GM-CSF+ Tc17 cells are required to bolster vaccine immunity against lethal fungal pneumonia without causing overt pathology

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SUMMARY

GM-CSF co-expressing T17 cells instigate pathologic inflammation during autoimmune disorders, but their function in immunity to infections is unclear. Here, we demonstrate the role of GM-CSF+ Tc17 cells for vaccine immunity against lethal fungal pneumonia and the cytokine requirements for their induction and memory homeostasis. Vaccine-induced GM-CSF+ Tc17 cells are necessary to bolster pulmonary fungal immunity without inflating pathology. Although GM-CSF expressing Tc17 cells preferentially elevate during the memory phase, their phenotypic attributes strongly suggest they are more like Tc17 cells than IFNγ-producing Tc1 cells. IL-1 and IL-23, but not GM-CSF, are necessary to elicit GM-CSF+Tc17 cells following vaccination. IL-23 is dispensable for memory Tc17 and GM-CSF+ Tc17 cell maintenance, but recall responses of effector or memory Tc17 cells in the lung require it. Our study reveals the beneficial, nonpathological role of GM-CSF+ Tc17 cells during fungal vaccine immunity.

Graphical abstract

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AUTHOR CONTRIBUTIONS
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DECLARATION OF INTERESTS
The authors declare no competing interests.
**In brief**

GM-CSF$^+$ and IL-17A$^+$ lineages of T cells are instrumental in controlling many fungal and bacterial infections and implicated in autoimmune pathology, host-microbial interactions at the mucosal surfaces, and neuro-immune nexus. Mudalagiriyappa et al. show that GM-CSF expressing Tc17 cells are necessary for mediating fungal vaccine immunity without augmenting pathology.

**INTRODUCTION**

T helper type 17 (Th17) cells were first described as a distinct subset during autoimmune disorders (Harrington et al., 2005; Langrish et al., 2005; Park et al., 2005), and their role in immunity against many bacterial and fungal pathogens is documented (Chen and Kolls, 2013; Cypowyj et al., 2012; Romani, 2011). IL-17A is beneficial in controlling respiratory infections caused by *Klebsiella, Mycobacteria, and Bordetella*, including defense against several systemic and pulmonary fungal infections (Chen and Kolls, 2013; Chen et al., 2011; Higgs et al., 2012; Romani, 2011) caused by *Cryptococcus, Candida, Coccidioides, Histoplasma*, and *Blastomyces* (Cypowyj et al., 2012; Hung et al., 2011). Similarly, IL-17A$^+$ CD8$^+$ T (Tc17) cells contribute to defense against infections and participate in autoimmunity (Huber et al., 2013; Luckel et al., 2019; Nanjappa et al., 2012a, 2017; Yeh et al., 2010). IL-17A-mediated effects during infections include induction of antimicrobial peptides and chemokines, enhancing proinflammatory cytokines expression, and activation of neutrophils (McGeachy et al., 2019).

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Like IL-17A, granulocyte-macrophage colony-stimulating factor (GM-CSF) has been linked to various inflammatory disorders, implying a potential nexus (Cornish et al., 2009; Hamilton, 2002; McGeachy, 2011). Consistent with this idea, studies have identified a non-redundant role for GM-CSF in cooperating with Th17 cells during autoimmune neuroinflammation (Codarri et al., 2011; El-Behi et al., 2011; Lee et al., 2012). A recent study showed that antigen-specific tissue-resident T<sub>H</sub>17 cells, co-expressing GM-CSF, are linked to the severity of COVID-19-induced pathology (Zhao et al., 2021). In contrast, GM-CSF offers protection to the host by defending against infections caused by bacteria, protozoa, and fungi (Bandera et al., 2008; Chen et al., 2007; Deepe et al., 1999; Gonzalez-Juarrero et al., 2005; LeVine et al., 1999; Morrissey and Charrier, 1990; Murray et al., 1995; Olivares Fontt et al., 1996; Richardson et al., 1992; Riopel et al., 2001; Rosen et al., 2013; Weiser et al., 1987). Immunity and immunopathology are two facets of the immune response; the first is required for protection against pathogens and tumors, whereas the latter, caused by exuberant responses, instigates pathology. Despite the collaborative role of GM-CSF and IL-17A in Th17-mediated autoimmunity (McGeachy, 2011), their potential immune or pathogenic role during infections has not been clear. However, IL-17A and GM-CSF are common defense cytokines during infections, including that caused by fungi, and both cytokines are involved in bolstering functions of neutrophils and monocytes/macrophages, essential controlling invading pathogens (Bandera et al., 2008; Chen et al., 2007; Deepe et al., 1999; Nanjappa et al., 2012a; Richardson et al., 1992).

The cytokines IL-1 and IL-23 are instrumental in the differentiation, stabilization, and homeostasis of IL-17A<sup>+</sup>T (T17) cells. IL-1 plays a key role in the induction of T17 cells by regulating the expression of IRF4, RORγt, and IL-23R, and its dysregulated expression is associated with autoinflammatory diseases (Lopalco et al., 2015; Masters et al., 2009). Whereas IL-6- and TGF-β-induced Th17 cells were non-pathogenic, the addition of IL-1β and IL-23 evoked pathogenicity, causing experimental autoimmune encephalomyelitis (EAE) (Ghoreschi et al., 2010; McGeachy et al., 2007) and suggesting distinct in vivo functions of lineage-defining cytokines. IL-23 bolsters the induction of T17 responses by stabilizing and expanding their numbers while also providing signals for memory cell homeostasis (Haines et al., 2013; Veldhoen et al., 2006). At the site of insult, IL-23 augments the severity of autoimmune disorders, and the clinical use of therapeutic blocking monoclonal antibody (mAb) ameliorates the condition (Noviello et al., 2021; Teng et al., 2015). Thus, IL-1 and IL-23 modulate T17 responses, but their functions in the homeostasis of GM-CSF<sup>+</sup>T17-cells during infection are unclear.

