Transcriptional profiling identifies caspase-1 as a T cell–intrinsic regulator of Th17 differentiation

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Dendritic cells (DCs) are critical for the differentiation of pathogen-specific CD4 T cells. However, to what extent innate cues from DCs dictate transcriptional changes in T cells remains elusive. Here, we used DCs stimulated with specific pathogens to prime CD4 T cells in vitro and found that these T cells express unique transcriptional profiles dictated by the nature of the priming pathogen. More specifically, the transcriptome of in vitro C. rodentium–primed Th17 cells resembled that of Th17 cells primed following infection in vivo but was remarkably distinct from cytokine-polarized Th17 cells. We identified caspase-1 as a unique gene up-regulated only in pathogen-primed Th17 cells and discovered a critical role for T cell–intrinsic caspase-1, independent of inflammasome, in optimal priming of Th17 responses. T cells lacking caspase-1 failed to induce colitis or confer protection against C. rodentium infection due to suboptimal Th17 cell differentiation in vivo. This study underlines the importance of DC-mediated priming in identifying novel regulators of T cell differentiation.

Introduction

CD4 T helper (Th) cells play a central role in adaptive immunity by producing specific effector cytokines (Zhu et al., 2010). The differentiation of Th in vivo in response to a pathogen (henceforth referred to as pathogen-specific Th cells) results in a heterogeneous effector population (Sallusto, 2016). The frequency of naive precursors for specific epitopes is extremely low, ranging from 0.8 to 10 epitope-specific cells per million naive CD4 T cells (Jenkins and Moon, 2012). Due to the absence of tools available to identify all responding effector T cells, it has been challenging to detect, track, and analyze newly differentiated pathogen-specific Th cells following infection in vivo (O’Shea and Paul, 2010). Functionally, high degrees of heterogeneity and plasticity are the hallmark of pathogen-specific Th cells (Sallusto, 2016). Phenotypically plastic T cell subtypes (e.g. Th1/Th17 dual-lineage cells) emerge following infection and can perform either pathogenic or regulatory roles (McGeachy et al., 2007; Harbour et al., 2015), but identifying and tracing these cells following pathogen-specific activation and understanding their development remain to be explored.

Although dendritic cells (DCs) are major drivers of Th cell activation and differentiation in vivo, lineage-specific polarization by using defined cytokine cocktails has been a major approach to study CD4 T cell biology. However, a broad range of DC-derived cues that might affect differentiating T cells is not present in such an approach (Jain and Pasare, 2017). For example, DCs have been reported to impact the fate of Th cell differentiation by altering TCR signaling strength or by licensing the expression of transcription cofactors and regulatory microRNAs (Zhang et al., 2016; Tubo et al., 2013). Importantly, the influence of DCs on T cell fate goes beyond the initial activation phase. The generation of T cell heterogeneity and the formation of T cell memory rely on DC-derived cues (Sallusto, 2016; Iborra et al., 2016; Shen et al., 2010; MacLeod et al., 2010), but whether DCs alone are sufficient to elicit these changes in T cells remains unclear.

To understand if DCs exposed to different pathogens regulate the transcriptional profile of CD4 T cells during differentiation, we used an in vitro approach to prime naive CD4 T cells. This approach allows for an unbiased assessment of pathogen-specific clonal expansion and differentiation of naive CD4 T cells. We have found that in vitro priming was able to generate pathogen-specific Th cells and that the effector lineage

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commitment was dictated by the nature of the priming pathogen. Comparison of the transcriptional profile of cytokine-polarized and Citrobacter rodentium (Cr)–primed Th17 cells led to the identification of a unique gene cluster associated with DC-mediated priming. Among this cluster, we identified caspase-1 to be one of the unique genes up-regulated in pathogen-primed Th17 (ppTh17) cells and discovered that it functions in a T cell–intrinsic fashion to promote Th17 differentiation. This study establishes that DCs provide critical cues for transcriptional programming of CD4 T cells, which are absent during cytokine-driven polarization, and furthermore provides a framework for identifying novel regulators of CD4 T cell differentiation.

Results and discussion

In vitro priming system generates pathogen-specific Th cell responses

We aimed to study the transcriptional regulation of pathogen-specific Th cell differentiation. Recent reports have highlighted a system of in vitro priming and de novo differentiation of pathogen-specific human CD4 T cells by autologous monocytes, although the specificity and functionality of the primed T cells were not fully established (Zielinski et al., 2012; Becattini et al., 2015). Building on these previous ideas, we sought to validate a murine in vitro priming system to mimic in vivo priming of naive CD4 T cells following microbial infections. As DCs play a central role in T cell differentiation (Iwasaki and Medzhitov, 2015), we designed the in vitro system using splenic CD11c+ DCs (Flt3L dependent, ex vivo) to prime naive CD4 T cells isolated from the spleen and peripheral lymph nodes of WT mice (Fig. S1 A). Whole-cell lysates of bacteria were used to activate DCs. This bacterial lysate containing diverse pathogen-associated molecular patterns and proteins acted as pattern recognition receptor stimuli and the source of antigen without the addition of any exogenous peptide or recombinant cytokine. Subsequently, DCs were cocultured with naive CD4 T cells to initiate their differentiation (Fig. 1 A). We argue that this simple, reductionist system that contains core elements required for T cell priming should allow the differentiation of pathogen-specific Th cells. Indeed, a proportion of cocultured naive CD4 T cells proliferated (CFSE$^+$CD90$^+$), up-regulated activation markers, and produced effector cytokines, while unexpanded T cells (CFSE$^+$CD90$^+$) remained in a naive state (Fig. 1 B). A large proportion of CFSE$^{hi}$ cells suggested a very low frequency of naive T cells undergoing clonal expansion, in contrast to polyclonal priming by CD3 ligation (Fig. S1 B). Accordingly, in vitro T cell priming required peptide MHC class II (MHCIi)/TCR interaction, costimulation, and the innate cytokine milieu, indicating a stringent requirement for the three signals for T cell activation (Fig. S1, C and D).

We tested whether this approach can differentiate functional pathogen-specific Th cells by using well-studied mouse pathogens: Listeria monocytogenes (Lm), Cr, and Staphylococcus aureus (Sa). All three induced significant T cell expansion (Fig. S1 E) but generated distinct cytokine profiles. Cr-primed T cells contained significantly more IL-17A$^+$ cells, while Lm generated predominantly IFNγ$^+$ T cells (Fig. 1, C and D). Sa-primed T cells contained both IFNγ$^+$ and IL-17A$^+$ subsets (Fig. 1, C and D).

We further examined the transcriptome of Lm- and Cr-primed CFSE$^-$ T cells (Fig. 1 E). Cr-primed T cells exhibited a Th17-associated gene signature, while Lm-primed T cells contained a high level of Th1-associated transcripts. We also identified genes uniquely expressed in Lm- or Cr-primed T cells, indicating a pathogen-dictated T cell transcriptome (Fig. 1 E and Table S3). Furthermore, DCs stimulated with gut pathobiont Bacteroides fragilis (Bf) were able to induce robust proliferation of T cells and prime high levels of Th1/Th17 differentiation. In contrast, nonpathogenic commensal Lactobacillus acidophilus (La) resulted in significantly lower T cell responses (Figs. 1 F and S1 F). These data establish that the in vitro priming system faithfully recapitulates the in vivo abilities of pathogens and commensal bacteria to induce differential T cell responses.

Importantly, pathogen-primed Th (ppTh) cells were able to secrete cytokines (IFNγ/IL-17A) or up-regulate T cell activation markers only when restimulated in vitro with the priming pathogen, indicating their specificity (Fig. S1, G–I). We further tested the specificity of these T cells in vivo by transferring Lm- or Cr-primed CD45.2$^+$ T cells into congenic recipients and challenged them with Lm 48 h after cell transfer (Fig. 1 G). Uninfected mice that received T cells served as baseline controls. Lm-primed, but not Cr-primed, donor T cells proliferated and up-regulated ICOS (inducible T cell costimulator) in response to Lm rechallenge (Fig. 1, H and I; and Fig. S1, J and K). In summary, these data demonstrate that the in vitro priming system generates a pathogen-specific Th cell response and, therefore, presents a reliable system to further interrogate the nature of these responses. Future work can be done to improve this in vitro priming system by including tissue-resident or migratory DC populations, such as lamina propria (LP) CD103$^+$ DCs or skin Langerhans cells, or through the addition of tissue-specific microenvironmental cues, when investigating CD4 T cell responses to tissue invading or compartmentalized pathogens.

