuates TCR-dependent IL-4 and IFN- γ secretion, regardless of genetic background.

Role of YY-1 in T_H2 differentiation and allergic immune responses

We focused the remaining experiments on IL-4 production because this gene was one of the most consistently inhibited by deficiency of *yy-1*. To determine whether partial *yy-1* deficiency affected IL-4 production during T_H cell differentiation, we isolated naive CD62L⁺CD4⁺ cells from WT and *yy-1* mice and polarized them in vitro under neutral (T_H0), T_H1, and T_H2 conditions (see the Methods section). These experiments revealed that IL-4 secretion was reduced, especially under T_H2-polarizing conditions (see Fig E4 in the Online Repository at www.jacionline.org), indicating that partial deficiency of *yy-1* attenuates the differentiation of naive T cells toward the T_H2 phenotype. To test the possibility that *yy-1* deficiency attenuated T_H2-driven immune responses *in vivo*, we used a well-established model of allergic airway inflammation characterized by antigen-driven T_H2 immunity to inhaled OVA (see the Methods section). When compared with WT littermates, *yy-1*^{+/-} mice demonstrated significantly lower influx of both eosinophils and lymphocytes into the lung after OVA sensitization and challenge (Fig 3, A and B), which is indicative of a reduced allergic immune response. Levels of the T_H2 cytokines IL-4 and IL-13 were at or below the detection limit of ELISA in lung lavage fluids from both WT and *yy-1*^{+/-} mice (data not shown), whereas recall IL-4 secretion and mRNA expression were both reduced in spleen

cells from *yy-1^{+/-}* mice compared with WT littermates (Fig 3, D). Expression of GATA-3, the master T_H2 transcription factor, was unchanged. Taken together, these data indicate that deficiency of YY-1 attenuates allergen-driven IL-4 gene expression independently of the T_H2 lineage marker GATA-3.

Because YY-1 deficiency attenuated allergic lung inflammation in mice, we wanted to study the expression of this factor in human subjects with and without asthma. We obtained endobronchial biopsy specimens from 6 subjects each with atopic and nonatopic asthma defined by using standard criteria and 6 nonasthmatic nonatopic control subjects (see the Methods section). By means of immunohistochemical analysis, we found that YY-1 expression was significantly increased in the airways in subjects with asthma compared with that seen in healthy control subjects (Fig 4). YY-1⁺ cells were equally increased in both atopic and nonatopic asthmatic subjects (8.8 vs 9.7 immunoreactive cells per square millimeter, respectively). The number of airway CD3⁺ T cells was not significantly different in asthmatic versus control subjects (data not shown), which is in keeping with numerous prior studies.²⁹ In ongoing studies we found that YY-1 expression is also increased in lung tissues from subjects with sarcoidosis and idiopathic pulmonary fibrosis (data not shown). Taken together, these data indicate that YY-1 expression is increased in the airways in asthma and that this is not simply due to an increased number of T cells.

YY-1 deficiency inhibits T-cell proliferation

Because partial YY-1 deficiency was recently shown to inhibit cell proliferation,⁸ we wondered whether T cells from *yy-1^{+/-}* deficient mice would proliferate normally. To address this, CFSE-labeled spleen cells from WT and heterozygous OTII mice were stimulated with antigen-loaded APCs, and CD4⁺ T cell proliferation was analyzed by means of immunofluorescence and flow cytometry. As shown in Fig 5, YY-1 deficiency inhibited antigen-driven T-cell proliferation.

DISCUSSION

YY-1 is a pleiotropic transcription factor that regulates the expression of a diverse array of genes. Although YY-1 can bind to the promoter regions of several cytokine genes, the role of this factor as a transcriptional repressor or activator during immune responses has been difficult to determine with certainty. Here we provide evidence that YY-1, in a gene dose-dependent manner, regulates the expression of effector cytokines and allergen-driven T_H2-dependent immune responses *in vivo*. In acutely stimulated CD4⁺ T cells, partial deficiency of *yy-1* attenuates the expression of both IL-4 and IFN- γ . In the case of IL-4, this was especially apparent at later stages of T-cell activation due in part to delayed occupancy of the IL-4 promoter by YY-1. YY-1 deficiency also attenuated IL-5 and IL-13 gene expression, as well as airway inflammation in an allergen-driven T_H2-dependent immune response.

