Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Mucosal Immunol. Author manuscript; available in PMC 2023 June 24.

Published in final edited form as:

Mucosal Immunol. 2023 June ; 16(3): 357–372. doi:10.1016/j.mucimm.2023.04.002.

A bifurcated role for c-Maf in Th2 and Tfh2 cells during helminth infection

Katherine Bao[1], **Uryan Isik Can**[2], **Mindy M. Miller**[2], **Ivy K. Brown**[2], **Mark Dell'Aringa**[1,2], **Hans Dooms**[2,3], **Max A. Seibold**[4,5,6], **James Scott-Browne**[2,3], **Richard Lee Reinhardt**[1,2,3,✉]

[1]Department of Immunology, Duke University Medical Center, Durham, USA.

[2]Department of Immunology and Genomic Medicine, National Jewish Health, Denver, USA.

[3]Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, USA.

[4]Center for Genes, Environment, and Health, National Jewish Health, Denver, USA.

[5]Department of Pediatrics, National Jewish Health, Denver, USA.

[6]Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado School of Medicine, Aurora, USA.

Abstract

Differences in transcriptomes, transcription factor usage, and function have identified T follicular helper 2 (Tfh2) cells and T helper 2 (Th2) cells as distinct clusters of differentiation 4+",(CD4) T-cell subsets in settings of type-2 inflammation. Although the transcriptional programs driving Th2 cell differentiation and cytokine production are well defined, dependence on these classical Th2 programs by Tfh2 cells is less clear. Using cytokine reporter mice in combination with transcription factor inference analysis, the b-Zip transcription factor c-Maf and its targets were identified as an important regulon in both Th2 and Tfh2 cells. Conditional deletion of c-Maf in T cells confirmed its importance in type-2 cytokine expression by Th2 and Tfh2 cells. However, while c-Maf was not required for Th2-driven helminth clearance or lung eosinophilia, it was required for Tfh2-driven Immunoglobulin E production and germinal center formation. This differential regulation of cell-mediated and humoral immunity by c-Maf was a result of redundant pathways in Th2 cells that were absent in Tfh2 cells, and c-Maf-specific mechanisms in Tfh2 cells that were absent in Th2 cells. Thus, despite shared expression by Tfh2 and Th2 cells, c-Maf serves as a unique regulator of Tfh2-driven humoral hallmarks during type-2 immunity.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

✉ ReinhardtL@NJHealth.org.

AUTHOR CONTRIBUTIONS

K.B. designed the study, performed experiments, analyzed data, and wrote the manuscript. M.A.S. directed and analyzed scRNA-sequencing studies. J.S-B. helped analyze bulk and scRNA-sequencing data. M.M.M. analyzed scRNA-sequencing and performed transcription factor inference analysis. U.I.C, I.K.B., and M.D. performed experiments and analyzed data. H.D. provided key reagents and advised STAT5 studies. R.L.R conceived the work, supervised the study, and wrote the manuscript.

DECLARATIONS OF COMPETING INTEREST

The authors have no competing interests to declare.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

INTRODUCTION

Type-2 immunity to parasitic helminth infection and the induction of allergic inflammation are orchestrated by type-2 cytokine-expressing clusters of differentiation ($CD4^+$) T cells[1,2]. Antigen-specific $CD4^+$ T helper (Th) 2 cells in mucosal tissues mediate barrier immunity through their release of interleukin (IL)-4, IL-5, and IL-13[3]. In contrast, $CD4^+$ T follicular helper (Tfh) cells act in the B cell follicles of lymphoid tissues to promote antibody production and humoral immunity. In settings of type-2 inflammation, a subset of Tfh cells producing IL-4, termed Tfh2 cells, promote the selection of high-affinity antibodies and class switching to immunoglobulin (Ig)E and IgG1 isotypes[4–7]. A subset of Tfh2 cells, termed Tfh13 cells, acquire the ability to produce IL-13 along with IL-4 and serve a critical role in the production of pathogenic IgE during allergic responses[8]. Together, Th2 and Tfh2 cells are instrumental in coordinating cellular and humoral hallmarks of type-2 inflammation.

The unique cell programming critical to the development of T helper cell subsets has greatly increased our understanding of Tfh2 and Th2 cells[9,10]. Distinct transcription factor usage in combination with the selective expression of type-2 cytokines can be used to functionally delineate these two subsets[3,6,11,12]. Th2 cell differentiation and commitment rely on the classical Th2-associated transcription factors GATA Binding Protein (GATA) 3, Signal Transducer And Activator Of Transcription (STAT) 6, STAT5, and c-Maf[13–19]. Although these Th2 factors work synergistically to promote Th2 lineage specification and function, some selectivity in how they promote type-2 cytokine expression has been observed. For instance, GATA-3 is critically important in the production of IL-5 and IL-13 in committed Th2 cells, but it has less influence on IL-4 expression[20,21]. The dependence on GATA-3 for IL-13 production but not IL-4 extends to other type-2 immune cells such as group 2 innate lymphoid (ILC2) cells[12,22–24]. Furthermore, basophils and natural killer T cells, which selectively produce IL-4 relative to IL-13, express little GATA-3 relative to IL-13-producing Th2 cells and ILC2 cells[25]. This indicates an important and selective role for GATA-3 in facilitating IL-13 competency. In contrast to GATA-3, the transcription factor c-Maf appears to have a greater impact on IL-4 transcription relative to IL-5 and IL-13 in Th2 cells[26,27]. The preferential production of IL-4 as compared to IL-5 and IL-13 is also a phenotype prevalent among Tfh2 cells[6,8,12,25,28,29]. Thus, while c-Maf has been described as important in the development of many CD4+ T-cell subsets including Th2 and Tfh cells, how and if this transcription factor differentially impacts Th2 versus Tfh2 biology in vivo remains ill-defined[30–35].

To better dissect the role of c-Maf in T cell-mediated orchestration of type-2 immunity, sensitive IL-4 and IL-13 reporter mice, single-cell analytics, and T cell-intrinsic deletion of c-Maf were used during parasitic helminth infection. Both Th2 cells in the lung and Tfh2 cells in the lung-draining mediastinal lymph nodes were investigated. These studies help refine an important but redundant role for c-Maf in Th2-mediated immunity while revealing nonredundant mechanisms by which c-Maf impacts Tfh2 cell function and humoral immunity. In sum, the data demonstrate how c-Maf contributes to the bifurcated role that Tfh2 and Th2 cells serve in type-2 inflammation.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

RESULTS

Transcriptome analysis reveals that c-Maf is broadly expressed by IL-4-producing Tfh2 and Th2 cells during helminth infection

To better understand the relationship between IL-4$^+$ Th2 and Tfh2 cells in the lymph nodes compared to those in the lung in settings of type-2 immunity, mice were infected with the helminth *Nippostrongylus brasiliensis*. An unbiased approach using bulk transcriptomes of IL-4$^+$ and IL-4$^-$ CD4$^+$ T-cell populations at the peak of the response was used to reveal Th2 and Tfh2 cells in these distinct tissues (Fig. 1A)[36]. Th2-associated genes such as *Gata3*, *Il1rl1* (IL-33R), *Pparg* (peroxisome proliferator-activated receptor γ), and *Cyp11a1* (cholesterol side-chain cleavage enzyme family 11, subfamily a, polypeptide 1) were enriched in IL-4-expressing populations compared to IL-4-negative cells in the lung[16,17,37–41]. In contrast, when comparing IL-4$^+$ T cells to their IL-4$^-$ counterparts in the lymph node, cells were enriched for the Tfh markers C-X-C chemokine receptor type 5 (*Cxcr5)*, B-cell lymphoma 6 (*Bcl6)*, Interferon Regulatory Factor 4 (*Irf4)*, Programmed Cell Death 1 (*Pdcd1* PD1), *Il4*, and *Maf*[10]. Similarly, the volcano plots indicated a selective enrichment for the Tfh markers *Bcl6*, Achaete-scute complex homolog 2 (*Ascl2)*, inhibitor of DNA binding 3 (*Id3)*, and *Cxcr5* in the lymph node-resident IL-4$^+$ population compared to IL-4$^+$ T cells in the lung (Fig. 1A)[42–47]. Of note, IL-4$^+$ CD4$^+$ T cells in the lung were significantly enriched for Nuclear Receptor Subfamily 4 Group A Member 1 (*Nr4a1* Nur77), *Cd69*, Fos-related antigen 2 (*Fosl2)*, and TNF alpha induced protein 3 (*Tnfaip3* A20) expression (Fig. 1A). Nur77 and CD69 expression are notable surrogates for T cell receptor (TCR) signaling and nonlymphoid tissue residency, findings suggestive of either recent or prolonged antigen engagement or a tissue-resident memory (Trm) phenotype within this population[48–50]. The enhanced expression of Fosl2 and A20 in the lung among IL-4-expressing T cells also indicated a likely repression of type-2 cytokine production by Th2 cells at this timepoint, consistent with control of the infection and modulation of inflammation[51–53].

To gain a better appreciation of the heterogeneity within different IL-4$^+$ T-cell populations residing in the lung and lymph nodes, we analyzed single-cell RNA-sequencing data of the same populations described in the bulk transcriptome analysis[36]. As expected, transcriptomes of IL-4$^+$ CD4$^+$ T cells isolated from the lung (aqua) and lymph node (purple) possessed largely unique profiles as shown by differential clustering on the t-stochastic neighbor embedding (SNE) plot (Fig. 1B). Consistent with the bulk transcriptome data, lung IL-4$^+$ cells were enriched for Th2 markers *Gata3* and *Il1rl1* (IL-33R) while lymph node IL-4$^+$ cells contained Tfh-associated transcripts *Id3* and *Cxcr5* (Fig. 1C). Of note, c-Maf (*Maf*) was expressed equally across IL-4$^+$ CD4$^+$ T cells regardless of tissue residence. Concomitant c-Maf expression was found in both *Id3*$^+$, *Cxcr5*$^+$ Tfh2 cells, and *Gata3*$^+$, *Il1rl1*$^+$ Th2 cells. C-Maf was also found to be expressed in some Ki67+ proliferating cells that corresponded to both the lung (aqua) and lymph nodes (purple) (Figs. 1B and 1C). This pattern of expression is consistent with a reported role for c-Maf in IL-4 production by Th2 cells and may indicate a similarly important role in the expression of IL-4 by Tfh2 cells[26,27,30–34,54].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

C-Maf protein is equivalently expressed in both Th2 and Tfh cells during helminth infection

Although the transcriptomic data illustrates that c-Maf messenger RNA (mRNA) is equivalently expressed by Tfh2 and Th2 cells, it was important to confirm these findings at the protein level. To verify that Th2 and Tfh2 cells represent unique $CD4^+$ T-cell subsets in the context of *N. brasiliensis* infection, intracellular staining for the Th2 factor GATA-3 and the Tfh factor BCL6 was performed in IL-$4^{KN2–4get}$ reporter mice[16,17,43,55,56]. IL-$4^{KN2–4get}$ mice reliably report both the presence of IL-4 mRNA (green fluorescent protein - GFP) and recent IL-4 production via surface expression of human CD2 (huCD2)[57,58]. Prior work with this model has shown that CD4+ $huCD2^+$ T cells in the lymph nodes but not the lung are PD-1^{high} and $CXCR5^{+12}$. Furthermore, BCL6 protein expression was independent of GATA-3 providing a clear demarcation of Tfh2 and Th2 cells, respectively[12]. Consistent with the bulk and single-cell RNA-sequencing data, two distinct populations were present representing a GATA3^{high}, $BCL6^–$ Th2 population and a GATA-3^{low}, $BLC6^{high}$ Tfh cell population (Fig. 2A). Both BCL6 and GATA3 protein expression were enriched among IL-4-expressing ($huCD2^+$) $CD4^+$ T cells in the lymph nodes relative to IL-$4^–$ ($huCD2^–$) $CD4^+$ T cells (Fig. 2B). However, consistent with Tfh2 enrichment in the lymph nodes, BCL6 staining was more enriched among IL-4 protein-expressing cells than GATA-3. This was confirmed when assessing BCL6 staining in GATA-3^{low} and GATA-3^{high} populations of IL-4-producing cells (Fig. 2C). Together the data indicate that while GATA-3 is likely critical for the maintenance and production of type-2 cytokines by Th2 cells, Tfh2 cells are less reliant on this transcription factor to elicit IL-4 production.

Given the enrichment of c-Maf mRNA among IL-4^+ $CD4^+$ T cells in both the lymph nodes and lung (Fig. 1C), intracellular staining for c-Maf protein was explored in both Tfh2 and Th2 cells. Intracellular staining for c-Maf relative to GATA-3 revealed a substantial increase in c-Maf^+ $CD4^+$ T cells in the lung compared to uninfected controls (Fig. 2D). Furthermore, within lung-resident $CD4^+$ T cells, c-Maf was enriched among populations expressing GATA-3. Both findings are consistent with c-Maf being expressed in Th2 cells. In contrast, there were two populations of $CD4^+$ T cells expressing c-Maf in the mediastinal lymph nodes. These two populations were distinguished by the presence or absence of BCL6 expression (Fig. 2E). Of note, all $BCL6^+$ Tfh cells co-expressed c-Maf, confirming its prominent role in Tfh fate and function[30–33].