In this study, using a mouse model of fungal vaccine immunity, we evaluated the role of GM-CSF<sup>+</sup>Tc17 cells for immunity or immunopathology. Furthermore, we investigated the role of IL-1 and IL-23 cytokines’ role in GM-CSF<sup>+</sup>Tc17 cell homeostasis; that is, during induction, memory homeostasis, recall responses, immunity, and immunopathology.
RESULTS

Fungal vaccination induces GM-CSF+ Tc17 cells that preferentially develop as memory cells

Vaccine-induced, long-lasting memory Tc17 cells mediate immunity to lethal fungal pneumonia in the absence of CD4+ T cells (Nanjappa et al., 2012a, 2017). Polycytokine-producing CD8+ T cells are functionally superior and are highly desirable for vaccine success (Seder et al., 2008). We investigated the expression of other cytokines produced by Tc17 cells and found induction of GM-CSF (GM-CSF+ Tc17) cells in response to fungal vaccination that persisted as long-lived memory. Consistent with previous observations (Hirota et al., 2011; Nanjappa et al., 2017), ~50% of eYFP+ (“fate-mapping” IL-17ACreRosa26eYFP mice) CD8+ T cells expressed functional protein, IL-17A (Tc17), at the peak of the vaccine response (3 weeks post-vaccination; Figures S1A and S1B, upper left panel), where ~30% of Tc17 cells co-expressed GM-CSF (GM-CSF+ Tc17; Figure 1A, upper second left panel). In contrast, the percentage of GM-CSF expressing cells among IFNγ+ cells (eYFP−; GM-CSF+ Tc1; Figure 1A, upper second right panel) was lower (~11%). We also noted induction of IL-17A−IFNγ−GM-CSF+CD8+ T cells at both effector and memory phases (Figures 1A and S1A).

Next, we assessed if the effector GM-CSF+Tc17 cells become long-lived memory cells. We found memory GM-CSF+Tc17 cells in increased frequencies (~50% of Tc17 cells), with a concomitant reduction in single IL-17A+ cells (Figure 1A, lower left panels). We also found enrichment of memory GM-CSF+ Tc1 cells, albeit at lower frequencies. As GM-CSF has been traditionally classified as a type I cytokine and grouped with IFNγ (Tc1)-producing cells, we asked if GM-CSF+ Tc17 cells behave more like Tc1 cells. Analysis of the profile of lineage-defining transcription factors suggested that GM-CSF+ Tc17 (only double positive) cells were closer to Tc17 (only single positive) lineage than Tc1, despite their relative reduced levels of RORγt expression (Figure 1B). To further validate the phenotype of GM-CSF+Tc17 cells and their association to Tc17, we used t-distributed stochastic neighbor embedding (tSNE) analysis, an algorithmic technique whereby similar populations tend to cluster on their phenotypic/functional relatedness (van der Maaten and Hinton, 2008). GM-CSF+ Tc17 cells were preferentially clustered with Tc17 cells rather than with the Tc1 subset during effector and memory phases (Figure 1C). Collectively, our data showed that fungal vaccine-induced GM-CSF+ Tc17 cells are distinct and develop as long-lived memory cells with the characteristics reminiscent of Tc17 cells than the Tc1 cells.

GM-CSF+ Tc17 cells are necessary for fungal vaccine immunity without causing inflated pathology

The foregoing data revealed that fungal vaccination induces the development of memory GM-CSF+Tc17 cells. The current dogma is that T17 cells co-expressing GM-CSF instigate severe tissue damage and disease during autoimmune disorders and COVID-19 infection (Zhao et al., 2021). We have previously shown that Tc17 cells are necessary for fungal vaccine immunity, and the role of GM-CSF in fungal infections has been documented (Deepe et al., 1999; Kasahara et al., 2016; Whitney et al., 2014). Additionally, CD4+ T cell-derived GM-CSF contributed to immunity against Pneumocystis (Elsegeiny et al., 2018).
Here, we evaluated the role of GM-CSF for CD8+ T cell-mediated vaccine immunity during CD4+ T cell deficiency. In a mouse model of vaccine immunity against lethal pulmonary blastomycosis during CD4+ T cell deficiency, all unvaccinated mice succumb to death following the challenge, and all vaccinated mice mount sterilizing immunity with fungal clearance beginning from days 5 to 20 post-challenge (Figure S1C). To assess the role of GM-CSF for vaccine immunity, cohorts of wild-type (WT) and GM-CSF−/− unvaccinated mice or vaccinated mice were infected intratracheally (30 days post-vaccination) with a virulent strain of B. dermatitidis. As expected, vaccinated WT mice showed enhanced immunity with significant reduction in fungal burden (p ≤ 0.05; 30-fold; Figure S2A). However, vaccine immunity was severely blunted in vaccinated GM-CSF−/− mice, despite the good induction of Tc17 and Tc1 cells following vaccination (p ≤ 0.05; Figures S2B and S2C). Blunted immunity in the vaccinated GM-CSF−/− mice could be confounded by alveolar proteinosis in this mutant condition (Kitamura et al., 1999; Tazawa et al., 2019). In line with this concern, we found significantly higher fungal burdens and severely compromised immunity in unvaccinated GM-CSF−/− compared with unvaccinated WT mice (p ≤ 0.001; 190-fold). Nevertheless, neutralization of GM-CSF during the effector phase (recall responses in the lung) curtailed the fungal control in the lung following the intratracheal challenge of vaccinated mice has been previously shown (Wuthrich et al., 2003).

We next asked if GM-CSF+ Tc17 cells participate in vaccine immunity or precipitate overt pathology. To address this, we generated mixed bone-marrow chimera mice using WT, GM-CSF−/−, and IL-17A−/− donor cells with TCRα−/− recipients. The premise is that co-transfer of IL-17A−/− and GM-CSF−/− donor cells, wherein the populations express one or the other but not both cytokines, will reveal the role of dualproducing cells (both GM-CSF and IL-17) characterized by the WT. After transfer, we vaccinated mice, rested them, and challenged by the intratracheal route with the isogenic virulent fungal strain. Typically, the vaccinated WT mice start clearing the infection as early as day 5 post-challenge whereas unvaccinated WT mice show uncontrolled fungal growth (Figure S1C). Here, at day 12 post-infection, as expected, unvaccinated recipients had significantly higher lung colony-forming units (CFU) than did the vaccinated WT cell recipients (Figure 2A). In line with our previous observations (Nanjappa et al., 2012a), vaccinated IL-17A−/− cell recipients also had significantly higher lung CFU than did the WT cell recipients (p ≤ 0.0001), suggesting an essential role for Tc17 cells in vaccine immunity. We further observed that vaccinated GM-CSF−/− cell recipients had lung CFU values higher than WT cell recipients, but lower than IL-17A−/− cell recipients, suggesting a dominant role for Tc17 cells. Importantly, the vaccinated recipients of both IL-17A−/− and GM-CSF−/− cells, where each CD8+ T cell can produce either of the cytokines but not both, had a significantly higher fungal burden compared with the vaccinated WT cell recipients (p ≤ 0.05). These data suggest that GM-CSF+ Tc17 cells bolster fungal vaccine immunity.

