

Article

Potential Roles of Gamma-Delta T Cells in a Bacterial Immun-Ization Model

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Abstract

Background/Objective: *Francisella tularensis* is a highly infectious intracellular pathogen that causes severe pulmonary tularemia following aerosol exposure, yet no licensed vaccine exists. Because infection initiates at the respiratory mucosa, understanding mechanisms of protective pulmonary immunity is critical for mucosal vaccine development. This study investigated the role of lung-resident $\gamma\delta$ T cells following intranasal immunization with inactivated *F. tularensis* (iFt) and subsequent lethal challenge with live vaccine strain (LVS). **Methods:** Mice were intranasally immunized with iFt and later challenged with lethal LVS. Pulmonary immune responses were evaluated using flow cytometry and cytokine analysis. Recruitment of $\gamma\delta$ and $\alpha\beta$ T cells, production of IL-17 and IFN- γ , neutrophil infiltration, and $\gamma\delta$ T cell memory phenotypes were assessed in naïve and immunized mice following infection. **Results:** Primary LVS infection induced rapid recruitment of $\gamma\delta$ T cells to the lung beginning on Day 2 post-infection, preceding significant $\alpha\beta$ T cell accumulation. Increased pulmonary IL-17 and IFN- γ correlated with expansion of IL-17- and IFN- γ -associated $\gamma\delta$ T cell populations. Following iFt immunization, mice demonstrated enhanced survival after lethal LVS challenge, accompanied by early increases in pulmonary IL-17 and IL-17 producing $\gamma\delta$ T cells. Immunized mice also exhibited expansion of effector memory and central memory $\gamma\delta$ T cell populations associated with IL-17 production. **Conclusions:** These findings identify IL-17 producing $\gamma\delta$ T cells as contributors to early mucosal immunity following intranasal vaccination against *F. tularensis* and suggest that targeting lung-resident $\gamma\delta$ T cells may support the development of next-generation mucosal vaccines against respiratory pathogens.

Keywords: gamma-delta ($\gamma\delta$) T cells; mucosal immunity; intranasal immunization; pulmonary immunity; lung-resident memory cell formation



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1. Introduction

Francisella tularensis (*F. tularensis*) is a highly infectious intracellular Gram-negative bacterium and the causative agent of tularemia. Pulmonary tularemia, which results from inhalation of aerosolized organisms, represents the most severe clinical manifestation and is associated with substantial mortality if untreated [1]. In natural settings, transmission occurs through contact with infected wildlife or inhalation of contaminated aerosols [1]. Disease severity varies depending on the route of infection, with pulmonary and typhoidal

forms exhibiting severe toxicity. Due to its low infectious dose, efficient airborne transmission, and potential for large-scale dissemination, *F. tularensis* has long been recognized as a significant biodefense concern [2]. Despite this, no licensed vaccine currently exists.

A major obstacle to vaccine development is the capacity of *F. tularensis* to evade early host immune responses. During the initial stages of infection, the bacterium suppresses inflammatory signaling and promotes anti-inflammatory cytokine production, thereby delaying activation of innate and adaptive immunity [3,4]. Under physiological conditions, pattern recognition receptors (PRRs) detect conserved microbial structures and initiate pro-inflammatory cytokine and chemokine responses that coordinate host defense [5]. However, *F. tularensis* either fails to robustly activate PRRs or engages pathways that limit inflammation, thereby enabling intracellular replication [5]. Consequently, early immune events at mucosal surfaces are likely critical determinants of disease outcome and protective immunity.

The live attenuated vaccine strain (LVS) of *F. tularensis* remains the most widely used experimental model for studying tularemia. LVS reproduces key aspects of human disease and is lethal in mice, allowing mechanistic investigation of pulmonary infection under biosafety level 2 conditions. This model provides a platform to define immune correlates of protection and to evaluate mucosal vaccination strategies.

