T-cell–intrinsic Tif1α/Trim24 regulates IL-1R expression on T_H2 cells and T_H2 cell-mediated airway allergy

Jimena Perez-Lloret[a], Isobel S. Okoye[a], Riccardo Guidi[a], Yashaswini Kannan[a], Stephanie M. Coomes[a], Stephanie Czieso[a], Gabrielle Mengus[b], Irwin Davidson[b], and Mark S. Wilson[a,1]

[a]Allergy and Anti-Helminth Immunity Laboratory, The Francis Crick Institute, Mill Hill Laboratories, London NW7 1AA, United Kingdom; and [b]Department of Functional Genomics and Cancer, Institut de Génétique et de Biologie Moléculaire et Cellulaire, 67400 Illkirch, France

Edited by Robert L. Coffman, Dynavax Technologies, Berkeley, CA, and approved December 16, 2015 (received for review November 16, 2015)

There is a paucity of new therapeutic targets to control allergic reactions and forestall the rising trend of allergic diseases. Although a variety of immune cells contribute to allergy, cytokine-secreting $\alpha\beta^+CD4^+$ T-helper 2 (T_H2) cells orchestrate the type-2–driven immune response in a large proportion of atopic asthmatics. To identify previously unidentified putative targets in pathogenic T_H2 cells, we performed in silico analyses of recently published transcriptional data from a wide variety of pathogenic T_H cells [Okoye IS, et al. (2014) Proc Natl Acad Sci USA 111(30):E3081–E3090] and identified that transcription intermediary factor 1 regulator-alpha (*Tif1α*)/tripartite motif-containing 24 (*Trim24*) was predicted to be active in house dust mite (HDM)- and helminth-elicited $Il4^{gfp+}\alpha\beta^+CD4^+$ T_H2 cells but not in T_H1, T_H17, or Treg cells. Testing this prediction, we restricted Trim24 deficiency to T cells by using a mixed bone marrow chimera system and found that T-cell–intrinsic Trim24 is essential for HDM-mediated airway allergy and antihelminth immunity. Mechanistically, HDM-elicited $Trim24^{-/-}$ T cells have reduced expression of many T_H2 cytokines and chemokines and were predicted to have compromised IL-1–regulated signaling. Following this prediction, we found that $Trim24^{-/-}$ T cells have reduced IL-1 receptor (IL-1R) expression, are refractory to IL-1β–mediated activation in vitro and in vivo, and fail to respond to IL-1β–exacerbated airway allergy. Collectively, these data identify a previously unappreciated Trim24-dependent requirement for IL-1R expression on T_H2 cells and an important nonredundant role for T-cell–intrinsic Trim24 in T_H2-mediated allergy and antihelminth immunity.

Trim24 | Th2 | allergy | inflammation | asthma

Allergic diseases, including allergic asthma, have continued to rise in the past 50 y. With few new drugs available to treat allergic diseases and many patients with severe asthma refractory to currently available drugs (1), there is a growing need for new molecular targets to curb symptoms and prevent exacerbations. Dysregulated T-cell responses underpin the hyperinflammatory allergic reaction leading to asthma. Although many T-cell populations contribute to the spectrum of allergic asthma phenotypes (2), cytokine-secreting T-helper 2 (T_H2) cells have the capacity to induce allergen-specific IgE (atopy) and invoke many of the pathophysiological manifestations associated with asthma, including airway eosinophilia, mucus hypersecretion, airway remodeling, and airway hyperreactivity. Targeting specific cytokine-signaling pathways in allergic asthma has had mixed efficacy (3–5), suggesting that additional targets and more focused approaches should be considered (6). Several T_H2 cell lineage-promoting transcriptional regulators, including GATA binding protein 3 (GATA-3) (7), STAT-3 (8), STAT-6 (9), and avian musculoaponeurotic fibrosarcoma (cMAF) (10), have been identified in T_H2 cells; however, it is unclear whether other transcriptional regulators are required for T_H2 cell-mediated responses.

The two-signal model of T_H2 cell differentiation, involving T-cell receptor (TCR) and costimulatory engagement coupled with secondary cytokine signaling, is well defined (11). However, the activation of differentiated T_H2 cells and the acquisition of cytokine-secreting effector function in the tissues is poorly understood. Tertiary cytokine signals by tissue-associated inflammatory cytokines, including members of the IL-1 family (12, 13), and the "alarmins" (IL-25 and TSLP) (14) have been proposed to activate T_H2 cells; however, the regulation and pathways involved are unclear. An IL-1R (IL-1 receptor)/ubiquitin C/Trim24 (tripartite motif-containing 24) axis has been identified previously (15–19), but the involvement of Trim24 in T_H2 biology has not been reported.

The tripartite motif (Trim) family of more than 60 proteins is highly conserved throughout metazoans and has been widely studied in innate antiviral immunity (20). However, Trim proteins have a variety of functions, including regulation of transcription and chromatin (21–23), tumor suppression (24), and cytokine signaling and secretion, in both innate and adaptive immune cells (25, 26). Specifically, the transcription intermediary factor 1 regulator-alpha, Tif1α (Trim24), which is structurally related to Tif1β (Trim28) and Tif1γ (Trim33) (22), has important roles in cancer (27, 28), gene regulation (29), and cytokine signaling (30), in part through the interaction of Trim24 with nuclear hormone receptors, vitamin D receptors, estrogen receptors, and retinoic acid receptors (30, 31). Unlike the closely related Trim28 (26), which regulates T_H17-mediated immunity, a role for Trim24 in T-cell biology, type-2 immunity, or allergic asthma has not been reported. In this study, we found that deletion of Trim24 in T cells did not lead to any overt autoimmune phenotype. In contrast, Trim24 is essential for T_H2 cell-mediated airway allergy and T_H2-dependent expulsion of intestinal helminths. Mechanistically, $Trim24^{-/-}$ T cells isolated from the lungs of allergic mice had a dampened IL-1–regulated transcriptome, suggesting that

Significance

The increasing number of patients presenting with severe asthma throughout the world present a clear unmet medical need. This study identified putative transcriptional regulators in T-helper 2 (T_H2) cells with the aim of identifying previously unidentified targets to inhibit T_H2-mediated allergy. Genetic deletion of *Trim24* (tripartite motif-containing 24) in T cells showed that Trim24 was essential for T_H2-mediated allergy. Transcriptional analysis showed that *Trim24* was required for many of the pathogenic properties of T_H2 cells and that IL-1–regulated signaling is compromised in $Trim24^{-/-}$ cells. In vivo, in vitro, and in silico approaches identified a previously overlooked role for Trim24 in T_H2-mediated allergy and validate a combined approach to interrogate transcriptional datasets to identify new therapeutic targets to prevent allergy and asthma.

Author contributions: J.P.-L. and M.S.W. designed research; J.P.-L., I.S.O., R.G., Y.K., S.M.C., S.C., and M.S.W. performed research; G.M. and I.D. contributed new reagents/analytic tools; J.P.-L. and M.S.W. analyzed data; and M.S.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: mark.wilson@crick.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1522287113/-/DCSupplemental.

Trim24 is required for IL-1–mediated T_H2 cell activation. Indeed, *Trim24*$^{-/-}$ T_H2 cells had reduced IL-1R expression. Furthermore, deletion of T-cell–intrinsic Trim24, similar to the effect of IL-1R deletion on T cells, rendered T cells refractory to the stimulatory effects of IL-1β and curtailed the manifestation of house dust mite (HDM)-induced airway allergy, identifying a critical and nonredundant role for Trim24 in IL-1–mediated T_H2 cell-mediated inflammation in vivo.

Results

Tif1α/Trim24 Is a Predicted Transcriptional Regulator in T_H2 Cells but Not in T_H1, T_H17, or Foxp3$^+$ Treg Cells. Dysregulated T_H2-cell responses following allergen exposure contribute directly to allergic disease (32). We recently reported that HDM- or helminth-elicited T_H2 cells have distinct transcriptional profiles, compared with ex vivo T_H1, T_H17, and natural Treg cells (33). Using these transcriptional datasets (more than twofold change relative to naive T cells, $P < 0.05$) we applied in silico upstream regulator analyses (Ingenuity Pathways Analysis, IPA) (34) and generated a z-score representing the likelihood of activity of putative transcriptional regulators. Briefly, this analysis examines how many known target genes of a transcription regulator are present in the dataset and compares their expression with the level expected from the literature to predict transcriptional regulator activity. If the observed expression of target genes is mostly consistent with a particular activation state of the transcriptional regulator, then a higher prediction (z-score) is made about that activation state. This in silico approach identified GATA-3,