DC-mediated priming dictates the distinct transcriptional profile of Th17 cells

Th17 cells are sensitive to microenvironmental cues and display a high degree of plasticity and heterogeneity (Hu et al., 2011; Ghoreschi et al., 2011; Gagliani et al., 2015). This intrinsic fine-tuning of Th17 cells prompted us to dissect their transcriptional regulation during pathogen-specific differentiation. We generated 17A-fm mice (Il17a$^{creER26}$tdTomato$^+$) to fate map IL-17A$^+$–producing cells (Hirotta et al., 2011). Cr-specific Th cells contained a higher percentage of tdTomato$^+$ population than Lm-specific Th cells, reflecting the same Th17 bias found previously (Fig. 1, C and D; and Fig. S2 A). Expression of Th17-associated cytokines and transcription factors (TFs) was highly enriched in the CFSE$^-$tdTomato$^+$ fraction, indicating consistent tracing of Th17 population (Fig. S2 B).

Th17 cells generated by TCR-activating antibodies and cytokine cocktails (cytokine differentiated, cdTh17), have been widely used for transcriptome profiling (Yosef et al., 2013;
Ciofani et al., 2012). To understand transcriptional programming of pathogen-specific Th17 cells, we isolated tdT+ populations from cdTh17 cells or Cr-primed Th17 cells (ppTh17 cells) using i7A-fm mice and subjected these cells to RNA sequencing (RNA-seq) analysis (Fig. 2 A). We also interrogated the relationship between these in vitro–generated Th17 cells and Th17 cells primed in vivo following Cr infection (ex vivo tdT+; Fig. 2 A). Such a comparison would serve as an important validation of the in vitro priming system.

Sample-to-sample Euclidean distance indicates that ppTh17 cells were closely related to ex vivo tdT+ cells but highly divergent from cdTh17s (Fig. 2 B). We next focused on differentially regulated genes between the aforementioned three types of Th17 cells. The majority (871 genes) of the 1,133 ppTh17 signature genes (compared with cdTh17 cells) were also shared by ex vivo tdT+ cells (Fig. 2 C and Table S4). Less than 50% (935 of 1,938 genes) of cdTh17 up-regulated genes overlapped with either ppTh17 or ex vivo tdT+ cells, confirming that it deviated significantly from the other two Th17 populations (Fig. 2 C). Pathway analysis of these gene sets indicated that ppTh17/ex vivo tdT+ cells expressed higher levels of genes related to diverse T cell effector functions, while cdTh17s up-regulated cell cycle–related genes (Fig. 2 C and Table S5).

ppTh17 and ex vivo tdT+ cells exhibited lineage plasticity by expressing Th1- and Th2-associated TFs and cytokines (Tbx21, Gatn3, Ifng, Il4, and Il10), while cdTh17 cells were characterized by polarized expression of Th17-lineage transcripts (Fig. 2 D). Indeed, ppTh17 and ex vivo tdT+ cells generated using 17-γ double reporter mice (Il17aR26tdT+IfngYFP) contained a significant population of tdT+YFP+ cells, indicating Th1 differentiation from committed Th17 lineage (Fig. S2, C and D). Overall, these data indicate a close functional relationship between ppTh17 and in vivo–primed Th17 cells and suggest that the cdTh17 does not resemble the physiologically generated Th17 population.

Additionally, ppTh17/ex vivo tdT+ cells expressed various chemokine receptor genes, potentially associated with their migration capacity (Fig. 2 E, Table S5, and Table S6). ppTh17/ex vivo tdT+ also highly expressed Il7r and Il15ra, suggesting that T cell memory formation was supported in these contexts (Fig. 2 E; Purton et al., 2007; Li et al., 2003). These cells also exhibited metabolic changes by switching to 5′ AMP-activated protein kinase (AMPK) pathway, fatty acid oxidation, and oxidative phosphorylation programs, whereas cdTh17 cells up-regulated c-Myc and amino acid metabolism genes that support proliferation (Fig. 2, F and G; Waickman and Powell, 2012; Wang et al., 2011). These transcriptional features indicate a lower activation amplitude in physiologically derived Th17 cells that typically maintain memory T cell functions, while cdTh17 cells resemble terminally differentiated effector T cells (Fig. 2, F and G; Patsoukis et al., 2016). Gene set enrichment analysis (GSEA) confirmed an enriched memory T cell signature in ppTh17s and an enriched effector signature in cdTh17s (Fig. 2 H and Table S7). The expression of specific ppTh17 and cdTh17 genes was validated by flow cytometry and quantitative RT-PCR (qRT-PCR; Figs. 2 I and S2 E). Cumulatively, these data suggest that DCs influence transcriptional programming of T cells that extends beyond the provision of activation signals and innate cytokines. CD4 T cells differentiated by pathogen-stimulated DCs, such as ppTh17s and ex vivo Th17s, showed transcriptional profiles that display lineage plasticity and features of memory differentiation, while Th17 differentiation driven by defined cytokines led to gene programs indicative of terminal differentiation and devoid of plasticity.

T cell–intrinsic caspase-1, independent of its enzymatic function, is critical for an optimal Th17 response

It remains unclear how DCs precisely regulate T cell differentiation beyond the provision of the classic three signals of T cell activation. To identify novel regulatory factors, we focused on genes uniquely expressed in ppTh17s but not in cdTh17s. Casp1 (caspase-1) emerged as a candidate, with 5–10-fold induction of expression in ppTh17/ex vivo tdT+ cells compared with cdTh17 or naive T cells (Fig. 3, A and B). Caspase-1 and caspase-11 are inflammatory caspases that have overlapping effector functions downstream of inflammasome activation, primarily in myeloid cells (Shi et al., 2014; Mariathasan et al., 2004). However, the role of these caspases in T cells is not well defined. Casp1 and Casp11 transcripts were up-regulated in memory T cells, but Casp11 levels were not significantly different between ppTh17s and cdTh17s, indicating that Casp11 may be up-regulated upon T cell activation, while Casp1 is specifically induced by interacting DCs (Figs. 3 A and S2 F). Therefore, we used Casp1A10 mice, which specifically lack caspase-1 but not caspase-11 (Rauch...
et al., 2017). We found defective Th17 differentiation in Casp1Δ10 T cells following in vitro priming with Cr- and Bf-stimulated WT DCs (Fig. 3, C and D; and Fig. S2 G). Interestingly, Th1/Th2 lineage commitments and proliferation were unaffected (Figs. 3 E and S2 H). These results prompted us to further examine the role for T cell–intrinsic caspase-1 in Th17 responses.

The increase of Casp1 mRNA over time correlated with the expansion of the tdT+ signal, with the highest caspase-1 protein...
Figure 3. **Caspase-1 promotes the differentiation of Th17 lineage independent of its enzymatic activity or inflammasome activation.** (A) Differentially expressed transcripts between ppTh17 and cdTh17 cells. Each dot represents the average of three independent experiments. Blue dots indicate differentially regulated genes (fold change >1.5, false discovery rate <0.05). Black dots indicate differentially regulated transcripts described in Fig. 2. Red dots indicate Casp1 and Casp11 transcripts. (B) mRNA expression of Casp1 relative to 18s rRNA in sorted naive (CFSE+), ppTh17, cdTh17, or ex vivo tdT+ cells from mLNs of Cr-infected 17A-fm mice (10 dpi), quantified by independent qRT-PCR experiments. n=2. (C) Naive CD4 T cells from WT or Casp1Δ10 (Δ10) mice were primed in vitro by Cr lysate–stimulated WT splenic CD11c+ DCs, and IL-17A– and IFNγ–producing cells were measured by intracellular cytokine staining and flow cytometry analysis of CD90+CFSE− live CD4 T cells (left); IL-17A+ percentages were quantified (right). n = 7. (D) IL-17A in the supernatant from experiments in C, measured by ELISA. n=3. Δ10, Casp1Δ10. (E) IFNγ+ and IL-13+ percentage of CD90+CFSE− live cells, quantified from experiments in C. n = 7. Δ10, Casp1Δ10. (F) Relative expression (normalized to 18s rRNA and relative to day 3) of Casp1 mRNA at indicated time points after Cr-priming. n = 4. (G) Western blot analysis of Casp1Δ10, Casp1Δ10.
of procasase-1 (p45) and cleaved caspase-1 (p20) from naive CD4 T cells, sorted CD90+CFSE- Cr-primed Th cells, or WT bone marrow–derived macrophages (MbD) that were unstimulated or under conventional inflammasome activation (4 h LPS + 30 min ATP [L+A]). (f) Naive WT, Casp1Δ10 (Δ10), Pycard+/−; Il1b+/−; CD4 T cells were primed with Cr-stimulated WT DCs. IL-17A+ percentage of CFSE CD90+ live cells was measured by intracellular cytokine staining and quantified. n = 5. (g) Casp1Δ10 CD4 T cells were differentiated to Th17 lineage and retrovirally reconstituted with MSCV-ires-hcd2 alone (Vector), full-length Casp1 (FL), Casp1 deficient of CARD (Casp1ΔCARD), or enzymatically (Enz) inactive form of Casp1 (EnzDead, C284A) and quantified for IL-17A+ percentage (gated on live, hCD2+ population). n = 4. (h) Log (fold change) of major Th cell cytokine and TF expression comparing WT and Casp1Δ10 Δ10-specific Th cells. WT or Casp1Δ10 naive CD4 T cells were primed with Cr-stimulated WT DCs for 10 d. CFSE CD90+ population was FACS-sorted and subjected to mRNA-seq and analysis. n = 2. (i) Heatmap and hierarchical clustering of differentially expressed (>1.5 fold) genes comparing WT and Casp1Δ10 Δ10-Cr-specific Th cells. Genes of interest were labeled by the heatmap. Each column represents one independent replicate. (j) Activation z-score of pathways enriched in WT or Casp1Δ10 Cr-specific Th cells. Pathway analysis was performed using Ingenuity Pathway Analysis. Data are representative of or combined from two to seven independent experiments. Statistics represent mean ± SEM, and P values were determined by paired Student’s t test (C–E and H) or one-way ANOVA with Tukey correction (I). ns, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Casp1Δ10 T cells exhibit increased fatty acid metabolism and decreased Th17 gene signature