Transcription factor inference analysis identifies the differential use of Th2 regulons by Tfh2 cells

Given the differential gene expression observed in IL-4^+ $CD4^+$ T cells from the lymph nodes compared to the lung after *N. brasiliensis* infection, we next wanted to assess whether this translated into the differential activity of gene sets in Tfh2 and Th2 cells. To do this, we used single-cell regulatory network inference and clustering (SCENIC) to identify regulons —groups of genes and upstream transcription factors that are regulated as a unit—that were active in IL-4^+ cells within the lymph node and lung[59]. Hierarchical clustering of the most differentially active regulons among IL-4^+ and IL-$4^–$ $CD4^+$ T cell populations revealed significant heterogeneity (Fig. 3A). Concerning IL-4^+ $CD4^+$ T cells in the lung and lymph nodes, regulon activity corresponded to genes known to be important in Th2 and Tfh fate and function, respectively (Figs. 3B and 3C). For example, gene sets linked to GATA-3 and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

JunB activity showed increased regulon activity among lung IL-4$^+$ cells, which are enriched for Th2 cells. This is consistent with GATA-3 and JunB being important transcription factors for IL-4 expression in this T helper cell subset[16,60]. These regulons were not active in IL-4$^+$ CD4$^+$ T cells obtained from the lymph nodes, a subset shown above to be enriched for Tfh2 cells. In contrast, IL-4$^+$ CD4$^+$ T cells from the lymph nodes showed increased T cell factor 7 (TCF7encoding TCF-1), and Nuclear Factor of Activated T-cells 1 (NFATc1) regulon activity. TCF7 and NFAT regulon activity match transcription factor motifs that are uniquely enriched among IL-4$^+$ CD4$^+$ T cells in the lymph nodes compared to IL-4$^+$ CD4$^+$ T cells from the lung[36]. Together, these findings confirm the understood role that these factors play in Tfh2 gene regulation and IL-4 expression[61–67].

Of note, c-Maf was expressed in both IL-4$^+$ populations and showed equivalent regulon activity in both tissues, similar to Basic leucine zipper transcription factor, ATF-like (BATF). This is consistent with a role for c-Maf in the active regulation of genes important in Tfh2 and Th2 cell function, including IL-4. In support of a role for c-Maf in regulating IL-4, two half-Maf recognition elements (MAREs) were found at the proximal *Il4* promoter and enhancer, which correlated with regions of open chromatin found in IL-4$^+$ CD4$^+$ T cells isolated from the lung and mediastinal lymph node after helminth infection (Fig. 3D)[36]. The half MARE site located at position −37 to −42 of the *Il4* transcription start site is essential for IL-4 production by Th2 cells[27,68].

c-Maf is required for optimal IL-4 production by Tfh2 and IL-4 and IL-13 by Th2 cells

Expression and regulon activity of c-Maf in both Tfh and Th2 cells implicated an important role in the production of IL-4 in both subsets during *N. brasiliensis* infection. Although c-Maf has long been proposed to promote IL-4 expression in T cells, a recent study concluded that c-Maf restricted IL-4 expression *in vivo*[26,30,34]. This discrepancy led us to explore how T cell-intrinsic deletion of c-Maf was modulating IL-4 expression in both Tfh2 and Th2 cells in helminth infection. To do this, we combined sensitive dual IL-4 reporter mice with T cell-specific deletion of c-Maf. In this IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} mouse model, loxP-flanked *c-Maf* is excised by Cre recombinase in all T cells[69]. Both IL-4 competency and recent IL-4 protein production in the draining lymph nodes of IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} and IL-4$^{4get\text{-}KN2}$c-Maf^{flox} mice 8 days after *N. brasiliensis* infection was quantified using the 4get and KN2 reporters. The results show a significant decrease in the percentage and number of both GFP$^+$ (IL-4 mRNA) and huCD2$^+$ (recent IL-4 protein) CD4$^+$ T cells in the lymph nodes of IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} mice (Figs. 4A and 4B). A significant reduction in the percentage of IL-4-competent and recent IL-4-producing CD4$^+$ T cells in IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} mice compared to IL-4$^{4get\text{-}KN2}$c-Maf^{flox} mice was also observed in the lungs of these animals (Figs. 4C and 4D). Despite a significant reduction in the percentage and total number of IL-4-protein$^+$ cells in the lung, no significant difference was observed in the total number of IL-4-competent CD4$^+$ T cells as marked by GFP expression (Fig. 4D). This may indicate an outgrowth of other T helper subsets in the absence of c-Maf. However, in total, the data conclusively show a T cell-intrinsic role for c-Maf in the optimal production of IL-4 by CD4$^+$ T cells in the lungs and lymph nodes after helminth infection.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

As noted above, Tfh2 and Th2 cells differentially express IL-4 and IL-13 in the context of helminth infection and allergic sensitization[8,12,25,29]. To investigate whether the loss of c-Maf in T cells similarly or differentially impacted IL-13 production in the context of *N. brasiliensis* infection, IL-4$^{4get\text{-}Smart13}$CD4^{cre}c-Maf^{flox} mice and IL-4$^{4get\text{-}Smart13}$c-Maf^{flox} were infected with *N. brasiliensis*. In these dual IL-4 and IL-13 reporter mice, IL-4 expression is reported by GFP, and IL-13 expression is reported by human CD4 expression[12,25]. As observed in IL-4$^{4get\text{-}KN2}$ mice, CD4^{+} T cells isolated from the mediastinal lymph nodes and lung of IL-4$^{4get\text{-}Smart13}$CD4^{cre}c-Maf^{flox} mice and IL-4$^{4get\text{-}Smart13}$c-Maf^{flox} showed a significant difference in the percentage of IL-4-competent (GFP +) T cells at the peak of the pulmonary response to *N. brasiliensis* infection (Figs. 4E–H). However, unlike what was observed for the IL-4 protein, no difference was observed in the production of IL-13 among c-Maf-sufficient or c-Maf-deficient T cells in the mediastinal lymph nodes (Figs. 4E and 4F). This finding is consistent with c-Maf serving a more selective role in the regulation of IL-4 compared to IL-13 in Tfh2 cells. Unexpectedly, the loss of c-Maf also impacted the ability of Th2 cells in the lung to produce IL-13 (Figs. 4G and 4H). This contrasts with c-Maf-deficient mice which show impaired IL-4 expression but normal IL-13 production in Th2 cells[21,26]. This may reflect a more general role for c-Maf in type-2 cytokine regulation in Th2 cells than previously believed at least as it pertains to a robust type-2 response to helminth infection.

T cell-intrinsic loss of c-Maf leads to a more selective impairment in humoral versus cell-mediated immunity to *N. brasiliensis* infection

Loss of IL-4 and IL-13 protein production by CD4^{+} T cells in the lung and IL-4 in the lymph nodes after *N. brasiliensis* infection suggested that these animals may exhibit an impaired type-2 immune response. As IL-4 and IL-13 differentially drive humoral and cell-mediated hallmarks of type-2 inflammation, we investigated whether these two distinct arms were functionally compromised in mice lacking c-Maf expression in T cells. Consistent with an important role for c-Maf in regulating Tfh2-derived IL-4, IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf$^{fl/fl}$ and IL-4^{4get}IL13$^{Smart\text{-}}$CD4^{cre}c-Maf$^{fl/fl}$ (combined groups denoted as IL4^{4get}CD4^{cre}c-Maf$^{fl/fl}$) mice were unable to generate CD95^{+} GL7^{+} germinal center B cells after helminth infection (Fig. 5A). Additionally, these mice exhibited a significant reduction in serum IgE (Fig. 5B). These findings are consistent with Tfh2-derived IL-4 being required for optimal germinal center generation and IgE isotype-switching in this infection model[6,12]. This further establishes an intrinsic role for c-Maf in Tfh2 function.

We next investigated whether similar defects could be observed in Th2-mediated outcomes. As Th2 cells are important in the recruitment of innate cells to the lung in response to *N. brasiliensis*, we quantified the number of eosinophils and basophils 8 days after infection. While no significant difference in basophils was observed between infected c-Maf-sufficient IL-4^{4get}c-Maf$^{fl/fl}$ mice and c-Maf-deficient IL-4^{4get}CD4^{cre}c-Maf$^{fl/fl}$ mice, eosinophilia was significantly reduced after T cell-intrinsic deletion of c-Maf (Fig. 5C). While significant, a substantial recruitment of eosinophils in IL-4^{4get}CD4^{cre}c-Maf$^{fl/fl}$ was still observed relative to uninfected controls. This is consistent with c-Maf-deficient Th2 cells being capable of generating enough IL-5 to mobilize eosinophils from the bone marrow[70].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Th2 cells are also actively involved in the rapid elimination of *N. brasiliensis* from the intestine. To investigate whether T cell-intrinsic c-Maf was critical in the clearance of adult worms in the intestine, we infected IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} and IL-4$^{4get\text{-}KN2}$c-Maf^{flox} mice with *N. brasiliensis* and scored their worm burden 9 days post-infection[71,72]. We found that both IL-4$^{4get\text{-}KN2}$c-Maf^{flox} mice and IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} expelled worms consistent with productive Th2-mediated immunity (Fig. 5D). This result either indicates that despite a significant impairment in IL-4 and IL-13 production in the absence of c-Maf, Th2 cells are still able to produce enough cytokine to mediate worm clearance, or it could suggest that the decreased cytokine production observed in Th2 cells is compensated by other type-2 immune cells resident in the intestine of IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} mice. This result is consistent with the contribution of ILC2 cells to worm clearance[12,72–74].

Taken together, T cell-intrinsic loss of c-Maf has a more dramatic impact on Tfh2-driven humoral immunity than Th2-driven cell-mediated immunity. Although diminished IL-4 and IL-13 may still reach a sufficient level to effectively promote Th2-driven hallmarks, this does not occur in Tfh2 cells as IL-13 is not expressed by these cells during helminth infection[8,12]. Additionally, unlike the intestine where other type-2 immune cells aid in worm clearance through their additional production of IL-4 and IL-13, Tfh2 cells are the dominant source of IL-4 in the lymph nodes with little contribution from other cell types[5–7]. Impaired humoral immunity that results from a Tfh2 cell-intrinsic loss of c-Maf is further exacerbated by the failure to generate productive germinal centers.

STAT5A and STAT6 deficiency selectively impairs IL-4 production in Th2 cells

The low expression of GATA-3 in Tfh2 cells combined with the essential role of c-Maf in the production of IL-4 by this subset supports the important function of c-Maf in the regulation of IL-4. However, transcription factors outside of GATA-3 and c-Maf have also been shown to modulate type-2 cytokine production and the differentiation of Th2 cells. As such, we wanted to investigate if other pathways might also work independently of GATA-3 to promote IL-4 production in Tfh2 cells. The signal transducer and activator of transcription (STAT) factors STAT5 and STAT6 play important roles in the differentiation of established Th2 cells[13–15,18,19]. However, the role that these factors serve in Tfh2 cytokine production is less clear as STAT6 signaling is not required to gain IL-4 competency by T cells in the lymph nodes during helminth infection, and STAT5 negatively regulates Tfh cell lineage specification via IL-2 receptor signaling[12,58,75–78].