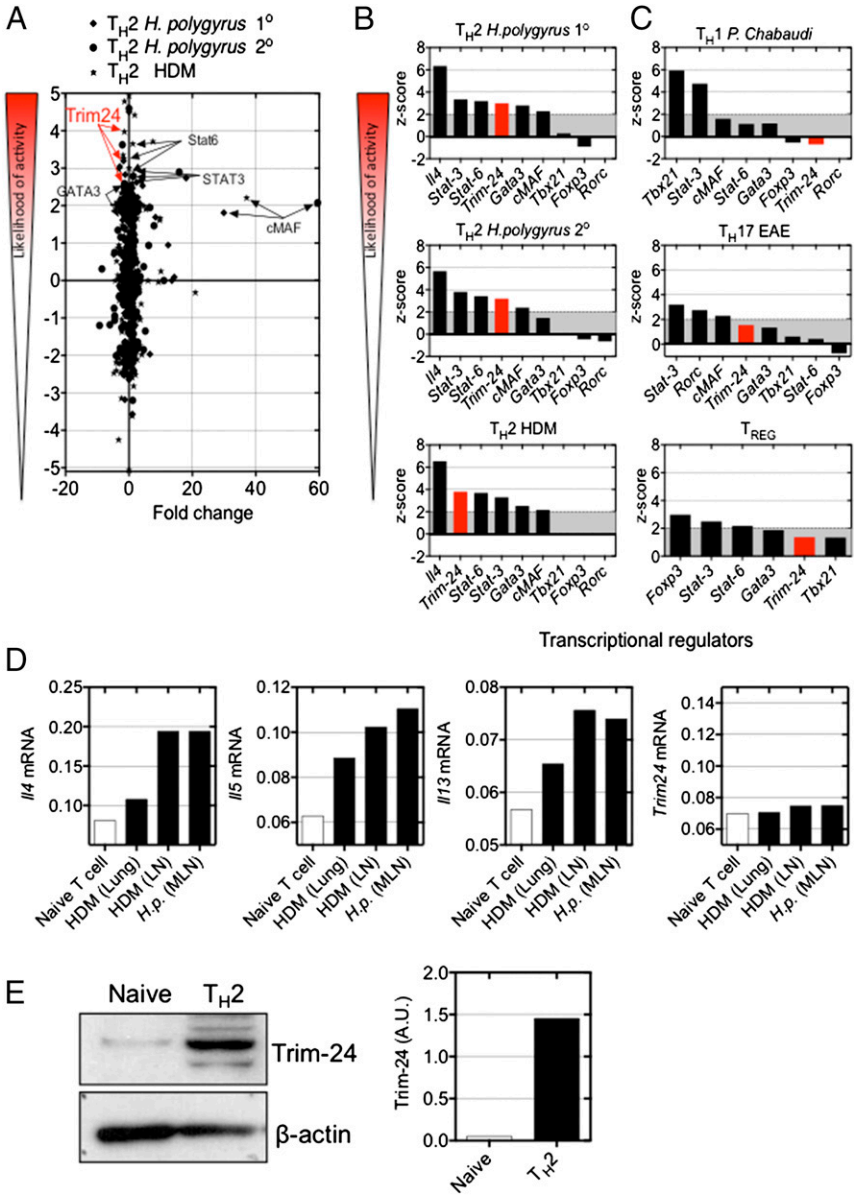

Fig. 1. Trim24 protein is elevated in T_H2 cells and is predicted to be functionally active in T_H2 cells but not in T_H1, T_H17, or Treg cells. (*A*) Transcriptional datasets from T_H2 cells purified from helminth (*H. polygyrus*)-infected or HDM-challenged mice (33) were analyzed using IPA upstream analysis software (34) to generate a z-score of the likelihood of activity. (*B* and *C*) Comparative analysis of predicted upstream transcriptional regulators in T_H2 cells (*B*) and T_H1, T_H17, and Treg cells (*C*). (*D*) Expression of *Il4*, *Il5*, *Il13*, and *Trim24* in *Il4*$^{gfp+}$ T_H2 cells isolated from pulmonary tissue [HDM (lung)] and local lymph nodes [HDM (LN)] of mice with HDM-induced airway allergy and from the local lymph nodes of *H. polygyrus*-infected mice [*H.p.* (MLN)]. Expression is shown relative to *Hprt*. (*E*) Expression of Trim24 protein in naive and in vitro-generated T_H2 cells. A.U., arbitrary units.

STAT-6, and cMAF as putative regulators of T_H2 cells (Fig. 1 *A* and *B*), in line with previous experimental observations. This analysis also predicted that Tif1α, belonging to the tripartite motif (Trim) family (i.e., Trim24), would be active in T_H2 cells but not in T_H1, T_H17, or Treg cells (Fig. 1 *A*–*C* and Table S1). The microarray analysis showed that Trim24, like Gata3, Stat6, and other Trim family members, was not significantly differentially regulated in T_H2 cells as compared with naive T cells (Fig. 1*A* and Fig. S1 *A* and *B*). Using *Il4*^{gfp}-reporter mice, we assessed the expression of *Trim24* mRNA by quantitative RT-PCR (qRT-PCR) in freshly isolated *Il4*$^{gfp+}$ T_H2 cells from the lung tissue or local lymph nodes of HDM-challenged mice and from the local lymph nodes of helminth-infected mice. *Il4*$^{gfp+}$ T_H2 cells had elevated *Il4*, *Il5*, and *Il13* mRNA but not *Trim24* mRNA (Fig. 1*D*), in line with the microarray data (Fig. S1*A*). Western blot analysis of *Il4*$^{gfp+}$ T_H2 cells generated in vitro identified increased Trim24 protein expression, as compared with naive T cells (Fig. 1*E*), supporting a role for Trim24 in T_H2 cells. Collectively, predictive in silico analyses coupled with the elevated levels of Trim24 protein in T_H2 cells suggest that Trim24 may contribute to T_H2 cell biology.

T-Cell–Intrinsic Tif1α/Trim24 Is Required for HDM-Induced Airway Allergy and Antihelminth Immunity. To test whether the predicted activity of Trim24 (Fig. 1*A*) and the elevated expression of Trim24 protein in T_H2 cells (Fig. 1*E*) is required for T_H2 cell-mediated