Recent work has highlighted the importance of immune populations capable of rapid effector function in barrier tissues. $\gamma\delta$ T cells represent a distinct T lymphocyte subset that bridges innate and adaptive immunity [6]. Unlike conventional $\alpha\beta$ T cells, $\gamma\delta$ T cells recognize antigen independently of major histocompatibility complex (MHC) restriction and respond rapidly without prior clonal expansion [7,8]. Although they constitute a small fraction of circulating T cells, $\gamma\delta$ T cells are enriched in epithelial and mucosal compartments, including the lung, where they contribute to tissue homeostasis and early immune responses [9,10]. Functionally, $\gamma\delta$ T cells modulate both naïve and memory $\alpha\beta$ T cell responses, help B cells, act as antigen-presenting cells, and produce cytokines associated with Th1, Th2, and Th17 profiles [11–13]. Their strategic localization and rapid cytokine production suggest that they may play a pivotal role in shaping pulmonary immunity during infection.

Clinical and experimental studies support this concept. Increased frequencies of circulating $\gamma\delta$ T cells have been reported during acute tularemia, with persistence following recovery [14,15]. Similarly, murine models of respiratory bacterial infection demonstrate rapid recruitment of $\gamma\delta$ T cells to the lungs, where they contribute to early pathogen control and inflammatory activation [16,17]. Mice lacking $\gamma\delta$ T cells exhibit increased bacterial burden, impaired neutrophil recruitment, and reduced survival, highlighting a non-redundant role in host defense. Similar $\gamma\delta$ T cell responses have been observed across various immunization models, including BCG-vaccinated non-human primates infected with *Mycobacterium tuberculosis*, cattle infected with *Leptospira borgpetersenii*, humans exposed to *Mycobacterium* BCG, mice infected with *Staphylococcus*, and chickens challenged with *Salmonella enteritidis*, where $\gamma\delta$ T cell activity increased upon reinfection [18–22]. These findings suggest that $\gamma\delta$ T cells act as early sentinels during pulmonary infection.

Mechanistically, $\gamma\delta$ T cell mediated protection is closely linked to cytokine production. These cells are potent sources of interferon- γ (IFN- γ) and interleukin-17 (IL-17), both of which are essential for macrophage activation, neutrophil recruitment, and bacterial containment [23]. In respiratory tularemia, early survival is dependent on innate immune pathways, including IFN- γ and neutrophil-mediated responses, rather than classical adaptive immunity [24,25]. CD4–CD8– T cells consistent with a $\gamma\delta$ phenotype have been identified as dominant early producers of these cytokines in the lung [26,27]. Importantly,

IL-17 signaling is required for neutrophil recruitment and effective bacterial clearance, underscoring the importance of this pathway in early defense [27–33].

Emerging evidence further indicates that $\gamma\delta$ T cells exhibit adaptive-like features. Memory-associated phenotypes defined by CD44 and CD62L expression have been described, and $\gamma\delta$ T cells expand upon secondary exposure to bacterial pathogens [18–22,34–38]. These observations suggest that $\gamma\delta$ T cells may contribute to both early innate responses and adaptive mucosal immunity.

Mucosal vaccination strategies against *F. tularensis* support this idea. Intranasal immunization with inactivated bacteria (iFt) induces partial protection and promotes IFN- γ production and inflammatory cell recruitment in the lung [39,40]. However, the cellular mechanisms underlying this protection remain incompletely defined. In particular, previous work identified a CD4⁺ non-CD8⁺ T cell population producing IFN- γ following immunization and challenge, suggesting a potential role for $\gamma\delta$ T cells in recall responses [4].

Based on these observations, we hypothesized that lung-resident $\gamma\delta$ T cells contribute to early and memory-like immune responses following mucosal immunization against *F. tularensis*. The present study therefore investigated $\gamma\delta$ T cell activation, cytokine production, and memory phenotypes following intranasal immunization with inactivated *F. tularensis* and subsequent LVS challenge. Defining the contribution of these cells to pulmonary immunity will provide insight into mechanisms of protection and may inform the rational design of next-generation vaccines targeting mucosal defense against intracellular pathogens.