We next evaluated if GM-CSF+Tc17 cells inadvertently caused pathology in the lung during vaccine immunity. As expected, all unvaccinated cell recipient groups had more pronounced pathology compared with the vaccinated mice (Figure 2B). We hypothesized that IL-17A−/− and GM-CSF−/− recipients would have less pathology than WT cell recipients if GM-CSF+ Tc17 cells were instigators of overt inflammation, but we found that was not the case.
We found either similar or more pathology in IL-17A−/− and GM-CSF−/− cell recipients compared with vaccinated WT cell recipients. Our data show that fungal vaccine-induced GM-CSF+Tc17 cells contribute to immunity without precipitating overt pathology.

**IL-1 is required for fungal vaccine-induced GM-CSF+ Tc17 cell responses**

Lineage-specific cytokines are key for the differentiation of T cells. Previously, we have shown that IL-1 was required for inducing Tc17 cells in a MyD88-dependent manner but was largely dispensable for the Tc1 lineage (Nanjappa et al., 2015). Here, we investigated the role of IL-1 for the elicitation of GM-CSF+Tc17 cells following vaccination. As expected, the percent Tc17 cells was significantly blunted in the absence of IL-1 signaling, whereas IFNγ+ CD8+ T (Tc1) cell responses were relatively unaffected in both draining LN (dLN) and spleen (Figures 3A, S3A, and S3B). However, the percentage of GM-CSF+ cells among Tc17 and Tc1 subsets was significantly reduced in Il1r1−/− mice compared with the Il1r1+/+ group, especially in the dLN. Similarly, enumeration of cytokine-producing cells suggested a significant reduction of Tc17 and GM-CSF+ Tc17 cells with relatively intact Tc1 and GM-CSF+ Tc1 cells in the absence of IL-1 signaling (Figure S3C).

Antigen-induced proliferation predominantly shapes the clonal burst size of effector CD8+ T cells during an immune response. We investigated if IL-1 is required for the expansion of GM-CSF+ Tc17 cells. Consistent with previous observations (Nanjappa et al., 2015), Tc17 cells proliferated at a significantly higher rate than Tc1 cells, but required IL-1 for this augmented rate (Figure 3B). Similarly, GM-CSF+ Tc17 cells required IL-1 signaling for higher proliferation. Interestingly, the proliferation rate of GM-CSF+ Tc17 cells was lower than that of Tc17 cells. This was not due to cell-intrinsic T cell developmental defects, as homeostatic proliferation was not affected in naive (CD44lo) Il1r1−/− CD8+ T cells compared with naive Il1r1+/+ CD8+ T cells (Figure 3B, left panels). The type I responses (Tc1; IFNγ+) were relatively unaffected, where GM-CSF+ Tc1 cells were modestly reduced in the absence of IL-1 cytokine (Figures S3B and S3C). Thus, IL-1 bolstered the proliferation of both Tc17 and GM-CSF+ Tc17 cells.

IL-6 and TGF-β are involved in the primary commitment of naive T cells into T17 cells. However, IL-1 has been shown to induce Th17 cells independent of IL-6 (Ikeda et al., 2014). We asked if IL-1 has a role in lineage differentiation of GM-CSF+ Tc17 cells. The expression of RORγt in Tc17 cells was uncompromised in the absence of IL-1, which was the same for GM-CSF+Tc17 cells (Figure 3C, upper panels). Also, IL-1 was largely dispensable for the induction of T-bet in Tc1 subset (Figure 3C, lower panels). Thus, IL-1 signaling is required for GM-CSF+ Tc17 cell responses following fungal vaccination, which is reminiscent of the Tc17 cell lineage.

**IL-1 signaling, not GM-CSF, is necessary for vaccine-induced GM-CSF+ Tc17 cell responses**

IL-1 can act on innate immune cells to potentiate inflammation-mediated immune responses and on T cells for their expansion. To delineate the cell-intrinsic effects of IL-1 signaling in CD8+ T cells, we used conditional mice deficient in T cell MyD88 (MyD88ΔT mice) and
II1r1−/− mice to assess vaccine responses. As in our previous studies (Nanjappa et al., 2015), we found significantly reduced Tc17 responses in MyD88ΔT mice compared with WT mice and II1r1−/− mice, suggesting some non-IL-1R signaling through MyD88 (Figure 4A, left panel). However, GM-CSF+ Tc17 responses required IL-1, which seems to be enough for MyD88-mediated effects as responses in MyD88ΔT mice mirrored the responses in II1r1 mice (Figure 4A, right panel). Thus, intrinsic signaling of IL-1 is required for augmenting GM-CSF+ Tc17 cells.

GM-CSF is a pleiotropic cytokine modulating immunity secreted by many cells, including T cells. Here we investigated the effect of GM-CSF on Tc17/Tc1 subset responses. We found that IL-17+ and IFNγ+ CD8+ T cells were significantly reduced in gmcsf−/− mice compared with WT mice (Figures 4A and S4A). As we could not address the role of GM-CSF for GM-CSF+ Tc17 cells using these mice, we used adoptive transfer of enriched naive congenic WT CD8+ T cells to assess vaccine responses. As expected from the above, Ly5.1+ Tc17 cell responses and endogenous Ly5.2+ (gm-csf−/−) Tc17 cells were significantly reduced in gmcsf−/− recipients compared with WT recipients (Figures 4B, 4C, and S4B). However, differentiated GM-CSF+ Tc17 cell responses were uncompromised in gmcsf−/− recipients and were similar to that in WT recipients. Thus, for GM-CSF+Tc17- but not Tc17-mediated vaccine responses, GM-CSF is dispensable.

**IL-23 is necessary for the formation of effector GM-CSF+ Tc17 cells, vaccine immunity, and mitigation of pathology**

IL-23 has been implicated in the programming and terminal differentiation of Th17 cells and diseases, including autoimmunity (Burkett et al., 2015). The *in vitro* differentiation of Tc17 cells was dependent on IL-6, TGF-β, and IL-1, whereas the addition of IL-23 modestly enhanced their numbers (Yeh et al., 2010; Yen et al., 2009) or augmented dual IL-17A and IFNγ cytokine-producing cells (Curtis et al., 2009). IL-23 drove pathogenic Tc17 cells to induce diabetes *in vivo* (Ciric et al., 2009). Previously, we have shown that IL-6 is requisite for Tc17 responses and immunity (Nanjappa et al., 2012a). Here, we investigated the role of IL-23 in the induction of both Tc17 and GM-CSF+ Tc17 cell responses to fungal vaccination. IL-23 knockout (KO) mice poorly control the attenuated live vaccine and dissemination occurs (Figure S5A), which may confound results on vaccine responses (Figure S5B) and immunity. Thus, we used killed yeast for vaccination. At the peak of T cell expansion after vaccination, both Tc17 and GM-CSF+Tc17 cells were significantly blunted in the IL-23 KO compared with WT mice (Figures 5A and S5C, left panels). We asked if the booster dose of the vaccine would help enhance responses. The anamnestic response to a booster dose was clearly noticeable in the WT group (Figures 5A and S5C, right panels). However, this response was significantly weaker in KO mice (p ≤ 0.05).