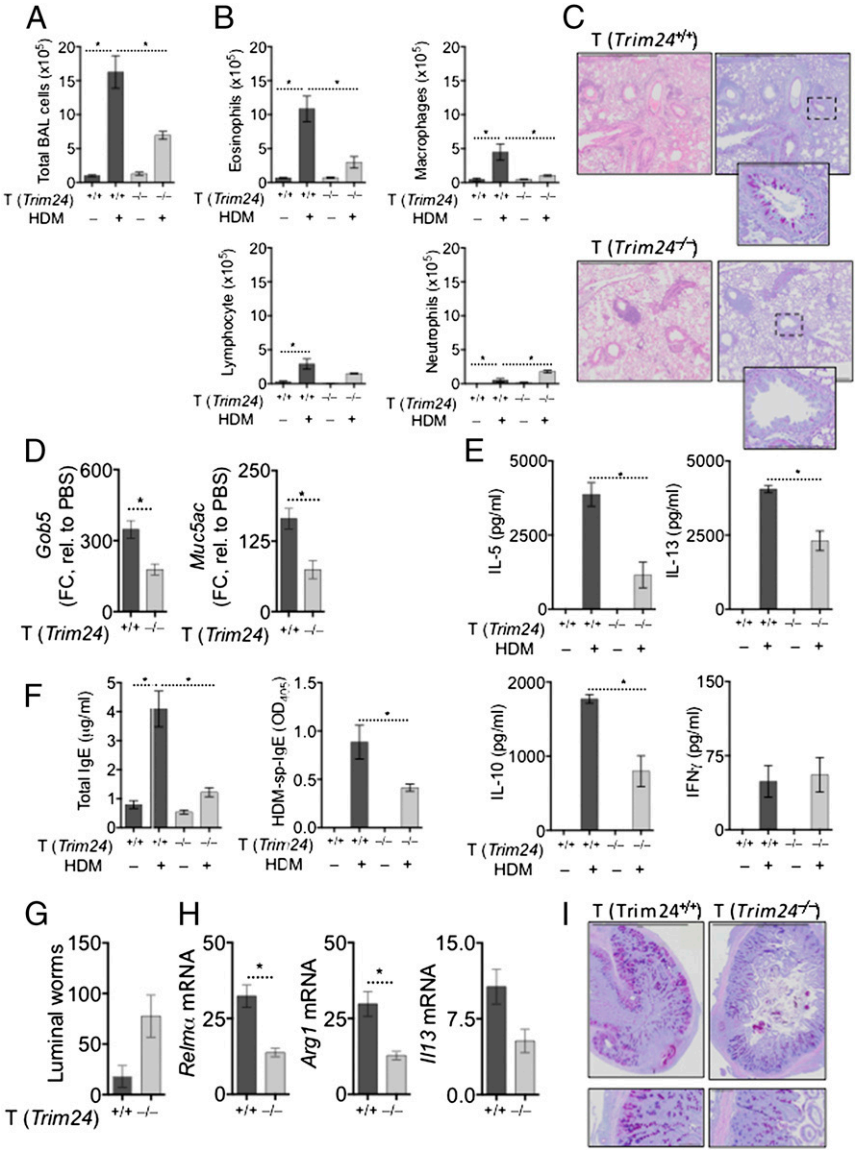

Fig. 2. T-cell–intrinsic Trim24 is essential for T_H2-mediated airway allergy and T_H2-dependent antihelminth immunity. Chimeric mice with WT or *Trim24*$^{-/-}$ T cells were sensitized and challenged with HDM, and airway allergy was assessed 1 d after the last airway challenge. *$P \leq 0.05$. One of three representative experiments is shown. (*A* and *B*) BAL cells were recovered, counted (*A*), and used for cytospins for differential analysis (*B*). (*C*) H&E- and AB-PAS–stained lung sections from chimeric mice with HDM-induced airway allergy. (*D*) Gene expression in the pulmonary tissue of mice with HDM-induced airway allergy. Expression is shown relative to PBS-treated mice. (*E*) Cells from local lymph nodes were restimulated with HDM. Secreted cytokines were measured in supernatants after 4 d. (*F*) Circulating total and HDM-specific IgE measured in the serum of mice with HDM-induced airway allergy. (*G*) Chimeric mice with WT or *Trim24*$^{-/-}$ T cells were infected, drug-treated on days 14 and 15, and given a challenge infection with *H. polygyrus* on day 21. Adult worms were counted in the small intestine 14 d after the challenge infection (day 35). (*H*) Gene expression in the small intestine 14 d after the challenge infection. Fold change (FC) is shown relative to uninfected mice. (*I*) AB-PAS–stained tissue sections from chimeric mice 14 d after the challenge infection.

airway allergy and T_H2-dependent antihelminth immunity in vivo, we generated chimeric mice in which Trim24-deficiency was restricted to T cells (Fig. S2*A*). T-cell–chimeric mice had a comparable number and frequency of naive, memory, and regulatory T cells with no overt immunopathology after 3 mo of bone marrow reconstitution (Fig. S2 *B–D*). This T-cell–chimera system relied upon on the fact that non-T cells from WT or *Trim24*$^{-/-}$ bone marrow repopulate at a similar rate in both WT and *Trim24*$^{-/-}$ chimeric mice and constitute only a minor fraction (20%) of the total bone marrow. To determine whether *Trim24*$^{-/-}$ cells displayed any potential growth advantage that might compromise the T-cell–chimeric system, we generated separate 50:50 chimeras with 50% WT and 50% *Trim24*$^{-/-}$ bone marrow. After 8 wk of reconstitution, the 50:50 chimeras retained the 50:50 ratio with comparable ratios of lymphocytes, myeloid cells, and granulocyte populations, indicating that neither the WT nor the *Trim24*$^{-/-}$ populations had an overt growth advantage (Fig. S2*E*). Following HDM sensitization and local airway challenge of T-cell–chimeric mice (Fig. S2*A*), mice with *Trim24*$^{-/-}$ T cells had significantly reduced T_H2-mediated airway allergy, with reduced airway inflammation (Fig. 2*A*) and reduced airway eosinophilia (Fig. 2*B*), as compared with mice with WT T cells. Mild perivascular infiltrates were present in mice with *Trim24*$^{-/-}$ T cells; however, the cells failed to migrate and cause interstitial or peribronchial inflammation or mucus secretion following HDM challenge (Fig. 2*C*). Reduced airway mucus was supported by reduced *Gob5* and *Muc5ac* expression in pulmonary tissue of mice with *Trim24*$^{-/-}$ T cells (Fig. 2*D*). Local lymph node cells restimulated with HDM indicated that Trim24 is required for optimal T_H2 responses, because the absence of T-cell–intrinsic Trim24 led to reduced HDM-induced IL-5, IL-13, and IL-10 secretion without any appreciable change in HDM-induced IFNγ (Fig. 2*E*). Mice with *Trim24*$^{-/-}$ T cells also had reduced total and HDM-specific IgE (Fig. 2*F*), confirming the requirement of T-cell–intrinsic Trim24 for T_H2-orchestrated type-2 immunity. Using an additional, adjuvant-free HDM-induced airway allergy model, we observed a similar reduction in airway infiltrates, eosinophilia, airway pathology, IgE production, and T_H2 cytokine secretions in chimeric mice with *Trim24*$^{-/-}$ T cells as compared with mice with WT T cells (Fig. S3), further confirming an important requirement for T-cell–intrinsic Trim24.

Expulsion of a challenge infection with the intestinal helminth *Heligmosomoides polygyrus* following drug cure of a primary infection requires T_H2-dependent orchestration of a type-2 inflammatory cascade around invading larvae (35). To test further whether T-cell–intrinsic Trim24 is required for T_H2-dependent antihelminth immunity in the gut, we gave a challenge infection of *H. polygyrus* to previously infected and drug-cured mice. Mice with *Trim24*-deficient T cells failed to expel the challenge infection (Fig. 2*G*), indicating that T-cell–intrinsic Trim24 also is required for proficient antihelminth immunity in the gut. The failure to expel *H. polygyrus* correlated with reduced expression of type-2 innate immune cell activation, including reduced *Relmα* and *Arg1*, as well as reduced *Il13* expression in the small intestine (Fig. 2*H*) and reduced mucus staining in the small intestine (Fig. 2*I*). Collectively, these data identify an important requirement for T-cell–intrinsic Trim24 for T_H2-mediated airway allergy and T_H2-dependent antihelminth immunity.

Tif1α/Trim24 Is Required for IL-1R Expression and IL-1β–Mediated Activation of T_H2 Cells in Vitro. To identify how T-cell–intrinsic Trim24 regulated T_H2 cell responses and T_H2-mediated immunity, we purified activated WT or *Trim24*$^{-/-}$ T cells (TCRβ$^+$CD4$^+$CD44$^+$) from the lungs of HDM-challenged mice, isolated total RNA, and subjected the RNA to transcriptional profiling (Fig. 3*A*). Microarray analysis showed that Trim24 regulated many genes in the purified T cells (Fig. 3*B*), with reduced expression of several chemokines (*Ccl1*, *Ccl4*, and *Ccl5*), chemokine receptors (*Ccr5*, *Ccr6*, *Ccr9*, and *Cxcr3*), cytokines (*Il4*, *Il10*, *Il21*, *Spp1*, and *Ccl3l3*), cytokine receptors (*Il1r2*, *Il1rl1*, *Il2ra*, *Il17re*, *Csf1r*, and *Csf2rb*), and regulators of cytokine secretion (*Socs2*) (Fig. 3*C*), compared with WT T cells. These data indicate that Trim24 is required for a spectrum of T_H2-associated characteristics and provide a mechanistic explanation for the reduced airway allergy and compromised antihelminth immunity observed in vivo (Fig. 2). To identify how Trim24 regulates such a broad spectrum of pathways, we again used an upstream analysis algorithm [IPA software (34)] similar to that used in Fig. 1 and found that an IL-1–regulated pathway was predicted to be inhibited or absent in *Trim24*$^{-/-}$ T cells as compared with WT T cells (Fig. 3*D* and Table S2). These data suggest that Trim24 is required for IL-1–activated pathways in HDM-elicited pulmonary T cells. Several lines of evidence support this Trim24/IL-1 pathway. First, it has been widely reported that IL-1 signaling is required for T_H2-mediated immunity and allergy (36–39). More specifically, IL-1 signaling is required to activate T_H2 cells directly in vivo (13, 40). Second, a relationship between IL-1R/ubiquitin C and Trim24 has been suggested previously (15–19); however, the requirement of Trim24 for IL-1R expression has not been reported. Thus, based on our in silico prediction (Fig. 3*D*) and the previous reports mentioned above, we hypothesized that Trim24 regulates IL-1R expression and/or IL-1–mediated T_H2 cell activation. To test this hypothesis, we isolated naive (CD4$^+$CD44$^-$CD62L^{hi}) WT and *Trim24*$^{-/-}$T cells and polarized these cells under T_H2 conditions. *Trim24*$^{-/-}$ T_H2 cells secreted slightly lower levels of IL-4, IL-5, and IL-13 in vitro, but these differences failed to reach statistical significance (Fig. 3*E*). However, in vitro *Trim24*$^{-/-}$ T_H2 cells had lower mRNA (Fig. 3*F*) and protein (Fig. 3*G*) expression of IL-1R1. Similarly, ex vivo *Trim24*$^{-/-}$ T cells had lower expression of IL-1R (Fig. S4*D*), indicating that Trim24 is required for IL-1R expression on T_H2 cells. To test whether the reduced IL-1R expression led to a functional reduction of T_H2 cell activation, at day 10 in vitro-generated WT or *Trim24*$^{-/-}$ T_H2 cells were washed, counted, and restimulated with anti-CD3 in the presence or absence of IL-1β for 24 h. *Trim24*$^{-/-}$ T_H2 cells proliferated less than WT cells (Fig. 3*H*) and transcribed (Fig. 3*I*) and secreted (Fig. 3*J*) slightly less IL-4 and less IL-13; however, this difference failed to reach statistical significance. In the presence of IL-1β, WT cells increased activation/proliferation (Fig. 3*H*) with increased *Il4* transcription (Fig. 3*I*) and IL-4 and IL-13 secretion (Fig. 3*J*). In contrast, *Trim24*$^{-/-}$ T_H2 cells were almost completely refractory to IL-1β–mediated activation/proliferation and cytokine production, suggesting that Trim24 is required for IL-1β–mediated T_H2 cell activation in vitro. Similarly, ex vivo *Trim24*$^{-/-}$ T cells (TCRβ$^+$CD4$^+$CD44$^+$) isolated from the lungs of HDM-challenged mice were refractory to IL-1β–mediated activation in vitro (Fig. S4*E*). Collectively, these data suggest that *Trim24* is required for IL-1R expression on T_H2 cells and for IL-1β–mediated activation of T_H2 cells in vitro.