To assess the impact of these STAT factors on Tfh2 generation and cytokine competency, a series of IL-4^{4get} reporter mice were generated that lacked either STAT5A, STAT6, or both transcription factors. Mice were infected with *N. brasiliensis* and the mediastinal lymph nodes were assessed 8 days later. Although not completely abolished, the percentage and number of Tfh cells co-expressing CXCR5 and PD-1 were compromised in the absence of STAT6 and further compromised in mice lacking both STAT5A and STAT6 (Figs. 6A and 6B). Next, we investigated whether IL-4 competency was impaired in the absence of STAT5A and STAT6. Mice lacking STAT6 exhibited a significant decrease in the percentage of IL-4-expressing CD4^{+} T cells (Figs. 6C and 6D). Interestingly, the impact on *Il4* transcript expression was not uniform. STAT6 deficiency resulted in a more substantial

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

loss of IL-4 expression among CXCR5^{-} relative to CXCR5^{+} CD4^{+} T cells (Figs. 6C and 6D). This was further exacerbated by the combined absence of both STAT6 and STAT5A. When comparing the number of IL-4-producing Tfh (CXCR5^{+}) cells to the number of IL-4-producing non-Tfh (CXCR5^{-}) cells, the ratio grew from 0.5 in wildtype animals, to 1 in STAT6-deficient, to 1.5 in STAT5A$^{-/-}$STAT6$^{-/-}$-deficient mice (Fig. 6E). Together these results indicate that, while IL-4-producing CD4^{+} T cells are decreased in their absence, STAT6 and STAT5A deficiency impacts IL-4^{+} expression in Th2 cells to a larger extent than Tfh2 cells.

c-Maf serves a nonredundant role in the production of IL-4 by Tfh2 cells

To determine if c-Maf could have a nonredundant impact on IL-4 expression in CXCR5^{+} Tfh2 cells compared to STAT5A and STAT6, wildtype and STAT5A$^{-/-}$STAT6$^{-/-}$ mice were infected with *N. brasiliensis* and c-Maf expression was measured by flow cytometry. Intracellular c-Maf was observed equally in CXCR5^{+} CD4^{+} T cells harvested from the mediastinal lymph nodes of both wildtype and STAT5A$^{-/-}$STAT6$^{-/-}$ mice (Fig. 6F). This confirmed that c-Maf remained a likely regulator of IL-4 competency in Tfh2 cells. To establish whether c-Maf served an essential and nonredundant role in the production of IL-4 in Tfh2 cells, we infected IL-4$^{KN2-4get}$CD4^{cre}c-Maf^{flox} and IL-4$^{KN2-4get}$c-Maf^{flox} mice with *N. brasiliensis* and quantified GFP expression in CXCR5^{+} and CXCR5^{-} CD4^{+} T cells from the mediastinal lymph nodes. Although the percentage and total number of GFP^{+} CD4^{+} T cells were reduced in mice lacking c-Maf in T cells (Figs. 6G–I), the reduction was again varied across the IL-4-competent T-cell compartment. Although a sizable population of CXCR5^{-} CD4^{+} T cells still expressed GFP in the absence of c-Maf, there was a near-complete loss of CXCR5^{+} GFP^{+} CD4^{+} T cells. The selective impact of c-Maf deficiency in CXCR5^{+} Tfh2 cells was even more striking when focused on IL-4-competent CD4^{+} T cells. In contrast to IL-4 being largely restricted to the CXCR5^{+} compartment in STAT5A$^{-/-}$STAT6$^{-/-}$ mice, IL-4 production was largely restricted to the CXCR5^{-} population in c-Maf-deficient T cells (Figs. 6E and 6J). Thus, unlike what was observed in Th2 cells, this confirms a critical and nonredundant role for c-Maf in the regulation of IL-4 by Tfh2 cells.

c-Maf is required for follicular migration and Tfh2-driven humoral immunity

The above data show a nonredundant role for c-Maf as a regulator of Tfh2 cell cytokine production and function. Although a direct role for c-Maf in IL-4 expression by Tfh2 cells is likely based on the significant reduction in IL-4 expression by Th2 cells lacking c-Maf, a more dominant mechanism for c-Maf in modulating the ability of Tfh2 cells to orchestrate humoral immunity may reside its requirement for efficient migration of T cells into the B cell follicles. This idea is supported by a known role for c-Maf in increasing CXCR5 expression, the chemokine receptor required for follicular migration[32,45,46,79,80]. To investigate a role for c-Maf in Tfh2 cell migration, we first determined whether there was a decline in CD4^{+} CXCR5^{+} PD1^{+} Tfh cells 8 days after *N. brasiliensis* infection of IL-4$^{4get-KN2}$CD4^{cre}c-Maf^{flox} compared to IL-4$^{4get-KN2}$c-Maf^{flox} mice. The data show that mice with T cells deficient for c-Maf display a near-complete loss of the CXCR5^{+} PD1^{+} Tfh population (Fig. 7A). This finding is consistent with a role for c-Maf in promoting CXCR5 expression and the downstream entry of T cells into the B cell follicles. This

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

mechanism is supported by the presence of a half MARE sequence located proximal to the *Cxcr5* promoter (Fig. 7B). The c-Maf binding site is present near open chromatin within the *Cxcr5* locus of IL-4$^+$ CD4$^+$ T cells isolated from the mediastinal lymph nodes 8 days after *N. brasiliensis* infection[36]. This finding supports a key role for c-Maf in driving the transcription of *Cxcr5*[32].

To confirm a role for c-Maf in the follicular migration of IL-4$^+$ Tfh2 cells, we performed immunofluorescent staining on tissue sections taken from the lung-draining, mediastinal lymph nodes of IL-4$^{4get\text{-}KN2}$c-Maf^{flox} and IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} mice 8 days after infection with *N. brasiliensis*. Wildtype IL-4$^{4get\text{-}KN2}$c-Maf^{flox} mice showed robust follicular migration and IL-4-expression (GFP$^+$) in both the B cell follicles and T-cell paracortex, indicative of IL-4-competency in both Tfh and non-Tfh cells (Fig. 7C). However, in mice with T cells that are deficient for c-Maf, no accumulation of IL-4$^+$ CD4$^+$ cells in the B cell follicles was observed, despite robust IL-4 expression in the T-cell paracortex (Fig. 7C). The absence of follicular localization by c-Maf-deficient CD4$^+$ T cells resembled the follicular phenotype observed in helminth-infected BATF-deficient animals (Fig. 7C)[81]. However, unlike BATF-deficient mice—which lack IL-4-expressing CD4$^+$ T cells in the B cell follicles and paracortex—IL-4$^{4get\text{-}KN2}$CD4^{cre}c-Maf^{flox} mice exhibited a more selective defect among IL-4 expression in the B cell follicles. This is consistent with the flow data indicating that CXCR5$^-$ GFP$^+$ Th2 cells were less impacted by the absence of c-Maf compared to CXCR5$^+$ Tfh2 cells. Together, this highlights an independent mechanism by which c-Maf regulates Tfh2 function that is not found in Th2 cells and works independently from a direct role c-Maf plays in IL-4 transcription.

Discussion

Th2 cells have long been considered orchestrators of humoral and cell-mediated immunity through their production of type-2 cytokines[82,83]. However, studies over the last decade have begun to define a more nuanced view regarding the role these cells serve in type-2 inflammation[1,2]. In a bifurcated model of type-2 immunity, Th2 cells function primarily in mucosal tissues to regulate the cell-mediated characteristics of type-2 inflammation, while Tfh2 cells reside in the B cell follicles of lymphoid tissues to promote humoral immunity[3]. In the context of a type-2 immune response, Th2 cells stimulate mucus production, smooth muscle contractility, eosinophil mobilization, and other hallmarks of type-2 inflammation through the production of IL-4, IL-5, and IL-13. In contrast, Tfh2 cells primarily produce IL-4 to promote the production of IgE and IgG1 antibody isotypes. The current study shows that c-Maf is a key factor in the production of both IL-4 and IL-13 by Th2 and IL-4 by Tfh2 cells. However, from a functional perspective, the absence of c-Maf in T cells had a more substantive impact on Tfh2-driven humoral immunity.

The differential role of c-Maf in regulating IL-4 and IL-13 suggests that c-Maf works differently in Th2 and Tfh2 cells. In Th2 cells, multiple transcription factors work with c-Maf to regulate IL-4, IL-5, and IL-13. Specifically, GATA3 is critical for the generation and maintenance of committed Th2 cells[16,20,84]. Importantly, while GATA-3 regulates IL-4, IL-5, and IL-13 expression, it appears most critical in the production of IL-5 and IL-13[12,17,20,21,85]. In addition to GATA-3, IL-4 receptor-mediated STAT6 signaling and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

IL-2 receptor-mediated STAT5A signaling is important for Th2 cell maintenance and function[13,19,58,86,87]. STAT6 signaling reinforces Th2 commitment through the induction of both GATA-3 and c-Maf, confirming that c-Maf does play a role in the typical regulation of cytokines during Th2 differentiation[27,34,88]. However, these factors can also work independently from one another suggesting that redundant pathways developed for the expression of type-2 cytokines in Th2 cells. For example, in the absence of STAT6, GATA-3 is sufficient to drive Th2 differentiation, likely, in part, through the increased expression and activity of c-Maf[89]. Similarly, c-Maf expression can be achieved independently from IL-4 receptor signaling (STAT6) through the actions of IL-6 and STAT3[90]. Another likely mechanism for how c-Maf is modulating IL-4 production in Th2 cells is through its ability to synergize with JunB[60]. This is supported herein via the use of unbiased transcription factor inference analysis of IL-4$^+$ CD4$^+$ Th2 cells from the lung following *N. brasiliensis* infection. This analysis revealed overlapping regulons for GATA-3, c-Maf, and JunB consistent with these factors involved in driving similar gene programs. In sum, optimal type-2 cytokine expression by Th2 cells involves the coordination of multiple transcription factors. However, redundancy has been built into this system to ensure adequate Th2 function remains if for example c-Maf regulons are disrupted.

C-Maf follows a more atypical path in Tfh2 cells. Analysis of IL-4$^+$ T cells in the mediastinal lymph nodes after *N. brasiliensis* infection, a population highly enriched for Tfh2 cells, showed active c-Maf regulons but no GATA-3 and JunB regulons. This highlights that neither signaling via the IL-4 receptor nor high GATA-3 expression is required for Tfh2 cells to acquire IL-4 competency[6]. In addition, our findings here show that while STAT5A complements IL-4 receptor signaling to achieve optimal IL-4 expression in Th2 cells, the impact of combined STAT6 and STAT5A deficiency on IL-4 expression in Tfh2 cells was significantly less than that observed in Th2 cells[19,91]. Instead, c-Maf appears to be the essential IL-4-driver in Tfh2 cells. This is highlighted by the observation that T cells lacking c-Maf showed greater impairment in IL-4 expression among CXCR5$^+$ Tfh2 cells than CXCR5$^-$ Th2 cells. The selective expression of IL-4—independent of IL-5 and IL-13—by Tfh2 cells and the lack of reliance on other classical Th2 transcription factors such as GATA-3, STAT6, and STAT5A to achieve IL-4 expression further implicates an important and nonredundant role for c-Maf in the regulation of type-2 cytokines in Tfh2 cells[12]. The lack of redundant pathways to promote IL-4 expression in Tfh2 cells marks a clear and important difference when compared to Th2 cells.

Currently, candidates required for expression of IL-4 in Tfh2 cells have concentrated on factors that bind at the 3' enhancer of *Il4*, a region essential for IL-4 expression by Tfh cells[64,92]. The intracellular domain of Notch and the AP-1 factor BATF are two such factors[3,31,93–95]. The transcription factor inference analysis confirms that both factors are active in IL-4$^+$ CD4$^+$ T cells residing in the lymph nodes after *N. brasiliensis* infection. This is consistent with both BATF and Notch (through Wnt signaling) mediating IL-4 expression by Tfh2 cells[31,81,96,97]. In addition, BATF and TCF-7 regulons overlapped with those of c-Maf, adding it to the evolving list of factors that impact Tfh2 cytokine production. Like Notch and BATF, c-Maf can bind at the 3' enhancer of the *Il4* locus[31]. Although c-Maf can also regulate IL-4 expression in Th2 cells by working synergistically with JunB upstream of the *Il4* promoter, this mechanism is likely not involved in Tfh2 cells as no

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

JunB regulon activity was observed in IL-4$^+$ CD4$^+$ T cells in the mediastinal lymph nodes after *N. brasiliensis* infection[60]. Instead, c-Maf regulons match those of the nuclear factor of activated T cells 1 (NFATc1) suggesting that these factors might work in concert in Tfh2 cells to promote IL-4 expression. In support, c-Maf and other AP-1 factors have been proposed to cooperate with NFAT in the regulation of IL-4[27,98,99]. Thus, while the data shown here confirm a key role for c-Maf in the expression of IL-4 in both Tfh2 and Th2 cells, the conditions in which c-Maf regulates IL-4 expression in Tfh2 cells are unique from classical pathways observed in Th2 populations.

The differential dependence on c-Maf for type-2 cytokine expression in Tfh2 cells and Th2 cells suggests that c-Maf may also differentially impact Th2-driven and Tfh2-driven hallmarks of type-2 immunity. In support of this and extending on previous findings showing that c-Maf-deficient T cells exhibit a defect in Tfh cell generation and humoral immunity, the current study helps to resolve why c-Maf deficiency has a more dramatic effect on Tfh-mediated hallmarks (e.g. IgE) compared to Th2-mediated hallmarks (eosinophilia and worm clearance) despite its equivalent expression by both of these cell types[30,32,33,54]. As shown here, these selective defects are the result of two key functions c-Maf serves in Tfh cells. First, the dependency on c-Maf for migration of Tfh cells into the B cell follicles would selectively impact IgE but not Th2 function in peripheral tissues. Second, while these studies show that c-Maf affects IL-4 production in both Th2 and Tfh2 cells, IL-13 production by Th2 cells, while significantly reduced, remained intact in the absence of c-Maf. This may allow IL-4 and IL-13 to combine to reach a threshold sufficient for the promotion of Th2-driven eosinophilia and intestinal worm clearance[100–103]. Alternatively, it may reflect compensation by other type-2 cytokine-expressing cells present in these mucosal sites. In contrast, IL-13 is unable to similarly compensate for the lack of IL-4 in Tfh2 cells as Tfh2 cells fail to produce IL-13 after helminth infection and appear to represent the dominant source of IL-4 in the B cell follicles[8,12]. As a result, Tfh2-driven humoral hallmarks are significantly impaired in the absence of c-Maf relative to Th2-driven immunity. It will be interesting to assess if c-Maf serves a similar role in Tfh13 cells, which arise in some settings of allergic sensitization but not others[8,29]. Given the higher GATA-3 expression in Tfh13 cells compared to Tfh2 cells, it is possible that IL-13 might compensate for the absence of c-Maf-driven IL-4 in those cells similar to what has been observed in Th2 cells found in other settings of allergic inflammation[12,70,102,104].