Expression of the IL-4 gene in T lymphocytes is tightly regulated at the level of gene transcription by numerous transcription factors that bind to a proximal promoter region, as well as to other regulatory cis elements.^{30,31} For example, in the promoter region alone, the rate of IL-4 transcription is activated by binding of NFAT,^{32,33} activator protein 1,³⁴ c-Maf,³⁵ nuclear factor Y,³³ and CCAAT enhancer binding protein- β ³⁶ to their cognate elements,

whereas IL-4 transcription is repressed by binding of signal transducer and activator of transcription 6,^{37,38} Oct-1,³⁹ and other still poorly defined factors^{40,41} (for recent review, see Li-Weber et al⁴⁰). IL-4 gene expression is also controlled at the level of chromatin remodeling, especially during T_H2 differentiation.^{42–45} An open chromatin structure is required for maximal IL-4 gene expression in T_H2 cells and appears to be inherited as an epigenetic trait (for review, see Lee et al⁴⁶). We previously reported that the IL-4 promoter contains 4 YY-1 binding sites that appear to act in an NFAT-independent manner to enhance IL-4 transcription.¹⁵ The data presented in the current report support the idea that YY-1 is a positive regulator of IL-4 gene expression, especially at later stages of T-cell activation. YY-1 is intimately involved in the regulation of chromatin structure at different genetic loci,² and although we cannot rule out the possibility that partial deficiency of *yy-1* attenuates IL-4 gene expression by affecting chromatin remodeling, we did not detect major differences in histone H3 or H4 lysine acetylation at the IL-4 promoter when comparing resting and activated *yy-1*^{+/+} versus *yy-1*^{+/-} T cells (data not shown). We currently favor a model in which YY-1 directly regulates IL-4 gene expression by regulating transcriptional activation.

Three lines of evidence support the idea that YY-1 is involved in TCR-dependent cytokine gene expression. First, we noticed significantly reduced IL-4 and IFN- γ production after T-cell activation with anti-CD3 and anti-CD28 antibodies (Fig 1). Second, TCR transgenic T cells secreted significantly less IL-4 and IFN- γ after stimulation with antigen (Fig 2). Third, when we used calcium ionophore plus PMA to bypass receptor-mediated signaling pathways and acutely stimulate cells, differences in IL-4 secretion from WT versus *yy-1*^{+/-} T cells were attenuated (data not shown). Therefore these results suggest that YY-1 integrates with physiologic signals emanating from the TCR or costimulatory receptors. YY-1 is generally considered to be a constitutively nuclear phosphoprotein, although it is subject to receptor-mediated posttranslational modifications in different cell types.^{47–50} It remains to be determined how T-cell signaling cascades affect YY-1 or its cofactors during T-cell activation. One potential mechanism for reduced IL-4 production in T cells from *yy-1*^{+/-} mice is due to attenuated cell proliferation. Indeed, we found that *yy-1*^{+/-} T cells stimulated with antigen or anti-CD3 plus anti-CD28 antibodies proliferated less efficiently compared with their WT counterparts (Fig 5). This was not due to defects in IL-2 secretion, which was equivalent between groups. The relationship between T-cell proliferation and cytokine gene expression is complex. Although T-cell proliferation can influence IL-4 expression probably by affecting chromatin structure at the T_H2 locus,^{51,52} cycle progression is not absolutely required for full effector cytokine gene expression.^{52–54} Future experiments in which the level of YY-1 can be inducibly regulated in T cells should provide new insights into the role of this factor in T-cell proliferation and activation.

Asthma is a complex inflammatory disease of the airways involving the secretion of numerous gene products from cells of both the innate and adaptive immune systems.⁵⁵ Interestingly, the promoter regions of several asthma-associated genes contain single nucleotide polymorphisms that generate YY-1 binding consensus sequences, including IL-13,¹⁴ IL-10,¹¹ monocyte chemoattractant protein 4,¹² and TGF- β 1.¹³ Using a well-established mouse model of allergic airway inflammation, we also found that partial YY-1 deficiency attenuated airway inflammation and a T_H2-dependent immune response *in vivo*. By means of immunohistochemistry, we also found that YY-1 expression was increased in

the asthmatic airway. Thus in addition to signal transducer and activator of transcription 6,⁵⁶ GATA-3,⁵⁷ and T-bet,⁵⁸ YY-1 provides another example of a transcription factor involved in allergic immune responses and asthma. Understanding how YY-1 expression is regulated and how it interacts with other transcription factors to reproduce different features of the asthmatic phenotype should prove worthwhile in future studies.

METHODS

Cell isolation and analysis by means of flow cytometry

Thymocytes and spleen and lymph node cells from *yy-I^{+/-}* heterozygous mice at age 6 to 8 weeks and sex-matched littermate WT control animals were obtained by using standard protocols. Single-cell suspensions were analyzed by means of immunofluorescence and flow cytometry with saturating concentrations of isotype-matched controls and anti-CD4 (no. 553049), CD8 (no. 553031) and B220 (no. 553090, all from BD Pharmingen) and NK1.1 (no. 11-5941-82; eBioscience, San Diego, Calif). To monitor TCR transgenic T cells from OTII mice, spleen cells were labeled with anti-CD4 antibodies together with anti-V α 2 (no. 553289) and anti-V β 5 (no. 553190, BD Pharmingen).

Cytokine ELISA or cytometric bead array

ELISA kits were purchased from eBioscience (IFN- γ) and Biolegend (San Diego, Calif; IL-2 and IL-4) and used according to the manufacturer's specification. A Bio-Plex mouse cytokine assay (Bio-Rad, Hercules, Calif) was performed by using a 96-well filtration plate and 50- μ L aliquots of premixed beads (Fig 2). Samples and controls were read with a Bio-Plex 200 suspension array system, and the data were analyzed with Bio-Plex Manager software with 5PL curve fitting.