T-Cell–Intrinsic Trim24 Is Required for IL-1β–Mediated Activation of T_H2 Cells in Vivo. IL-1R signaling is essential for the effector phase of T_H2-mediated airway allergy (38, 39), and IL-1 can directly activate T_H2 cells in vivo (13, 40). However, it is unclear how this process is regulated and whether Trim24 is required for IL-1–mediated T_H2 cell activation in vivo. First, using chimeric mice with an IL-1R deficiency restricted to T cells (Fig. S5*A*), we confirmed that IL-1R-signaling in T cells is required for a fulminant type-2 allergic airway response. In line with previous reports (38, 39), mice with *Il1r*$^{-/-}$ T cells had reduced airway inflammation (Fig. S5*B*), eosinophilia (Fig. S5*C*), and pulmonary inflammation (Fig. S5*D*) as compared with chimeric mice with *Il1r*-sufficient T cells. Furthermore, T-cell expression of IL-1R is required for IL-1β–mediated exacerbation of airway allergy, because intratracheal challenge with HDM and IL-1β had little impact in chimeric mice with *Il1r*$^{-/-}$ T cells (Fig. S5). These data suggest both that IL-1R signaling in T cells is required for

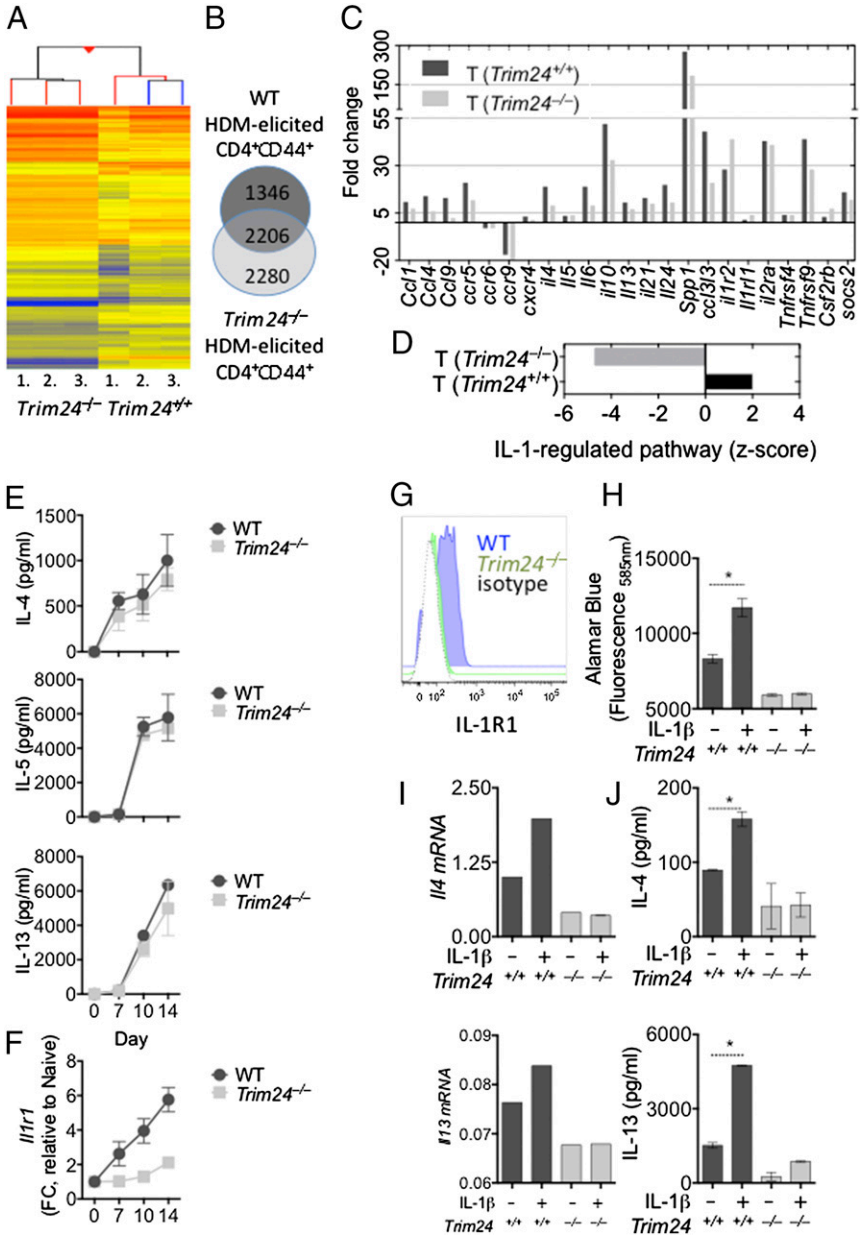

Fig. 3. Transcriptional analysis of activated (CD44$^+$) *Trim24*$^{-/-}$ T cells from the lungs of mice with identified airway allergy, reduced cytokine and chemokine expression, and reduced IL-1–regulated pathways. Chimeric mice with WT or *Trim24*$^{-/-}$ T cells were sensitized and challenged with HDM. Activated (CD4$^+$CD44$^+$) WT or *Trim24*$^{-/-}$ T cells were isolated from the lungs and FACS-purified for transcriptional analysis 1 d after the final airway challenge. *$P \leq 0.05$. (*A*) Heat map of differentially expressed genes in WT and *Trim24*$^{-/-}$ T cells, relative to naive T cells. (*B*) Venn diagram showing common and unique genes regulated in WT and *Trim24*$^{-/-}$ T cells. (*C*) Noteworthy T_H2 effector genes involved in airway allergy. (*D*) Z-score of the likelihood of activity for IL-1–regulated pathways in WT and *Trim24*$^{-/-}$ T cells. (*E*) Naive (CD4$^+$CD44$^-$CD62L^{hi}) WT and *Trim24*$^{-/-}$ T cells were FACS-purified and cultured under T_H2 conditions for the indicated days. IL-4, IL-5, and IL-13 were measured in supernatant. (*F*) WT and *Trim24*$^{-/-}$ T cells cultured under T_H2 conditions were harvested at the indicated days. RNA was extracted, and *Il1r1* expression was determined by qRT-PCR. (*G*) IL-1R1 expression on CD4$^+$CD44^{hi} WT and *Trim24*$^{-/-}$ T_H2 cells at day 10. (*H*) Day 10 WT and *Trim24*$^{-/-}$ T_H2 cells were washed, counted, and replated at 2×10^5 cells per well. Cells were stimulated with anti-CD3 in the presence or absence of IL-1β (10 ng/mL), as indicated. Cell viability/activity was determined by Alamar blue fluorescence. (*I*) *Il4* and *Il13* mRNA expression in day 10 WT and *Trim24*$^{-/-}$ T_H2 cells. Expression is shown relative to *Hprt*. One of three representative experiments is shown. (*J*) IL-4 and IL-13 protein expression in supernatant from WT or *Trim24*$^{-/-}$ T cells. Expression is shown relative to *Hprt*. One of three representative experiments is shown.

fulminant airway allergy and that T cells are a major target of IL-1β–exacerbated airway allergy.