To gain insight into how caspase-1 is regulating Th17 differentiation, we profiled in vitro–differentiated Cr-specific WT or Casp1Δ10 CD4 T cells by mRNA-seq. Consistent with the previous observation, we found reduced expression of Th17 cytokines and TFs in Casp1Δ10 T cells (Fig. 3 J). Ifng expression was also lower in Casp1Δ10 T cells, possibly due to a reduced IFNgIL-17A population, while Th1 TF Tbx21 was not significantly affected (Fig. 3 J). Th2 and Tr reg genes were increased, suggesting a shift in Th cell balance in the absence of caspase-1 (Fig. 3 J). We analyzed differentially expressed transcripts (Table S8), and found that genes involved in fatty acid uptake (Cd36) and utilization (Slc27a2, Slc27a5, Acat3, Cpt1b, and Acaulb) were up-regulated in Casp1Δ10 T cells (Fig. 3 K). Indeed, PPARα and AMPK targets were enriched in Casp1Δ10 CD4 T cells (Fig. 3 L), suggesting a metabolic change to exogenous fatty acid metabolism that potentially inhibits Th17 differentiation (Berod et al., 2014). Additionally, iNOS response, reported to negatively regulate Th17 response (Yang et al., 2013), was also elevated in Casp1Δ10 T cells (Fig. 3 L). In summary, we find that Casp1Δ10 T cells exhibit an altered metabolic state that may negatively regulate Th17 differentiation. The detailed mechanism will require further investigation.

T cell–intrinsic caspase-1 is required for Th17-mediated host protection and autoinflammatory disease

Although the absence of T cell–intrinsic caspase-1 impaired the generation of ppTh17 cells, cdTh17 cells were unaffected, thus highlighting the differential regulation present in cdTh17 versus physiologically generated Th17 cells (Fig. S3, A and B). This prompted us to examine the in vivo function of T cell–intrinsic caspase-1 in host protection against Cr infection. To this end, we transferred CFSE-labeled WT or Casp1Δ10 naive CD4 T cells to Rag1−/− mice and subsequently challenged them with Cr (Fig. 4 A).
Transferred WT or CaspΔ10 T cells proliferated at a similar level (Fig. S3 C). However, CaspΔ10 T cells showed defective commitment to Th17/Th22 cells as well as fewer IFNγ+IL-17A+ cells in the mesenteric lymph nodes (mLNAs), but normal Th1 (IFNγ+IL-17A+) differentiation (Fig. 4, B and C). Consequently, transfer of WT CD4 T cells, but not CaspΔ10 T cells, protected mice from Cr colonization compared with nontransferred mice (Fig. 4 D). Transfer of WT, but not CaspΔ10 T cells, was also able to prevent stool inconsistency and increased stool fluidity (Fig. 4 E), a reported consequence of Cr-induced diarrhea (Viswanathan et al., 2009). Cytokine responses from the CD90+CD4+ population (presumably innate lymphoid cells [ILCs]) were not significantly different between WT or CaspΔ10 T cell transferred mice (Fig. S3 D), suggesting that T cell–intrinsic caspase-1 was specifically mediating protection against Cr infection.

To examine the broad role of caspase-1 in Th17-mediated immune responses, we transferred WT, CaspΔ10, or Il1β−/−CD45RBΔ− CD4 T cells to Rag1−/− mice to test their ability to induce colitis (Ostanin et al., 2009). In this model, transferred naïve CD4 T cells differentiate in response to gut microbiota (Matsuda et al., 2000). Recipients of CaspΔ10 T cells developed a mild disease, while WT or Il1β−/− T cell recipient mice developed measurable colitis, including significant weight loss (Fig. 5, A and B) and colon shortening (Fig. 5 C). CaspΔ10 recipients also exhibited ameliorated colonic pathology, with less transmural infiltration of leukocytes and fewer submucosal morphological changes compared with WT (Figs. 5 D and S3 E). We isolated ex vivo CD90+CD4+ T cells from colonic LP 3 wk after transfer and did not find cleaved caspase-1 (detected by FAM−ILCS staining) in these cells (Fig. S3, F and G). Previous reports have suggested that caspase-1, in some cases, can contribute to the secretion of IL-1α as well as IL-1β that is independent of its cleavage and catalytic activity (Conos et al., 2016; Gross et al., 2012). We have excluded the role of IL-1α and IL-1β in vitro (Fig. S2 M) and T cell–derived IL-1β in vivo (Fig. 5 A). However, it is possible that IL-1α produced by T cells in vivo could contribute to colitis, and this remains to be investigated. Furthermore, the reported profile of caspase-1 substrates includes a broad range of cellular proteins and enzymes (Shao et al., 2007). Therefore, uncleaved caspase-1 could also mediate the release of unconventional substrates from T cells that act in cis and lead to Th17 differentiation.

In the mLN and colon LP, we found a reduced frequency of pathogenic IL-17A−IFNγ− cells in CaspΔ10 compared with WT T cell recipients, consistent with the established role of these cells in the induction of T cell–mediated colitis and the development of pathology (Fig. 5 E; Harbour et al., 2015). Of note, there was no difference in the proportion of IL-17A−IFNγ− nonpathogenic Th17 or IL-17A−IFNγ− Th1 cells between WT and CaspΔ10 recipients (Fig. S3, H and I). The total percentage and numbers of CD4+CD90+ cells were comparable between the two genotypes (Fig. S3 J), confirming that caspase-1 deficiency did not affect T cell proliferation or cell death. Additionally, we observe significantly less splenic expansion of total IL-17A+ CaspΔ10 cells (Fig. 5 F), consistent with the reduced circulating IL-17A levels throughout the course of the disease (Fig. 5 G). Collectively, these data extend the function of caspase-1 to the differentiation of autoinflammatory Th17 cells. Earlier studies have reported ameliorated autoimmune disease in caspase-1–deficient animals and attributed it to abrogated IL-1β and IL-18 production during inflammasome activation (Furlan et al., 1999; Sieg mund et al., 2001). Our results suggest that impaired Th17 commitment, resulting from T cell–intrinsic deficiency of caspase-1, could also contribute to these phenotypes. Since the majority of these studies used whole-body knockout mice, it would be necessary to dissect the specific roles of caspase-1 in innate and adaptive immune compartments in regulating Th17 differentiation and associated disease outcomes.

Taken together, our work highlights a novel workflow for studying pathogen-specific Th cell differentiation. By combining the in vitro priming system with genetic fate mapping and mRNA-seq, we were able to profile the transcriptome of Cr-specific Th17 cells. Conceptually, the dataset from this study provides experimental evidence for the importance of DCs in dictating global transcriptional programming of pathogen-specific Th cells. Integration of a systems biology approach into this in vitro priming system will empower high-throughput analysis of antimicrobial T cell responses to discover novel players, such as caspase-1, in CD4 T cell differentiation.

