NIP45 controls the magnitude of the type 2 T helper cell response

John W. Fathman[a], Michael F. Gurish[b], Saskia Hemmers[a], Kevin Bonham[a], Daniel S. Friend[c], Michael J. Grusby[d], Laurie H. Glimcher[b,d,1], and Kerri A. Mowen[a,1]

[a]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037; [b]Department of Medicine, Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; [c]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; and [d]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115

Contributed by Laurie H. Glimcher, January 7, 2010 (sent for review December 5, 2009)

Nuclear factor of activated T cell (NFAT) transcription factors are key regulators of gene transcription within immune cells. The NFAT-interacting protein, (NIP45), augments NFAT-driven IL-4 expression by a mechanism that relies on arginine methylation. To establish the function of NIP45 in vivo, we generated mice with a targeted deletion of the gene encoding this cofactor. *NIP45*-deficient T helper cells displayed profound defects in the expression of NFAT-regulated cytokine genes, including IL-4. Whereas *NIP45* deficiency does not interfere with T helper cell NFAT activation or lineage-specific transcription-factor expression, NIP45 acts as an enhancer for the assembly of protein arginine methyltransferase 1 and the protein arginine methyltransferase 1-linked histone 4 arginine 3 methylation with the IL-4 promoter. Our study reveals an essential role for NIP45 in promoting robust cytokine expression in vivo, which is required for the efficient handling of parasites. We propose that NIP45 acts as a molecular rheostat serving to amplify the type-2 immune response.

arginine methylation | cytokines | PRMT1 | chromatin | NFAT

On encounter with cognate antigen, the T helper precursor (Thp) cell is instructed by signals from the environment and antigen-presenting cells to specialize by committing to a T helper (Th) 1, Th2, Th17, T regulatory (Treg), or T follicular (Tfh) cell fate. This T helper cell fate decision is reinforced by the expression of lineage-specific transcription factors (1–5).

Although members of the nuclear factor of activated T cell (NFAT) family transcription factors are expressed in all subsets, they are vital for translating the T cell receptor (TCR)/antigen interaction into lineage-specific gene-expression patterns (6). There are five NFAT family members, NFATc1-c5, of which three (NFATc1-c3) are expressed within the lymphoid system (7, 8). NFAT resides in the cytoplasm of unstimulated cells as a highly phosphorylated molecule (9, 10). TCR ligation induces calcium mobilization that results in activation of the phosphatase calcineurin (11, 12). Calcineurin dephosphorylates NFAT, unmasking the nuclear localization sequence, allows NFAT to enter the nucleus where it interacts with many different binding partners to induce immunoregulatory genes (6).

Multiple lines of evidence support T helper cytokine gene-regulatory function for NFAT. NFATc1-deficient T helper cells exhibit impaired proliferative and Th2 responses (13, 14). In contrast, NFATc2-deficient mice express enhanced Th2 development caused by prolonged maintenance of IL-4 transcription (15–18). Whereas NFATc3-deficient mice do not present any defects in cytokine production, expression of a constitutively active NFATc3 protein enhances the production of Th1 cytokines and suppresses Th2 cytokine genes (19, 20). NFATc2/NFATc3 doubly deficient mice produce extremely large amounts of Th2 cytokines and are resistant to activation-induced cell death, which leads to severe allergic and inflammatory disease (7, 18). Although these observations suggest that NFATc2 and NFATc3 play negative immunoregulatory roles, NFATc1/NFATc2 doubly deficient mice have a global impairment of cytokine gene expression, indicating a critical role for these two NFAT family members in T helper cell cytokine production (21). Thus, it seems that the proportion of active NFAT isoforms determines the range of T helper cell cytokine gene expression.

The heterodimerization of NFAT with different nuclear proteins serves to modify transcriptional activity. Mutual contacts between activator protein-1 (AP-1) factors and NFAT facilitate the coordinate binding to composite sites within the IL-2 and IL-4 promoters (22, 23). Similarly, interferon regulatory factor - 4 (IRF-4) cooperates with NFATc1 and NFATc2 to activate IL-4 transcription (24, 25). NFAT-interacting protein (NIP45) was cloned by virtue of its ability to interact with the Rel-homology domain of the NFAT proteins (26). NIP45 does not act as a transcriptional activator on its own but instead, potently synergizes with NFAT and the Th2-specific transcription factor c-Maf to induce IL-4 production from the endogenous IL-4 locus (26). Although NIP45 does not itself contain enzymatic activity, it recruits the arginine methyltransferase- and histone-modifier protein arginine methyltransferase 1 (PRMT1) to the NFAT complex, likely facilitating NFAT-driven transcriptional activity (27).

To examine the function of NIP45 in regulating endogenous cytokine expression, we disrupted the NIP45 gene in mice by homologous recombination. We asked whether or not the deletion of this single coregulator protein would be sufficient to disrupt the type-2 immune response. We focused our analysis on the effects that the NIP45 deletion had on Th1 and Th2 cells, two subsets that have been classically described to oppose one another in the immune response (28). Deletion of NIP45 results in reduced expression of specific NFAT-regulated T helper cell cytokine genes, including IL-4. NFAT expression and activation is not affected by *NIP45* deletion. Instead, we linked diminished IL-4 expression by *NIP45*$^{-/-}$ Th2 cells with reduced recruitment of PRMT1 and concomitant methylation of arginine 3 on histone 4 with the IL-4 promoter. Subtle changes in IL-4 promoter-associated chromatin remodeling and ensuing IL-4 expression in *NIP45*$^{-/-}$ Th2 cells are amplified in vivo and result in a profoundly compromised type-2 immune response to the gastrointestinal parasite *Trichinella spiralis*.