In summary, c-Maf serves a bifurcated role in type-2 immunity through its impact on IL-4 expression in Tfh2 and IL-4 and IL-13 expression in Th2 cells. Although IL-4 and IL-13 production by Th2 cells were compromised in T cells lacking c-Maf, the T cell-intrinsic loss of this factor had a significantly more profound impact on Tfh2-mediated immunity. Mechanistically, c-Maf exhibits control of Tfh2-mediated humoral hallmarks in two ways. First, c-Maf is likely required for optimal transcription of IL-4 by Tfh2 cells. Second, c-Maf is required for Tfh2 cell migration into the B cell follicles. As a result, deletion of c-Maf limits the hosts' ability to mount a productive germinal center and IgE response to helminth infection. Thus, while Th2 cells have evolved pathways to maintain type-2 cytokine expression in the absence of c-Maf, Tfh2 cells lack such redundancy. The lack of redundant pathways in Tfh2 cells makes these cells more reliant on this single transcription factor for their function. These pathways are well conserved between mice and humans.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

As such, c-Maf and its associated pathways may represent targets that can selectively alter humoral hallmarks of type-2 inflammation and IgE-associated disease outcomes in humans.

MATERIALS AND METHODS

Mice

IL4$^{\text{get}}$, IL4$^{\text{KN2}}$, and IL13$^{\text{Smart}}$ mice have been previously described[12,57,58]; STAT5A$^{-/-}$ mice on the Balb/c background were provided by Hans Dooms[105]; c-Maf$^{\text{fl/fl}}$ mice were rederived at Duke University with permission from Christina Birchmeier (Max Delbrück Center for Molecular Medicine)[69]; CD4$^{\text{cre}}$ [106]; and STAT6$^{-/-}$ mice were purchased from Jackson laboratories. Mice were bred and maintained on reporter C57BL/6 and Balb/c genetic backgrounds at Duke and National Jewish Health. To minimize confounders, littermates were used when possible and mice were randomized between treatment and control groups. When littermates were not available, sex and age-matched (6–12 weeks of age) control and treatment groups were randomized by genotype. All experiments were performed with male and female mice of each genotype. Mice were maintained in specific pathogen-free animal facilities in accordance with animal guidelines established by the Institutional Animal Care & Use Committee (IACUC), the Division of Laboratory Animal Resources at Duke University Medical Center, and the Biological Resource Center at National Jewish Health.

Infections

N. brasiliensis was prepared as previously described[107]. Mice were infected with 500 L3 larvae in 200 ul phosphate-buffered saline (PBS) subcutaneously in the lower back.

Tissue digestion for flow cytometry and sorting

Lungs were chopped with a razor blade or underwent automated digestion in C-Tubes using a heated OctoMACS (Miltenyi Biotech, Bergisch Gladbach, North Rhine-Westphalia, Germany), digested with 250 μg/ml Collagenase XI (C7657; Sigma, St. Louis, MO, USA), 50 μg/ml Liberase (145495; Roche, Basel, Switzerland), 1 mg/ml Hyaluronidase (h3506; Sigma, St. Louis, MO, USA), and 200 μg/ml DNase I (DN25; Sigma, St. Louis, MO, USA) in RPMI 1640 at 37°C for 30 minutes. Tissues were vortexed and pipetted vigorously every 15 minutes until fully digested and filtered through an 80-micron mesh, washed, lysed for 2 minutes with ammonium-chloride-potassium (ACK) buffer to reduce red blood cells, washed with PBS, and resuspended in 2% fetal bovine serum in PBS. Single-cell suspensions of lymph nodes were prepared by mechanical dissociation. All tissues were filtered immediately prior to data collection.

Staining for flow cytometry and sorting

Fc receptors were blocked (1:100; Trustain FcX Biolegend) for 15 minutes prior to antibody staining. All stains were performed on ice for 30 minutes unless otherwise stated with the following antibodies in 2% fetal bovine serum in PBS: anti-mouse CD3ε (145–2C11), anti-mouse CD4 (RM4–5), anti-mouse CD8α (53–6.7), anti-mouse CD11c (N418), anti-mouse CD11b (M1/70), anti-mouse/human CD45R/B220 (RA3–6B2), anti-mouse CD279/PD-1 (RMP1–30), anti-mouse CD49b (DX5), anti-mouse/human GL7 (GL7), anti-mouse Siglec-F

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(E50–2440), anti-mouse CD131 (JORO50), anti-mouse NK1.1 (PK136), anti-mouse Ter119 (Ter 119), anti-human CD4 (RPA-T4), and streptavidin from Biolegend (San Diego, CA, USA); anti-human CD2 (S5.5) from Invitrogen; and anti-mouse CD95 (JO2) and anti-rat/mouse CD185/CXCR5 (2G8) from BD biosciences (Franklin Lakes, NJ, USA). Prior to analysis, cells were resuspended in 2% fetal calf serum in PBS containing DAPI (4', 6-diamidine-2'-phenylindole dihydrochlo-ride; 0.5 μg/ml) to discriminate live cells. Lymphocyte and singlet gates were performed by size and granularity based on forward and side scatter. Cells were sorted using a FACSAria (BD Biosciences, Franklin Lakes, NJ, USA) and data were collected on a Fortessa or LSR II (BD Biosciences, Franklin Lakes, NJ, USA) cytometer and analyzed using FlowJo (Tree Star, Inc, Ashland, OR, USA).

Transcription factor staining

Surface stains were performed followed by staining of dead cells with a Fixable Violet Dead Cell Stain kit (L34964; ThermoFisher, Waltham, MA, USA). A Foxp3 transcription factor staining buffer kit (eBioscience) was used to fix and permeabilize cells. Antibodies used for transcription factor staining were phycoerythrin (PE) or allophycocyanin APC/alexa647-conjugated anti-mouse/human GATA-3 (TWAJ; ThermoFisher, Waltham, MA, USA), anti-mouse/human c-MAF (SymOF1; ThermoFisher, Waltham, MA, USA), and anti-mouse BCL6 (K112–91; BD Biosciences, Franklin Lakes, NJ, USA).

Enzyme-linked immunosorbent assay

We coated 96-well plates with Rat-anti-mouse-IgE (R35–72, BD Biosciences, Franklin Lakes, NJ, USA). Two-fold dilutions of serum were added to the plate, and total serum IgE was detected by biotinylated anti-mouse-IgE (R35–118, BD Biosciences, Franklin Lakes, NJ, USA), followed by streptavidin-horseradish peroxidase and o-phenylenediamine. Concentrations of total IgE were determined using an Emax Precision Microplate Reader (Molecular Devices, San Jose, CA, USA) and compared to standard curves generated with purified IgE (MEB-38, Biolegend, San Diego, CA, USA).

Immunohistochemistry

The draining mediastinal lymph nodes were isolated, fixed in 4% paraformaldehyde, placed in 30% sucrose, embedded, and frozen in optimum cutting temperature embedding compound (Sakura, Jefferson, MA, USA). Sections were cut at 6 microns and quenched of endogenous peroxidase activity with 1% H_2O_2 and 0.5% sodium azide in PBS. Slides were subsequently blocked for non-specific Fc receptor binding (TruStain Fc block, clone 93; Biolegend, San Diego, CA, USA) and endogenous biotin (Avidin/Biotin Block; Vector Labs, Newark, CA, USA). eGFP was detected by anti-mouse-GFP (NB600–308; Novus Biologicals, Centennial, CO, USA), followed by biotinylated F(ab')2 donkey anti-rabbit (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Tyramide amplification was applied to maximize detection of biotinylated anti-eGFP. Briefly, following addition of biotinylated F(ab')2 antibodies, tissue sections were incubated with streptavidin-HRP followed by fluorescein isothiocyanate–tyramide from the Tyramide Signal Amplification (TSA) Fluorescein System according to the manufacturer's instructions (NEL 701001KT; PerkinElmer, Watham, MA, USA). Prior to CD4 detection, a second round of peroxidase quenching and blocking of biotin (Avidin/Biotin Block;

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Vector labs, Newark, CA, USA) was performed. CD4 was detected using biotinylated anti-CD4 antibody (RM4–5; Biolegend, San Diego, CA, USA), and signal was amplified with tyramide–Alexa Fluor 555 (Invitrogen, Watham, MA). After a third round of blocking and quenching of biotin and peroxidase, B220 was detected by biotinylated anti-B220 (RA3–6B2; Biolegend, San Diego, CA, USA), followed by Dylight649 conjugated to anti-Streptavidin (Biolegend, San Diego, CA, USA). Nuclei were counterstained with DAPI (Roche) in PBS before mounting using Vectashield (Vector Laboratories, Newark, CA, USA) on coverslips. Digital images were collected with the Zeiss Axio Imager (Zeiss, Oberkochen, Baden-Württemberg Germany). Images were converted to red, green, blue (RGB), colored, and overlaid with Photoshop CS5 software (Adobe Systems, San Jose, CA, USA).

Transcriptome and transcription factor inference analysis

Bulk and single-cell transcriptomes derived from IL-4^{+} and IL-4^{-} CD4^{+} T cells isolated from the lungs and lymph nodes of IL4^{4get} C57BL/6 mice infected with *N. brasiliensis* were compiled and processed as described (GSE125788)[36]. Transcription factor inference analysis was performed using the SCENIC package[59]. The mouse mm9 database for RcisTarget was downloaded from aert-slab.org. Filtering was performed to retain only genes consisting of at least six reads, those that were detected in at least 1% of the cells, and that were also available in the RcisTarget database. After running the standard SCENIC pipeline, area under the curve data was added back to the original Seurat object as meta-data to visualize regulons in a consistent format.

Statistical analysis

All analyses were performed as two-tailed paired or unpaired t-tests where indicated. All comparisons were made using Student's t test, paired t test with Prism 7 (GraphPad, La jolla, CA, USA) where possible exact p values are provided in figures.

Sample size and rigor criteria

Unless otherwise noted, group size was based on significance achieved in outcome measures from prior experiments as stated below. If basis from prior experiments were not possible, group size was based on Meads Equation (E = N-B-T). Within designated group size, consistent results reflecting substantial differences between outcome measure of experimental and control groups were required. Data from each independent experiment was independently calculated to determine whether significance existed between each group. The cutoff for publication was a result in which the difference between control and experimental group outcome measures attained a significance level of $p < 0.05$ with X animals/group in 2–3 experiments. If in two experiments the significance level did not attain at least $p < 0.05$, a third repeat was always performed. Individual outcome measures are defined in figure legends. All data were included, and group allocation was known throughout the experiment unless exclusion criteria/blinding was directly noted in the figure legend.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

FUNDING

This work was supported by the National Institutes of Health Grant AI119004, AI156901 (R.L.R.); T32 AI074491(U.I.C).

ACKNOWLEDGMENTS

Thank you to Richard Locksley (UCSF), Dan Littman (NYU), and Carmen Birchmeier (Max Delbrück Center) for mice and reagents; Traci Reddick (Duke University) for mouse rederivation; Shirley Sobus and Josh Loomis and the National Jewish Health Flow Core expertise in cell sorting; and Ann Miller and Julio Barrera for technical assistance.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available in the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) under the accession numbers GSE125788 and are available from the corresponding author on reasonable request.

REFERENCES

1. Pulendran B & Artis D New paradigms in type 2 immunity. Science 337, 431–435 (2012). [PubMed: 22837519]
2. Lambrecht BN & Hammad H The immunology of asthma. Nat. Immunol 16, 45–56 (2015). [PubMed: 25521684]
3. Bao K & Reinhardt RL The differential expression of IL-4 and IL-13 and its impact on type-2 immunity. Cytokine 75, 25–37 (2015). [PubMed: 26073683]
4. Yusuf I et al. Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). J. Immunol 185, 190–202 (2010). [PubMed: 20525889]
5. Glatman Zaretsky AG et al. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. J. Exp. Med 206, 991–999 (2009). [PubMed: 19380637]
6. Reinhardt RL, Liang HE & Locksley RM Cytokine-secreting follicular T cells shape the antibody repertoire. Nat. Immunol 10, 385–393 (2009). [PubMed: 19252490]
7. King IL & Mohrs M IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. J. Exp. Med 206, 1001–1007 (2009). [PubMed: 19380638]
8. Gowthaman U et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE. Science 365, eaaw6433 (2019).
9. Shih HY et al. Transcriptional and epigenetic networks of helper T and innate lymphoid cells. Immunol. Rev 261, 23–49 (2014). [PubMed: 25123275]
10. Crotty S T follicular helper cell differentiation, function, and roles in disease. Immunity 41, 529–542 (2014). [PubMed: 25367570]
11. Chtanova T et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. J. Immunol 173, 68–78 (2004). [PubMed: 15210760]
12. Liang HE et al. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nat. Immunol 13, 58–66 (2011). [PubMed: 22138715]
13. Kaplan MH, Schindler U, Smiley ST & Grusby MJ Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity 4, 313–319 (1996). [PubMed: 8624821]
14. Shimoda K et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 380, 630–633 (1996). [PubMed: 8602264]
15. Takeda K et al. Essential role of Stat6 in IL-4 signalling. Nature 380, 627–630 (1996). [PubMed: 8602263]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