Materials and methods
Animals
C57BL/6J (WT), B6.SJL-Ptprc<sup>−/−</sup> Pepc<sup>b</sup>/Boj/ (CD45.1<sup>+</sup>), and B6.129S7-Rag1tm1Mom/J (Rag1<sup>−/−</sup>) mice were obtained from Jackson Laboratory and maintained in the University of Texas (UT) Southwestern mouse breeding core facility. STOCK Il17atm1.1(cre)Stck/J (Il17a<sup>Cre</sup>) and B6.129S4-Ifngtm3.1Lky/J (Ifng<sup>YFP</sup>) mice were obtained from Jackson Laboratory and bred in-house to B6.Cg-Gt(Rosa26Sor<sup>tm14(CAG-tdTomato)Hze</sup>/J) (R26<sup>tdTomato</sup>) mice (a gift from Morrison Laboratory, UT Southwestern). CaspΔ10 mice were a kind gift from Drs. Russell Vance and Isabella Rauch (University of California, Berkeley, CA) and were maintained on Jackson Laboratory C57/BL6J background. PyCARD<sup>−/−</sup> mice were provided by Dr. Vishva Dixit (Genentech, South San Francisco, CA). Il1b<sup>−/−</sup> mice were provided by Dr. Fayyaz S. Sutterwala (Cedars Sinai Medical Center, Los Angeles, CA). Unless specified, mice were bred and maintained at the specific pathogen–free facility of UT Southwestern Medical Center and provided with sterilized food and water ad libitum. Mice used for infection experiments were kept at a conventional animal facility and provided with nonautoclaved food and water ad libitum. Age- and sex-matched mice 6–12 wk of age were used for all experiments. Both female and male mice were used in experiments. All mouse experiments were performed as per protocols approved by Institutional Animal Care and Use Committee at UT Southwestern Medical Center.

Bacterial strains and infection
Lm (LM 10403 serotype 1, a gift from Dr. James Forman, UT Southwestern Medical Center, Dallas, TX), Cr (strain ICC168, Nalidixic acid-resistant) and Sa (ATCC-25923) were cultured in
agar plate of brain-heart infusion, Luria-Bertani, with 30 µg/ml nalidixic acid, and tryptic soy broth, respectively. A single colony was chosen and secondarily expanded in the respective liquid broth with appropriate antibiotics. Bf and La were cultured as described previously (Fan et al., 2015). For Lm infection, bacteria were grown to log phase (OD600 = 0.6–1) on the day of infection, extensively washed, and resuspended in PBS. Mice were injected i.p. with 2 × 10^4 CFU of Lm. Tissues were harvested 5 d postinfection (dpi). For Cr infection, mice were intragastrically administered 1% sodium bicarbonate and, 20–30 min later, infected with 2–5 × 10^8 CFU of Cr. mLNs were harvested at 10 dpi.

**Extraction of bacterial lysates and protein quantification**

Bacterial cultures were grown to late log phase (OD600 = 0.8–1.2), washed extensively, and resuspended in PBS. Bacteria suspensions were subjected to three cycles of snap-freeze-thaw in liquid nitrogen and lysed by repeated sonication. Lysates were centrifuged at 4,000 rpm for 20 min to remove insoluble components, and the supernatant was sterilized through a 0.22-µm
membrane filter. Protein concentration was measured for cleared lysate by Bradford assay.

Expansion of mouse splenic DCs by B16-FLT3L implant
B16-FLT3L mouse melanoma cell lines were maintained in 10% complete RPMI (RPMI [Hyclone], 10% FBS [Sigma-Aldrich], L-glutamine, penicillin-streptomycin, sodium pyruvate, and β-mercaptoethanol [Sigma-Aldrich]). For the generation of spleen-derived DCs, 5 × 10⁶ B16-FLT3L cells were injected subcutaneously into the flank of mice. After 10-16 d, spleens were harvested and used for CD11c⁺ DC isolation. B16-FLT3L injection did not induce CD11c⁺ DC maturation, as reported previously (Dudziak et al., 2007).

Figure 5. T cell–intrinsic caspase-1 is required for Th17-mediated colitis. (A) Weight change of Rag1−/− mice that received WT, Casp1Δ10, or Il1b−/− naive CD4 T cells (CD45RBhi) at indicated time points (n = 6–10 mice for each group). NT, nontransferred (i.p. PBS). (B) Percentage of initial body weight 4 wk after transfer. n = 12–19 mice per group. (C) Representative image of colon (left) and measured colon length (right) of Rag1−/− mice at 4–5 wk after transfer. n = 4–8 mice per group. (D) Representative H&E staining of colon sections from NT, WT, or Casp1Δ10Δ (Δ10) naive T cell–transferred Rag1−/− mice (left) and histology score (right). Images are displayed at 20× magnification. Ruler bar represents 100 µm. n = 12–14 mice per group. (E) Representative flow plots showing the percentages of IL-17A⁺ IFNγ⁺ CD4⁺ CD90⁺ CD44⁺ T cells in the mLNs or colonic LP (Colon-LPL) of Rag1−/− mice 4 wk after transfer of WT or Casp1Δ10 (Δ10) naive CD4 T cells. n = 8–13 mice per group. (F) Serum IL-17A levels at the indicated time points from the mice in Fig. 5 F. n = 5 mice per group. Data are representative of or combined from two to four independent experiments. Each data point represents one biological replicate. Statistics represent mean ± SEM and P values were determined by two-way repeated ANOVA with Bonferroni correction (A), one-way ANOVA (B and C), Mann-Whitney U test (D), unpaired Student’s t test (E and F) or multiple t tests with Holm-Sidak correction (G). *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.
Flow cytometry and FACS
Antibodies used for flow cytometry are listed in Table S2. For surface marker staining, cells were blocked with Fc block (anti-mouse CD16/CD32, BioLegend) for 10 min and then incubated with antibodies for 30 min, with extensive washes with FACS buffer (PBS, 2% FCS, and 2 mM EDTA). For intracellular cytokine analysis, cells were stimulated with 50 ng/ml PMA (Sigma-Aldrich) and 1 µM ionomycin (Sigma-Aldrich) in the presence of 5 µg/ml brefeldin A (BioLegend) for 4–6 h. Cell permeabilization and intracellular staining were performed according to manufacturer’s protocol (eBioscience Foxp3/TF staining buffer set). Samples were analyzed using an LSR Fortessa flow cytometer (BD) or Novocye 3001 (ACEA Biosciences). Cells were gated on singlets, and dead cells were excluded using Zombie Yellow live/dead staining (BioLegend). Data were analyzed using FlowJo software (BD). For FACS sorting, cells were stained in sterile FACS buffer. Dead cells were excluded by DAPI or propidium iodide staining. Cells were sorted by FACS Aria (BD) into FACS buffer or directly into TriZol LS Reagent (Invitrogen) for RNA extraction.

Isolation of mouse lymphocyte populations
Spleen and lymph nodes were harvested from 6–12-wk-old mice. Single-cell suspension was obtained by dissociating using sterile frosted slides and passing through a 70-µm cell strainer. Red blood cell lysis was performed as needed. Naive CD4 T cells were isolated according to MoJoSort kit protocol (BioLegend). The purity of naive CD4 T cells was constantly monitored and maintained at >95% CD4-MHCII-CD62L-CD44-. Splenic DCs were isolated from the spleen of a mouse injected with B16-FL/T3L melanoma. Splenocytes were blocked with Fc block (anti-CD32/CD16) and stained with CD11c-biotin (BioLegend), then with anti-Biotin beads (Miltenyi), and isolated using AutoMacs magnetic selection (Miltenyi). The purity of isolated splenic DCs was maintained at >98% CD11c+. For all experiments, DC donor mice and naive CD4 T cell donor mice were sex and age matched. B cells were isolated from sex- and age-matched naive mouse spleen by isolating the CD19+ population using CD19-biotin (BD) and AutoMacs (Miltenyi). LP lymphocytes (LPLs) were isolated as previously described (Hu et al., 2011).

Pathogen-specific Th cell priming
X-VIVO15 serum-free media (Lonza) was used to avoid T cell activity to bovine serum proteins. CD11c+ DCs were pulsed with pathogen lysate (10 µg/ml, dose titrated to induce the maximum response and minimum cell death across the panel) at 10^6/ml for 5 h and then extensively washed. Naïve T cells were labeled with CFSE (5 µM, BioLegend). DCs and T cells were cocultured at a 1:5 ratio for 5–12 d, depending on the experiments.

Recall of pathogen-specific T cell response by B cell–mediated restimulation
Pathogen-specific T cells generated using the approach described previously were rested with the provision of a low dose of IL-2 (10 units/ml, BioLegend) for an additional 2 d until active cytokine production waned. B cells were either pulsed with pathogen lysate or blasted with CpG (Keck Oligonucleotide Synthesis Facility, Yale University, New Haven, CT) for 18–24 h in X-VIVIO15 serum-free medium, extensively washed, irradiated at a dose of 12 Gy using an x-ray irradiator (X-RAD320, Precision X-Ray), and cocultured with T cells at a 2:1 ratio. T cell responses were assessed 48 h later.