We next asked if memory T cell formation was influenced by IL-23 signaling. The vaccinated mice were rested for ~10 weeks. The memory T cell development recapitulated the effector phase responses, i.e., IL-23 KO mice had significantly lower frequencies and numbers of both Tc17 and GM-CSF+Tc17 cytokine-producing cells than WT mice (Figures 5B and S5D). We next asked if this phenotype affects vaccine immunity. We lethally challenged cohorts of vaccinated mice by the intratracheal route and measured fungal
resistance at day 10 post-challenge. Vaccinated WT mice mounted near sterilizing immunity, whereas vaccinated KO mice did not and had a significantly higher fungal burden (Figure 5C; p ≤ 0.0001). Of note, unvaccinated KO mice had a higher fungal load than unvaccinated WT mice, suggesting an innate role for IL-23. Of interest, vaccination did significantly help reduce fungal burden in vaccinated KO mice compared with unvaccinated mice, but not as robustly as in WT mice (93-fold versus 3,372-fold; p ≤ 0.01 versus p ≤ 0.0001, respectively).

We investigated the impact of recall responses and fungal load on lung histopathology. IL-23 KO mice had greater pathology and seemingly futile granulomatous inflammation compared with WT controls (Figure 5D), suggesting that the higher fungal load instigated unwanted immune pathology.

**IL-23 is dispensable for maintenance of memory GM-CSF+ Tc17 cells**

It is increasingly evident that IL-23 imparts effector T17 cell terminal differentiation. However, the active role of IL-23 for maintenance of the memory T17 phenotype is not clear. We investigated if IL-23 plays a role in the sustenance of both memory Tc17 and GM-CSF+Tc17 cells. The previous data (Figure 5B) did not eliminate lingering antigen or the influence of IL-23 on the expansion of Tc17 subsets, both of which influence the number of memory cells. To address this, we enriched the effector/memory CD8+ T cells from vaccinated WT mice and transferred equal numbers of cells into congenic naive WT or IL-23 KO mice. Use of the congenic marker Ly5.1 on donor WT CD8+ T cells helped us track the cells in Ly5.2 recipients by flow cytometry. We noticed relatively intact persistence of memory CD8+ T cell in the absence of IL-23 one month after the transfer (Figures 6 and S6A, left panels). We next asked if there is slow attrition of memory T cells. To address this, we transferred enriched WT memory CD8+ T cells (8 weeks post-vaccination) and rested for 2 months (Figures 6 and S6A, middle panels). We still did not see any significant change in the numbers of memory Tc17 cells in the presence or absence of IL-23. To check if the effectors/memory precursors need IL-23 for long-lasting memory, we enriched WT effector cells at 3 weeks post-vaccination, transferred them, and then rested for 4 months. Memory Tc17 cells were comparable in WT and KO recipients (Figures 6 and S6A, right panels). However, there was a modest but significant reduction in GM-CSF expressing Tc17 cells, whereas memory Tc1 cell subsets were intact. We evaluated the effect of IL-23 on the quality of cytokine-producing memory CD8+ T cells. The mean fluorescence intensities (MFI) of cytokines among the different memory CD8+ T cells were largely unaffected in the absence of IL-23 (Figure S6B). Collectively, our data suggest that once the effectors are fully matured, IL-23 plays a minimal role in the maintenance of memory Tc17/GM-CSF+Tc17 cells.

**IL-23 bolsters recall responses but not the redifferentiation of GM-CSF+ Tc17 cells**

One characteristic phenotype of memory T cells is their ability to extravasate into the peripheral tissues to mediate immunity following reinfection. This recruitment is dynamic and requires the inflammatory milieu and signals. In Figure 5B above, we found an indispensable role of IL-23 for vaccine immunity but with a caveat of poor effector Tc17 cell responses in KO mice, leading to poor vaccine immunity. To investigate the role of IL-23 at subsequent stages of the vaccine response, we used adoptive transfer studies (as in Figure 6). We vaccinated congenic (Ly5.1+) WT mice, rested them for nine weeks, enriched
the memory CD8+ T cells, and adoptively transferred equal numbers of cells into Ly5.2 recipients (WT or KO). We rested the recipients for two months, as we know that memory CD8+ T cells do not undergo attrition in the absence of IL-23. We challenged these mice by the intratracheal route and assessed the recall responses in the lung. The responses of the Tc17 subset were significantly lower in the absence of IL-23 (Figures 7A and S7A). However, the proportion of GM-CSF co-expressing Tc17 cells was uncompromised in the KO recipients compared with WT recipients, suggesting dispensability of IL-23 for their re-differentiation. Nevertheless, it should be noted that the numbers of GM-CSF+Tc17 cells was considerably lower despite the unaltered frequency in KO recipients compared with WT recipients. We did not observe a significant effect of IL-23 on recall responses of Tc1 subsets. We also determined the influence of Tc17 responses on neutrophil responses. The activation of neutrophils was significantly impaired in IL-23 KO mice compared with WT mice, although the frequency was similar between the groups (Figure 7B).

We next evaluated the effect of IL-23 on memory T cell immunity. Recipients of adoptively transferred memory WT CD8+ T cells were challenged by the intratracheal route and assessed fungal control. In line with the poor recall responses and defective activation of neutrophils, the fungal burden was significantly higher on day 4 post-infection in IL-23 KO recipients than in WT controls (Figures 7C and S7B; p ≤0.05). We asked if there was also delayed fungal clearance in the IL-23 recipients by measuring the lung fungal burden on day 7 post-challenge. We found a further significant increase of CFU in KO recipients, whereas WT recipients started to clear the fungal infection by this time point (Figure 7D; p ≤0.05). To evaluate if fungal clearance depends on the numbers of memory CD8+ T cells, we adoptively transferred low numbers of cells into WT and KO recipients. Following intratracheal infection, we found a significant increase in the fungal burdens in KO recipients compared with WT recipients (Figure 7E; p ≤0.05). Notably, the granulomatous inflammation and pathology were directly correlated with the fungal burden in the lung and was higher in KO recipients. Thus, IL-23 is required for the recall response of Tc17 cells, the activation of neutrophils, fungal vaccine immunity, and mitigation of pathology.