Results

NIP45-Deficient Mice. The observation that overexpression of NIP45, together with NFATc2 and c-Maf, endows a B cell line with the capability of IL-4 production led us to create a mouse line lacking NIP45 expression. The first and second exons were

---

Author contributions: J.W.F., L.H.G., and K.A.M. designed research; J.W.F., M.F.G., S.H., K.B., and K.A.M. performed research; and J.W.F., M.F.G., S.H., K.B., D.S.F., M.J.G., and K.A.M. analyzed data K.A.M. wrote the paper.

Conflict of interest statement: L.H.G. is on the Board of Directors of Bristol Myers Squibb Pharmaceutical Industry and holds equity therein.

[1]To whom correspondence may be addressed. E-mail: lglimche@harvard.edu or kmowen@scripps.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0914700107/DCSupplemental.

IMMUNOLOGY

targeted for deletion and replaced by a neomycin resistance cassette flanked by *loxP* sites (Fig. S1*A*). The targeting construct was electroporated into a murine protamine 1 promoter driving Cre recombinase transgene (*Prm1*^{Cre}) bearing embryonic stem-cell line to mediate *Neo* deletion within the male founder germline (29), and the NIP45 deletion was confirmed (Figs. S1 *B–E*). NIP45$^{-/-}$ mice were born at the expected Mendelian ratio and appeared healthy and viable. No alteration in thymic T cell populations, T cell proliferation, or peripheral lymphocyte development was observed in *NIP45*$^{-/-}$ mice compared with *NIP45*$^{+/+}$ mice (Figs. S2 and Figs. S3). Importantly, deletion of NIP45 did not alter the stability of its binding partners, NFATc1, NFATc2, and NFATc3 (Fig. S2*D*).

Delayed Expulsion of *T. spiralis* in *NIP45*-Deficient Mice. Because NIP45 cooperates with NFATc2 to drive the activity of a Th2-selective region of the IL-4 expression, we examined whether or not NIP45 is essential for an efficient type-2 antiparasitic response to the intestinal nematode *T. spiralis*. The adult stage of *T. spiralis* resides within the intestine and requires the proper functioning of both T helper cells and mast cells (30). We inoculated wild-type and *NIP45*$^{-/-}$ mice with *T. spiralis* larvae and evaluated the immune response after 12 days of infection. A 4-fold reduction of serum IL-4 levels in *NIP45*$^{-/-}$ mice supported our hypothesis that NIP45 regulates IL-4 production during an in vivo immune response (Fig. 1*A*). Consistent with the observation that IL-4 is a critical factor in mediating protective immunity to *T. spiralis* (31), NIP45$^{-/-}$ mice also displayed an elevated intestinal worm burden after *T. spiralis* infection (Fig. 1*B*). To determine if the diminished serum IL-4 levels and increased worm burden found in *NIP45*$^{-/-}$ mice were a reflection of a disabled T helper cell response, we isolated mesenteric T helper cells from infected animals and measured cytokine production after anti-CD3 stimulation by ELISA. An effective immune response against *T. spiralis* requires both IL-4 and IL-13 (30). Blocking either IL-4 or IL-13 is not sufficient to interfere with the antiparasitic response, but a persistent infection develops in the absence of both cytokines (32). Indeed, *NIP45*$^{-/-}$ mesenteric T helper cells were markedly impaired in both IL-4 and IL-13 production (Fig. 1*C* and Fig. S4*A*). Decreased NIP45$^{-/-}$ T helper cell IL-4 and IL-13 production did not result in a reciprocal up-regulation of Th1 cytokines, because IFNγ levels were equivalent between wild-type and *NIP45*$^{-/-}$ T helper cells (Fig. 1*C*). These data support the notion that a diminished Th2 response contributed to the ineffective expulsion of *T. spiralis* in *NIP45*$^{-/-}$ mice.

Mast cells are an essential component of an effective *T. spiralis* immune response (33–37). Therefore, we investigated intestinal mast-cell recruitment and activity in infected mice. Histological