ChIP assays

For more information, see Fig 1. Solubilized chromatin was prepared from CD4⁺ splenic T cells (1×10^6 per antibody condition) isolated from WT and *yy-I^{+/-}* heterozygous littermates by using an Acetyl H3 Histone ChIP assay kit (Upstate Biotechnologies, Lake Placid, NY), as previously described.^{E1} After reversion of cross-links at 65°C for 4 hours, the immunoprecipitated DNA was extracted, and PCR was performed with the following primers specific for the murine IL-4 promoter: mIL4p263F sense, tctgaaggccgattatggtg; antisense, taacaatgcaatgctggcaga.

T_H polarization

For more information, see Fig E4. Naive CD62L⁺CD4⁺ T cells were isolated from WT and *yy-I^{+/-}* heterozygous splenocytes by means of positive (anti-CD62L) and negative (anti-CD4) immunomagnetic selection (Miltenyi Biotec). Cells (0.5×10^6 per well in 6-well plates precoated with 2 μ g/mL anti-CD3 antibodies) were incubated with recombinant mIL-2 (50 ng/mL) under the following conditions: neutral (no other added cytokines or antibodies), T_H1 (5 ng/mL mIL-12 plus 3 mg/mL anti-mIL-4), or T_H2 (10 ng/mL mIL-4 plus 3 μ g/mL anti-mIFN- γ). All recombinant murine cytokines were purchased from R&D Systems, and anti-cytokine antibodies were from Biolegend. After 7 days, cells were washed

3 times and restimulated with A23187 plus PMA for 18 hours, followed by cytokine secretion by means of ELISA.

PCR and RT-PCR

For more information, see Fig E1. Genomic DNA and total cellular RNA from splenocytes was obtained by using TRIzol reagent (Life Technologies, Grand Island, NY), respectively, as per the manufacturer's instructions. RNA samples were reverse transcribed, and gene-specific primers (Invitrogen, Carlsbad, Calif) were used to amplify selected regions of each gene. To verify that equal amounts of RNA were added in each RT-PCR, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. Amplified PCR products were detected by using ethidium bromide gel electrophoresis. Primers used for PCR were as follows: neomycin forward, 5' ATGAAGTGCAGGACGAGGCAGCG3'; neomycin reverse, 5' GGCGATAGAAGGCGATGCGCTG3'; YY-1 forward, 59TCGCGCTGCAGCCGCTGGTGAC39; YY-1 reverse, 5' CGCCACGGTGA CCAGCGTCTGC3'; IL-4 forward, 5' CACTTGAGAGAGATCATCGGC3'; IL-4 reverse, 5' GAGTCTCTGCAGCTCCATGAG3'; GATA-3 forward, 5' GCC GAGGACATGGAGGTGA3'; GATA-3 reverse, 5' CTAACCCATGGCGGTG ACCATG3'; GAPDH forward, 5' CAATTCAACGGCACAGTCAAG3'; GAP DH reverse, 5' CCTCACCCATTGATGTTAGTG3'.

Western blotting

For more information, see Fig E1. Whole-cell lysates (30 µg per lane) were separated on 4% to 20% SDS-polyacrylamide gels, electroblotted onto nitro-cellulose membranes (Hybond-C extra; Amersham Pharmacia Biotech, Little Chalfont, Bucks, United Kingdom), blocked for 1 hour at room temperature in blocking buffer (1× PBS, 0.1% Tween 20, and 5% [wt/vol] Carnation non-fat dry milk), and washed 3 times for 10 minutes each in washing buffer PBST (PBS and 0.1% Tween 20). Blots were incubated with a 1:1000 dilution of primary antibody in PBST buffer overnight at 4°C with anti-YY-1 (H-10, Santa Cruz Biotechnology) or anti-GAPDH antibodies (AB8246, Abcam, Cambridge, Mass) followed by washing in PBST buffer at room temperature. The secondary antibody, (anti-mouse IgG-horseradish peroxidase, NA931V, Amersham Pharmacia Biotech, and anti-goat IgG horseradish peroxidase, R&D Systems) were diluted 1:10,000 in PBST buffer, incubated for 30 minutes at room temperature, and then washed 3 times in PBST buffer. Secondary antibody–horseradish peroxidase was detected by means of chemiluminescence (Western Blotting Luminol Reagent sc-2048, Santa Cruz Biotechnology).