To determine whether T-cell–intrinsic Trim24 is required for IL-1β–mediated T-cell activation in vivo, we challenged mice with WT or *Trim24*$^{-/-}$ T cells with HDM supplemented with IL-1β. After HDM/IL-1β challenge chimeric mice with WT T cells developed significantly enhanced airway inflammation (Fig. 4*A*) with elevated eosinophilia, lymphocyte recruitment, and, to a lesser extent, increased neutrophilia (Fig. 4 *A* and *B*). In contrast, mice with *Trim24*$^{-/-}$ T cells had only a modest increase in airway inflammation with a small increase in airway neutrophils after HDM/IL-1β challenge (Fig. 4 *A* and *B*). HDM/IL-1β challenge also increased systemic IgE in mice with WT cells but not in mice with *Trim24*$^{-/-}$ T cells (Fig. 4*C*). Within pulmonary tissue, the expression of mucus-associated genes (*Muc5ac* and *Gob5*) and type-2 cytokine genes (*Il5* and *Il13*) also was increased in mice

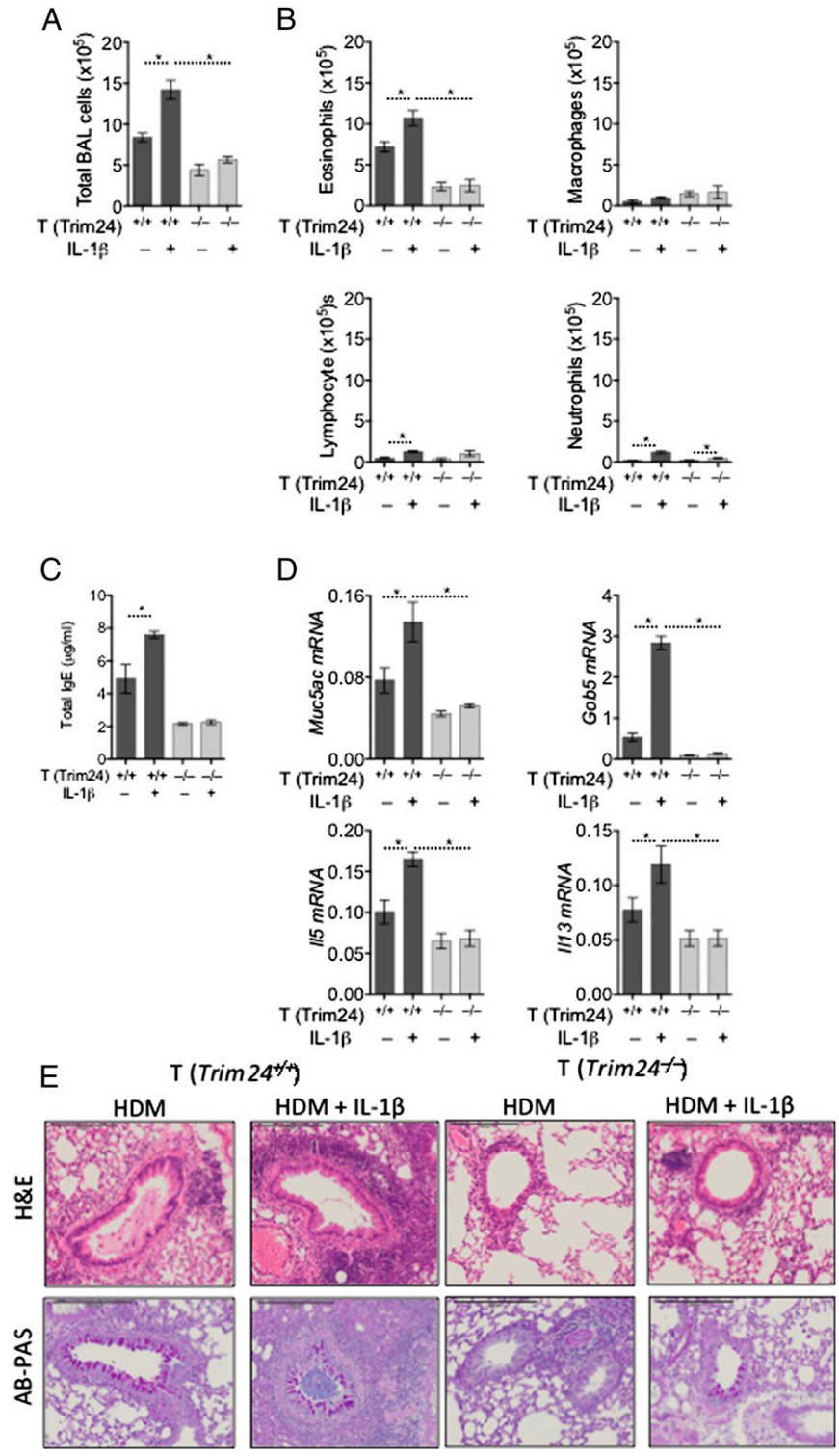

Fig. 4. IL-1β–exacerbated airway allergy is dependent on T-cell–intrinsic Trim24. Chimeric mice with WT or *Trim24*$^{-/-}$ T cells were sensitized with HDM and challenged with HDM supplemented with IL-1β or unsupplemented as indicated. One of two representative experiments is shown. *$P \leq 0.05$. (*A* and *B*) BAL cells were recovered, counted (*A*), and used for cytospins for differential analysis (*B*). (*C*) Circulating IgE was measured in the serum of chimeric mice. (*D*) Gene expression in pulmonary tissue of mice with HDM-induced airway allergy. Expression is shown relative to *Hprt*. (*E*) H&E- and AB-PAS–stained lung sections from chimeric mice with HDM-induced airway allergy.

with WT cells but not in those with *Trim24*$^{-/-}$ T cells (Fig. 4*D*). Most strikingly, pulmonary inflammation was increased in dramatically HDM/IL-1β–challenged mice with WT T cells and was accompanied by increased mucus staining (Fig. 4*E*). Supporting the requirement of T-cell–intrinsic Trim24 for T_H2-mediated airway allergy and the mechanistic requirement of T-cell–intrinsic Trim24 for IL-1β–mediated exacerbation, mice with *Trim24*$^{-/-}$ T cells showed very little pulmonary inflammation with only a

modest increase in mucus staining following HDM/IL-1β challenge. In summary, we have identified an unappreciated and nonredundant role for T-cell–intrinsic Trim24 in the manifestation of T_H2-mediated allergy and immunity and have identified mechanistically that Trim24 is required for IL-1R expression on T_H2 cells and for IL-1β–mediated T_H2 cell activation in vivo.

Discussion

In this study we found that T-cell–intrinsic Trim24, which was predicted to be active in T_H2 cells but not in T_H1, T_H17, or Treg cells, is essential for HDM-mediated airway allergy and T_H2-dependent expulsion of the intestinal helminth *H. polygyrus*. Genome-wide transcriptional analysis of activated, tissue-derived *Trim24*$^{-/-}$ T cells showed that Trim24 is required for cytokine, chemokine, and chemokine receptor expression. In silico upstream analyses of transcriptional data from WT and *Trim24*$^{-/-}$ T cells suggested that IL-1 signaling is compromised in *Trim24*$^{-/-}$ T cells. Both in vitro and in vivo experiments supported this mechanistic pathway, with *Trim24*$^{-/-}$ T cells being refractory to IL-1β–mediated T_H2 cell activation. Thus this study has identified a previously unappreciated role for Trim24 in T_H2 cells and highlights a previously unidentified therapeutic target to forestall hyperactive T_H2-mediated inflammatory diseases.

A large proportion of allergic asthmatic patients mount dysregulated type-2 immune responses to otherwise innocuous allergens. An increasing number of these patients—indeed, the majority of asthmatics—respond poorly to currently available drugs (1), increasing the need to identify new targetable pathways to treat allergic asthma. Cytokine and chemokine-secreting T_H2 cells orchestrate many of the pathophysiological features of allergic asthma. We therefore interrogated the transcriptional profile of highly purified T_H2 cells that contribute directly to HDM-induced airway allergy, in addition to T_H2 cells isolated from helminth-infected mice (33). Rather than focusing on differentially regulated genes in the datasets, we focused on putative upstream pathways that could contribute to the transcriptional profiles. Validating this approach, we identified IL-4 (41) and several transcriptional factors/regulators including Gata3 (7), Stat3 (8), Stat6 (9), and cMaf (10), all of which are necessary for T_H2 cell differentiation and/or effector function. This approach also predicted that Trim24 is functionally active in T_H2 cells, even though its expression does not change significantly. Trim24 was not transcriptionally regulated in vitro in T cells after 3 d of stimulation with IL-4 and TCR engagement, but the protein levels of Trim24 were increased as compared with naive T cells, supporting the involvement of Trim24 in T_H2 cells. The precise mechanisms of posttranscriptional regulation of Trim24, which could explain the disparity in mRNA and protein levels, are currently unclear. There are many miRNAs that are predicted to target Trim24, which, if down-regulated in T_H2 cells, could permit the translation of Trim24 without altering the transcription of Trim24. For example, miR-384 and miR-539 are predicted to target Trim24 and are down-regulated in T_H2 cells (33). Whether these or other miRNA-mediated pathways regulate Trim24 expression in T cells requires further validation.

Trim24 is expressed early in the developing embryo (42) in the developing nervous system (43), liver (28, 44), intestine (45), lung (46), and microglia (47). Unlike Trim28 (26) and Trim33 (48), which have reported roles in T cells, Trim24 has not been studied within the immune system. However, IL-4 can up-regulate *Trim24* expression in microglia as early as 4 h poststimulation (47), and combinatorial transcription factor predictions identified that Trim24 interacts with Stat-6 (49), an IL-4–mediated signal transduction component. Furthermore, elevated Trim24 (28, 44, 45, 50–52) and constitutively active IL-4/STAT6 signaling (53–56) have been observed in many forms of human cancer. However, whether observation describes a causal relationship is currently unclear.