**DISCUSSION**

Significant strides have been made to understand the elements of T cell subsets during infections and autoimmune disorders. Although multifunctional and polycytokine expressing T cells are highly desirable for vaccine immunity, these could harm the host because of pleiotropic and redundant effects of cytokines causing pathology over immunity. For example, IL-17A provides immunity to bacterial and fungal infections by inducing proinflammatory cytokines and chemokines and activating myeloid cells (Stockinger and Omenetti, 2017). Similar, often overlapping, effects have been associated with GM-CSF functions in the production, recruitment, and activation of myeloid cells (Lang et al., 2020). However, these cytokines’ inadvertent or overt production leads to pathology over immunity (Ingelfinger et al., 2021; Lee et al., 2020; McGeachy et al., 2019; Zambrano-Zaragoza et al., 2014). Although GM-CSF+T17 cells are pathogenic during autoimmune disorders, their role during infections is unclear. However, the accumulation of GM-CSF+ Th17 cells were strongly correlated with the severity of COVID-19 (Zhao et al., 2021). These cells
seem to be viral antigen-specific and their immune role early in the infection is unknown. Furthermore, whether their induction during COVID-19 is stochastic, and the disease severity or chronicity is due to suppression of protective T cell responses not explained. Our study revealed that GM-CSF expression by Tc17 cells is necessary to mediate vaccine immunity and alleviate pathology. One can appreciate the antigen specificity of T cells dictating the outcome of defense against different infections, although overt inflammation and pathology during coinfections are possible.

GM-CSF, a pleiotropic cytokine produced by multiple cells, plays an important role during T cell-mediated pathogenesis. Although various mechanisms of EAE have been documented, the existing dogma suggests the essentiality of GM-CSF-mediated pathogenesis (Lotfi et al., 2019; Noster et al., 2014). Notably, GM-CSF production by CD4+ T cells, not by dendritic cells (DCs), was necessary for the disease by supporting DCs’ expression of IL-6 and IL-23, both for further differentiation of Th17 cells (El-Behi et al., 2011; Sonderegger et al., 2008). IL-23 further enhanced GM-CSF production suggesting a positive feedback loop between them (Poppensieker et al., 2012). We found an essential role of GM-CSF and IL-23 for Tc17 cell responses, but it was not required for differentiation of GM-CSF+Tc17 cells (Figure 5), although the autocrine effect of GM-CSF is possible. In line with the previous reports, our data suggested that GM-CSF+Tc17 cells expressed both RORγt and T-bet, which is required to produce GM-CSF from T cells (Codarri et al., 2011; Noster et al., 2014). However, consistent with our previous observations (Nanjappa et al., 2015), IL-1 did not enhance RORγt but promoted the proliferation of effector, including GM-CSF+Tc17 subsets. Nevertheless, the mechanistic insights of co-expression of GM-CSF and IL-17A, not an independent expression by two subsets, for autoimmunity or immunity to infections are unclear.

Immunological memory is the hallmark of vaccination. Despite the differences in homeostatic turnovers of different subsets, memory T cell homeostasis is chiefly governed by IL-7 and IL-15, independent of cognate antigen. Whether additional cytokines are integral for the different subsets of memory T cells, including T17, is not well understood. However, superfluous IL-23 induced certain levels of IL-17 from Th17 cells and loss of IL-23R signaling led to low IL-7 receptor expression that is necessary for memory maintenance (Kondrack et al., 2003; McGeachy et al., 2009). Our data showed dispensability of IL-23 for memory Tc17 and GM-CSF+ Tc17 cell homeostasis without the loss of their numbers. Nevertheless, IL-23 was required to form fully matured memory precursor cells during the expansion phase as early transfer of effector cells into IL-23 deficient mice led to reduced GM-CSF+ Tc17. In line with the prior observations, where IL-23R blocking antibody reduced the IL-17A+ Th17 cells and disease severity in an EAE model during memory recall responses (Haines et al., 2013), our data suggested that IL-23 is needed for recall Tc17 responses in the lung following intratracheal infection. However, we found no significant effect of IL-23 on the redifferentiation of GM-CSF+ Tc17 cells. Thus, memory GM-CSF+Tc17 cells are stably maintained independent of IL-23 but these cells required it for memory recall responses.

In summary, in this study, we have reported the generation and cellular functions of GM-CSF+ Tc17 subsets for vaccine immunity against fungal infection. GM-CSF+ Tc17 cells
bolstered fungal vaccine immunity and were dependent on IL-1 and IL-23 for their enhanced responses. However, IL-23 was dispensable for memory homeostasis of Tc17 and GM-CSF+ Tc17 cells.

Limitations of the study

Our study showed the essential role of IL-23 for Tc17 cell recall responses and immunity, but a limitation of our study is the direct function of IL-23 on effector Tc17 cells. Our studies showed the essential role of IL-1 and IL-23 for GM-CSF+ Tc17 cell vaccine responses and that they were enriched in the memory Tc17 cell compartment, but the mechanisms of the latter phenotype could not be addressed. We believe there is a selective advantage in the survival of multi-cytokine-producing cells during memory homeostasis.

STAR★METHODS

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Som G. Nanjappa (nanjappa@illinois.edu).

Materials availability—Reagents are commercially available and/or contacts for materials are listed in the key resources table.

Data and code availability

- The data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice—Homozygous breeding pairs of B6.129S2-TCRa\textsuperscript{tm1Mom}/J (Stock #002115), B6.129S1-Il1r\textsuperscript{tm1Roml}/J (Stock #003018), and B6.129S-Csf2\textsuperscript{tm1Mlg}/J (Stock #026812) were purchased from Jackson Laboratories. Breeding pairs of IL17a\textsuperscript{tm1.1((icre)Stck)/J (Stock #016879) and B6.129X1-Gt(Rosa)26Sor\textsuperscript{tm1(EYFP)Cos}/J (Stock #006148), purchased from Jackson Laboratories, were intercrossed to generate for fate-mapping of eYFP+ve IL-17A-expressing cells. Breeder pairs of IL17a\textsuperscript{−/−} and p19\textsuperscript{−/−} were from Yoichiro Iwakura (Tokyo University of Science, Tokyo, Japan) and Nico Ghilardi (Genentech Inc., San Francisco, USA), respectively. Wild type C57BL/6 and B6.SJL-Ppcre\textsuperscript{Pepe-β}BoyCrl (Ly5.1 congenic) mice were purchased from National Cancer Institute/Charles River Laboratories. Mice were bred, housed, and cared for following the strict guidelines of the University of Wisconsin-Madison (UW-Madison) and the University of Illinois at Urbana-Champaign (UIUC) Animal Care Committees, who approved all aspects of this work.