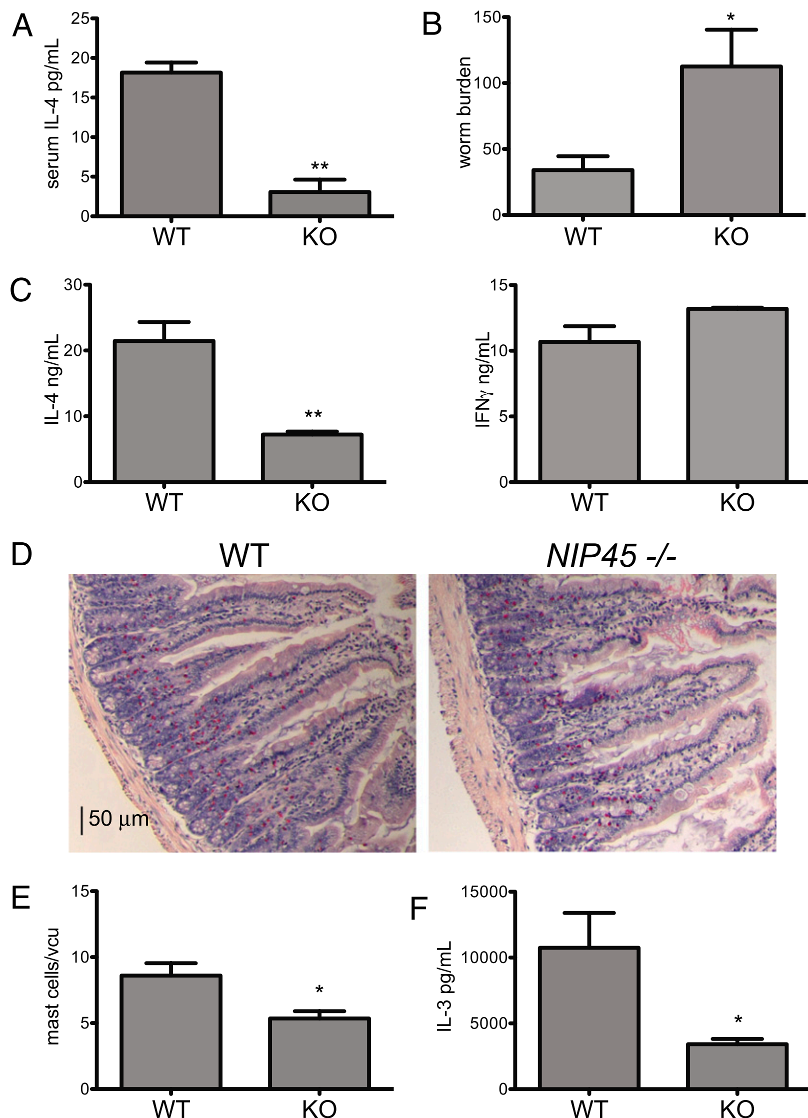

Fig. 1. Increased worm burden in *T. spiralis*-infected *NIP45*$^{-/-}$ mice. (*A*) Wild-type (WT) control or *NIP45*$^{-/-}$ knockout (KO) mice were inoculated orally with 500 *T. spiralis* muscle larvae. Mice were killed, and small intestine-residing adult worms were counted on day 12. (*B*) Serum IL-4 levels were measured on day 12 after *T. spiralis* inoculation of WT and *NIP45*$^{-/-}$ (KO) mice. (*C*) Cytokine responses from activated T helper cells. T helper cells were isolated from the draining mesenteric lymph node of *T. spiralis*-infected mice, and cell supernatants were analyzed by cytokine ELISA for IL-4 (*Left*) or IFNγ (*Right*). (*D*) Histological analysis of mast cells in the jejunum of infected WT and *NIP45*$^{-/-}$ mice. (Scale bar: 50 μm.) Mast cells were identified as cells with CAE activity. (*E*) Mast cells per villus crypt unit (vcu; small intestine) were counted. (*F*) IL-3 production by mesenteric T helper cells. *$P \leq 0.05$; **$P \leq 0.01$ (unpaired Student's *t* test). Data are presented as mean ± SEM (*n* = 5). Data depicted are representative of three independent experiments.

analysis revealed decreased jejunal mastocytosis in $NIP45^{-/-}$ mice ($P = 0.03$) (Fig. 1 *D* and *E*). The reduced intestinal mast-cell numbers in $NIP45^{-/-}$ mice were not caused by a mast-cell developmental deficiency, because uninfected $NIP45^{-/-}$ mice had mast-cell numbers equal to wild-type controls in ear tissue samples (Fig. S4*B*). Further, decreased intestinal mastocytosis was unlikely caused by an intrinsic mast-cell development or proliferation defect, because bone marrow-derived mast cells (BMMC) developed normally in culture and exhibited normal proliferative responses to the mast-cell growth-factor IL-3 (Fig. S4 *C* and *D*). IL-3 production by mesenteric lymphocytes is critical for maintaining intestinal mastocytosis in response to parasitic infection, whereas the addition of exogenous IL-3 during infection accelerates *T. spiralis* expulsion (38–42). Thus, we examined the production of IL-3 by mesenteric T helper cells after infection. Mesenteric T helper cells from *T. spiralis*-infected $NIP45^{-/-}$ mice displayed significantly impaired IL-3 production (Fig. 1*F*). These results suggest that defective T helper cell function in $NIP45^{-/-}$ mice within the mesenteric lymph nodes of $NIP45^{-/-}$ accounts for the impaired type-2 immune response.

NIP45 Is Required for Robust IL-4 Expression in Vitro. The diminished IL-4 levels produced by mesenteric T helper cells could be caused by an intrinsic defect in T helper cells or factors upstream of Th2 differentiation. Similar to mesenteric T helper cells isolated after *T. spiralis* infection, *NIP45* deficiency resulted in a greater than 2-fold reduction in IL-4 production by in vitro-derived Th2 cells (Fig. S5*A*). In gene-reporter assays, NIP45 also cooperates with the Th1 lineage-specific transcription factor T-bet to induce IFNγ promoter activity (27). Indeed, $NIP45^{-/-}$ Th1 cells displayed a modest but significant decrease in IFNγ levels (Fig. S5*A*). The frequency of IFNγ-producing Th1 cells was not significantly affected by the NIP45 deficiency ($P = 0.0848$), although intracellular IFNγ mean fluorescence intensity (MFI) levels in $NIP45^{-/-}$ Th1 cells were decreased compared with wild-type controls (Fig. S5*B*). The frequency and the MFI of IL-4–producing Th2 cells were diminished, suggesting that NIP45 regulates the quantity of IL-4–producing cells ($P = 0.0128$) (Fig. S5*B*). In addition, the intracellular IL-4 MFI levels in $NIP45^{-/-}$ Th2 cells were reduced compared with wild-type Th2 cells (Fig. S5*B*). Decreased IL-4 protein levels in $NIP45^{-/-}$ Th2 cells were accompanied by a reduction in IL-4 transcript, showing IL-4 dysregulation was at the level of transcription (Fig. S5*C*). These results further support the notion that NIP45 regulates the magnitude of NFAT-driven transcription.