2. Materials and Methods

2.1. Murine Model

C57BL/6 mice were purchased from Taconic Laboratories (Hudson, NY, USA). All mice were housed at Seton Hall University (South Orange, NJ, USA) in the Animal Research Facility (McNulty Hall). The female mice were used at 6–10 weeks of age. Mice were housed and cared for according to IACUC protocol CB1401, starting date 7 February 2019.

2.2. *F. Tularensis* Bacteria

The live vaccine strain of *F. tularensis* (LVS) was provided by Dr. Edmund Goselin (Albany Medical College, Albany, NY). The organisms grew to a concentration of 2.6×10^9 CFU/mL at 37 °C to a concentration of 2.6×10^9 CFU/mL, using Mueller-Hinton broth supplemented with 2% IsovitaleXTM (Fisher Scientific, Waltham, MA, USA). Live *F. tularensis* samples were stored at −80 °C in liquid nitrogen until needed.

2.3. Inactivation of Bacteria

Inactivated *F. tularensis* LVS (iFt) was generated by growing *F. tularensis* LVS in Mueller Hinton broth (MHB) media (BD Biosciences, San Jose, CA, USA) up to a density of 1×10^9 CFU/mL. The culture was then centrifuged at $22,000 \times g$ for 20 min at 4 °C, washed 3 times with phosphate-buffered saline (PBS), resuspended in 2% Paraformaldehyde (Sigma, Seoul, Republic of Korea) and incubated on a rocker for 2 h at room temperature. Bacteria were then washed three more times with PBS. In total, 1×10^9 organisms were placed on a chocolate agar plate (BD Biosciences) at 37 °C and incubated for 7 days to confirm inactivation. The final concentration of iFt organisms was determined by OD at 610 nm.

2.4. Dose Titration: Inflammation Study

Initial studies were conducted to understand the in vivo response of $\gamma\delta$ T cells to *F. tularensis* LVS through intranasal dosing in mice and monitored for cell changes and cytokine production. In the first set of experiments, naïve C57/BL6 mice were intranasally dosed with 20 μ L of 10,000 colony-forming units (CFUs) or 20,000 CFUs of *F. tularensis* LVS,

the control animals were kept naïve. On days 2, 5, or 7 post-dosing, three mice from each treatment group were euthanized, and the lungs were collected. The PBS group served as a single baseline control. Figure 1 demonstrates the procedures used for dose titration.

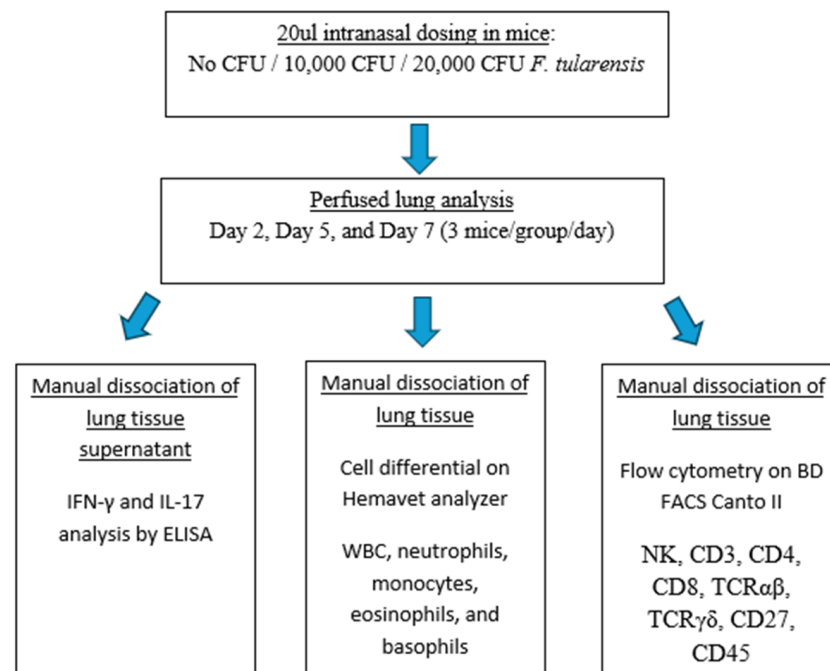


Figure 1. Dose Titration Procedures: Inflammation Study—The dose titration/inflammation study consisted of three groups of animals (one control and two test groups), days of perfused lung analysis, and cytokine testing, cell differentials, and flow cytometry testing on lung tissue.