Quantitative RT-PCR targeted array

For more information, see Fig E2. CD4⁺ T cells from 6-to 9-week-old WT and *yy-1*^{+/-} mice (n = 3 each) were incubated with immobilized anti-CD3 on magnetic beads (2 µg/mL) and soluble anti-CD28 (2 µg/mL) for 48 hours, and total RNA was isolated with RNA Easy kits (Qiagen, Valencia, Calif). RT-PCR was performed with a FirstStrand cDNA Synthesis Kit (SuperArray, Gaithersburg, Md), and quantitative gene expression was analyzed with a 96-well RT-PCR array (Mouse T_H1/T_H2/T_H3 Pathway Array, SuperArray) and an IQ5 thermocycler (Bio-Rad). Relative gene expression was calculated by using the 2^{-Ct} method, in which Ct indicates the fractional cycle number at which the fluorescent signal

reaches the detection threshold. The method uses the normalized Ct value of each sample calculated by using a total of 5 endogenous control genes (18S rRNA, HPRT1, RPL13A, GAPDH, and ACTB). Values are presented as an average fold change of $2^{-(\text{average Ct})}$ for genes in CD4⁺ T cells from *yy-1*^{+/-} versus WT mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr Mark Boothby for helpful suggestions and advice, Dr Vincenzo Casolaro for guidance, and Jessica Roman and Krystal Matthews for expert technical assistance.

Supported by research grants from the National Institutes of Health/National Heart, Lung, and Blood Institute (R01HL073952) and the American Lung Association.

Abbreviations used

APC	Antigen-presenting cell
BAL	Bronchoalveolar lavage
CFSE	5, 6-Carboxyfluorescein <i>diacetate</i> succinimidyl ester
ChIP	Chromatin immunoprecipitation
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
NFAT	Nuclear factor of activated T cells
OVA	Ovalbumin
PMA	Phorbol 12-myristate 13-acetate
TCR	T-cell receptor
WT	Wild-type
YY-1	Yin-Yang 1

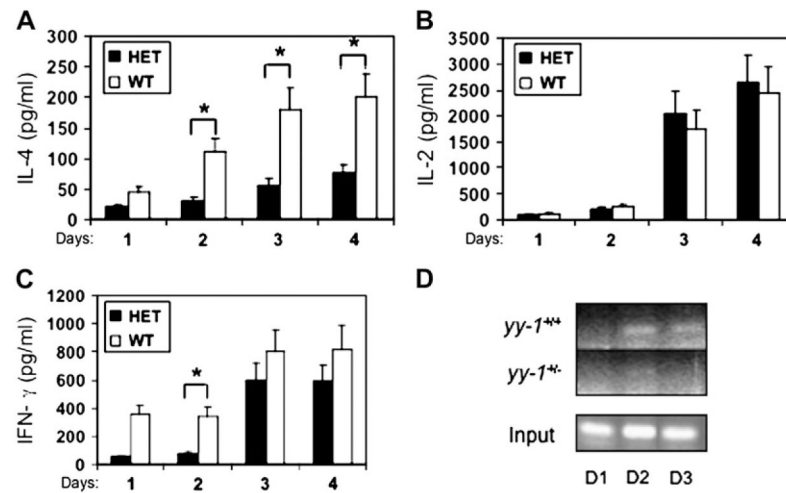
REFERENCES

1. Shi Y, Lee JS, Galvin KM. Everything you have ever wanted to know about Yin Yang 1. *Biochim Biophys Acta* 1997;1332:F49–66. [PubMed: 9141463]
2. Thomas MJ, Seto E. Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? *Gene* 1999;236:197–208. [PubMed: 10452940]
3. Kim JD, Hinz AK, Bergmann A, Huang JM, Ovcharenko I, Stubbs L, et al. Identification of clustered YY1 binding sites in imprinting control regions. *Genome Res* 2006;16:901–11. [PubMed: 16760423]
4. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* 2007;450:736–40. [PubMed: 18046414]