Fungal strains—The wild-type virulent strain of Blastomyces dermatitidis is obtained from American Type Culture Collection, #26199. The isogenic strain #55, an attenuated
mutant lacking BAD1 (kindly provided by Bruce Klein, UW-Madison), was used for vaccination. Isolates of both strains were maintained as yeast on Middlebrook 7H10 agar supplemented with oleic acid-albumin complex (Sigma-Aldrich) at 39°C.

**METHOD DETAILS**

**Vaccination and infection**—All mice of both sexes were six to eight weeks of age at the time of vaccination, unless stated. Mice were vaccinated with attenuated strain of #55 (~10^5 CFU) by subcutaneous (s.c.) route at each of two sites, dorsally and at the base of tail. For challenge studies, mice were inoculated with 4–6x10^3 CFU yeast of the isogenic virulent strain, ATCC #26199 by the intratracheal route. To assess the fungal burden, infected lungs were homogenized before plating on Brain Heart Infusion (BHI; Difco) agar supplemented with oleic acid-albumin complex.

**Depletion of CD4^+ T cells**—All the experimental animals were depleted of CD4^+ T cells with mAb, GK1.5 (BioXCell Inc. Lebanon, NH), using a weekly intravenous (i.v.) dose of 100μg. The efficiency of CD4^+ T cell depletion was ≥95% (Nanjappa et al., 2012b).

**Flow cytometry**—Single cell suspensions were re-stimulated with anti-CD3ε (0.1 μg/mL) and anti-CD28 (1 mg/mL) monoclonal antibodies in the presence of GolgiStop at 37°C for 5 h. Cells were preincubated with FcR block antibodies before staining for surface phenotypic markers with antibodies and cell-permeable Live/Dead fixable viable dye (Thermofisher) in FACS buffer (2% BSA and 0.05% NaN_3 in PBS). After 30′ incubation, cells were washed, fixed with Fix/Perm buffer (BD Biosciences), and stained with antibodies for intracellular cytokines in Perm/Wash buffer (BD Biosciences). In some experiments, cells were stained directly with antibodies without re-stimulation for phenotyping the neutrophils. Cells were analyzed either by BD LSRII or by Cytek Aurora flow analyzer. All fluorochrome-conjugated antibodies and dyes were purchased from BD Biosciences, Biolegend, and Thermofisher Scientific sources.

**t-SNE analysis**—t-SNE (t-distributed stochastic neighbor embedding) analysis of cytokine-producing cells was performed as described elsewhere (van der Maaten and Hinton, 2008). Single-cell suspensions were re-stimulated and analyzed by Cytek Aurora flow analyzer. Activated (CD44^{hi}) CD8^+ population were selected, concatenated from the samples after excluding dead cell population, and were identically gated for analysis using FlowJo. Selected CD8^+ CD44hi population was subjected to t-SNE module (parameters: Iterations-600, Perplexity-20, and Theta-0.5). Phenotypic expressions of CD44, CD8, CD122, CD127, CD43, and CD45.2 among activated CD8^+ T cells were considered to generate the formation of cell clusters mapped in a dimensionally reduced t-SNE space, where near distance indicated closeness of the cell clusters.

**Transcription factors staining**—Single cell suspensions, after re-stimulation, were stained for surface markers followed by intracellular cytokine-staining using Fix/Perm buffer kit (BD Biosciences). Cells were washed and stained for transcription factors (T-bet and RORγt) using FoxP3 buffer kit (eBioscience) according to manufacturer’s protocol. In some
experiments, cells were stained with anti-GFP antibody to detect eYFP\(^{+}\)ve cells during intra-cellular cytokine staining.

**Measuring in vivo CD8\(^{+}\) T-cell proliferation**—The thymidine-analog, BrdU (MP Biomedicals), was administered to vaccinated mice in drinking water (DW) @0.8 mg/mL. BrdU-DW was replaced daily. At ~3-wk post-vaccination, the draining lymph nodes were harvested and single-cell suspensions prepared. Cells were re-stimulated with anti-CD3ε/CD28 mAbs for 5 h in the presence of GolgiStop (BD Biosciences). Cells were washed and surface stained for phenotypic markers in FACS buffer. Cells were washed, fixed with BD Cytofix/Cytoperm kit for 20’ at 4°C, and stained for intracellular cytokines (ICS) in BD Perm/Wash buffer for 30’ at 4°C. Following manufacturer’s instructions, cells were then stained with anti-BrdU mAb using BrdU Flow Kit (BD Biosciences).

**Adoptive transfers**—Naïve or effector T cells (~3-wk post-vaccination) or memory T cells (>5-wk post-vaccination) were negatively enriched using BD IMag CD8\(^{+}\) Cell Enrichment Set (BD Biosciences) or EasySep Mouse CD8\(^{+}\) T Cell Isolation Kit (StemCell). Cells were analyzed by flow cytometry for percent purity and equal numbers were adoptively transferred into recipient mice. In some experiments, enriched congenic naive CD8\(^{+}\) T cells (10\(^{6}\)) were transferred into disparate recipient mice to study vaccine-responses.

**Mixed bone-marrow chimera experiments**—Bone marrow cells from donor mice (WT, GM-CSF KO, IL-17A KO) were transferred into lethally irradiated TCR\(^{α-/-}\) recipient mice for generation of mixed bone-marrow chimera mice. The experimental donor cells were mixed in 1:1 ratio with TCR\(^{α-/-}\) BM cells for the transfer. After 2 months rest, mice were vaccinated and challenged by the intratracheal route with a lethal isogenic strain to assess the vaccine-immunity and pathology.