Trim24 can promote glucose metabolism and cellular proliferation (28, 44, 45, 50–52, 57) in a variety of cells, contributing to its involvement in tumorigenesis. T_H2 cells are dependent on glucose metabolism (58); thus deletion of Trim24 in T cells also may compromise glucose metabolism, providing an additional explanation for reduced T_H2 cell responses in the absence of Trim24. In addition, Trim24 interacts with several nuclear receptors, including the estrogen and retinoic acid receptors (28, 59), regulating a variety of transcriptional programs. Both estrogen receptor (60) and retinoic acid receptor signaling (61) have been widely studied in the context of allergy and T-cell biology; however, our transcriptional analysis of *Trim24*$^{-/-}$ T cells (Fig. S4) did not indicate that these pathways are dysregulated in the absence of Trim24. Instead, *Trim24*$^{-/-}$ T cells had dysregulated expression of the genes involved in amino acid transport, lipid metabolism, and glucogenesis (*Lat-1*, *Trib3*, *Gpt2*, *Arsb*, *Chpt1*, and *Galc*). However, the most notable transcriptional changes observed in *Trim24*$^{-/-}$ T cells included chemokines, cytokines, and their receptors (Fig. 3), many of which have critical functions in airway allergy. For example, *Trim24*-deficient T cells had reduced expression of *Ccl1* and *Ccl5*, chemokines involved in the recruitment of eosinophils (62) and T_H2 cells (63), respectively; reduced expression of *Il4*, *Il21*, and *Spp1* (Osteopontin), all of which contribute significantly to airway allergy (64–66); and reduced expression of *Il1rl1* (*Il33r*) and *il17re* (*Il25r*), both of which contribute to T_H2 cell activation and recruitment (67–69). These data highlight the importance of Trim24 for T_H2 cell effector function and provide multiple mechanistic explanations for the reduced airway allergy observed in mice with *Trim24*$^{-/-}$ T cells.

To identify an upstream mechanistic link that could underpin the various deficiencies observed in *Trim24*$^{-/-}$ T cells, we performed upstream analyses of transcriptional datasets from WT and *Trim24*$^{-/-}$ T cells. These analyses predicted that IL-1R signaling would be compromised in *Trim24*$^{-/-}$ T cells. In line with this prediction, IL-1R signaling is required for T_H2-dependent immunity (36, 37) and T_H2-mediated airway allergy (38, 39), supporting observations presented here with *Trim24*$^{-/-}$ T cells. Specifically, IL-1 signaling is required to activate T_H2 cells in vivo (13, 40), and, as we show here, IL-1R signaling in T cells can exacerbate T_H2-driven airway allergy. In support of this upstream mechanistic link, *Tim24*$^{-/-}$ T_H2 cells were refractory to IL-1β–induced activation/proliferation and cytokine production in vitro, and mice with *Tim24*$^{-/-}$ T cells failed to develop exacerbated disease when activated with allergen and IL-1β. Whether integrated IL-1R and IL-4R signaling pathways converge on Trim24 in T cells is unclear; however, a series of protein interactions involving an IL-1R1–ubiquitin C–Trim24–STAT6 axis (15, 18, 49) has been independently described but as yet not validated. In addition, IL-1 signaling in lymphocytes potently activates NFκB (70), which is regulated by several Trim family members (71). Whether Trim24 regulates IL-1–mediated NFκB also is currently unknown but may explain the requirement of Trim24 for IL-1–mediated T_H2 cell activation.

Therapeutically, anakinra, an IL-1R antagonist, has been approved and is used to treat rheumatoid arthritis when there are no signs of infection (72); however, inhibiting IL-1R signaling may compromise continuous immunosurveillance, particularly in the allergic lung. Instead, targeting Trim24 may not disarm the host as much as blocking IL-1R but still may curtail T_H2-mediated airway disease. Bromodomain inhibitors targeting Trim24 have been developed (73, 74) and also may prevent dysregulated T_H2-mediated diseases. In summary, we have identified an important and previously unappreciated role for T-cell–intrinsic Trim24, which is required for T_H2 cell-mediated airway allergy and for IL-1R–mediated exacerbation of allergic airway disease.

Materials and Methods

Animals. C57BL/6, C57BL/6 *Il4*^{gfp}, C57BL/6.*Tcra*$^{-/-}$, *Rag2*$^{-/-}$, and *Il1r*$^{-/-}$ mice were bred and kept in the specific pathogen-free facility at The Francis Crick Institute. *Trim24*$^{-/-}$ bone marrow was kindly provided by Gabrielle Mengus and Irwin Davidson (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France). Mice were randomly housed five mice per cage before the experimental procedure. A minimum of five mice per group was used for each experiment, unless indicated.

Generation of Mixed T-Cell Bone Marrow Chimeric Mice. *Rag2*$^{-/-}$mice (6–8 wk old) were irradiated (2 × 450 rad) followed by adoptive transfer of 2–5 × 10^6 bone marrow cells (20% bone marrow from *Trim24*$^{-/-}$, *Il1r*$^{-/-}$, or WT donor mice and 80% bone marrow from C57BL/6.*Tcra*$^{-/-}$ mice) for 6–8 wk before any experimental procedure. Mice were given water ad libitum that was supplemented with Baytril for 3 wk after radiation. For 1:1 chimeric mice, *Rag2*$^{-/-}$ mice were lethally irradiated (2 × 450 rad) and given 2 × 10^6 WT and *Trim24*$^{-/-}$ bone marrow cells for 8 wk before analysis.

CD4 T-Cell Isolation, Flow Cytometry, and T_H2 Polarization. $CD4^+$ T cells were isolated from tissues by mechanical disruption followed by red blood cell lysis and Percoll gradient separation followed by positive or negative cell enrichment using magnetic beads (L3T4; Miltenyi Biotec). Cells then were stained with anti-mouse CD4 (clone RM4-5), CD44 (clone IM7), CD25 (clone PC61), TCR-β (clone H57-597), Vβ5 (clone MR9-4), Vα2 (clone B20.1), and IL-1R1 (JAMA-147). Dead cells were excluded from sorting and analysis with propidium iodide or Live/Dead stain (Invitrogen). Anti-CD16/32 was used in all staining. Cells were acquired using a BD LSR II flow cytometer and were analyzed with FlowJo software (Tree Star). For cell sorting, BD Influx, FACS Aria (BD), or MoFlo XDP (Beckman Coulter) sorters were used, with a purity of sorted cells >95%. For T_H2 polarization, naive T cells were stimulated with anti-CD3 (1 μg/mL) and anti-CD28 (10 μg/mL) and were cultured with IL-4 (10 ng/mL), IL-2 (5 ng/mL), and anti-IFNγ (10 μg/mL) for 10 d or as indicated in figures.

Airway Allergy and *H. polygyrus* Infection Models.

H. polygyrus. Mice were infected with 200 *H. polygyrus* infective larvae (stage 3) by oral gavage. On day 14 and 15 mice were treated with an oral dose of pyrantel embonate (2 mg) to clear the helminth infection. On day 28 mice were given a challenge infection. T_H2-dependent immunity to *H. polygyrus* determined 14 d after the secondary infection by counting luminal worms in the small intestine.

HDM/alum-induced airway allergy. For HDM/alum-induced airway inflammation, mice were sensitized by i.p. injection with 10 μg HDM (Greer) with 2 mg of Imject Alum (Thermo Scientific) on day 0 and day 14 followed by intratracheal challenge with 10 μg of HDM in PBS on day 21 and day 24. The severity of airway inflammation and pathology was determined on day 25.

HDM-induced airway allergy, without alum. Alternatively, mice were sensitized by intranasal (i.n.) delivery of 10 μg HDM (Greer) in PBS on day 0 followed by i.n. challenge with 10 μg of HDM in PBS on days 7–11, with airway allergy determined on day 14. Cytospins of bronchoalveolar lavage (BAL) recoveries were performed to determine cellular infiltrates. Pulmonary tissue was recovered in RNAlater (Thermo Fisher) for gene-expression analysis or in 10% neutral-buffered formalin for histopathology. Tissues were stained with Alcian blue Periodic acid-Schiff (AB-PAS) stain and H&E to detect mucus production and leukocyte infiltration, respectively. Local lymph nodes were recovered, and 2 × 10^5 cells were restimulated in 96-well plates with 10 μg of HDM to determine antigen-specific cytokine secretions. Serum was recovered for IgE analysis.

Western Blot for Trim24. For immunoblotting, cells were lysed in 1× RIPA buffer [500 mM Tris·HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 0.5% deoxycholate, 1% Nonidet-P40] containing protein inhibitors per the manufacturer's instructions (Roche), including 50 mM sodium flouride (NaF), 1 mM sodium orthovanadate, 100 nM Okadaic acid, and 2 mM sodium pyrophosphate tetrabasic in MilliQ water. Cell lysates were normalized to equal total protein content using the Pierce BCA Protein Assay Kit (Life Technologies) and were resolved on NuPAGE 4–12% Bis-Tris Gel (Life Technologies). Separated proteins were transferred onto Amersham Hybond-P PVDF membranes (GE Healthcare) via the semidry Trans-Blot Turbo system (Bio-Rad). Membranes were blocked with 0.1% TBS and Tween 20 (TBST) containing 5% milk (Sigma) and were incubated with primary Rabbit anti-TIF1α/TRIM24 (A300-815A; Bethyl) in 0.1% TBST containing 5% BSA and secondary antibodies (Rabbit IgG; GE Healthcare) in 0.1% TBST (Sigma) containing 5% milk (Sigma). Membranes were washed in 0.1% TBST, and specific bound antibodies were visualized by chemiluminescence (Immobilon; Merck Millipore).

