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

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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.)

Key words: *T lymphocyte, cytokine gene regulation, transcription factors, allergic inflammation, asthma*

Abbreviations used
APC: Antigen-presenting cell
BAL: Bronchoalveolar lavage
CFSE: 5, 6-Carboxyfluorescein diacetate 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

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 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 al[14] 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 al[17] 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 al[20] 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 al[21] used site-directed

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Supported by research grants from the National Institutes of Health/National Heart, Lung, and Blood Institute (R01HL073952) and the American Lung Association.
Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.
Received for publication October 23, 2007; revised March 6, 2008; accepted for publication March 10, 2008.
Available online April 23, 2008.
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0091-6749/$34.00

doi:10.1016/j.jaci.2008.03.012

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 C57BL6x129 background or backcrossed more than 10 generations to a BALB/c background. OTII.2[25] 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

Form 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-1*$^{+/-}$ 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 μL of 20 μg 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 μL of saline. On day 14, mice were challenged with intratracheal OVA solution (2.5 μg in 10 μL 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-1*$^{+/-}$ OTII.2 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 Côeur 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 guidelines[26]; 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-μm 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