**Histopathology**—Lung tissues were harvested and stored in 10% neutral buffered formalin (Fisherbrand). Tissues were paraffin-embedded, and sections were mounted on slides for hematoxylin and eosin (H&E) staining. Histopathological readings were made by a board-certified anatomic pathologist in a double-blinded manner.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical significance of differences in fungal lung CFU was measured by non-parametric Kruskal-Wallis (one-way ANOVA). All other statistical analysis was performed using a two-tailed unpaired Student’s t-test. Prism 8.4 (GraphPad Software, LLC) software was used for all statistical analysis. The values shown in the figures are mean ± SD. A two-tailed p value of ≤0.05 was considered statistically significant. All other statistical details are found in the figure legends. All cytometric data was analyzed using FlowJo v10.8.1 (BD Biosciences) and the graphical abstract was generated using bioRENDER software (Biorender, 2022).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.
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INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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GM-CSF+ Tc17 cells are required for fungal vaccine immunity without causing pathology
- IL-1 and IL-23 cytokines are necessary for GM-CSF+ Tc17 cells
- IL-23 is dispensable for memory homeostasis of GM-CSF+ Tc17 cells
- IL-23 is essential for recall responses and vaccine immunity against fungal infection
Figure 1. Development of fungal vaccine-induced GM-CSF+ Tc17 cells

(A–C) Naive IL-17ACreRosa26ReYFP mice were vaccinated subcutaneously with ~10^5 CFU of attenuated #55 strain of B. dermatitidis (B.d. #55). Single-cell suspensions from draining lymph nodes (dLNs) and spleens were restimulated with αCD3/CD28 mAbs, stained with antibodies, and analyzed using flow cytometry. Data show the percentage of cytokine-producing cells from spleen during effector (3 weeks post-vaccination) and memory (~10 months post-vaccination) phases. GM-CSF+ Tc17/Tc1 cells were gated on IL-17+/IFNγ+ cells, respectively (A). Shown are mean fluorescence intensity of transcription factors in the cytokine+ cells in dLN (3 weeks post-vaccination; B) and the t-SNE analysis of respective cytokine-producing cells from spleen gated on activated (CD44hl; grey) CD8+ T cells (C). n = 4 or 5 mice.

Data are representative of at least two-independent experiments. See also Figure S1.
Figure 2. GM-CSF⁺ Tc17 cells are required for vaccine immunity

(A and B) Equal numbers (2 × 10⁶) of mixed bone marrow cells from respective WT or mutants and TCRA⁻/⁻ donors (50:50) were co-transferred into lethally irradiated TCRA⁻/⁻ recipients to generate mixed bone marrow chimeric mice. Mice were rested (~2 months) for lymphoid recovery before being subcutaneously vaccinated with B.d. #55 (~10⁵ CFU). After three months post-vaccination, mice were lethally challenged by the intratracheal route (~6 × 10³ CFU of WT virulent B.d. #26199), and lungs were harvested after 12 days. Whisker plots show fungal burdens in the lungs (A), and H&E-stained images of lung sections show inflammation (B). CD4⁺ T cells were depleted throughout the experiment, starting from vaccination.

Data are from 2 independent experiments. n = 4–8 mice/unvaccinated groups, and n = 16–24 mice/vaccinated groups. **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. Scale bar, 100 μm.

See also Figures S1 and S2.
Figure 3. Role of IL-1 for differentiation, expansion, and proliferation of GM-CSF+ Tc17 responses

(A–C) Naive 6- to 8-week-old Il1r1+/+ and Il1r1−/− mice were vaccinated with B.d. #55 (~10^5 CFU). At 3 weeks post-vaccination, single-cell suspensions of spleens were restimulated with αCD3/CD28 mAbs, and frequencies of cytokines (A) and expression of transcription factors (C) expressions in CD8+ T cells were analyzed using flow cytometry. Dotted lines (C) represent the expression in CD8+ CD44lo populations from respective group samples. Cohorts of vaccinated mice were pulsed daily with BrdU in drinking water starting from day 8 post-vaccination. At ~3 weeks, single-cell suspensions were prepared, and percentage BrdU+ cytokine+ cells in dLNs were analyzed using flow cytometry (B). n = 5 mice/group.
Data are representative of 2 or 3 independent experiments. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. See also Figure S3.
Figure 4. Intrinsic/extrinsic role of IL-1R and GM-CSF for GM-CSF+ Tc17 cells

Naive 6- to 8-week-old WT, \textit{Il1r1}−/−, \textit{MyD88}Δ\textit{T} (a kind gift from Laurence Turka), and \textit{gmcsf}−/− mice were vaccinated with \textit{B. d.} #55 (~10^5 CFU). At 3 weeks post-vaccination, single-cell suspensions were prepared from the tissues, restimulated with αCD3/CD28 mAbs, stained, and analyzed using flow cytometry.

(A) The percentage of activated CD8^+ T cells expressing respective cytokines in dLNs.

(B and C) Enriched naive congenic WT Ly5.1^+ cells (7.22 × 10^6) were adoptively transferred into the recipients a day before the vaccination. At 3 weeks post-vaccination, single-cell suspensions from dLN and spleen were restimulated, stained, and analyzed using flow cytometry. Bar graphs show percentage (B) and absolute numbers (C) of WT Ly5.1^+ T cells expressing the cytokines. n = 4–6 mice/group.

Data are representative of two independent experiments. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001. See also Figure S4.
Figure 5. Role of IL-23 for vaccine-induced GM-CSF+ Tc17 cell sustenance and immunity

(A–D) Naive p19+/+ and p19−/− mice were vaccinated with heat-killed B.d. #55 strain (~10^6 CFU). Single-cell suspensions from the tissues at 3-wk post-vaccination (A) and at 72 days post-vaccination (B) were restimulated with αCD3/CD28 mAbs and cytokine-producing cells were analyzed by flow cytometry. Flow plots depict percentage of cytokine+ CD8+ T cells. n = 4 or 5 mice/group. At three weeks post-boost (~6 weeks post-vaccination), mice were lethally challenged by the intratracheal route. Whisker plots show fungal CFUs in the lung at day 10 post-challenge (n = 7–14 mice/group; C) and images of H&E staining show granulomas (D).

*p ≤0.05, **p ≤0.01, ***p ≤0.001, and ****p ≤0.0001. Scale bar, 100 mm. See also Figure S5.
Figure 6. IL-23 for maintenance of memory GM-CSF+ Tc17 cells

Naive Ly5.1 WT mice were vaccinated with attenuated live *B. d*. #55 strain (~10^5 CFU). DLN and spleens were harvested after at least three weeks (in brackets), CD8+ T cells were enriched, and comparable numbers (31.4 x 10^6, left; 9.8 x 10^6, middle; 24.7 x 10^6, right) were adoptively transferred into naive Ly5.2+ p19++ and p19−/− recipients. At indicated time post-transfers, single-cell suspensions from spleens were restimulated with αCD3/CD28 mAbs to assess the cytokine+ CD8+ T cells by flow cytometry. Flow plots depict percentage of cytokine+ Ly5.1+ CD8+ T cells. n = 4–5 mice/group.