RNA, qRT-PCR, Microarray, and in Silico Analysis. RNA was isolated from tissues and cells using RNeasy mini spin columns according to the manufacturer's instructions (Qiagen). For microarray analysis, cDNA was generated from 5 ng of total RNA using the WT-Ovation Pico (version 1) RNA Amplification System followed by double-stranded cDNA synthesis using the WT-Ovation Exon Module. cDNA quality was determined using an Agilent BioAnalyzer and through hybridization performance on Affymetrix GeneChip mouse Genome 430A 2.0 microarray (Affymetrix) by the Systems Biology Unit at The Francis Crick Institute. Microarray data were quantile-normalized and analyzed using GeneSpring software (Agilent). Differentially expressed genes were determined using ANOVA and *t* tests. Genes with false discovery rate-corrected *P* values <0.1 and fold-change values ≥1.5 were considered significant, as indicated in figure legends. Three to five biological replicates were used, as indicated. Data then were uploaded into IPA (Ingenuity Systems; www.ingenuity.com) for pathways and upstream analysis (34). For qRT-PCR, RNA was reverse-transcribed using miScript II RT Kit (Qiagen). Real-time RT-PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with relative quantities of mRNA determined using SYBR Green PCR Master Mix (Applied Biosystems) and by the comparative threshold cycle method as described by Applied Biosystems for the ABI Prism 7700/7900HT Sequence Detection Systems. mRNA levels were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) or GAPDH and expressed as a relative increase or decrease compared with levels in controls or relative to HPRT, as indicated. The following primer pairs were used: *Il4*, forward: ACGAGGTCACAGGAGAAGGGA, reverse: AGCCCTACAGACGAGCTCACTC; *Il5*, forward: TGACAAGCAATGAGACGATGAGG, reverse: ACCCCCACGGACAGTTTGATTC; *Il13*, forward: CCTCTGACCCTTAAGGAGCTTAT, reverse: CGTTGCACAGGGGAGTCTT; *Relma*, forward: CCCTCCACTGTAACGAAGACTC, reverse: CACACCCAGTAGCAGTCATCC; *Muc5ac*, forward: CAGGACTCTCTGAAATCGTACCA, reverse: AAGGCTCGTACCACAGGGA; *Gob5*, forward: CATCGCCATAGACCACGACG, reverse: TTCCAGCTCTCGGGAATCAAA; *Arg1*, F-GGAAAGCCAATGAAGAGCTG, reverse: GCTTCCAACTGCCAGACTGT; *Trim24*, forward: TTACCAAACCCTAGAATGCAG, reverse: ACATTCTGGCTTGGTGAATATC.

ELISA. Cytokines and IgE were measured by ELISA. Capture and biotinylated detection antibodies for IL-4, IL-5, IL-13, IFNγ, and IL-10 were from R&D Systems. Total IgE was measured in serum using Purified Rat Anti-Mouse IgE (R35-72; BD Pharmingen) at 2 μg/mL For measurement of HDM-specific IgE, IgG was adsorbed to protein G Sepharose beads before measurement of HDM-specific IgE on HDM-coated (10 μg/mL) plates, as previously described (75) followed by Biotin Rat Anti-Mouse IgE at 1 μg/mL (R35-118; BD Pharmingen) with IgE, k isotype standard (BD Pharmingen), and was detected with Streptavidin HRP at 1:000 (BD Pharmingen) and ABTS One Component HRP Microwell Substrate (SurModics). The concentration of analytes in the sample was determined from a serial-fold diluted standard curve with OD read at 405 nm in an ELISA reader (Tecan II Safire).

Statistical Analysis. Datasets were compared by Mann–Whitney test using GraphPad Prism (V.5.0). Differences were considered significant at $P \leq 0.05$.

Ethics Statement. All animal experiments were carried out following United Kingdom Home Office regulations (project license 80/2506) and were approved by the Francis Crick Institute Ethical Review Panel.

ACKNOWLEDGMENTS. We thank Abdul Sesay, Harsha Jani, and Leena Bhaw-Rosun in the Systems Biology Department for help with microarray experiments; Radma Mahmood and Radika Anand for help with histology; Graham Preece, Wayne Turnbull, Bhavik Patel, and Dr. Phil Hobson for assistance with flow cytometry; Trisha Norton, Keith Williams, Adebambo Adekoya, and the staffs of buildings B1, B2, and C for animal husbandry; Rose-Marie Vesin for technical assistance in Illkirch; and members of the M.S.W. laboratory for critical feedback, discussion, and reading of the manuscript. This work was supported by the Francis Crick Institute (Grant FCI01) which receives its core funding from Cancer Research UK, the UK Medical Research Council (MRC reference no. MC_UP_A253_1028), and the Wellcome Trust. Work in the I.D. laboratory was supported by grants from the CNRS, the INSERM, the Equipes Labellisées program of the Ligue Nationale contre le Cancer, and the ANR-10-LABEX-0030-INRT French state fund through the Agence Nationale de la Recherche (ANR) under the Future Investments Program labeled "ANR-10-IDEX-0002-02."