The *IL-4* gene is located within a coordinately regulated cluster of Th2 cytokine genes also containing the *IL-13* and *IL-5* loci (43). Both IL-13 and IL-5 levels were diminished in $NIP45^{-/-}$ Th2 cells (Fig. 2 *A* and *B*). In conjunction with NFAT, NIP45 is required for T cell-specific IL-3 enhancer activity in over-expression analysis. Indeed, $NIP45^{-/-}$ Th2 cells were impaired in IL-3 production (Fig. 2*C*). Importantly, not all NFAT-regulated cytokine expression was attenuated in $NIP45^{-/-}$ T helper cells, because IL-2 levels were comparable between wild-type and $NIP45^{-/-}$ Th1 and Th2 cells (Fig. 2 *D* and *E*). Together, these results indicate that NIP45 modulates a specific subset of NFAT-regulated cytokine genes.

NIP45 Enhances IL-4–Promoter Chromatin Modifications. Because NIP45 is an NFAT-binding partner (26), we examined whether or not the integrity of the NFAT-activation pathway was disrupted in $NIP45^{-/-}$ T helper cells. Purified naïve T helper cells were skewed for 7 days under Th1 or Th2 polarizing conditions. After stimulation with phorbol myristate acetate (PMA) and ionomycin, we analyzed the localization of NFATc1, NFATc2, and NFATc3 isoforms in wild-type and $NIP45^{-/-}$ Th1 and Th2 cells. Immunoblotting cytoplasmic and nuclear extracts revealed no deficiency in NFAT translocation to the nucleus in $NIP45^{-/-}$ cells (Fig. 3). Nuclear NFAT in $NIP45^{-/-}$ Th2 cells retained the capacity to bind DNA, which was revealed by gel shift analysis using a probe corresponding to a NFAT-binding region (−88

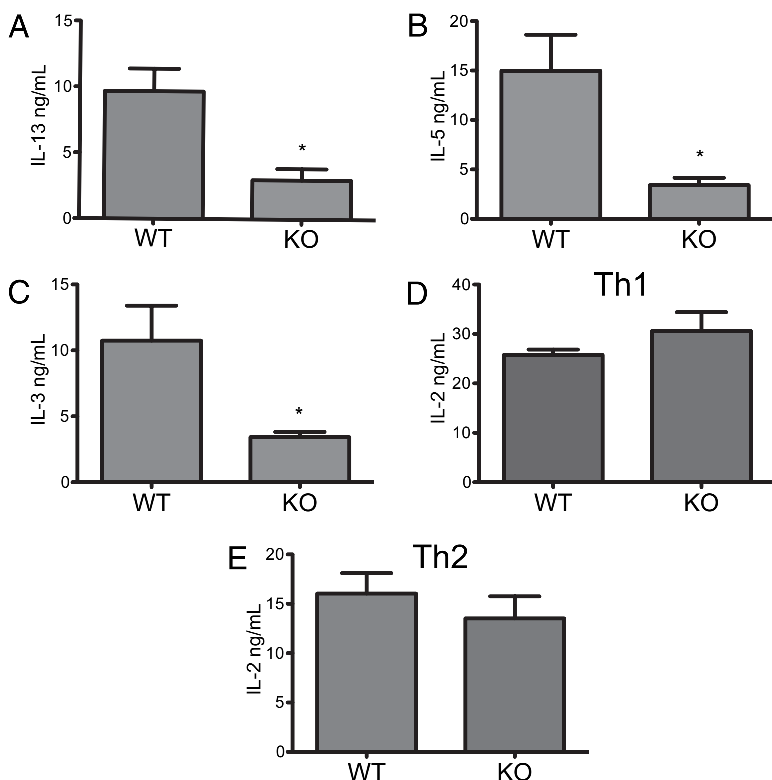

Fig. 2. NIP45 deletion does not alter all NFAT-regulated cytokine genes. Th2 cell supernatants were analyzed by cytokine ELISA for (*A*) IL-4, (*B*) IL-5, or (*C*) IL-3. Supernatants from (*D*) Th1 or (*E*) Th2 cells were analyzed for IL-2 levels. Graphs depict mean ± SD and are representative of at least five independent experiments. *$P \leq 0.05$ (unpaired Student's *t* test).

IMMUNOLOGY

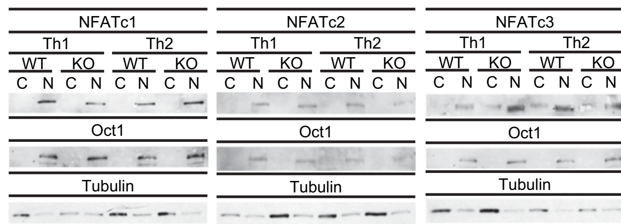

Fig. 3. *NIP45* deficiency does not alter NFAT activation or lineage-commitment program. NFAT is translocated in *NIP45*$^{+/+}$ (WT) and *NIP45*$^{-/-}$ (KO). Th2 cells were stimulated with PMA (50 ng/mL) and ionomycin (1 μM) for 2 h. Cytoplasmic and nuclear extracts from WT and KO Th2 cells were analyzed by Western blot with antibodies to NFATc1, NFATc2, and NFATc3. Integrity of nuclear and cytoplasmic extracts was determined using antibodies to Oct1 and Tubulin, respectively. Data are representative of at least three independent experiments.

to −60) of the murine IL-4 promoter (Fig. S6*A*) (44). Thus, NIP45 is not required for NFAT activation.

Signals from the TCR and cytokine receptors converge to induce a genetic program, which gives rise to a specific T helper effector lineage (45). To gain insight into the molecular events leading to the IL-4 deficiency seen in *NIP45*$^{-/-}$ Th2 cells, we examined the expression of the Th1 and Th2 lineage-specific transcription factors T-bet and GATA-3 (46). *NIP45*$^{-/-}$ cells expressed equivalent levels of T-bet and GATA-3 (Fig. S6*B*). Therefore, NIP45 is not required for the expression of Th1- or Th2-programming factors.