*p ≤ 0.05. See also Figure S6.
Figure 7. IL-23 is required for GM-CSF+ Tc17 cell recall responses and immunity

(A–E) Naive congenic Ly5.1 WT mice were vaccinated with *B. d*. #55 strain (~10^5 CFU). dLN and spleens were harvested at least after 5–6 weeks, CD8+ T cell were enriched, and equal numbers of donor cells (A–C, 31.4 × 10^6; D, 24.3 × 10^6; E, 2.7 × 10^6) were adoptively transferred into naive Ly5.2^+ p19^+/+ and p19^−/− recipients. Following the rest (4–5 weeks), mice were lethally challenged by the intratracheal route. (A and B) At ~4 days post challenge, lungs were harvested to assess the cytokine+ WT (Ly5.1^+^) CD8+ T cells (following restimulation) and percentage and activated neutrophils by flow cytometry. Flow plots depict percentage or MFI of the cells. (C–E) Fungal burden and H&E staining readings of lungs were assessed at indicated days post-challenge. n = 4 or 5 mice/group.

*p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001. Scale bar, 100 μm. See also Figure S7.
## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Anti-mouse CD4 (RM4-5) | BD Biosciences | Cat# 563727, RRID:AB_312718 |
| Anti-mouse CD8a (53-6.7) | BD Biosciences | Cat# 558106, RRID:AB_397029 |
| Anti-mouse CD44 (IM7) | BD Biosciences | Cat# 563970, RRID:AB_2738517 |
| Anti-mouse CD45.2 (104) | BD Biosciences | Cat# 562129, RRID:AB_10897142 |
| Anti-mouse CD122 (TM-β) | BD Biosciences | Cat# 564764, RRID:AB_2738937 |
| Anti-mouse CD127 (SB/199) | BD Biosciences | Cat# 562419, RRID:AB_11153131 |
| Anti-mouse CD45.1 (A20) | BD Biosciences | Cat# 560120, RRID:AB_1727490 |
| Anti-mouse Ly6G (1A8) | BD Biosciences | Cat# 560603, RRID:AB_1727564 |
| Anti-mouse T-bet (O4-46) | BD Biosciences | Cat# 561266, RRID:AB_10562570 |
| Anti-mouse RORγt (Q31-378) | BD Biosciences | Cat# 562684, RRID:AB_2651150 |
| Anti-mouse TNFα (MP6-XT22) | BD Biosciences | Cat# 557644, RRID:AB_396761 |
| Anti-mouse IL-17A (TC11-18H10) | BD Biosciences | Cat# 561848, RRID:AB_1645204 |
| Anti-mouse IFNγ (XMG1.2) | BD Biosciences | Cat# 557998, RRID:AB_396979 |
| Anti-mouse GM-CSF (MPI-22E9) | BD Biosciences | Cat# 554406, RRID:AB_395371 |
| Anti-Mouse CD11a (M17/4) | BD Biosciences | Cat# 563668, RRID:AB_2738362 |
| Anti-mouse CD90.2 (30-H12) | BD Biosciences | Cat# 566074, RRID:AB_2738184 |
| Anti-mouse CD11b (M1/70) | BioLegend | Cat# 563402, RRID:AB_2738184 |
| InVivoMAb anti-Mouse CD4 (GK1.5) | BioXCell | Cat# BE0003-1, RRID:AB_1107636 |
| **Chemicals, peptides, and recombinant proteins** | | |
| RPMI 1640 medium | Corning | Cat# 10-040-CV |
| Fetal Bovine Serum | Gibco | Cat# 10437-028 |
| Penicillin-Streptomycin | Corning | Cat# 30-002 CI |
| Fixable Near IR Dead Cell Stain | Invitrogen | Cat# L34976 |
| Sodium Azide | Fisher | Cat# 52271 |
| Parafomaldehyde (16%) | Electron Microscopy Science | Cat# 15710 |
| Bovine Serum Albumin Fraction V | Chem-Implex Int. Inc | Cat# 00039 |
| **Critical commercial assays** | | |
| Cytofix/Cytoperm Kit | BD Biosciences | Cat# 554722 |
| GolgiStop Protein Transport Inhibitor | BD Biosciences | Cat# 554724 |
| eBioscience Transcription Factor Fixation/Permeabilization | Invitrogen | Cat# 00-5521-00 |
| Mouse CD8 T Lymphocyte Enrichment Set – DM | BD Biosciences | Cat# 558471, RRID:AB_2869195 |
| Easy Step Mouse CD8+ T cell Isolation Kit | STEMCELL Technologies | Cat# 19853 |
| 5-Bromo-2’-deoxyuridine | MP Biomedicals | Cat# 02100171 |
| BrdU Flow Kit | BD Biosciences | Cat# 559619 RRID:AB_2617060 |
| **Experimental models: Organisms/strains** | | |
| C57BL/6 | Charles River Laboratories | 566NCI |
| B6.SJL-129Ptek+*Pepc+*BoyCrl | Charles River Laboratories | Strain #494 |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| B6.129S2-Tcra<sup>tm1Mom</sup>/J | Jackson Laboratories | Stock #002115 |
| B6.129S-Cx2<sup>tm1Mlg</sup>/J | Jackson Laboratories | Stock #026812 |
| B6.129S1-Il1r1<sup>tm1Roml</sup>/J | Jackson Laboratories | Stock #003018 |
| B6.129X1-Gt(ROSA)26Sor<sup>tm1(EYFP)Cos</sup>/J | Jackson Laboratories | Stock #006148 |
| B6.129S2-Tcra<sup>tm1Mon</sup>/J | Jackson Laboratories | Stock #002115 |
| IL17a<sup>−/−</sup> | Yoichiro Iwakura, University of Science, Tokyo, Japan | N/A |
| p19<sup>−/−</sup> | Nico Ghilardi, Genentech Inc., San Francisco, USA | N/A |
| Isogenic mutant *Blastomyces dermatitidis* #55 | Wuthrich et al. (2003) | N/A |
| *Blastomyces dermatitidis* (#26199) | ATCC | #26199 |

**Software and algorithms**

| Name | Source | Identifier |
|------|--------|------------|
| SpectroFlo v3.0 | Cytek Biosciences | N/A |
| FlowJo v10.8.1 | BD Biosciences | N/A |
| Prism v9.2.0 | GraphPad | N/A |
| bioRENDER | BioRender 2022 | N/A |

**Other**

| Name | Source | Identifier |
|------|--------|------------|
| Tissue Homogenizer | Omni-International | TH115-PCF7H |
| Bullet Blender | Next Advance | N/A |
| Cytek Aurora flow cytometer | Cytek Biosciences | N/A |