5. Gordon S, Akopyan G, Garban H, Bonavida B. Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. *Oncogene* 2006;25:1125–42. [PubMed: 16314846]
6. Wilkinson FH, Park K, Atchison ML. Polycomb recruitment to DNA *in vivo* by the YY1 REPO domain. *Proc Natl Acad Sci U S A* 2006;103:19296–301. [PubMed: 17158804]
7. Cai Y, Jin J, Yao T, Gottschalk AJ, Swanson SK, Wu S, et al. YY1 functions with INO80 to activate transcription. *Nat Struct Mol Biol* 2007;14:872–4. [PubMed: 17721549]
8. Sui G, Affar el B, Shi Y, Brignone C, Wall NR, Yin P, et al. Yin Yang 1 is a negative regulator of p53. *Cell* 2004;117:859–72. [PubMed: 15210108]
9. He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, et al. The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. *Neuron* 2007;55:217–30. [PubMed: 17640524]
10. Liu H, Schmidt-Supprian M, Shi Y, Hobeika E, Barteneva N, Jumaa H, et al. Yin Yang 1 is a critical regulator of B-cell development. *Genes Dev* 2007;21:1179–89. [PubMed: 17504937]
11. Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998;158:1958–62. [PubMed: 9847292]
12. Kalayci O, Birben E, Wu L, Oguma T, Storm Van's Gravesande K, Subramaniam V, et al. Monocyte chemoattractant protein-4 core promoter genetic variants: influence on YY-1 affinity and plasma levels. *Am J Respir Cell Mol Biol* 2003;29:750–6. [PubMed: 12805085]
13. Silverman ES, Palmer LJ, Subramaniam V, Hallock A, Mathew S, Vallone J, et al. Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. *Am J Respir Crit Care Med* 2004;169:214–9. [PubMed: 14597484]
14. Cameron L, Webster RB, Stempel JM, Kiesler P, Kabesch M, Ramachandran H, et al. Th2 cell-selective enhancement of human IL13 transcription by IL13–1112C>T, a polymorphism associated with allergic inflammation. *J Immunol* 2006;177:8633–42. [PubMed: 17142763]
15. Guo J, Casolaro V, Seto E, Yang WM, Chang C, Seminario MC, et al. Yin-Yang 1 activates interleukin-4 gene expression in T cells. *J Biol Chem* 2001;276:48871–8. [PubMed: 11687571]
16. Ye J, Ghosh P, Cippitelli M, Subleski J, Hardy KJ, Ortaldo JR, et al. Characterization of a silencer regulatory element in the human interferon-gamma promoter. *J Biol Chem* 1994;269:25728–34. [PubMed: 7929277]
17. Ye J, Cippitelli M, Dorman L, Ortaldo J, Young H. The nuclear factor YY1 suppresses the human gamma interferon promoter through two mechanisms: inhibition of AP1 binding and activation of a silencer element. *Mol Cell Biol* 1996;16:4744–53. [PubMed: 8756632]
18. Mordvinov VA, Schwenger GT, Fournier R, De Boer ML, Peroni SE, Singh AD, et al. Binding of YY1 and Oct1 to a novel element that downregulates expression of IL-5 in human T cells. *J Allergy Clin Immunol* 1999;103:1125–35. [PubMed: 10359895]
19. Schwenger GT, Fournier R, Hall LM, Sanderson CJ, Mordvinov VA. Nuclear factor of activated T cells and YY1 combine to repress IL-5 expression in a human T-cell line. *J Allergy Clin Immunol* 1999;104:820–7. [PubMed: 10518827]
20. Soutto M, Zhang F, Enerson B, Tong Y, Boothby M, Aune TM. A minimal IFN-gamma promoter confers Th1 selective expression. *J Immunol* 2002;169:4205–12. [PubMed: 12370350]
21. Sweetser MT, Hoey T, Sun YL, Weaver WM, Price GA, Wilson CB. The roles of nuclear factor of activated T cells and yin-yang 1 in activation-induced expression of the interferon-gamma promoter in T cells. *J Biol Chem* 1998;273:34775–83. [PubMed: 9857002]
22. Donohoe ME, Zhang X, McGinnis L, Biggers J, Li E, Shi Y. Targeted disruption of mouse Yin Yang 1 transcription factor results in peri-implantation lethality. *Mol Cell Biol* 1999;19:7237–44. [PubMed: 10490658]
23. Lorente M, Perez C, Sanchez C, Donohoe M, Shi Y, Vidal M. Homeotic transformations of the axial skeleton of YY1 mutant mice and genetic interaction with the Polycomb group gene Ring1/Ring1A. *Mech Dev* 2006;123:312–20. [PubMed: 16624538]
24. Affar el B, Gay F, Shi Y, Liu H, Huarte M, Wu S, et al. Essential dosage-dependent functions of the transcription factor yin yang 1 in late embryonic development and cell cycle progression. *Mol Cell Biol* 2006;26:3565–81. [PubMed: 16611997]