1. Olin JT, Wechsler ME (2014) Asthma: Pathogenesis and novel drugs for treatment. *BMJ* 349:g5517.
2. Lloyd CM, Hessel EM (2010) Functions of T cells in asthma: More than just T(H)2 cells. *Nat Rev Immunol* 10(12):838–848.
3. Busse WW, et al. (2013) Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 188(11):1294–1302.
4. Corren J, et al. (2010) A randomized, controlled, phase 2 study of AMG 317, an IL-4Ralpha antagonist, in patients with asthma. *Am J Respir Crit Care Med* 181(8):788–796.
5. Ortega HG, et al.; MENSA Investigators (2014) Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 371(13):1198–1207.
6. Arron JR, Scheerens H, Matthews JG (2013) Redefining approaches to asthma: Developing targeted biologic therapies. *Adv Pharmacol* 66:1–49.
7. Zheng W, Flavell RA (1997) The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89(4):587–596.
8. Stritesky GL, et al. (2011) The transcription factor STAT3 is required for T helper 2 cell development. *Immunity* 34(1):39–49.
9. Kaplan MH, Schindler U, Smiley ST, Grusby MJ (1996) Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 4(3):313–319.
10. Ho IC, Hodge MR, Rooney JW, Glimcher LH (1996) The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* 85(7):973–983.
11. Okoye IS, Wilson MS (2011) CD4+ T helper 2 cells–microbial triggers, differentiation requirements and effector functions. *Immunology* 134(4):368–377.
12. Sims JE, Smith DE (2010) The IL-1 family: Regulators of immunity. *Nat Rev Immunol* 10(2):89–102.
13. Ben-Sasson SZ, et al. (2009) IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc Natl Acad Sci USA* 106(17):7119–7124.
14. Papazian D, Hansen S, Wurtzen PA (2015) Airway responses towards allergens - from the airway epithelium to T cells. *Clin Exp Allergy* 45(8):1268–1287.
15. Kim W, et al. (2011) Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol Cell* 44(2):325–340.
16. Wagner SA, et al. (2011) A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol Cell Proteomics* 10(10):M111.013284.
17. Danielsen JM, et al. (2011) Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level. *Mol Cell Proteomics* 10(3):M110.003590.
18. Brissoni B, et al. (2006) Intracellular trafficking of interleukin-1 receptor I requires Tollip. *Curr Biol* 16(22):2265–2270.
19. Teague TK, et al. (1999) Activation changes the spectrum but not the diversity of genes expressed by T cells. *Proc Natl Acad Sci USA* 96(22):12691–12696.
20. Ozato K, Shin DM, Chang TH, Morse HC, 3rd (2008) TRIM family proteins and their emerging roles in innate immunity. *Nat Rev Immunol* 8(11):849–860.
21. Cammas F, Khetchoumian K, Chambon P, Losson R (2012) TRIM involvement in transcriptional regulation. *Adv Exp Med Biol* 770:59–76.
22. Herquel B, Ouararhni K, Davidson I (2011) The TIF1α-related TRIM cofactors couple chromatin modifications to transcriptional regulation, signaling and tumor suppression. *Transcription* 2(5):231–236.
23. Zhou XF, et al. (2012) TRIM28 mediates chromatin modifications at the TCRα enhancer and regulates the development of T and natural killer T cells. *Proc Natl Acad Sci USA* 109(49):20083–20088.
24. Cambiaghi V, et al. (2012) TRIM proteins in cancer. *Adv Exp Med Biol* 770:77–91.
25. Versteeg GA, Benke S, García-Sastre A, Rajsbaum R (2014) InTRIMsic immunity: Positive and negative regulation of immune signaling by tripartite motif proteins. *Cytokine Growth Factor Rev* 25(5):563–576.
26. Chikuma S, Suita N, Okazaki IM, Shibayama S, Honjo T (2012) TRIM28 prevents autoinflammatory T cell development in vivo. *Nat Immunol* 13(6):596–603.
27. Jiang S, et al. (2015) TRIM24 suppresses development of spontaneous hepatic lipid accumulation and hepatocellular carcinoma in mice. *J Hepatol* 62(2):371–379.
28. Khetchoumian K, et al. (2007) Loss of Trim24 (Tif1alpha) gene function confers oncogenic activity to retinoic acid receptor alpha. *Nat Genet* 39(12):1500–1506.
29. Herquel B, et al. (2013) Trim24-repressed VL30 retrotransposons regulate gene expression by producing noncoding RNA. *Nat Struct Mol Biol* 20(3):339–346.
30. Tisserand J, et al. (2011) Tripartite motif 24 (Trim24/Tif1α) tumor suppressor protein is a novel negative regulator of interferon (IFN)/signal transducers and activators of transcription (STAT) signaling pathway acting through retinoic acid receptor α (Rarα) inhibition. *J Biol Chem* 286(38):33369–33379.
31. Khetchoumian K, et al. (2008) Trim24 (Tif1 alpha): An essential 'brake' for retinoic acid-induced transcription to prevent liver cancer. *Cell Cycle* 7(23):3647–3652.
32. Lambrecht BN, Hammad H (2015) The immunology of asthma. *Nat Immunol* 16(1):45–56.
33. Okoye IS, et al. (2014) Transcriptomics identified a critical role for Th2 cell-intrinsic miR-155 in mediating allergy and antihelminth immunity. *Proc Natl Acad Sci USA* 111(30):E3081–E3090.
34. Krämer A, Green J, Pollard J, Jr, Tugendreich S (2014) Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* 30(4):523–530.
35. Reynolds LA, Filbey KJ, Maizels RM (2012) Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. *Semin Immunopathol* 34(6):829–846.
36. Humphreys NE, Grencis RK (2009) IL-1-dependent, IL-1R1-independent resistance to gastrointestinal nematodes. *Eur J Immunol* 39(4):1036–1045.
37. Helmby H, Grencis RK (2004) Interleukin 1 plays a major role in the development of Th2-mediated immunity. *Eur J Immunol* 34(12):3674–3681.
38. Ritter M, et al. (2014) Functional relevance of NLRP3 inflammasome-mediated interleukin (IL)-1β during acute allergic airway inflammation. *Clin Exp Immunol* 178(2): 212–223.
39. Broide DH, Campbell K, Gifford T, Sriramarao P (2000) Inhibition of eosinophilic inflammation in allergen-challenged, IL-1 receptor type 1-deficient mice is associated with reduced eosinophil rolling and adhesion on vascular endothelium. *Blood* 95(1):263–269.
40. Nakae S, et al. (2003) IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. *Int Immunol* 15(4):483–490.
41. Kopf M, et al. (1993) Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 362(6417):245–248.
42. Torres-Padilla ME, Zernicka-Goetz M (2006) Role of TIF1alpha as a modulator of embryonic transcription in the mouse zygote. *J Cell Biol* 174(3):329–338.
43. Niederreither K, Remboutsika E, Gansmuller A, Losson R, Dollé P (1999) Expression of the transcriptional intermediary factor TIF1alpha during mouse development and in the reproductive organs. *Mech Dev* 88(1):111–117.
44. Liu X, et al. (2014) Overexpression of TRIM24 is associated with the onset and progress of human hepatocellular carcinoma. *PLoS One* 9(1):e85462.
45. Miao ZF, et al. (2015) TRIM24 is upregulated in human gastric cancer and promotes gastric cancer cell growth and chemoresistance. *Virchows Archive: European Journal of Pathology* 466(5):525–532.
46. Li H, et al. (2012) Overexpression of TRIM24 correlates with tumor progression in non-small cell lung cancer. *PLoS One* 7(5):e37657.
47. Freilich RW, Woodbury ME, Ikezu T (2013) Integrated expression profiles of mRNA and miRNA in polarized primary murine microglia. *PLoS One* 8(11):e79416.
48. Doisne JM, et al. (2009) iNKT cell development is orchestrated by different branches of TGF-beta signaling. *J Exp Med* 206(6):1365–1378.
49. Ravasi T, et al. (2010) An atlas of combinatorial transcriptional regulation in mouse and man. *Cell* 140(5):744–752.
50. Xue D, et al. (2015) Clinical significance and biological roles of TRIM24 in human bladder carcinoma. *Tumour Biol* 36(9):6849–6855.
51. Cui Z, et al. (2013) TRIM24 overexpression is common in locally advanced head and neck squamous cell carcinoma and correlates with aggressive malignant phenotypes. *PLoS One* 8(5):e63887.
52. Tsai WW, et al. (2010) TRIM24 links a non-canonical histone signature to breast cancer. *Nature* 468(7326):927–932.
53. Guiter C, et al. (2004) Constitutive STAT6 activation in primary mediastinal large B-cell lymphoma. *Blood* 104(2):543–549.
54. Ni Z, et al. (2002) Selective activation of members of the signal transducers and activators of transcription family in prostate carcinoma. *J Urol* 167(4):1859–1862.
55. Skinnider BF, et al. (2002) Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 99(2):618–626.
56. Qin JZ, et al. (2001) Constitutive and interleukin-7- and interleukin-15-stimulated DNA binding of STAT and novel factors in cutaneous T cell lymphoma cells. *J Invest Dermatol* 117(3):583–589.
57. Pathiraja TN, et al. (2015) TRIM24 links glucose metabolism with transformation of human mammary epithelial cells. *Oncogene* 34(22):2836–2845.
58. Michalek RD, et al. (2011) Cutting edge: Distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* 186(6):3299–3303.
59. Thénot S, Henriquet C, Rochefort H, Cavaillès V (1997) Differential interaction of nuclear receptors with the putative human transcriptional coactivator hTIF1. *J Biol Chem* 272(18):12062–12068.
60. Bonds RS, Midoro-Horiuti T (2013) Estrogen effects in allergy and asthma. *Curr Opin Allergy Clin Immunol* 13(1):92–99.
61. Hall JA, Grainger JR, Spencer SP, Belkaid Y (2011) The role of retinoic acid in tolerance and immunity. *Immunity* 35(1):13–22.
62. Bishop B, Lloyd CM (2003) CC chemokine ligand 1 promotes recruitment of eosinophils but not Th2 cells during the development of allergic airways disease. *J Immunol* 170(9):4810–4817.
63. Schuh JM, Blease K, Hogaboam CM (2002) The role of CC chemokine receptor 5 (CCR5) and RANTES/CCL5 during chronic fungal asthma in mice. *FASEB J* 16(2):228–230.
64. Lajoie S, et al. (2014) IL-21 receptor signalling partially mediates Th2-mediated allergic airway responses. *Clin Exp Allergy* 44(7):976–985.
65. Müller KM, Jaunin F, Masouyé I, Saurat JH, Hauser C (1993) Th2 cells mediate IL-4-dependent local tissue inflammation. *J Immunol* 150(12):5576–5584.
66. Xanthou G, et al. (2007) Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets. *Nat Med* 13(5):570–578.
67. Corrigan CJ, et al. (2011) T-helper cell type 2 (Th2) memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma. *Proc Natl Acad Sci USA* 108(4):1579–1584.
68. Tamachi T, et al. (2006) IL-25 enhances allergic airway inflammation by amplifying a TH2 cell-dependent pathway in mice. *J Allergy Clin Immunol* 118(3):606–614.
69. Löhning M, et al. (1998) T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc Natl Acad Sci USA* 95(12):6930–6935.
70. Stylianou E, et al. (1992) Interleukin 1 induces NF-kappa B through its type I but not its type II receptor in lymphocytes. *J Biol Chem* 267(22):15836–15841.
71. Tomar D, Singh R (2015) TRIM family proteins: Emerging class of RING E3 ligases as regulator of NF-kappaB pathway. *Biol Cell* 107(1):22–40.
72. Wang D, Li Y, Liu Y, Shi G (2014) The use of biologic therapies in the treatment of rheumatoid arthritis. *Curr Pharm Biotechnol* 15(6):542–548.
73. Palmer WS, et al. (2015) Structure-guided design of IACS-9571, a selective high-affinity dual TRIM24-BRPF1 bromodomain inhibitor. *J Med Chem*, 10.1021/acs.jmedchem.5b00405.
74. Bennett J, et al. (2015) Discovery of a chemical tool inhibitor targeting the bromodomains of TRIM24 and BRPF. *J Med Chem*, 10.1021/acs.jmedchem.5b00458.
75. Wilson MS, et al. (2005) Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J Exp Med* 202(9):1199–1212.