16. Zheng W & Flavell RA The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 89, 587–596 (1997). [PubMed: 9160750]
17. Zhang DH, Cohn L, Ray P, Bottomly K & Ray A Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J. Biol. Chem 272, 21597–21603 (1997). [PubMed: 9261181]
18. Cote-Sierra J et al. Interleukin 2 plays a central role in Th2 differentiation. Proc. Natl Acad. Sci. U. S. A 101, 3880–3885 (2004). [PubMed: 15004274]
19. Zhu J, Cote-Sierra J, Guo L & Paul WE Stat5 activation plays a critical role in Th2 differentiation. Immunity 19, 739–748 (2003). [PubMed: 14614860]
20. Zhu J et al. Conditional deletion of Gata3 shows its essential function in T(H) 1-T(H)2 responses. Nat. Immunol 5, 1157–1165 (2004). [PubMed: 15475959]
21. Kishikawa H, Sun J, Choi A, Miaw SC & Ho IC The cell type-specific expression of the murine IL-13 gene is regulated by GATA-3. J. Immunol 167, 4414–4420 (2001). [PubMed: 11591766]
22. Klein Wolterink RG et al. Essential, dose-dependent role for the transcription factor Gata3 in the development of IL-5+ and IL-13+ type 2 innate lymphoid cells. Proc. Natl Acad. Sci. U. S. A 110, 10240–10245 (2013). [PubMed: 23733962]
23. Mjösberg J et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. Immunity 37, 649–659 (2012). [PubMed: 23063330]
24. Hoyler T et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. Immunity 37, 634–648 (2012). [PubMed: 23063333]
25. O'Brien TF et al. Cytokine expression by invariant natural killer T cells is tightly regulated throughout development and settings of type-2 inflammation. Mucosal Immunol. 9, 597–609 (2016). [PubMed: 26349658]
26. Kim JI, Ho IC, Grusby MJ & Glimcher LH The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity 10, 745–751 (1999). [PubMed: 10403649]
27. Ho IC, Hodge MR, Rooney JW & Glimcher LH The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. Cell 85, 973–983 (1996). [PubMed: 8674125]
28. Meli AP, Fontés G, Leung Soo C & King ILT T Follicular helper cell-derived IL-4 is required for IgE production during intestinal helminth infection. J. Immunol 199, 244–252 (2017). [PubMed: 28533444]
29. Hondowicz BD et al. Interleukin-2-dependent allergen-specific tissue-resident memory cells drive asthma. Immunity 44, 155–166 (2016). [PubMed: 26750312]
30. Andris F et al. The transcription factor c-Maf promotes the differentiation of follicular helper T cells. Front. Immunol 8, 480 (2017). [PubMed: 28496444]
31. Sahoo A et al. BATF is important for IL-4 expression in T follicular helper cells. Nat. Commun 6, 7997 (2015). [PubMed: 26278622]
32. Kroenke MA et al. Bcl6 and maf cooperate to instruct human follicular helper CD4 T cell differentiation. J. Immunol 188, 3734–3744 (2012). [PubMed: 22427637]
33. Ise W et al. The transcription factor BATF controls the global regulators of class-switch recombination in both B cells and T cells. Nat. Immunol 12, 536–543 (2011). [PubMed: 21572431]
34. Ho IC, Lo D & Glimcher LH c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and - independent mechanisms. J. Exp. Med 188, 1859–1866 (1998). [PubMed: 9815263]
35. Gabryšová L et al. c-Maf controls immune responses by regulating disease-specific gene networks and repressing IL-2 in CD4+ T cells. Nat. Immunol 19, 497–507 (2018). [PubMed: 29662170]
36. Brown IK et al. Single cell analysis of host response to helminth infection reveals the clonal breadth, heterogeneity, and tissue-specific programming of the responding CD4+ T cell repertoire. PLoS Pathog. 17, e1009602 (2021). [PubMed: 34106992]
37. Xu D et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. J. Exp. Med 187, 787–794 (1998). [PubMed: 9480988]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

38. Löhning M et al. 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. U. S. A 95, 6930–6935 (1998). [PubMed: 9618516]
39. Nobs SP et al. PPARgamma in dendritic cells and T cells drives pathogenic type-2 effector responses in lung inflammation. J. Exp. Med 214, 3015–3035 (2017). [PubMed: 28798029]
40. Chen T et al. PPAR-gamma promotes type 2 immune responses in allergy and nematode infection. Sci. Immunol 2, eaal5196 (2017).
41. Gelfand EW, Joetham A, Wang M, Takeda K & Schedel M Spectrum of T-lymphocyte activities regulating allergic lung inflammation. Immunol. Rev 278, 63–86 (2017). [PubMed: 28658551]
42. Liu X et al. Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. Nature 507, 513–518 (2014). [PubMed: 24463518]
43. Johnston RJ et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science 325, 1006–1010 (2009). [PubMed: 19608860]
44. Kim CH et al. Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. J. Exp. Med 193, 1373–1381 (2001). [PubMed: 11413192]
45. Schaerli P et al. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J. Exp. Med 192, 1553–1562 (2000). [PubMed: 11104798]
46. Breitfeld D et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J. Exp. Med 192, 1545–1552 (2000). [PubMed: 11104797]
47. Shaw LA et al. Id2 reinforces TH1 differentiation and inhibits E2A to repress TFH differentiation. Nat. Immunol 17, 834–843 (2016). [PubMed: 27213691]
48. Moran AE et al. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. J. Exp. Med 208, 1279–1289 (2011). [PubMed: 21606508]
49. Turner DL et al. Lung niches for the generation and maintenance of tissue-resident memory T cells. Mucosal Immunol. 7, 501–510 (2014). [PubMed: 24064670]
50. Sathaliyawala T et al. Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. Immunity 38, 187–197 (2013). [PubMed: 23260195]
51. Hu R et al. miR-155 promotes T follicular helper cell accumulation during chronic, low-grade inflammation. Immunity 41, 605–619 (2014). [PubMed: 25367574]
52. Ferreira MAR et al. Eleven loci with new reproducible genetic associations with allergic disease risk. J. Allergy Clin. Immunol 143, 691–699 (2019). [PubMed: 29679657]
53. Simpson LJ et al. A microRNA upregulated in asthma airway T cells promotes TH2 cytokine production. Nat. Immunol 15, 1162–1170 (2014). [PubMed: 25362490]
54. Bauquet AT et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat. Immunol 10, 167–175 (2009). [PubMed: 19098919]
55. Poholek AC et al. In vivo regulation of Bcl6 and T follicular helper cell development. J. Immunol 185, 313–326 (2010). [PubMed: 20519643]
56. Nurieva RI et al. Bcl6 mediates the development of T follicular helper cells. Science 325, 1001–1005 (2009). [PubMed: 19628815]
57. Mohrs K, Wakil AE, Killeen N, Locksley RM & Mohrs M A two-step process for cytokine production revealed by IL-4 dual-reporter mice. Immunity 23, 419–429 (2005). [PubMed: 16226507]
58. Mohrs M, Shinkai K, Mohrs K & Locksley RM Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. Immunity 15, 303–311 (2001). [PubMed: 11520464]
59. Aibar S et al. SCENIC: single-cell regulatory network inference and clustering. Nat. Methods 14, 1083–1086 (2017). [PubMed: 28991892]
60. Li B, Tournier C, Davis RJ & Flavell RA Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. EMBO J 18, 420–432 (1999). [PubMed: 9889198]
61. Krishnamoorthy V et al. The IRF4 gene regulatory module functions as a read-write integrator to dynamically coordinate T helper cell fate. Immunity 47, 481–497.e7 (2017). [PubMed: 28930660]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

62. Kwon H et al. Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. Immunity 31, 941–952 (2009). [PubMed: 20064451]

63. Martinez GJ et al. Cutting edge: NFAT transcription factors promote the generation of follicular helper T cells in response to acute viral infection. J. Immunol 196, 2015–2019 (2016). [PubMed: 26851216]

64. Vijayanand P et al. Interleukin-4 production by follicular helper T cells requires the conserved Il4 enhancer hypersensitivity site V. Immunity 36, 175–187 (2012). [PubMed: 22326582]

65. Choi YS et al. LEF-1 and TCF-1 orchestrate T(FH) differentiation by regulating differentiation circuits upstream of the transcriptional repressor Bcl6. Nat. Immunol 16, 980–990 (2015). [PubMed: 26214741]

66. Xu L et al. The transcription factor TCF-1 initiates the differentiation of T(FH) cells during acute viral infection. Nat. Immunol 16, 991–999 (2015). [PubMed: 26214740]

67. Wu T et al. TCF1 is required for the T follicular helper cell response to viral infection. Cell Rep. 12, 2099–2110 (2015). [PubMed: 26365183]

68. Hodge MR, Rooney JW & Glimcher LH The proximal promoter of the IL-4 gene is composed of multiple essential regulatory sites that bind at least two distinct factors. J. Immunol 154, 6397–6405 (1995). [PubMed: 7759876]

69. Wende H et al. The transcription factor c-Maf controls touch receptor development and function. Science 335, 1373–1376 (2012). [PubMed: 22345400]

70. McKenzie GJ, Fallon PG, Emson CL, Grencis RK & McKenzie AN Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. J. Exp. Med 189, 1565–1572 (1999). [PubMed: 10330435]

71. Urban JF Jr, Maliszewski CR, Madden KB, Katona IM & Finkelman FD IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. J. Immunol 154, 4675–4684 (1995). [PubMed: 7722320]

72. Barner M, Mohrs M, Brombacher F & Kopf M Differences between IL-4R alpha-deficient and IL-4-deficient mice reveal a role for IL-13 in the regulation of Th2 responses. Curr. Biol 8, 669–672 (1998). [PubMed: 9635196]

73. Jarick KJ et al. Non-redundant functions of group 2 innate lymphoid cells. Nature 611, 794–800 (2022). [PubMed: 36323785]

74. Tsou AM et al. Neuropeptide regulation of non-redundant ILC2 responses at barrier surfaces. Nature 611, 787–793 (2022). [PubMed: 36323781]

75. Nurieva RI et al. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. J. Biol. Chem 287, 11234–11239 (2012). [PubMed: 22318729]

76. Johnston RJ, Choi YS, Diamond JA, Yang JA & Crotty S STAT5 is a potent negative regulator of TFH cell differentiation. J. Exp. Med 209, 243–250 (2012). [PubMed: 22271576]

77. Pepper M, Pagán AJ, Igyártó BZ, Taylor JJ & Jenkins MK Opposing signals from the bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. Immunity 35, 583–595 (2011). [PubMed: 22018468]

78. DiToro D et al. Differential IL-2 expression defines developmental fates of follicular versus nonfollicular helper T cells. Science 361, eaao2933.

79. Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG & Cyster JG In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. J. Exp. Med 190, 1123–1134 (1999). [PubMed: 10523610]

80. Förster R, Emrich T, Kremmer E & Lipp M Expression of the G-protein–coupled receptor BLR1 defines mature, recirculating B cells and a subset of T-helper memory cells. Blood 84, 830–840 (1994). [PubMed: 7913842]

81. Bao K et al. BATF modulates the Th2 locus control region and regulates CD4+ T cell fate during antihelminth immunity. J. Immunol 197, 4371–4381 (2016). [PubMed: 27798167]

82. Holgate ST Innate and adaptive immune responses in asthma. Nat. Med 18, 673–683 (2012). [PubMed: 22561831]

83. Kim HY, DeKruyff RH & Umetsu DT The many paths to asthma: phenotype shaped by innate and adaptive immunity. Nat. Immunol 11, 577–584 (2010). [PubMed: 20562844]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