Arginine methylation of histone H4 by PRMT1 (H4R3) facilitates histone H4 acetylation and correlates with transcriptionally active chromatin structure (47, 48). Because NFAT and NIP45 form a ternary complex with the arginine methyltransferase PRMT1 (27), we performed chromatin immunoprecipitation analysis of H4R3 methylation and H4 acetylation associated with the IL-4 promoter. In NIP45$^{-/-}$ Th2 cells, IL-4 promoter histone 4 acetylation was decreased (Fig. 4*A*), correlating with diminished IL-4 levels (Fig. S5). Importantly, NIP45 deficiency did not cause a global reduction in histone acetylation, because acetylation at the IFNγ promoter was unaffected in *NIP45*$^{-/-}$ Th2 cells (Fig. 4*B*).

Consistent with NIP45-mediated recruitment of PRMT1 to the NFATc2 complex, *NIP45*$^{-/-}$ Th2 cells displayed impaired H4R3 methylation (Fig. 4*C*) and recruitment of PRMT1 to the IL-4 promoter (Fig. 4*D*; Fig. S7). PRMT1 specifically associates with the IL-4 promoter, because PRMT1 was not recruited to the brain-specific neurofilament (Nfm) control (Fig. 4*D*). The reduced association of methyl-H4R3 and PRMT1 with the IL-4 promoter is not caused by a defect in PRMT1 expression, because wild-type and *NIP45*$^{-/-}$ cells expressed equivalent amounts of PRMT1 (Fig. 4*E*). These findings confirm the role of NIP45 in recruiting PRMT1 to participate in chromatin remodeling at the *IL-4* locus.

Discussion

Here, we have developed a *NIP45*$^{-/-}$ mouse line to investigate the role of this NFAT transcriptional cofactor in the type-2 immune response and to understand the mechanism by which NIP45 regulates Th2-driven immunity. In the absence of NIP45, the immune response to the parasitic nematode *T. spiralis* was severely impaired as a result of diminished levels of the Th2 cytokines IL-4 and IL-13 as well as the mast-cell growth-factor IL-3. NIP45 deficiency did not alter the general transcription-factor profile of Th1 or Th2 cells nor was NFAT activation affected. Instead, the absence of NIP45 hindered recruitment of PRMT1 to the IL-4 promoter, thereby diminishing PRMT1-associated chromatin modification and IL-4 transcription.

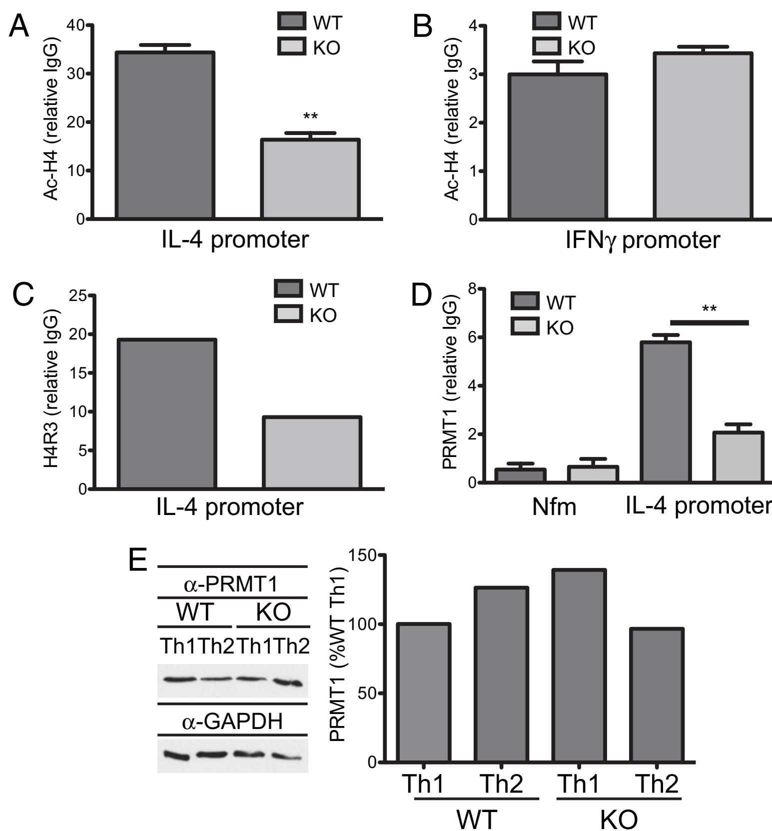

Fig. 4. Impaired chromatin modification and PRMT1 association in *NIP45* null Th2 cells. Th2 cells from *NIP45*$^{+/+}$ (WT) and *NIP45*$^{-/-}$ (KO) were stimulated with PMA (50 ng/mL) and ionomycin (1 μM) for 2 h. ChIP was performed for histone 4 acetylation (Ac-H4) associated with the (*A*) IL-4 or (*B*) IFNγ promoter, (*C*) dimethyl-histone 4 arginine 3 associated with the IL-4 promoter, and (*D*) PRMT1 associated with the IL-4 or neurofilament (Nfm) promoter. Data are expressed relative to IgG controls (n = 3) except *C*, which is a representative experiment (n = 2). (*E*) PRMT1 expression in *NIP45*$^{+/+}$ (WT) and *NIP45*$^{-/-}$ (KO) Th1 and Th2 cells. Th2 cell lysates were analyzed by Western blot using an antibody-recognizing PRMT1. GAPDH was used as a loading control. The bar graph depicts normalization of denisitometry to the WT Th1 sample. Data are representative of three independent experiments.