25. Barnden MJ, Allison J, Heath WR, Carbone FR. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol Cell Biol* 1998;76:34–40. [PubMed: 9553774]
26. National Asthma Education and Prevention Program. Expert Panel Report: guidelines for the diagnosis and management of asthma. Bethesda (MD): National Institutes of Health; 1997.
27. American Thoracic Society. Guidelines for fiberoptic bronchoscopy in adults. Medical Section of the American Lung Association. *Am Rev Respir Dis* 1987;136:1066. [PubMed: 3662229]
28. Bergeron C, Boulet LP, Page N, Laviolette M, Zimmermann N, Rothenberg ME, et al. Influence of cigarette smoke on the arginine pathway in asthmatic airways: increased expression of arginase I. *J Allergy Clin Immunol* 2007;119: 391–7. [PubMed: 17291856]
29. Djukanovic R, Roche W, Wilson J, Beasley C, Twentyman O, Howarth P, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;142:434–57. [PubMed: 2200318]
30. Li B, Tournier C, Davis RJ, Flavell RA. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *EMBO J* 1999;18:420–32. [PubMed: 9889198]
31. Ho IC, Glimcher LH. Transcription: tantalizing times for T cells. *Cell* 2002; 109(suppl):S109–20. [PubMed: 11983157]
32. Bruhn K, Nelms K, Boulay J, Paul W, Lenardo M. Molecular dissection of the murine interleukin-4 promoter. *Proc Natl Acad Sci U S A* 1993;90:9707–11. [PubMed: 8415766]
33. Szabo SJ, Jacobson NG, Dighe AS, Gubler U, Murphy KM. Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. *Immunity* 1995;2:665–75. [PubMed: 7796298]
34. Rooney J, Hodge M, McCaffrey P, Rao A, Glimcher L. A common factor regulates both Th1- and Th2-specific cytokine gene expression. *EMBO J* 1994;13:625–33. [PubMed: 8313907]
35. Ho AM, Jain J, Rao A, Hogan PG. Expression of the transcription factor NFATp in a neuronal cell line and in the murine nervous system. *J Biol Chem* 1994;269:28181–6. [PubMed: 7961754]
36. Davydov I, Krammer P, Li-Weber M. Nuclear factor-IL-6 activates the human IL-4 promoter in T cells. *J Immunol* 1995;155:5273–9. [PubMed: 7594540]
37. Georas SN. Inhaled glucocorticoids, lymphocytes, and dendritic cells in asthma and obstructive lung diseases. *Proc Am Thorac Soc* 2004;1:215–21. [PubMed: 16113437]
38. Dorado B, Jerez MJ, Flores N, Martin-Saavedra FM, Duran C, Ballester S. Autocrine IL-4 gene regulation at late phases of TCR activation in differentiated Th2 cells. *J Immunol* 2002;169:3030–7. [PubMed: 12218118]
39. Pfeuffer I, Klein-Hebling S, Heinfling A, Chuvpilo S, Escher C, Brabletz T, et al. Octamer factors exert a dual effect on the IL-2 and IL-4 promoters. *J Immunol* 1994;153:5572–85. [PubMed: 7989759]
40. Li-Weber M, Davydov I, Krafft H, Krammer P. The role of NF-Y and IRF-2 in the regulation of human IL-4 gene expression. *J Immunol* 1994;153:4122–33. [PubMed: 7930616]
41. Georas S, Cumberland J, Burke T, Chen R, Park E, Ono S, et al. Characterization of a novel repressor element in the interleukin 4 promoter. *Leukemia* 2000;14:629–35. [PubMed: 10764148]
42. Agarwal S, Viola JP, Rao A. Chromatin-based regulatory mechanisms governing cytokine gene transcription. *J Allergy Clin Immunol* 1999;103:990–9. [PubMed: 10359875]
43. Valapour M, Guo J, Schroeder JT, Keen J, Cianferoni A, Casolaro V, et al. Histone deacetylation inhibits IL4 gene expression in T cells. *J Allergy Clin Immunol* 2002; 109:238–45. [PubMed: 11842291]
44. Fields PE, Lee GR, Kim ST, Bartsevich VV, Flavell RA. Th2-specific chromatin remodeling and enhancer activity in the Th2 cytokine locus control region. *Immunity* 2004;21:865–76. [PubMed: 15589174]
45. Spilianakis CG, Lalioti MD, Town T, Lee GR, Flavell RA. Interchromosomal associations between alternatively expressed loci. *Nature* 2005;435:637–45. [PubMed: 15880101]
46. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by cis elements and epigenetics. *Immunity* 2006;24:369–79. [PubMed: 16618596]
47. Garban HJ, Bonavida B. Nitric oxide inhibits the transcription repressor Yin-Yang 1 binding activity at the silencer region of the Fas promoter: a pivotal role for nitric oxide in the up-

- regulation of Fas gene expression in human tumor cells. *J Immunol* 2001;167:75–81. [PubMed: 11418634]
48. Santiago FS, Lowe HC, Bobryshev YV, Khachigian LM. Induction of the transcriptional repressor Yin Yang-1 by vascular cell injury. Autocrine/paracrine role of endogenous fibroblast growth factor-2. *J Biol Chem* 2001;276:41143–9. [PubMed: 11487577]
49. Yao YL, Yang WM, Seto E. Regulation of transcription factor YY1 by acetylation and deacetylation. *Mol Cell Biol* 2001;21:5979–91. [PubMed: 11486036]
50. Hiromura M, Choi CH, Sabourin NA, Jones H, Bachvarov D, Usheva A. YY1 is regulated by O-linked N-acetylglucosaminylation (O-glcNAcylation). *J Biol Chem* 2003;278:14046–52. [PubMed: 12588874]
51. Bird J, Brown D, Mullen A, Moskowitz N, Mahowald M, Sider J, et al. Helper T cell differentiation is controlled by the cell cycle. *Immunity* 1998;9:229–37. [PubMed: 9729043]
52. Richter A, Lohning M, Radbruch A. Instruction for cytokine expression in T helper lymphocytes in relation to proliferation and cell cycle progression. *J Exp Med* 1999;190:1439–50. [PubMed: 10562319]
53. Laouar Y, Crispe IN. Functional flexibility in T cells: independent regulation of CD4+ T cell proliferation and effector function *in vivo*. *Immunity* 2000;13:291–301. [PubMed: 11021527]
54. Ben-Sasson SZ, Gerstel R, Hu-Li J, Paul WE. Cell division is not a “clock” measuring acquisition of competence to produce IFN-gamma or IL-4. *J Immunol* 2001;166:112–20. [PubMed: 11123283]
55. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;22:789–815. [PubMed: 15032597]
56. Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *J Exp Med* 1998;187:939–48. [PubMed: 9500796]
57. Zhang DH, Yang L, Cohn L, Parkyn L, Homer R, Ray P, et al. Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3. *Immunity* 1999;11:473–82. [PubMed: 10549629]
58. Finotto S, De Sanctis GT, Lehr HA, Herz U, Buerke M, Schipp M, et al. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J Exp Med* 2001;193:1247–60. [PubMed: 11390432]
- E1. Valapour M, Guo J, Schroeder JT, Keen J, Cianferoni A, Casolaro V, et al. Histone deacetylation inhibits IL4 gene expression in T cells. *J Allergy Clin Immunol* 2002;109:238–45. [PubMed: 11842291]