T cell polarization (cytokine-differentiated T cells)
Tissue culture-treated plates were coated with 5 µg/ml of anti-mouse CD3 (BioLegend) and anti-mouse CD28 (Tonbo) for 2–4 h. 1 × 10^6/ml naïve T cells were polarized for 5 d under Th17 polarization (cdTh17) conditions with 10 µg/ml anti-IFNγ (BioLegend), 10 µg/ml anti-IL-4 (BioLegend), 20 ng/ml IL-6 (Peprotech), 5 ng/ml TGFβ1 (Peprotech), 10 ng/ml IL-1β (Peprotech), and 20 ng/ml IL-23 (BioLegend) or Th1 polarization conditions with 10 µg/ml anti-IL-4, 50 units/ml IL-2, and 10 ng/ml IL-12 (Peprotech). For some experiments, polarized cells were removed from all polarizing cytokines and plate-bound anti-CD3/CD28, washed, and cultured with 10 units/ml IL-2 for an additional 5 d.

Retroviral transduction of Th17 cells
Retrovirus was prepared from 10^6 Platinum-E cells transfected with 2.5 µg vector and 0.63 µg pCCL-Eco using Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific). Viral supernatant was harvested from Platinum-E cultures after 48 and 72 h of transfection. 50 units/ml of IL-2 (BioLegend) and 10 µg/ml of protamine sulfate (Sigma-Aldrich) were added to the viral supernatant before transduction. Naïve CD4 T cells were prepared and differentiated as described earlier. 24 and 48 h after activation, 10^6 T cells were transduced with 1 ml of viral supernatant under spin-infection of 2,500 rpm for 90 min at 32°C. T cells were returned to the original activation medium after spin-infection. 5 d after T cell activation, cells were harvested and stained for hCD2 as a transduction efficiency marker, as well as intracellular staining.

In vitro neutralization and recombinant cytokine supplement
In vivo-grade anti-mouse IA/IE, anti-mouse CD86, anti-mouse CD80, or anti-mouse IL-12/23p40 antibodies (BioLegend) are listed in Table S2. Purified DCs were pulsed and extensively washed as indicated previously. DCs were preincubated with Fc block for 10 min and subsequently with blocking antibodies (10 µg/ml) for 30 min. Then DCs were cocultured, without further washing steps, with labeled naïve T cells as described above.

Adoptive transfer of in vitro-primed, pathogen-specific T cells
ppTh cells were extensively washed with plain RPMI 1640 without serum or additives and injected intravenously into the recipient mice (2 × 10^6 CD25+ICOS+ cells/mouse). Recipient mice were cohoused with their respective controls and experimental groups for ≥2 wk before the experiment and during the experimental period. Typically, one pair of mice represents one transferred-uninfected and one transferred-infected (cohoused and transferred at the same time) subject. Fold expansion was calculated as normalized to the cohoused uninfected control.

Naïve T cell transfer and Cr infection
Naïve T cells (CD4+MHCII-CD62L+CD44+) from indicated genotypes were injected into recipient (age- and sex-matched)
Detection antibodies were used according to manufacturer's instructions. Mice were then gavaged with 2 × 10^8 CFU/mouse Cr as described in the previous section. At 10 dpi, mLNs were harvested for measuring CD4 T cell responses. Cecae were dissected, weighed, and subjected to blood cell lysis. Cecae contents were homogenized in sterile PBS and plated on Luria-Bertani plates with nalidixic acid in serial dilution. Bacterial burden was calculated by averaging colony counts from countable dilutions. Mouse fecal pellet from the distal colon was collected and weighed as initial stool weight. The feces were allowed to completely dry for 24 h at 37°C and weighed as dry stool weight. Stool water content was calculated as [(initial stool weight − dry stool weight)/initial stool weight] × 100%.

**T cell transfer model of colitis**

5 × 10^5 CD4^+CD62L^hiCD44^loCD45RB^hiCD25^− T cells were FACs sorted from spleens and lymph nodes of mice of each genotype, washed, and injected i.p. into Rag1^−/− mice. Weights were measured weekly when weight >100% initial weight and biweekly when weight loss progressed (weight <100% initial weight). Serum was collected every week via submandibular bleeding. Mice were sacrificed 4–5 wk after transfer or when weight loss exceeded 20% of initial weight, and colon length was measured at the time of sacrifice. Colonos were removed from the cecum to anus, photographed, fixed with formalin, and submitted to the UT Southwestern Molecular Pathology Core for paraffin embedding, sectioning, and H&E staining. Digital images were obtained using a Zeiss Axiovert 100 Inverted Microscope with Jenoptik Gryphax Camera. Histology scores were blind-scored by Dr. Bret Evers at UT Southwestern. The scoring criteria are modified from Ostanin et al. (2009): (a) the degree of inflammatory infiltrate in the LP, range 0–3; (b) goblet cell loss as a marker of mucin depletion, range 0–2; (c) reactive epithelial hyperplasia/ataxia with nuclear changes, range 0–3; (d) the number of intraepithelial lymphocytes in the epithelial crypts, range 0–3; (e) abnormal crypt architecture (distortion, branching, atrophy, crypt loss), range 0–3; (f) number of crypt abscesses, range 0–2; (g) mucosal erosion to frank ulcerations, range 0–2; and (h) submucosal spread to transmural involvement, range 0–2; for a maximum score of 20. LPL cells were isolated from colonos of diseased mice, and intracellular staining was performed as described in Flow cytometry and FACS.

**ELISA**

Briefly, coating antibodies were diluted and coated in an ELISA plate overnight at 4°C, then blocked with PBS containing 10% FCS or 1% BSA (Sigma-Aldrich). Samples were loaded in duplicate, diluted in blocking buffer, and incubated overnight. Detection antibodies were used according to manufacturer’s instructions. Protein concentrations were quantified using 3,3′,5,5′-tetramethylbenzidine or o-phenylenediamine dihydrochloride colorimetric assay. Plates were washed extensively in between steps with PBS and 0.05% Tween-20.

**Western blot analysis**

Cells were extensively washed with PBS on ice and directly lysed in boiled SDS-containing 2× Laemmli buffer. Protein concentration was measured by detergent-resistant Bradford assay. 5–10 μg of each lysate was loaded to SDS-PAGE, and immunoblotting was performed using standard protocols. Antibodies used for Western blot are listed in Table S2.

**Inflammasome activation assay and FAM-FLICA staining**

For detection of caspase-1 processing following inflammasome activation, bone marrow–derived macrophages were incubated with LPS (100 ng/ml) for 4 h and pulsed with ATP (5 mM) during the last 30 min. Following stimulation, cells were immediately incubated on ice and washed with cold PBS, and boilded 2× Laemmli SDS sample buffer was directly added to each well to lyse cells. The lysate was harvested and boiled for 10 min before Western blot analysis.

For detection of caspase-1 processing by flow cytometry, bone marrow–derived DCs were incubated with 100 ng/ml LPS at 5 × 10^6/500 μl in complete medium in a polypropylene tube for 4 h at 37°C. Cells were spun down, 200 μl medium was removed, and cells were resuspended in the remaining 300 μl medium. 5 mM ATP and FAM-FLICA dye were directly added to the medium and incubated at 37°C for an additional 30 min. Cells were extensively washed in apoptosis buffer provided in the FAM-FLICA staining kit and resuspended in propidium iodide–containing FACS buffer for flow cytometry analysis. For staining of LPL T cells for FAM-FLICA, purified LPL cells were not pretreated with LPS or ATP. LPL cells were directly costained with FAM-FLICA and antibodies against CD90 and CD4 to discriminate LPL CD4 T cells.

**Plasmids**

MSCV-RES-hCD2tm* vector was described previously (Furlan et al., 2014). Full-length caspase-1 or caspase-1 deficient of CARD was cloned into backbone using XhoI and NotI loci and primers listed in Table S1. Enzymatically inactive caspase-1 was cloned by mutating cysteine (TGC) 284 to alanine (GCT) of the full-length caspase-1.

**RNA isolation and quantification and qRT-PCR**

Cells were sorted into either Trizol LS reagent or complete medium and lysed with Trizol reagent. RNA was extracted using miRNeasy (Qiagen) and treated with DNase I (Qiagen), according to the manufacturer’s instructions. Quantities of extracted RNA were determined using NanoDrop2000 for cDNA synthesis or Agilent Bioanalyzer 2100 before RNA-Seq Library preparation. All RNA sent for library preparation qualified for RNA integrity number >8.

cDNA synthesis was performed using Moloney murine leukemia virus reverse transcription (Invitrogen) in the presence of RNase inhibitor (Promega). qRT-PCR primers used in this study are listed in Table S1. qRT-PCR was performed using SYBR Green MasterMix (Applied Biosystems) and QuantStudio 7 Real-Time PCR System.