84. Pai SY, Truitt ML & Ho IC GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc. Natl Acad. Sci. U. S. A 101, 1993–1998 (2004). [PubMed: 14769923]
85. Zhang DH, Yang L & Ray A Differential responsiveness of the IL-5 and IL-4 genes to transcription factor GATA-3. J. Immunol 161, 3817–3821 (1998). [PubMed: 9780145]
86. Yamane H, Zhu J & Paul WE Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. J. Exp. Med 202, 793–804 (2005). [PubMed: 16172258]
87. Takatori H et al. Indispensable role of Stat5a in Stat6-independent Th2 cell differentiation and allergic airway inflammation. J. Immunol 174, 3734–3740 (2005). [PubMed: 15749913]
88. Kurata H, Lee HJ, O'Garra A & Arai N Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity 11, 677–688 (1999). [PubMed: 10626890]
89. Ouyang W et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity 12, 27–37 (2000). [PubMed: 10661403]
90. Yang Y, Ochando J, Yopp A, Bromberg JS & Ding Y IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. J. Immunol 174, 2720–2729 (2005). [PubMed: 15728480]
91. Kagami S et al. Stat5a regulates T helper cell differentiation by several distinct mechanisms. Blood 97, 2358–2365 (2001). [PubMed: 11290598]
92. Harada Y et al. The 3' enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells. Immunity 36, 188–200 (2012). [PubMed: 22365664]
93. Dell'Aringa M & Reinhardt RL Notch signaling represents an important checkpoint between follicular T-helper and canonical T-helper 2 cell fate. Mucosal Immunol. 11, 1079–1091 (2018). [PubMed: 29467447]
94. Auderset F et al. Notch signaling regulates follicular helper T cell differentiation. J. Immunol 191, 2344–2350 (2013). [PubMed: 23918982]
95. Tanaka S et al. The interleukin-4 enhancer CNS-2 is regulated by Notch signals and controls initial expression in NKT cells and memory-type CD4 T cells. Immunity 24, 689–701 (2006). [PubMed: 16782026]
96. Betz BC et al. BATF coordinates multiple aspects of B and T cell function required for normal antibody responses. J. Exp. Med 207, 933–942 (2010). [PubMed: 20421391]
97. Weber BN et al. A critical role for TCF-1 in T-lineage specification and differentiation. Nature 476, 63–68 (2011). [PubMed: 21814277]
98. Rooney JW, Hoey T & Glimcher LH Coordinate and cooperative roles for NF-AT and AP-1 in the regulation of the murine IL-4 gene. Immunity 2, 473–483 (1995). [PubMed: 7749982]
99. Tanaka Y, So T, Lebedeva S, Croft M & Altman A Impaired IL-4 and c-Maf expression and enhanced Th1-cell development in Vav1-deficient mice. Blood 106, 1286–1295 (2005). [PubMed: 15845902]
100. Urban JF Jr et al. IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite Nippostrongylus brasiliensis. Immunity 8, 255–264 (1998). [PubMed: 9492006]
101. McKenzie GJ, Bancroft A, Grencis RK & McKenzie AN A distinct role for interleukin-13 in Th2-cell-mediated immune responses. Curr. Biol 8, 339–342 (1998). [PubMed: 9512421]
102. Grünig G et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 282, 2261–2263 (1998). [PubMed: 9856950]
103. Bancroft AJ, McKenzie AN & Grencis RK A critical role for IL-13 in resistance to intestinal nematode infection. J. Immunol 160, 3453–3461 (1998). [PubMed: 9531306]
104. McKenzie GJ et al. Impaired development of Th2 cells in IL-13-deficient mice. Immunity 9, 423–432 (1998). [PubMed: 9768762]
105. Liu X et al. Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev. 11, 179–186 (1997). [PubMed: 9009201]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

106. Sawada S, Scarborough JD, Killeen N & Littman DR A lineage-specific transcriptional silencer regulates CD4 gene expression during T lymphocyte development. Cell 77, 917–929 (1994). [PubMed: 8004678]

107. Voehringer D, Reese TA, Huang X, Shinkai K & Locksley RM Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. J. Exp. Med 203, 1435–1446 (2006). [PubMed: 16702603]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

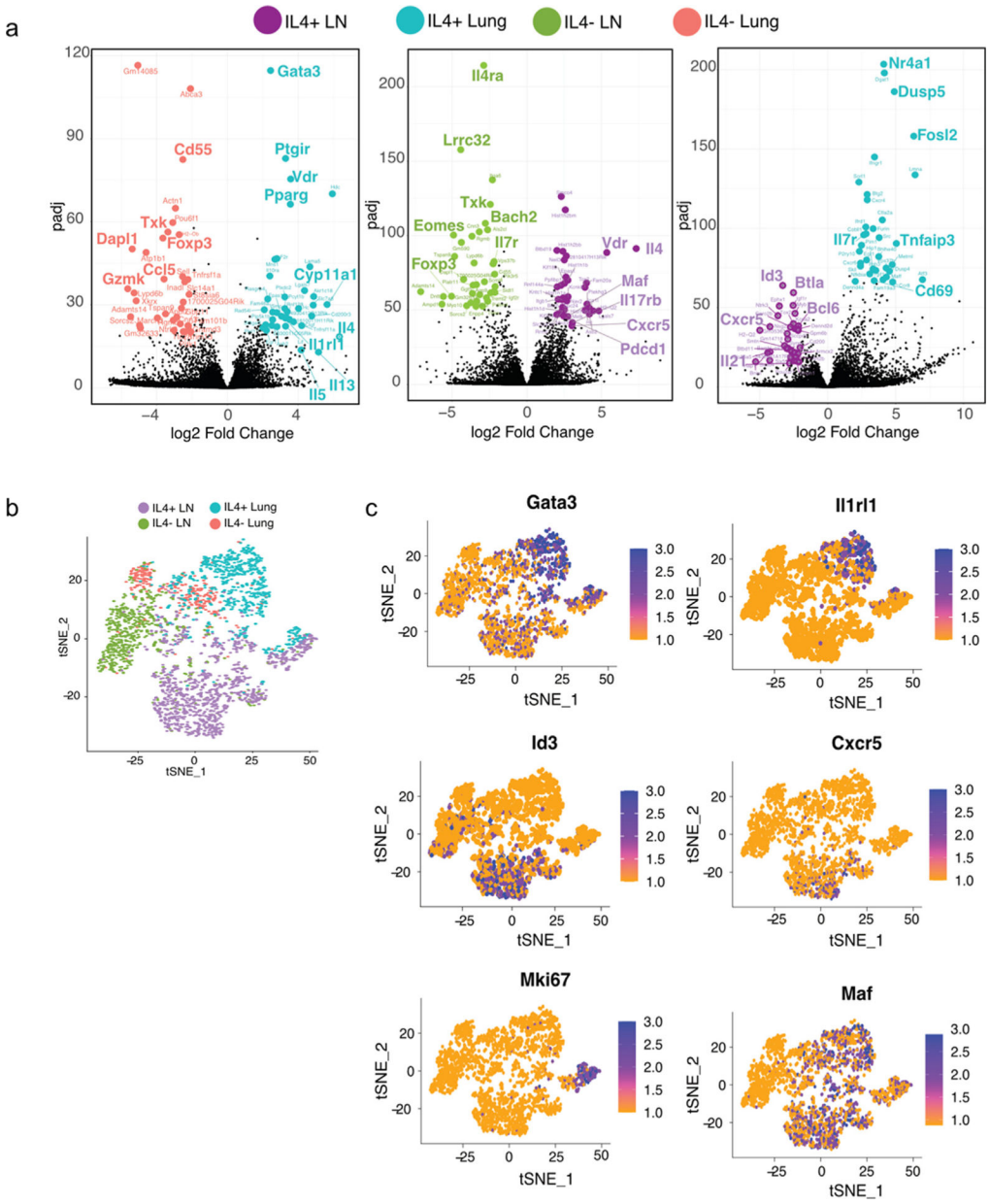

Fig. 1.

c-Maf mRNA is expressed equivalently in IL-4^{+} CD4^{+} T cells in the lymph nodes and lung after *N. brasiliensis* infection. Bulk and single-cell transcriptomic data of CD4^{+} T cells isolated from the lung and lymph nodes of IL4^{4get} C57BL6 mice infected with *N. brasiliensis* were assessed 9 days post-infection. (A) volcano plots represent the differentially expressed genes in the indicated bulk CD4^{+}-T cell population. Colored circles represent the top 30 most significant and differentially expressed genes within the indicated population. Genes of particular interest are indicated by increased font size; (B) t-SNE analysis of differentially expressed genes (p_{adj} <0.05) between GFP^{+} and GFP^{-} CD4^{+} T cells in the lung and mediastinal lymph node. Colors represent the four individual cell types isolated from the indicated tissues. Each dot represents an individual cell; C, accompanying

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

t-SNE plots show the relative co-expression of indicated gene. Orange to blue transition represents the relative expression of each gene. (A) $n = 6$, one mouse from six different infections; (B, C) $n = 1$ mouse that is paired with the above bulk RNA-sequencing data. CD = clusters of differentiation; GFP = Green Fluorescent Protein ; IL = interleukin; LN = lymph node; SNE = stochastic neighbor embedding.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

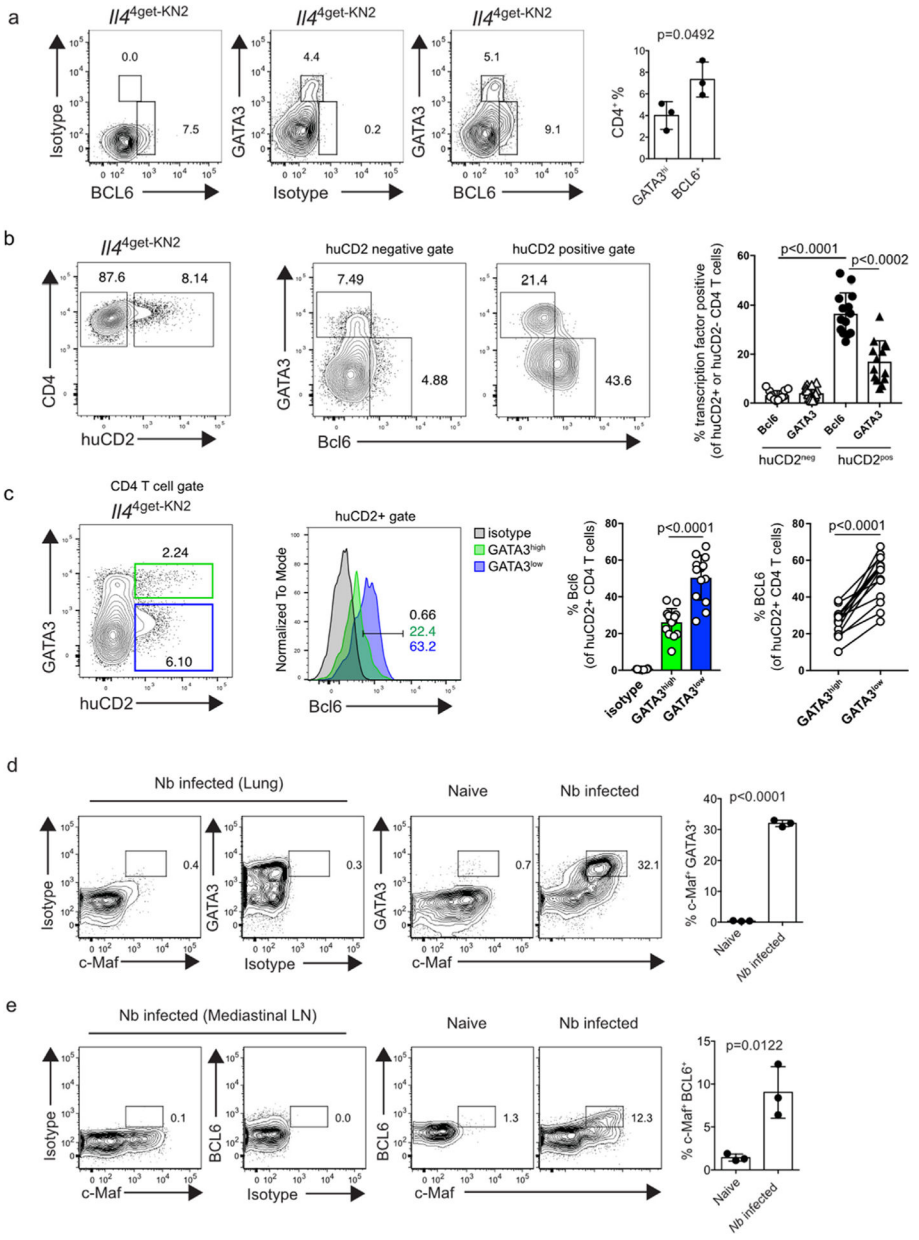

Fig. 2.