**FIG 1.**

Effector cytokine secretion, YY-1 protein expression, and promoter occupancy are reduced in CD4⁺ T cells from *yy-1^{-/-}* mice. CD4⁺ T cells were purified from WT and *yy-1^{+/-}* heterozygous (HET) spleen cells and stimulated with anti-CD3/CD28 antibodies as indicated, followed by analysis of cytokine secretion by means of ELISA (**A-C**) or ChIP assay and PCR primers spanning the proximal IL-4 promoter (**D**). Results are the means ± SEMs of 4 to 5 mice per genotype analyzed in duplicate (Fig 1, **A-C**) or from 1 experiment representative of 3 (Fig 1, **D**).

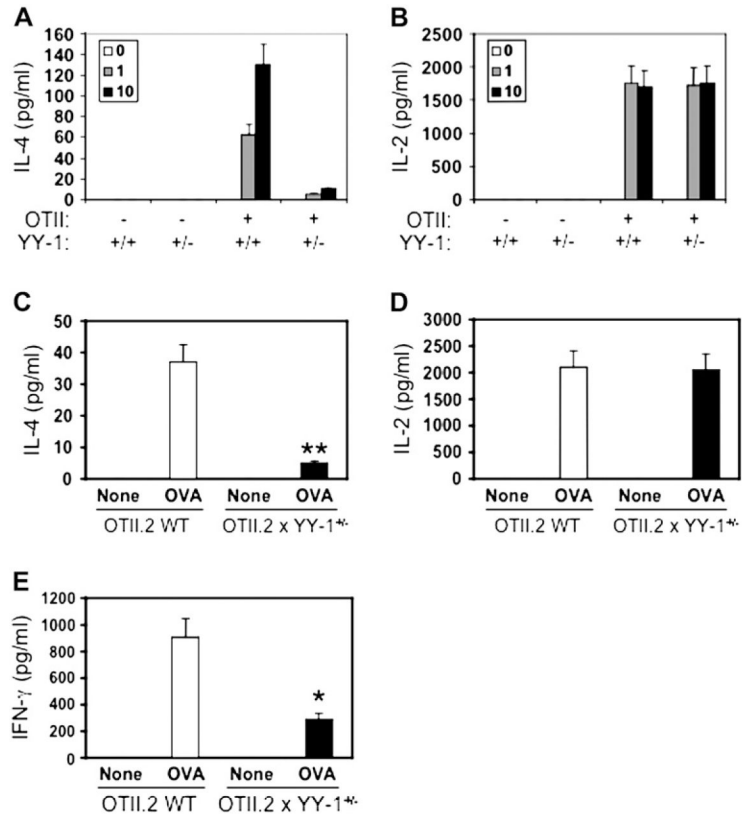


FIG 2. Antigen-driven CD4⁺ T-cell cytokine secretion is attenuated by partial *yy-1* deficiency. Spleen CD4⁺ T cells were purified from WT or *yy-1*^{+/-} (heterozygous) OTII.2 TCR transgenic mice and incubated with WT APCs and OVA₃₂₃₋₃₃₉ peptide for 48 hours (see the Methods section). After 48 hours, supernatants were harvested, and cytokine secretion was analyzed by means of ELISA for IL-4 (**A and C**), IL-2 (**B and D**), and IFN- γ (**E**). Data in Fig 2, **A and B**, are from 1 experiment run in triplicate (representative of 3), whereas data in Fig 2, **C through E**, are the means \pm SEMs of 4 mice per genotype. **P* < .05 and ***P* < .01 for differences between genotypes.