Both Th1 and Th2 hallmark cytokines are targets for members of the NFAT transcription-factor family (6, 49). Indeed, NFATc1/NFATc2 doubly deficient T helper cells are severely impaired in IL-2, IL-5, IL-4, and IFNγ production, whereas constitutively active forms of NFATc1 and NFATc2 can drive endogenous IL-4 production (21, 50, 51). Here, we have described the function of NIP45 in Th1 and Th2 cells. NIP45 plays a limited role in regulating IFNγ production by Th1 cells. In contrast, NIP45 contributes significantly to inducing Th2 IL-4 production both in vitro and in vivo during *T. spiralis* infection.

Histone arginine methylation facilitates histone acetylation and has been correlated with elevated transcriptional activity (47, 48). In contrast to acetylation, PRMT-mediated histone modifications have not been previously characterized within the *IL-4* locus (43). We have recently described the ability of NIP45 to recruit the arginine methyltransferase PRMT1 to NFAT complexes and postulated that one function of NIP45 might be to recruit PRMT1 to participate in chromatin remodeling at cytokine loci (27). In support of this model, the level of both PRMT1 recruitment to the IL-4 promoter and its methyl-histone mark is reduced in *NIP45*$^{-/-}$ Th2 cells. Because PRMT1 *IL-4* promoter association is still enriched relative to a nonspecific control in *NIP45*$^{-/-}$ Th2 cells, NIP45 by itself is not required for PRMT1 *IL-4* promoter recruitment, suggesting that PRMT1 can be recruited v through non-NIP45–containing transcriptional complexes. Because IL-5 and IL-13 levels are also altered in *NIP45*$^{-/-}$ Th2 cells, NIP45 may influence epigenetic chromatin modifications within other critical cis-regulatory elements that control IL-4, IL-13, and IL-5 production. Nevertheless, we propose that the adjustment to the coregulator complex in *NIP45* null Th2 cells is sufficient to dial down the magnitude of NFAT-driven IL-4 expression, which then cascades into an inefficient antiparasite immune response.

Expulsion of *T. spiralis* is critically dependent on Th2 cell and mast-cell function (30). As is the case during infection with many parasitic nematodes, the hallmark Th2 cytokine IL-4 is protective during infection with *T. spiralis* (32). Mast cells are also IL-4 producers (52). Indeed, IL-4 production by mast cells is almost entirely dependent on NFATc2 (53, 54). Whether or not IL-4 expression is also reduced in *NIP45*-deficient mast cells in response to FcεRI stimulation is currently being investigated. Although the primary cellular source of IL-4 during *T. spiralis* infection is unidentified, reduced expression of IL-4 by Th2 cells and possibly, other cellular sources contributes to the impaired antiparasitic response in *NIP45*$^{-/-}$ mice (32). IL-4 and IL-13 act in concert to mediate antiparasitic responses by cells not derived from bone marrow, inducing smooth-muscle contractility, intestinal epithelial permeability, intestinal secretory responses to prostaglandin E2 (PGE_2), and mucus secretion.

Our results provide in vivo confirmation of our original description of NIP45 as a factor that acts to augment IL-4 gene transcription by synergizing with NFAT and c-Maf transcription factors (26). As an NFAT coregulator, NIP45 coordinates many NFAT-driven cytokine-expression responses, in some cases paralleling results found with NFATc1 and NFATc2 knockouts (13, 14, 21, 26). We speculate that non–NIP45-regulated NFAT-responsive genes may also require a unique complex of transcriptional coregulator proteins. These findings indicate that, although NIP45 regulates the magnitude of expression for a subset of NFAT-driven genes, it is critically important for mediating a productive type-2 immune response.

Materials and Methods

Mice and Cell Culture. The targeting construct was designed to delete exon 1 and 2 of *NIP45* by replacement with a neomycin cassette flanked by lox P sites, and it was electroporated into PrmCre ES cells (29). To screen for homologous recombination, genomic DNA from drug-resistant clones was digested with XbaI and analyzed by Southern blot hybridization. For PCR genotyping, the 400-bp *NIP45*$^{-/-}$ PCR product was detected using forward (5′GTCTACAATCACGAGCATGAACC3′) and reverse (5′GATGTCACAGAGTCT-CAGAACTCC3′) PCR primers flanking exon deletion, and the 120-bp *NIP45* wild-type allele was detected using forward (5′-TAGGCTCATTCCAGACACCG-3′) and reverse (5′-CTGTCCTGCTCAGGTTTAGC-3′) primers corresponding to exon 1. Mice were backcrossed onto C57BL/6 or BALB/c for 6–12 generations. All animal protocols were in accordance with The Scripps Research Institute and Harvard School of Public Health Institutional Animal Care and Use Committee policies.

$CD4^+CD62L^{hi}$ naïve T cells were purified by FACS (MoFlo; Becton Dickinson) or by magnetic selection using the Naïve T cell Purification kit (Miltenyi). Th1 and Th2 cultures were grown as described (31).

Immunoblots. Cytoplasmic and nuclear fractions were prepared using hypotonic and hypertonic lysis buffers (55). Specific antibody clones are listed in *SI Materials and Methods*.

Flow Cytometry. Antibodies used for flow cytometry are listed in *SI Materials and Methods*. Cells were analyzed with a FACSCanto cytometer (BD Biosciences). Data were analyzed with FlowJo software (TreeStar). Intracellular cytokine staining was performed as described (56).

Histology. Ear pinna mast cells were identified by reactivity to chloroacetate esterase (CAE) (57) and were counted in 8–12 consecutive fixed fields (58). Examination of slides was performed by an observer who was not aware of individual section identities. For *T. spiralis* infections, the distribution of mast cells within the jejunum was compared between 5 mice in each wild-type and *NIP45*$^{-/-}$ group and expressed per villus crypt unit.