**RNA-seq analysis**

RNA-seq libraries were prepared with the Illumina TruSeq Stranded RNA Sample Preparation kit (Illumina) according to the manufacturer’s protocol. Libraries’ quality was validated on an Agilent Bioanalyzer 2100. This included using standard protocols for cDNA synthesis, fragmentation, addition of adaptors,
size selection, amplification, and quality control (Illumina). The amplified libraries were size selected, and libraries were quantified by PicoGreen assay (Life Technologies). SE85 single-end sequencing was performed using NextSeq SE-75 High Output V2 flow cell with an average of 23 million reads/sample. Alignment of RNA-seq reads on mouse reference genome mm10 was performed on a CLC Genomics Workbench 7 (Qiagen). The expression of the genes in all samples was calculated as reads/fragments per kilobase of transcript per million mapped reads. Methods for data normalization and analysis are based on the use of internal standards that characterize some aspects of the system’s behavior, such as technical variability, as presented elsewhere (Dozmorov and Lefkovits, 2009; Dozmorov and Centola, 2003). The comparison of these methods with some other normalization and analysis procedures was described previously (Dozmorov et al., 2010). Created initially for the analysis of microarray data, they were slightly modified to the needs of RNA-seq data analysis. The two-step normalization procedure and the associative analysis functions were implemented in MatLab (Mathworks). These algorithms are also obtainable from an R package diffGeneAnalysis, available as a part of Bioconductor packages (http://www.bioconductor.org/packages/2.5/bioc/html/diffGeneAnalysis.html).

Global hierarchical clustering and differential expression presented in Fig. 2 (B and C) were performed with DESeq2 and clustered by Euclidean distance. Heatmaps and hierarchical clustering were further generated using GenePattern with list of clustered by Euclidean distance. Heatmaps were visualized by HierarchicalClusteringImage with global z-score scale. Functional annotation of differentially regulated genes was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID) or Ingenuity Pathway Analysis, as described in each figure. Activation z-score or fold enrichment >1.5 were considered significantly enriched pathways.

GSEA analysis
The GSEA method has been described previously (Subramanian et al., 2005). 11,868 genes that were considered significantly expressed were used to create a ranked list ordered by comparing differential expression between ppTh17 and cdTh17 cells. This list was then used to compare with established memory T cell gene signatures downloaded from the Molecular Signature Database in GSEA server. The molecular signature dataset used by this study was derived from a publication by Luckey et al. (2006) (GSE1000002_1582_200_UP and GSE1000002_1582_200_UP), where the author extracted the top 200 enriched genes in effector versus memory CD8 T cells. The table containing common genes between query list and molecular signature dataset can be found in Table S7.

Quantification and statistical analysis
Statistical analyses performed are indicated in the figure legends, analyzed by GraphPad Prism 7. P values <0.05 were considered statistically significant. Significantly differentially expressed genes in RNA-seq experiments were determined using encoded MatLab or Gene Pattern DESeq2 function described in RNA-seq analysis. Sample sizes were not predetermined by statistical methods.

Data and software availability
Illustrations were created with Biorender (https://biorender.com). Matlab code used for RNA-seq analysis is described in RNA-seq analysis and is available on request. The sequencing data reported in this paper have been deposited to the Gene Expression Omnibus with the accession nos. GSE141054, GSE141061, and GSE141062.

Online supplemental material
Fig. S1 demonstrates the de novo differentiation of pathogen-specific Th cells using the in vitro-priming system, their dependence on three-signal activation, and specificity to the priming pathogen. Fig. S2 shows the divergent transcriptional profile between ppTh17/ex vivo Th17 and cdTh17 cells and also specific up-regulation of caspase-1 in DC-primed T cells and its role in Th17 differentiation independent of catalytic activity. Fig. S3 shows that T cell–intrinsic caspase-1 is required for host protection and Th17–mediated autoimmune inflammatory disease. Table S1 and Table S2 list the primers and reagents used in this study. Table S3 lists differentially expressed genes between Lm- and Cr-primed CD90+CFSE+ Th cells shown in Fig. 1. Table S4 and Table S5 shows a list of genes differentially expressed between ppTh17/ex vivo tdT+ and cdTh17 cells and their functional annotations determined by DAVID. Table S6 lists normalized expression values for genes shown in the heatmaps in Fig. 2. Table S7 shows a GSEA-determined list of differentially expressed genes in ppTh17 and cdTh17 cells that are enriched in memory or effector T cell signatures. Table S8 shows differentially regulated genes and their expression in WT and Casp1Δ10 Cr-specific Th cells.

Acknowledgments
We thank all members of the Pasare laboratory, especially Ms. Margaret McDaniel, for helpful discussions and critical reading of the manuscript and Mrs. Linley Riediger Pierce for mouse colony management and genotyping. We thank the members of Genomics and Microarray Core at UT Southwestern Medical Center for their help with the RNA-seq experiments and analysis. Russell E. Vance, PhD, at University of California, Berkeley (Berkeley, CA), generously provided Casp1Δ10 mice. Fayyaz S. Sutterwala, MD, PhD, at Cedars-Sinai Medical Center (Los Angeles, CA), kindly provided Il1b−/− mice. Vishva Dixit, MD, kindly provided Pycard−/− mice.

This work was supported by grants from the National Institutes of Health (AI13125, GM120196, and AI123176) to C. Pasare and the Edwin L. Cox Distinguished Chair in Immunology and Genetics to E.K. Wakeland. K. Deason was supported by American Heart Association grant 17PRE33410075.

Author contributions: Y. Gao, E.K. Wakeland, and C. Pasare conceived and designed the study and interpreted all the data;
Y. Gao performed all in vitro experiments and in vivo experiments with the help of K. Deason; Y. Gao and I. Dozmorov analyzed the RNA-seq data; A. Jain and R.A. Irizarry-Caro provided experimental support; L.A. Coughlin and A.Y. Koh provided the commensal microbes; I. Rauch provided the CaspΔ10 mice; B.M. Evers performed blind histopathology scoring; Y. Gao and C. Pasare wrote the manuscript; E.K. Wakeland and C. Pasare acquired and provided funding sources for this study.

Disclosures: The authors declare no competing interests exist.

Submitted: 15 March 2019
Revised: 7 October 2019
Accepted: 9 December 2019

References

Arboe, G., E.E. West, R. Spolski, A.A.B. Robertson, A. Klos, C. Rheinheimer, P. Dutow, T.M. Woodruff, Z.X. Yu, L.A. O’Neill, et al. 2016. T helper 1 immunity requires complement-driven NLPR3 inflammasome activity in CD4+ T cells. Science. 352:aad1210. https://doi.org/10.1126/science.aad1210

Becattini, S., D. Latore, F. Mele, M. Foglierini, M. Delinglade, F. Mele, J. Guo, L. Wang, S.M. Srinivasula, M.D. Jacobson, J.-L. Poyet, S. Ciofani, M., A. Madar, C. Galan, M. Sellars, K. Mace, F. Pauly, A. Agarwal, W. Collins, J.W., K.M. Keeney, V.F. Crepin, V.A.K. Rathinam, K.A. Fitzgerald, B.B. Bruchard, M., C. Rebé, V. Derangère, D. Togbé, B. Ryffel, R. Boidot, E. Furlan, A., and M. Centola. 2003. An associative analysis of gene expression and microarray data using Z score transformation. J. Mol. Diagn. 7:632–633. https://doi.org/10.1016/S1525-1578(10)60455-2

Bruchard, M., C. Rebé, V. Derangère, D. Togbé, B. Ryffel, R. Boidot, E. Furlan, A., and M. Centola. 2015. The receptor requirement for the protease function of caspase-1. Cell Death Differ. 22:1827–1838. https://doi.org/10.1038/cdd.2016.69

Dozmorov, I., and I. Lefkovits. 2009. Internal standard-based analysis of microarray data. Part I: analysis of differential gene expressions. Nucleic Acids Res. 37:6323–6339. https://doi.org/10.1093/nar/gkp706

Dozmorov, M.G., J.M. Guthridge, R.E. Hurst, and L.M. Dozmorov. 2010. A comprehensive and universal method for assessing the performance of differential gene expression analyses. PLoS One. 5:e12657. https://doi.org/10.1371/journal.pone.0012657