GATA3 and BCL6 expression delineate distinct IL-4-expressing Th2 and Tfh2 cells and c-Maf is expressed by both subsets of CD4$^+$ T cells. IL4$^{4get\text{-}KN2}$ or reporter negative control mice were infected with *Nippostrongylus brasiliensis* and the mediastinal lymph nodes were harvested and stained 9 days post-infection. A, contour plots and graph show the percentages of GATA3^{high} Th2 cells and BCL6$^+$ Tfh cells within CD4$^+$ T cells isolated from the mediastinal lymph node; (B) contour plots show the percentage of GATA3^{high} and Bcl6$^+$ cells among CD4$^+$ T cells falling into the huCD2$^+$ (IL-4$^+$) and huCD2$^-$ (IL-4$^-$) gates indicated in the plot to the left. Graph quantifies the percentage of transcription factor positive cells in each population; (C) contour plot of CD4$^+$ T cells from the draining mediastinal lymph node. Gates represent GATA3^{high} (green gate) and GATA3^{low} (blue gate)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

cells. Histogram and bar graph show the percentage of $Bcl6^{+}$ cells in each gate relative to isotype. Connected dots represent $GATA3^{high}$ and $GATA3^{low}$ populations from same lymph node. (D, E): Contour plots and graph represent the percentage of $GATA3^{high}$ Th2 cells in the lung (D) and $BCL6^{+}$ Tfh cells in the mediastinal lymph nodes (E) that co-express c-Maf. (A) Representative of four independent experiments (SD, $n = 3$/group); (B–C) combined data from six independent experiments (SD, $n = 1–3$ mice/group); (D, E) representative of two independent experiments (SD, $n = 3$/group). CD = clusters of differentiation; IL = interleukin; LN = lymph node; Th = T helper; Tfh = T follicular helper.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

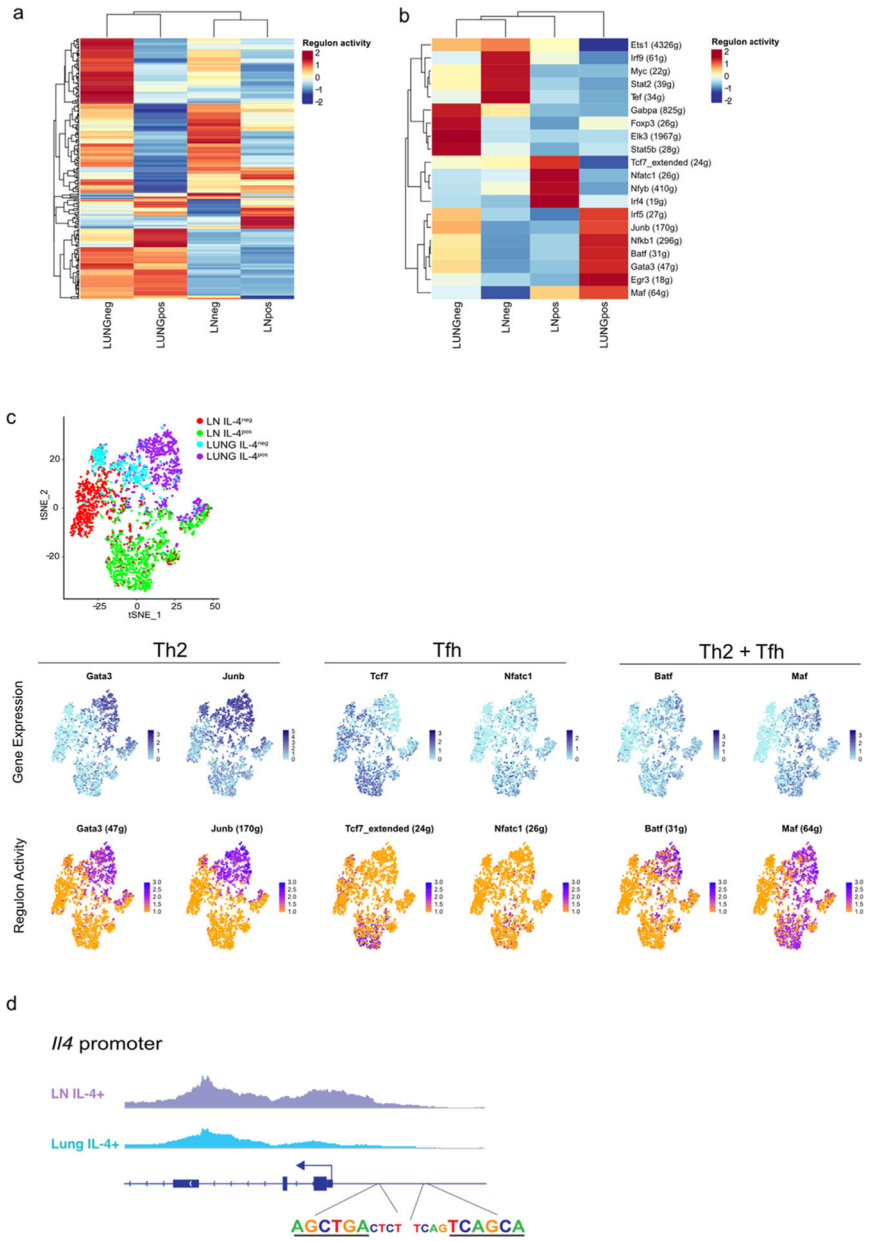

Fig. 3.

Transcription factor inference analysis identifies c-Maf as a key regulator of Tfh2 and Th2 cell fate and function. Single-cell regulatory network inference and clustering transcription factor analysis of single-cell RNA-sequencing data obtained from IL-4^{+} CD4^{+} T cells isolated from the lung and mediastinal lymph nodes 9 days after *Nippostrongylus brasiliensis* infection. (A) hierarchal clustering of the most differential regulons found IL-4^{+} and IL-4^{-} CD4^{+} T cell populations in the lung and lymph node; (B) heat map of selected regulons from (A) that associate with Tfh and Th2 fate and function; (C) selected gene expression and associated gene regulon activity in single cells as highlighted by tissue location provided in t-SNE plot; (D) association of half MARE c-Maf binding sites near open chromatin region surrounding the Il4 promoter in IL-4^{+} cells from the lymph node

(purple) and lung (aqua) 8 days after helminth infection. CD = clusters of differentiation; IL = interleukin; LN = lymph node; SNE = ; Th = T helper; Tfh = T follicular helper.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

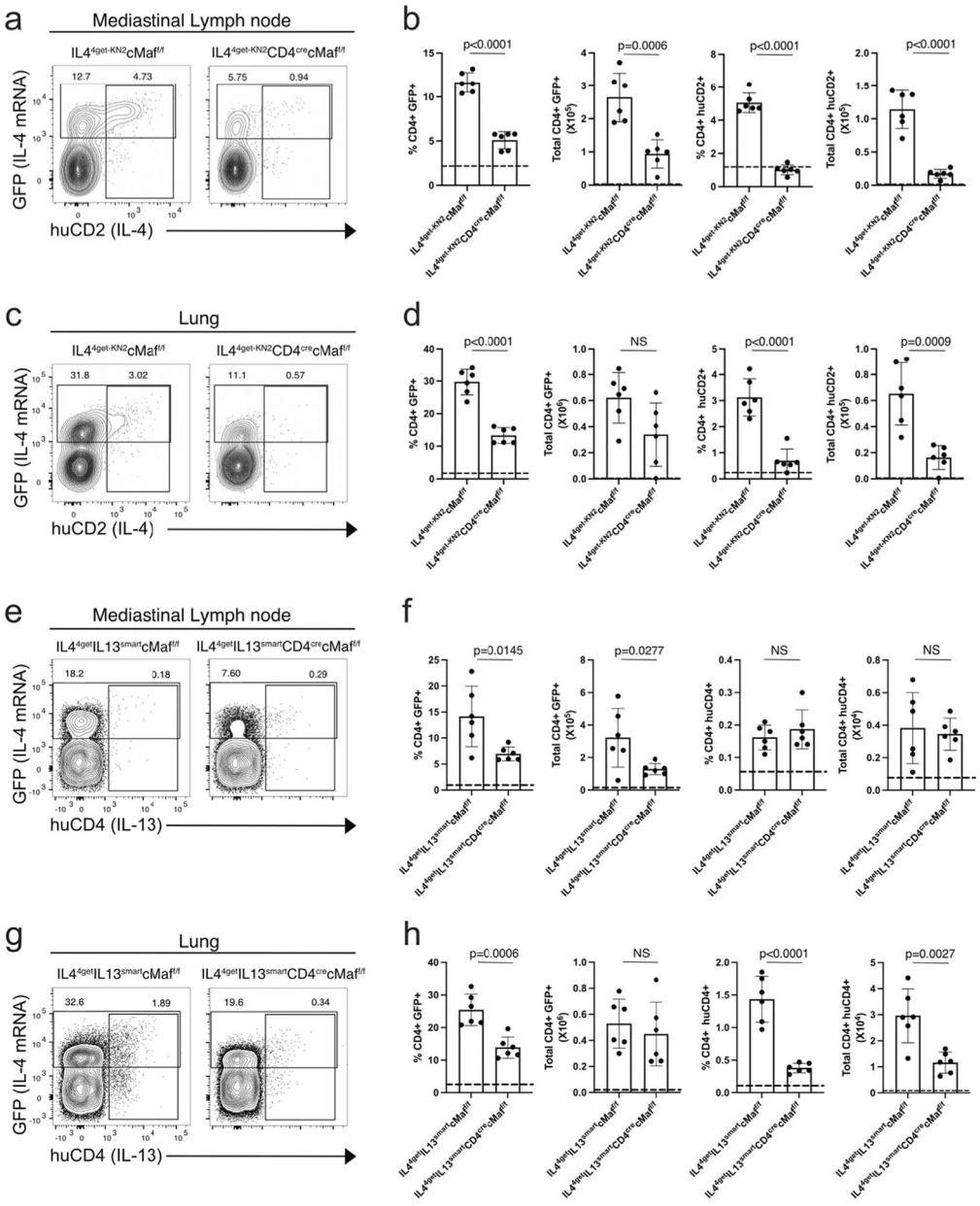

Fig. 4.

Deletion of c-Maf in T cells impairs Tfh2 and Th2 cell production of type-2 cytokines in response to *Nippostrongylus brasiliensis* infection. Mediastinal lymph nodes and lung were harvested 8 or 9 days after *N. brasiliensis* infection from mice with T cells sufficient ($IL4^{4get\text{-}KN2}cMaf^{fl/fl}$; $IL4^{4get}IL13^{smart}cMaf^{fl/fl}$) or deficient ($IL4^{4get\text{-}KN2}CD4^{cre}cMaf^{fl/fl}$; $IL4^{4get}IL13^{smart}CD4^{cre}cMaf^{fl/fl}$) in c-Maf expression. (A, E): Gates in contour plots represent the percentage of GFP^+; human $CD2^+$; or human $CD4^+$ $CD4^+$ T cells in the MedLN. (B, F): Graphs show the percentage and total number of IL-4-reporter$^+$ or IL-13-reporter$^+$ $CD4^+$ T cells gated in (A, E). (C, G) Gates in contour plots represent the percentage of GFP^+; human $CD2^+$; human $CD4^+$ $CD4^+$ T cells in the lung. (D, H) Graphs show the percentage and total number of IL-4-reporter$^+$ and IL-13-reporter$^+$ $CD4^+$

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

T cells gated in (C, G). (A-H) representative of two independent experiments (SD, n = 3–4 mice/group/experiment). (B, D, F, H) dashed line represents mean value of uninfected IL4$^{4get\text{-}KN2}$cMaf$^{fl/fl}$ or IL4^{4get}IL13^{Smart}cMaf$^{fl/fl}$ animals. CD = clusters of differentiation; IL = interleukin; NS = not significant; SNE = ; Th = T helper; Tfh = T follicular helper.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

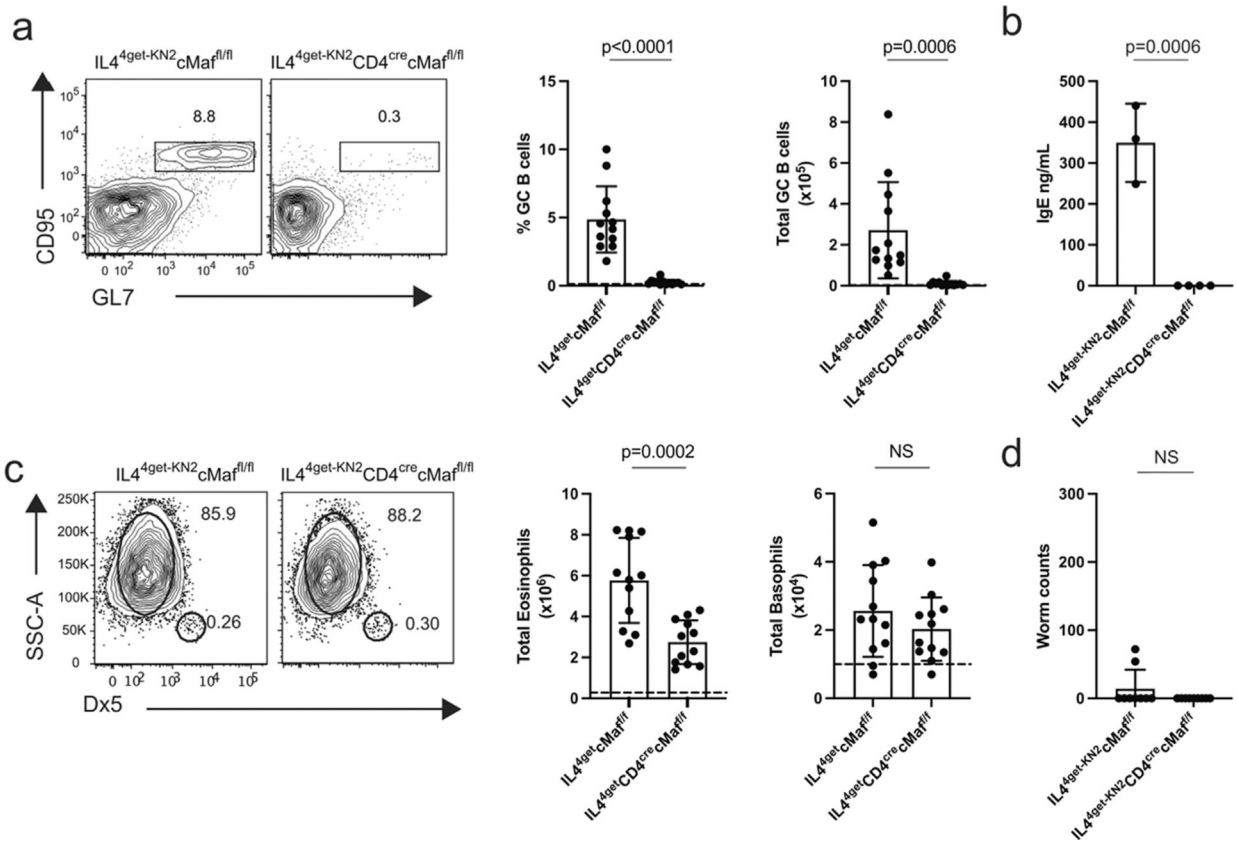

Fig. 5.