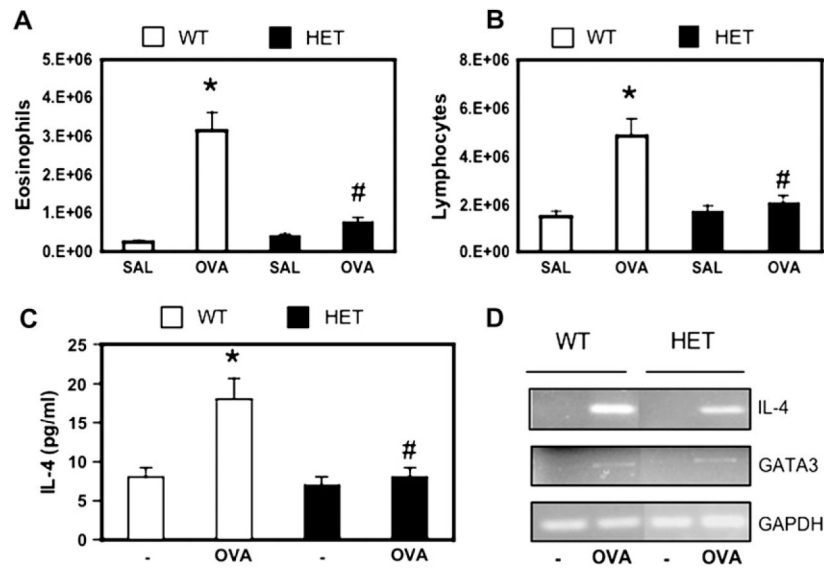


FIG 3. Airway inflammation and allergen-driven cytokine production are attenuated in *yy-I^{+/-}* mice in a mouse model of asthma. WT and *yy-I^{+/-}* (HET) mice were sensitized and challenged with OVA as indicated (see the Methods section), followed 48 hours later by analysis of lung lavage fluids by means of cytopspin for eosinophils (**A**) and lymphocytes (**B**). Splenocytes were incubated with or without OVA (20 μ g/mL) for 48 hours, and recall IL-4 expression was measured by means of ELISA (**C**) or RT-PCR compared with GATA-3 and GAPDH (**D**). Data are the means \pm SEMs of 15 (Fig 3, *A* and *B*) or 20 (Fig 3, *C*) mice per genotype.

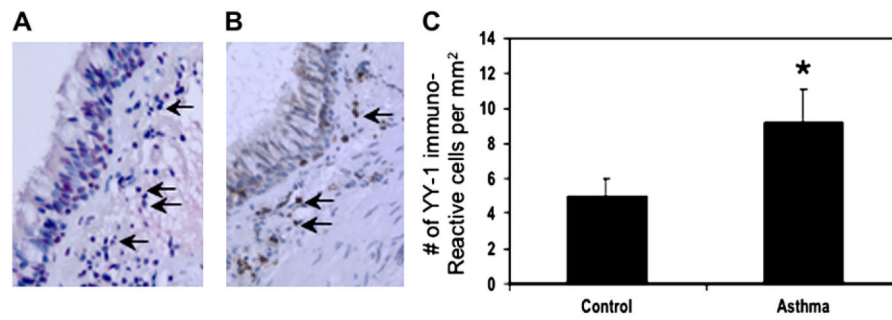


FIG 4. YY-1 expression is increased in the airways in asthmatic subjects. Endobronchial biopsy specimens were obtained from healthy and asthmatic subjects and analyzed by using immunohistochemistry for expression of YY-1⁺ and CD3⁺ cells (see the Methods section). **A** and **B** show representative staining of YY-1 and CD3, respectively, from a subject with asthma. **C** shows the mean \pm SEMs of 6 healthy and 12 asthmatic subjects, respectively.

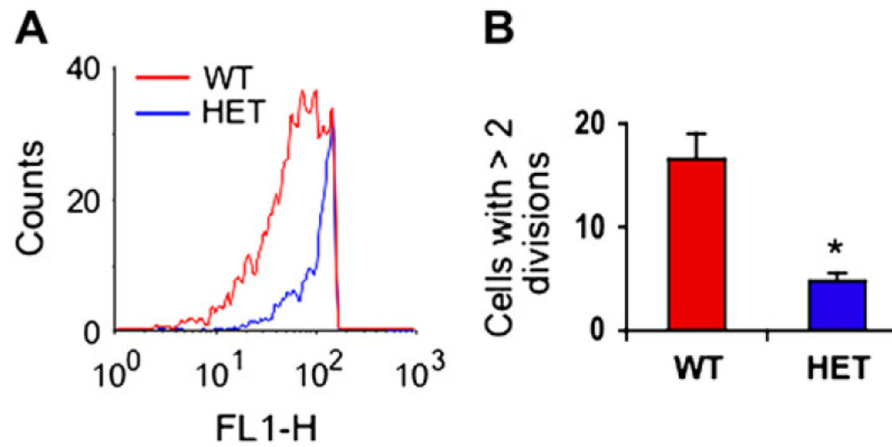


FIG 5. Partial YY-1 deficiency inhibits T-cell proliferation. Proliferation of spleen CD4⁺ T cells from WT and heterozygous (*HET*) mice was analyzed by monitoring CFSE dilution by means of immunofluorescence and flow cytometry. **A** shows representative data from 1 experiment, and **B** is the average \pm SEMs of 3 experiments using antigen-stimulated TCR transgenic T cells from WT (red) and *yy-1*^{+/-} (*HET*; blue) mice studied after 72 hours. **P* < .05.