Dudziak, D., A.O. Kamphorst, G.F. Heidkamp, V.R. Buchholz, C. Trumpf-eller, S. Yamazaki, C. Cheong, K. Liu, H.-W. Lee, C.G. Park, et al. 2007. Differential antigen processing by dendritic cell subsets in vivo. Science. 315:107–111. https://doi.org/10.1126/science.1136080

Fan, B., L.A. Coughlin, M.M. Neubauer, J. Kim, M.S. Kim, X. Zhan, T.R. Simms-Waldrip, Y. Xie, L.V. Hooper, and A.Y. Koh. 2015. Activation of HIF-1α and IL-37 by commensal bacteria inhibits Candida albicans colonization. Nat. Med. 21:808–814. https://doi.org/10.1038/nm.3837

Ferguson, B.J., C. Alexander, S.W. Rossi, I. Liv, A. Rebane, C.L. Worth, J. Wong, M. Laan, P. Peterson, E.J. Jenkinson, et al. 2008. AIRE’s CARD revealed, a new structure for central tolerance provokes transcriptional plasticity. J. Biol. Chem. 283:1723–1731. https://doi.org/10.1074/jbc.M707211200

Furlan, R., G. Martino, F. Galbiati, P.L. Poliani, S. Smiroldo, A. Bergami, G. Desina, G. Comi, R. Flavell, M.S. Su, and L. Adorini. 1999. Caspase-1 regulates the inflammatory process leading to autoimmune demyelination. J. Exp. Med. 192:2403–2409.

Gallon, N.L., G. Doith, K.M. Monroe, Z. Yang, I. Muñoz-Arias, D.N. Levy, and W.C. Greene. 2015. Cell-to-Cell Transmission of HIV-1 Is Required to Trigger Pyroptotic Death of Lymphoid Tissue-Derived CD4 T Cells. Cell Reports. 12:1565–1563. https://doi.org/10.1016/j.celrep.2015.08.011

Ghoreschi, K., A. Laurence, X.-P. Yang, K. Hirahara, and J.J. O’Shea. 2011. T helper 17 cell heterogeneity and pathogenicity in autoimmune disease. Trends Immunol. 32:395–401. https://doi.org/10.1016/j.it.2011.06.007

Harbour, S.N., C.L. Maynard, C.L. Zindl, T.R. Schoeb, and C.T. Weaver. 2015. Th17 cells give rise to Th1 cells that are required for the pathogenesis of colitis. Proc. Natl. Acad. Sci. USA. 112:7061–7066. https://doi.org/10.1073/pnas.1415657112

Iborra, S., M. Martín-Llobet, S. Conde-Garrosa, C. Del Fresno, and D. Sancho. 2016. Optimal Generation of Th17 Lineage Commitment. J. Immunol. 197:204–211. https://doi.org/10.4049/jimmunol.1500261

Iwasaki, A., and R. Medzhitov. 2015. Control of adaptive immunity by the innate immune system. Cell. 163:2403–2416. https://doi.org/10.1016/j.cell.2015.10.018

Jain, A., and C. Pasare. 2017. Innate Control of Adaptive Immunity: Beyond the Three-Signal Paradigm. J. Immunol. 198:3791–3800. https://doi.org/10.4049/jimmunol.1602020

Jenkins, M.K., and J.J. Moon. 2012. The role of naive T cell precursor frequency and recruitment in dictating immune response magnitude. J. Immunol. 188:4135–4140. https://doi.org/10.4049/jimmunol.1102661

Li, J., G. Huston, and S.L. Swain. 2003. IL-7 promotes the transition of CD4 effectors to persistent memory cells. J. Exp. Med. 198:1807–1815. https://doi.org/10.1084/jem.20030725

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https://doi.org/10.1084/jem.20190476

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Journal of Experimental Medicine
Shao, W.-G. Ye, R. J. Doiron, S.N. Hussain, and M. Saleh. 2007. The caspase-1 digestome identifies the glycosylation pathway as a target during infection and septic shock. J. Biol. Chem. 282:36321–36329. https://doi.org/10.1074/jbc.M708182200

Shen, C.-H., O. Talay, V.S. Mahajan, I.B. Leskov, H.N. Eisen, and J. Chen. 2010. Antigen-bearing dendritic cells regulate the diverse pattern of memory CD8 T-cell development in different tissues. Proc. Natl. Acad. Sci. USA. 107:22587–22592. https://doi.org/10.1073/pnas.100350108

Shi, J., Y. Zhao, Y. Wang, W. Gao, J. Ding, P. Li, L. Hu, and F. Shao. 2014. Inflammatory caspases are innate immune receptors for intracellular LPS. Nature. 514:187–192. https://doi.org/10.1038/nature13683

Shimada, K., R.A. Porritt, J.L. Markman, J.G. O’Rourke, D. Wakita, M. Noval Rivas, C. Ogawa, L. Kozhaya, G.A. Martins, D. Unutmaz, et al. 2018. T-Cell-Intrinsic Receptor Interacting Protein 2 Regulates Pathogenic T Helper 17 Cell Differentiation. Immunity. 49:873–885.e7. https://doi.org/10.1016/j.immuni.2018.08.022

Siegmund, B., H.A. Lehr, G. Fantuzzi, and C.A. Dinarello. 2001. IL-1 beta-converting enzyme (caspase-1) in intestinal inflammation. Proc. Natl. Acad. Sci. USA. 98:13249–13254. https://doi.org/10.1073/pnas.211379988

Subramanian, A., P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, and J.P. Mesirov. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA. 102:15545–15550. https://doi.org/10.1073/pnas.0506580102

Tubo, N.J., A.J. Pagán, J.J. Taylor, R.W. Nelson, J.L. Linehan, J.M. Ertelt, E.S. Huseby, S.S. Way, and M.K. Jenkins. 2013. Single naive CD4+ T cells from a diverse repertoire produce different effector cell types during infection. Cell. 153:785–796. https://doi.org/10.1016/j.cell.2013.04.007

Viswanathan, V.K., K. Hodges, and G. Hecht. 2009. Enteric infection meets intestinal function: how bacterial pathogens cause diarrhoea. Nat. Rev. Microbiol. 7:110–119. https://doi.org/10.1038/nrmicro2053

Waickman, A.T., and J.D. Powell. 2012. mTOR, metabolism, and the regulation of T-cell differentiation and function. Immunol. Rev. 249:43–58. https://doi.org/10.1111/j.1600-065X.2012.01152.x

Wang, R., C.P. Dillon, L.Z. Shi, S. Milasta, R. Carter, D. Finkelstein, L.L. McCormick, F. Fitzgerald, H. Chi, J. Munger, and D.R. Green. 2011. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity. 35:871–882. https://doi.org/10.1016/j.immuni.2011.09.021

Yang, J., R. Zhang, G. Lu, Y. Shen, L. Peng, C. Zhu, M. Cui, W. Wang, P. Arbabioldi, M. Tang, et al. 2013. T cell-derived inducible nitric oxide synthase switches off Th17 cell differentiation. J. Exp. Med. 210:1447–1462. https://doi.org/10.1084/jem.20122494

Yosef, N., A.K. Shalek, J.T. Gaublomme, H. Jin, Y. Lee, A. Awasthi, C. Wu, K. Karwacz, S. Xiao, M. Jorgolli, et al. 2013. Dynamic regulatory network controlling Th17 cell differentiation. Nature. 496:461–468. https://doi.org/10.1038/nature12081

Zhang, B., S.-Q. Liu, C. Li, E. Lykken, S. Jiang, E. Gong, Z. Tao, B. Zhu, Y. Wan, and Q.-J. Li. 2016. MicroRNA-23a Curbs Necrosis during Infection and septic shock. J. Exp. Med. 210:1969–1988. https://doi.org/10.1084/jem.2016142003

Zhu, J., H. Yamane, and W.E. Paul. 2010. Differentiation of effector CD4+ T cells populations (*). Annu. Rev. Immunol. 28:445–489. https://doi.org/10.1146/annurev-immunol-030410-120056