T cell-intrinsic deletion of c-Maf has a more pronounced impact on humoral versus cell-mediated hallmarks of type-2 inflammation. Mediastinal lymph nodes and lungs were harvested 8 or 9 days after *Nippostrongylus brasiliensis* infection from mice with T cells sufficient ($IL4^{4get}cMaf^{fl/fl}$) or deficient ($IL4^{4get}CD4^{cre}cMaf^{fl/fl}$) in c-Maf expression. $IL4^{4get\text{-}KN2}$ and $IL4^{4get}IL13^{Smart}$ backgrounds were combined unless otherwise stated to ensure results were not due to differences in IL-4 and IL-13 reporter backgrounds. A, Contour plots and graphs represent the percentage and total number of germinal center B cells among total B cells in the MedLN of indicated mice; B, graph represents the amount of IgE in the serum of mice 9 days post-*N. brasiliensis* infection; C, contour plots and graphs represent the total number of eosinophils and basophils in the lungs of indicated mice. (A, C) Combined data from four independent experiments (SD, $n = 3–4$ mice/group/experiment). (B) Representative of two independent experiments (SD, $n = 3–4$ mice/group). (D) Combined data from three independent experiments (SD, $n = 3–4$ mice/group/experiment). (A, C) Dashed line represents mean value of uninfected $IL4^{4get\text{-}KN2}cMaf^{fl/fl}$ and $IL4^{4get}IL13^{Smart}cMaf^{fl/fl}$ mice. CD = clusters of differentiation; IL = interleukin; NS = not significant; Th = T helper; Tfh = T follicular helper.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

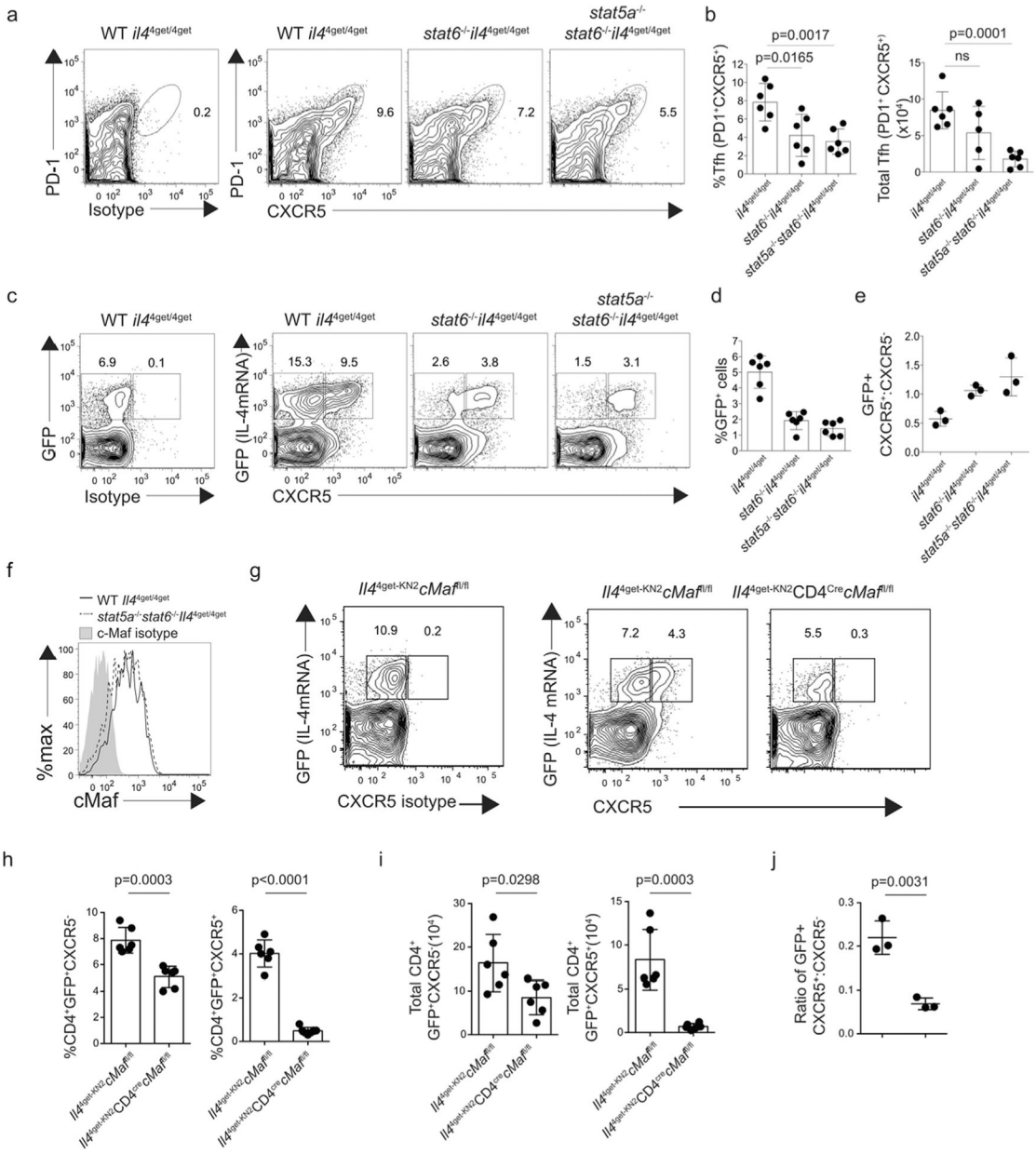

Fig. 6.

c-Maf regulates noncanonical IL-4 production in Tfh2 cells during *Nippostrongylus brasiliensis* infection. Wildtype, STAT6$^{-/-}$, and STAT5A$^{-/-}$STAT6$^{-/-}$ mice on the IL4^{4get} background were infected with *N. brasiliensis* and mediastinal lymph nodes were harvested 9 days after infection. (A) gating in contour plots reveals the percentage of CXCR5^{+} PD1^{+} Tfh cells of CD4^{+} T cells from indicated mice; (B) graphs show the percent and total number of CD4^{+} Tfh cells as gated in (A); (C) gates in contour plots show the percentage of CD4^{+} CXCR5^{+} GFP^{+} Tfh2 or CD4^{+} CXCR5^{-} GFP^{+} Th2 cells from the indicated MedLNs; (D) graph represents the percent of GFP^{+} CD4^{+} T cells among total CD4^{+} T cells in the MedLN of indicated mice. (D) Graph shows the ratio of CXCR5^{+} and CXCR5^{-} within GFP^{+} CD4^{+} T cells as gated populations in (C). (F) Histogram reveals

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the expression of intracellular c-Maf within GFP^+ $CD4^+$ T cells from the mediastinal LN of indicated mice. (G–I): Mediastinal lymph nodes were harvested 9 days after *N. brasiliensis* infection of $cMaf^{fl/fl}IL4^{4get\text{-}KN2}$ and $CD4^{cre}cMaf^{fl/fl}IL4^{4get\text{-}KN2}$ mice. (G) gates in contour plots represent the percentage of $CXCR5^+$ and $CXCR5^-$ $CD4^+$ T cells expressing GFP (IL-4) in the MedLN; (H, I) graphs represent the percentage and total number of $CXCR5^+$ or $CXCR5^-$ GFP^+ $CD4^+$ T cells in (A); (J) graph shows the ratio of $CXCR5^+$ and $CXCR5^-$ cells within GFP^+ $CD4^+$ T cells as gated in (G). (A-D) Data combined from two independent experiments (SD, $n = 3$ mice/group). (E, F) Representative of three and two independent experiments, respectively (SD, $n = 3$ mice/group). (G-I) Data combined from two independent experiments (SD, $n = 3$–4 mice/group). (J) Data representative of two independent experiments (SD, $n = 3$ mice/ group). CD = clusters of differentiation; GFP = ; IL = interleukin; NS = not significant; Th = T helper; Tfh = T follicular helper; WT, wild type.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

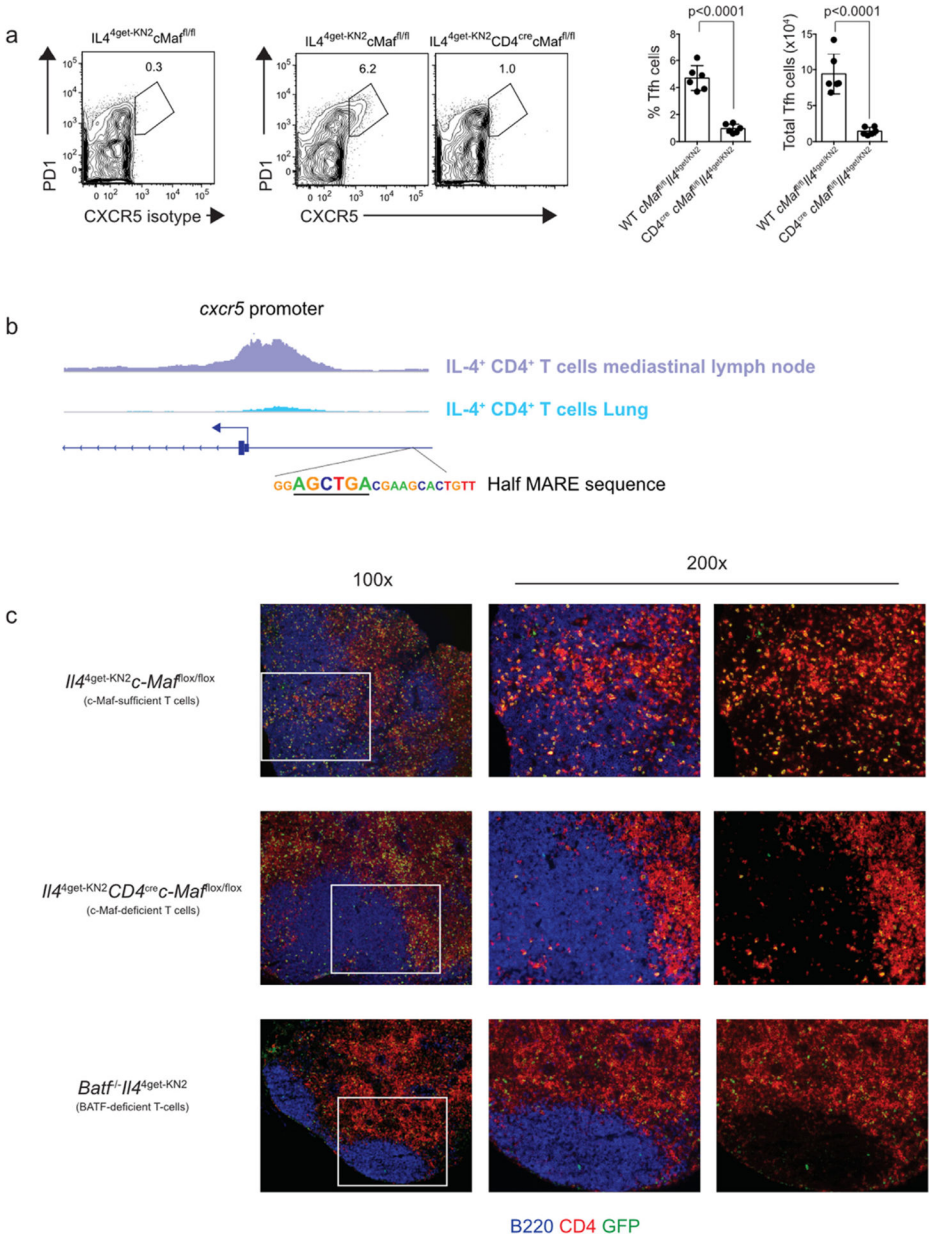

Fig. 7.

c-Maf is required for optimal Tfh cell generation, follicular migration, and humoral immunity to *Nippostrongylus brasiliensis* infection. Mediastinal lymph nodes were harvested 9 days after *N. brasiliensis* infection from mice with T cells sufficient ($IL4^{4get\text{-}KN2}cMaf^{fl/fl}$) or deficient ($IL4^{4get\text{-}KN2}CD4^{cre}cMaf^{fl/fl}$) in c-Maf expression. (A) contour plot and graphs show the percentage and total number of Tfh cells among $CD4^+$ T cells in the MedLN of indicated mice; (B) c-Maf binding sites and chromatin accessibility peaks located at the *Il4* promoter region. Peak represents mean ATAC-seq coverage for *Il4* loci in IL-4-expressing $CD4^+$ T cells from the lymph node. Line designates half MARE sequence; (C) images of tissue sections from mediastinal lymph nodes of indicated mice nice days after *N. brasiliensis* infection. Sections stained for B220 (blue), CD4 (red), and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

GFP (IL4; green). Left column 100x; middle and right columns 200x image of boxed insert shown in 100x image. (A) representative of two independent experiments (SD, n = 3–4 mice/group). (B) Images representative of 2–4 mice/group). CD = clusters of differentiation; GFP = ; IL = interleukin; NS = not significant; Th = T helper; Tfh = T follicular helper.