2.5. Immunization

To investigate the role of $\gamma\delta$ T cells in this immunization, studies were conducted on naïve C57BL/6 mice, following established protocols [3,4,40]. Mice were treated with either PBS (control) or inactivated *F. tularensis* LVS (iFt) on Day 0, followed by a booster dose of iFt on Day 21. On Day 34, thirteen days post-booster, all groups received a lethal challenge dose of 10,000 CFU *F. tularensis* LVS. Our lab has previously observed clinical symptoms in non-immunized mice seven days post-infection with this concentration, and we have confirmed activation of $\gamma\delta$ T cells at this dose.

C57BL/6 mice were divided into three groups consisting of 15 animals per group, 6–10 weeks of age. The mice were intranasally dosed on Day 0 and again on Day 21 with 20 μ L of 2×10^7 iFt. The control group was not immunized and considered naïve. On Day 34 (Day 0 of challenge), the mice in all groups were challenged intranasally with 20 μ L containing 10,000 CFU of live *F. tularensis* LVS. Three mice from each group were euthanized and the lungs collected: prior to the challenge, 2 h (Day 0), 24 h (Day 1), 48 h (Day 2), and seven days (Day 7) post-challenge with *F. tularensis* LVS. The chosen time points were informed by previous research within the Bitsaktsis lab [3,4,40]. The 2-h post-challenge collection was based on Ojeda [41], which demonstrated the detection of radiolabeled *F. tularensis novicida* in lung and digestive tissues as early as 15 min post-infection, with increased detection through the 2-h mark [41]. Figure 2 demonstrates the procedures used for immunization studies.