***T. spiralis* Infection and Worm-Burden Determination.** *T. spiralis* larvae were obtained from BALB/c mice as described previously (57). Mice were infected with ∼500 larvae by direct gastric installation (n = 5). Worm burden was determined by excising the small intestine, slicing it open longitudinally, and then, cutting it into 4-mm-long sections. Intestine sections were incubated in PBS for 3 h at 37 °C with gentle agitation, and worms were enumerated using an inverted microscope.

EMSA. EMSAs were performed using nuclear extracts and an end-labeled oligonucleotide corresponding to an NFAT-binding region of the IL-4 promoter (5′-CTGGTGTAATAAAATTTTCCAATGTAAAC-3′) as described (55).

ChIP Assay. ChIP assay was performed as described and is detailed in *SI Materials and Methods* (59).

Statistics. Statistical significance was determined by the Student's t test (for parametric data) or the Mann–Whitney test (for nonparametric data) using Graphpad Prism 5 software. P values of less than 0.05 were considered statistically significant.

ACKNOWLEDGMENTS. We thank S. Arandjelovic, D. Nemazee, M. McHeyzer-Williams, and J. Jameson for suggestions. We thank A. Sepulveda (Memorial Sloan-Kettering Cancer Center), N. Iwakoshi (Emory University, Atlanta, GA), S.-Y. Pai (Children's Hospital, Dana Farber Cancer Institute, Boston, MA), A. Wurster (National Institute of Aging, Bethesda, MD), and M. Pazin (NIA, Bethesda, MD) for technical expertise. This is manuscript # 20559 from TSRI. This work is supported by the National Institutes of Health Grants AI083516 (to M.F.G.), AI43953 (to L.H.G.), P01 NS038037 (to L.H.G.), AI067460 (to K.A.M.), and GM085117 (to K.A.M.).

1. Yu D, et al. (2009) The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity 31:457–468.
2. Johnston RJ, et al. (2009) Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 325:1006–1010.
3. Nurieva RI, et al. (2009) Bcl6 mediates the development of T follicular helper cells. *Science* 325:1001–1005.
4. Josefowicz SZ, Rudensky A (2009) Control of regulatory T cell lineage commitment and maintenance. *Immunity* 30:616–625.
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. Macian F (2005) NFAT proteins: Key regulators of T-cell development and function. *Nat Rev Immunol* 5:472–484.
7. Rengarajan J, Tang B, Glimcher LH (2002) NFATc2 and NFATc3 regulate T(H)2 differentiation and modulate TCR-responsiveness of naïve T(H)cells. *Nat Immunol* 3:48–54.
8. Hogan PG, Chen L, Nardone J, Rao A (2003) Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* 17:2205–2232.