Zielinski, C.E., F. Mele, D. Aschbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanavecchia, and F. Sallusto. 2012. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. Nature. 484:514–518. https://doi.org/10.1038/nature10957
**Figure S1. An in vitro priming approach to generate functional pathogen-specific Th cells.** (A) Top: Purity of isolated naive CD4 T cells (CD4+MHCII-CD62L+CD44-) from two independent experiments. Bottom: Purity of CD11c+ DCs before or after isolation. (B) Overlay histograms of CFSE dilution curves from naive CD4 T cells primed with Cr-stimulated DCs (pathogen primed) or LPS stimulated DC + TCR ligation (LPS + anti-CD3). (C) CFSE-C90+ percentage in Cr-primed CD4 T cells where 10 µg/ml blocking antibodies to mouse IA/IE (MHCII) or CD80/CD86 were added to the coculture. n = 2–3. (D) IFNγ+ percentage of Cr-primed CFSE-C90+ T cells in the presence or absence of anti-IL-12p40 antibodies during differentiation. n = 3. (E) Proliferation of T cells in the priming system with the indicated pathogens, shown by CFSE dilution. Un, unstimulated. (F) Proliferation of pathogen- or commensal-specific T cells primed by DCs stimulated with heat-killed commensal bacteria Bf, La (MOI = 3), or 10 µg/ml Lm/Cr lysate. n = 2–3. (G and H) IFNγ (G) and IL-17A (H) quantities in the culture supernatant of CD4 T cells previously primed with Lm (left) or Cr (right) that were restimulated for 48 h with unstimulated (Un) or Lm/Cr-fed, irradiated B cells. Lm and Cr concentrations used for restimulation were titrated at 3, 10, and 30 µg/ml. Culture supernatants from anti-CD3 (30 ng/ml)-stimulated T cells were also assessed for IFNγ production as a positive control. n = 2 technical replicates. Data are representative of three independent experiments. (I) Histogram showing CD25+ percentage or mean fluorescence intensity (MFI) of CD69, ICOS, and CD44 (percentage and MFI shown on upper left corner) on the CD90+ T cells from the same experiments in G and H denoting up-regulation of indicated activation markers in response to Lm/Cr rechallenge. Lysate concentration = 10 µg/ml. (J) Quantified fold expansion of transferred (in vitro-primed with Lm or Cr) T cells (CD45.2+) in the peripheral lymph nodes (pLNs) of mice from the same experiments shown in Fig. 1 G. n = 7 mice per group. (K) Quantified MFI of surface ICOS on recipient endogenous CD45.1+ T cells from the same experiments shown in Fig. 1 G. n = 7 mice per group. Data are pooled from or representative of two to four independent experiments. Statistics represent mean ± SEM, and P values were determined by repeated-measures two-way ANOVA with Tukey correction (C), one-way ANOVA with Tukey correction (F), or paired Student’s t test (D and J). *, P < 0.05; **, P < 0.01.
Comparative transcriptional analysis reveals caspase-1 as a T cell–intrinsic regulator of Th17 differentiation. (A) tdTomato+ percentage of unstimulated (Un), Lm-primed, Cr-primed, or Th17-polarized 17A-fm CD4 T cells. Un, n = 4; Lm and Cr, n = 5; Th17, n = 2. (B) Heatmap of Th17–associated cytokine and TF expression in CFSE−, CFSE−tdTomato−, and CFSE−tdTomato+ populations from Cr-primed T cells. (C) Representative flow plots showing IFNγ-YFP+ percentage of CFSE−tdTomato+ population, from 17-γ double reporter T cells under ppTh17 (Cr-primed) or cdTh17 conditions at early (day 5) and late (day 10) stages of differentiation. (D) Representative flow plots showing IFNγ-YFP+ percentage of CD4+CD90+tdTomato+ population, from mLN of 17-γ double reporter mice at 10 dpi of Cr. 17-fm mice that do not carry the YFP allele were used as negative signal control. (E) Confirmation of representative differentially expressed genes shown in Fig. 2 by qRT-PCR, n = 2. (F) Expression levels of Casp1 and Casp11 in murine T cell populations, extracted from the Immgen database. n = 2–3, heat map is shown as mean expression value. SPN, Spleen; sLN, skin-draining lymph node; PP, Peyer’s patches. (H) WT or Casp1 Δ10 naive T cells were primed for 10 d with WT DCs stimulated with heat-killed Bf (MOI = 3). Flow plot shows the percentage of IL-17A− and IFNγ-producing cells in live CFSE−CD90+ population. n = 2–3. (I) Overlay of CFSE dilution histograms (left) 10–12 d after Cr-priming and quantified CD4 T cell proliferation (CFSE−; right), n = 5, Δ10, Casp1Δ10. (J) tdTomato+ percentage in CFSE−CD90+ CD4 T cell population primed with Cr-stimulated DCs in vitro for the indicated time. (K) Western blot analysis of procaspase-1 (p45) and cleaved caspase-1 (p20) of Cr-primed CD4 T cells. Cells are sorted into CFSE-high (hi), CFSE-intermediate (int), and CFSE-negative (neg) populations and compared with WT macrophages (MΦ) undergoing inflammasome activation (4 h LPS + 30 min ATP [L+A]). (L) WT naive CD4 T cells were primed with Cr-stimulated WT DCs. At day 7, indicated inhibitors were added to the culture. Casp1 expression in FACS-sorted CFSE−CD90+ T cells was determined at day 10. Concentration of the inhibitors used were: p38i (SB203580), 5 µM; JNKi (JNK inhibitor, 1 µM); MEKi (U0126), 2 µM; JAKi (Ruxolitinib), 500 nM; IRAK1/4i (CAS 509093–47-4), 5 µM; IKKi (CAS 873225–46-8), 500 nM. n = 2–3. (M) IL-17A+ percentage of CFSE−CD90+ WT or Casp1Δ10 CD4 T cells primed with Cr-stimulated WT DCs in vitro. Cultures were supplemented with 10 ng/ml IL-1α or IL-1β. n = 4. (N) Bicistronic hCD2 expression on the surface of Th17 cells transduced with indicated vector. Data are pooled from or representative of at least two independent experiments. Statistics represent mean ± SEM, and P values were determined by paired Student’s t test (A and H) or paired two-way ANOVA (L and M). ns, not significant; *, P < 0.05.
Figure S3. T cell–intrinsic caspase-1 is required for host protection and Th17-mediated autoinflammatory disease. (A) Naive CD4 T cells from WT or Casp1Δ10 (Δ10) mice were isolated and polarized to cdTh17 cells. Representative flow cytometry plot (left) and quantifications of IL-17A+ percentage (right) are shown. n = 4. (B) Representative histogram overlay of CFSE dilution from experiments in A. (C) CFSE− percentage of transferred CD4 T cells, examined at 10 dpi. n = 8–9 mice per group. Δ10, Casp1Δ10. (D) Percentage of cytokine-positive cells in CD90+CD4+ population (ILCs) from the same experiments in Fig. 4. n = 5 mice per group. (E) Colonic crypt length measured from histology images in Fig. 5 D. n = 3–5 mice per group. NT, nontransferred (i.p. PBS). Δ10, Casp1Δ10. (F and G) WT and Casp1Δ10 CD45RBhi CD4 cells were transferred to Rag1−/− mice. 3 wk after transfer, LPL cells were isolated and stained with FAM-FLICA to detect activation of caspase-1. FLICA+PI+ indicates inflammasome-activated cells. WT and Casp1Δ10 bone marrow–derived DCs (BMDCs) that were treated with inflammasome-activating ligands (4 h LPS [100 ng/ml] and 30-min 5 mM ATP) were used as positive and negative controls for FLICA staining. Representative flow plot is shown in F and quantified in G; n = 2–4/group. (H and I) IL-17A+IFN− γ percentage (H) and IFN− γ+IL-17A− percentage (I) of CD4+CD90+ T cells in the mLN or colonic LP (Colon-LPL) of Rag1−/− mice 4 wk after transfer of WT or Casp1Δ10 naive CD4 T cells. n = 8–13 mice per group. (J) Percentages and numbers of CD4+CD90+ T cells from Colon-LPL of Rag1−/− mice 4 wk after transfer of WT or Casp1Δ10 naive CD4 T cells. n = 6–7 mice per group. Data are representative of or pooled from two to four independent experiments. Statistics represent mean ± SEM, and P values were determined by paired Student’s t test (A), one-way ANOVA with Tukey correction (E), or unpaired Student’s t test (G–I). ns, not significant; *, P < 0.05; ***, P < 0.001.
Tables S1–S8 are provided online as Word files (Tables S1 and S2) and Excel files (Tables S3–S8). Table S1 lists primers used in this study. Table S2 lists reagents. Table S3 is a list of genes differentially expressed between Lm-primed and Cr-primed CD90^+^CFSE^−^CD4 T cells, along with normalized expression values. Table S4 is a list of genes differentially expressed between ppTh17, cdTh17, and ex vivo tdT^+^ cells. Table S5 shows significantly enriched functional annotations in differentially expressed genes in ppTh17/ex vivo tdT^+^ or cdTh17 cells, determined by DAVID. Table S6 lists normalized expression values for genes shown in Fig. 2. Table S7 is a list of differentially expressed genes in ppTh17 and cdTh17 cells that are enriched in memory or effector T cell signatures, determined by GSEA. Table S8 is a list of differentially expressed genes in WT and CaspΔ10 Cr-specific Th cells.