9. Rao A, Luo C, Hogan PG (1997) Transcription factors of the NFAT family: Regulation and function. *Annu Rev Immunol* 15:707–747.
10. Crabtree GR, Clipstone NA (1994) Signal transmission between the plasma membrane and nucleus of T lymphocytes. *Annu Rev Biochem* 63:1045–1083.
11. Clipstone NA, Crabtree GR (1992) Identification of calcineurin as a key signaling enzyme in T-lymphocyte activation. *Nature* 357:695–697.
12. Clipstone NA, Crabtree GR (1993) Calcineurin is a key signaling enzyme in T lymphocyte activation and the target of the immunosuppressive drugs cyclosporin A and FK506. *Ann N Y Acad Sci* 696:20–30.
13. Yoshida H, et al. (1998) The transcription factor NF-ATc1 regulates lymphocyte proliferation and Th2 cytokine production. *Immunity* 8:115–124.
14. Ranger AM, et al. (1998) Delayed lymphoid repopulation with defects in IL-4-driven responses produced by inactivation of NF-ATc. *Immunity* 8:125–134.
15. Schuh K, et al. (1997) NF-ATp plays a prominent role in the transcriptional induction of Th2-type lymphokines. *Immunol Lett* 57:171–175.
16. Kiani A, Viola JP, Lichtman AH, Rao A (1997) Down-regulation of IL-4 gene transcription and control of Th2 cell differentiation by a mechanism involving NFAT1. *Immunity* 7:849–860.
17. Xanthoudakis S, et al. (1996) An enhanced immune response in mice lacking the transcription factor NFAT1. *Science* 272:892–895.
18. Hodge MR, et al. (1996) Hyperproliferation and dysregulation of IL-4 expression in NF-ATp-deficient mice. *Immunity* 4:397–405.
19. Chen J, et al. (2003) Role of NFATx (NFAT4/NFATc3) in expression of immunoregulatory genes in murine peripheral CD4+ T cells. *J Immunol* 170:3109–3117.
20. Oukka M, et al. (1998) The transcription factor NFAT4 is involved in the generation and survival of T cells. *Immunity* 9:295–304.
21. Peng SL, Gerth AJ, Ranger AM, Glimcher LH (2001) NFATc1 and NFATc2 together control both T and B cell activation and differentiation. *Immunity* 14:13–20.
22. Ullman KS, Northrop JP, Admon A, Crabtree GR (1993) Jun family members are controlled by a calcium-regulated, cyclosporin A-sensitive signaling pathway in activated T lymphocytes. *Genes Dev* 7:188–196.
23. Li B, Tournier C, Davis RJ, Flavell RA (1999) Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *EMBO J* 18:420–432.
24. Rengarajan J, et al. (2002) Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. *J Exp Med* 195:1003–1012.
25. Hu CM, Jang SY, Fanzo JC, Pernis AB (2002) Modulation of T cell cytokine production by interferon regulatory factor-4. *J Biol Chem* 277:49238–49246.
26. Hodge MR, et al. (1996) NF-AT-Driven interleukin-4 transcription potentiated by NIP45. *Science* 274:1903–1905.
27. Mowen KA, Schurter BT, Fathman JW, David M, Glimcher LH (2004) Arginine methylation of NIP45 modulates cytokine gene expression in effector T lymphocytes. *Mol Cell* 15:559–571.
28. Mowen KA, Glimcher LH (2004) Signaling pathways in Th2 development. *Immunol Rev* 202:203–222.
29. O'Gorman S, Dagenais NA, Qian M, Marchuk Y (1997) Protamine-Cre recombinase transgenes efficiently recombine target sequences in the male germ line of mice, but not in embryonic stem cells. *Proc Natl Acad Sci USA* 94:14602–14607.
30. Urban JF, Jr, et al. (2000) Stat6 signaling promotes protective immunity against Trichinella spiralis through a mast cell- and T cell-dependent mechanism. *J Immunol* 164:2046–2052.
31. Urban JF, Jr, Maliszewski CR, Madden KB, Katona IM, Finkelman FD (1995) IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *J Immunol* 154:4675–4684.
32. Finkelman FD, et al. (2004) Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol Rev* 201:139–155.
33. Grencis RK, Else KJ, Huntley JF, Nishikawa SI (1993) The in vivo role of stem cell factor (c-kit ligand) on mastocytosis and host protective immunity to the intestinal nematode Trichinella spiralis in mice. *Parasite Immunol* 15:55–59.
34. Kamiya M, Oku Y, Itayama H, Ohbayashi M (1985) Prolonged expulsion of adult Trichinella spiralis and eosinophil infiltration in mast cell-deficient W/Wv mice. *J Helminthol* 59:233–239.
35. Itayama H, Oku Y, Kamiya M (1987) Longevity and fecundity of Trichinella spiralis in mast cell-deficient Sl/Sld mice. *Jpn J Vet Res* 35:195–207.
36. Oku Y, Itayama H, Kamiya M (1984) Expulsion of Trichinella spiralis from the intestine of W/Wv mice reconstituted with haematopoietic and lymphopoietic cells and origin of mucosal mast cells. *Immunology* 53:337–344.
37. Ha TY, Reed ND, Crowle PK (1983) Delayed expulsion of adult Trichinella spiralis by mast cell-deficient W/Wv mice. *Infect Immun* 41:445–447.
38. Korenaga M, Abe T, Hashiguchi Y (1996) Injection of recombinant interleukin 3 hastens worm expulsion in mice infected with Trichinella spiralis. *Parasitol Res* 82: 108–113.
39. Lantz CS, et al. (1998) Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* 392:90–93.
40. Abe T, Nawa Y (1988) Kinetic study of mast-cell growth factor production by lymphocytes during the course of Strongyloides ratti infection in mice. *Parasitol Res* 74:484–488.
41. Kobayashi T, et al. (1998) Intestinal mast cell response and mucosal defense against Strongyloides venezuelensis in interleukin-3-hyporesponsive mice. *Parasite Immunol* 20:279–284.
42. Else KJ, Finkelman FD, Maliszewski CR, Grencis RK (1994) Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med* 179:347–351.
43. Ansel KM, Djuretic I, Tanasa B, Rao A (2006) Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol* 24:607–656.
44. Brogdon JL, Leitenberg D, Bottomly K (2002) The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells. *J Immunol* 168:3825–3832.
45. Wilson CB, Rowell E, Sekimata M (2009) Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol* 9:91–105.
46. Glimcher LH (2001) Lineage commitment in lymphocytes: Controlling the immune response. *J Clin Invest* 108:s25–s30.
47. Stallcup MR, et al. (2000) Co-operation between protein-acetylating and protein-methylating co-activators in transcriptional activation. *Biochem Soc Trans* 28:415–418.
48. Wang H, et al. (2001) Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* 293:853–857.
49. Luo C, et al. (1996) Recombinant NFAT1 (NFATp) is regulated by calcineurin in T cells and mediates transcription of several cytokine genes. *Mol Cell Biol* 16:3955–3966.
50. Monticelli S, Rao A (2002) NFAT1 and NFAT2 are positive regulators of IL-4 gene transcription. *Eur J Immunol* 32:2971–2978.
51. Kiani A, et al. (2001) Regulation of interferon-gamma gene expression by nuclear factor of activated T cells. *Blood* 98:1480–1488.
52. Galli SJ, et al. (2005) Mast cells as "tunable" effector and immunoregulatory cells: Recent advances. *Annu Rev Immunol* 23:749–786.
53. Monticelli S, Lee DU, Nardone J, Bolton DL, Rao A (2005) Chromatin-based regulation of cytokine transcription in Th2 cells and mast cells. *Int Immunol* 17:1513–1524.
54. Solymar DC, Agarwal S, Bassing CH, Alt FW, Rao A (2002) A 3′ enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells. *Immunity* 17:41–50.
55. Schreiber E, Matthias P, Müller MM, Schaffner W (1989) Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res* 17:6419.
56. Szabo SJ, et al. (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 100:655–669.
57. Friend DS, et al. (1996) Mast cells that reside at different locations in the jejunum of mice infected with Trichinella spiralis exhibit sequential changes in their granule ultrastructure and chymase phenotype. *J Cell Biol* 135:279–290.
58. Grimbaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ (2007) Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat Immunol* 8:1095–1104.
59. Wurster AL, Pazin MJ (2008) BRG1-mediated chromatin remodeling regulates differentiation and gene expression of T helper cells. *Mol Cell Biol* 28:7274–7285.