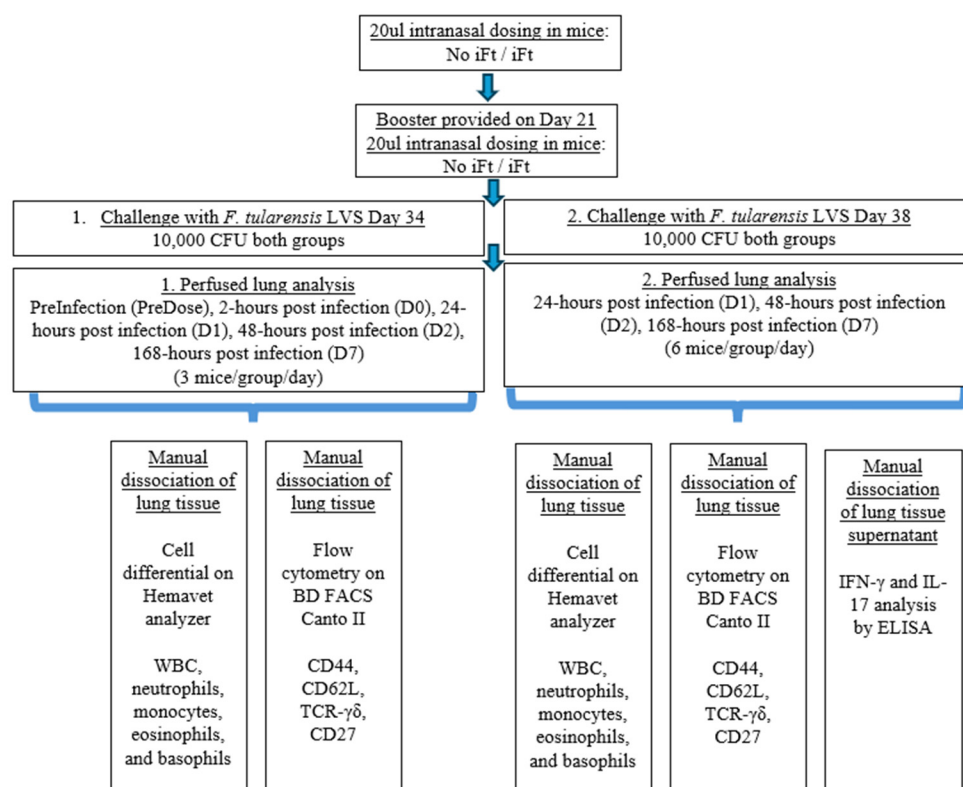


Figure 2. Immunization Studies Procedures: Immunization studies consisted of two groups (one control and one test group), an immunization booster for the test group, a *F. tularensis* LVS challenge in both groups, sample collection, and lung tissue analysis through cell differential, flow cytometry, and cytokine analysis.

2.6. Flow Cytometry Screening

Lungs were collected and perfused with 0.02% EDTA (Teknova, Hollister, CA, USA) in DPBS (Corning, Corning, NY, USA). The perfusion was discarded. The sample was then shipped on ice packs for same-day processing and analysis to Novartis Pharmaceuticals, East Hanover, NJ. Lung tissue was processed by manual homogenization (syringe plunger and Petri dish) and pushed through a 70 µm filter. The single-celled suspension was transferred to a 5 mL polystyrene 12 × 75 tube and centrifuged at 200× *g* for 5 min at 4 °C. The supernatant was saved for future cytokine analysis, and the cell pellet was then reconstituted in 1 mL red blood cell lysis buffer (house-made: Ammonium chloride, Potassium phosphate, EDTA) for approximately two minutes (until lysis occurs) and then centrifuged at 200× *g* for 5 min at 4 °C. The supernatant was discarded, and the cell pellet was adjusted to 1 × 10⁶ cells for flow cytometer analysis.

Analysis of the washed cell pellet was conducted using a Drew Scientific Hemavet 950 analyzer which was validated for mouse blood and tissue. From the washed cell pellet, 30 µL of the concentrated lung cells was analyzed for the initial concentration of white blood cells. The pellet was then adjusted with DPBS (Corning) to a final concentration of 1 × 10⁶ of white blood cells prior to complete cell differentiation and FACS analysis.

Lung cells for cell surface marker staining were added to a titrated antibody cocktail or corresponding isotype control and incubated on wet ice, protected from light, for at least 30 min. Two milliliters of DPBS were added to each tube and centrifuge at 400× *g* for 5 min at 4 °C. The supernatant was decanted, and 0.2 mL of 2% formaldehyde was added to each tube. Tubes were gently mixed and incubated on wet ice or refrigerated, protected from light, for at least 30 min. A singlet gate was established on the FACS Canto II flow cytometer, and 10,000 events were collected. The following antibodies were purchased from

BD Biosciences (San Jose, California) for FACS analysis: CD3-FITC (145-2C11 or 17A2), CD4-APC (RM4-5), CD8a-APC-Cy7 (53-6.7), NK1.1-PE (PK136), TCR $\alpha\beta$ -Pacific Blue (H57.597), TCR $\gamma\delta$ -PE/Cy7 (GL3), CD27-PerCP/Cy5.5 (LG.3A10), and CD45-BV510 (30-F11).

Immunophenotyping via fluorescence activated cell sorting (FACS) analysis of key cell types, including NK cells, T cells (CD3, CD4, CD8, TCR $\alpha\beta$, TCR $\gamma\delta$), and leukocytes (CD45) was performed. Further investigation of $\gamma\delta$ T cells through FACS analysis included examination of CD27 expression, a marker known to differentiate cytokine profiles within $\gamma\delta$ T cells. CD27+ $\gamma\delta$ T cells typically produce IFN- γ , while CD27− $\gamma\delta$ T cells are more likely to produce IL-17, as shown in Ribot [38]. For flow cytometry, $\gamma\delta$ T cells producing IFN- γ will be identified as CD27+TCR $\gamma\delta$ +, and those producing IL-17 will be CD27-TCR $\gamma\delta$ +. The cytokine profile will aid in further understanding the $\gamma\delta$ -T cell response due to infection from *F. tularensis* LVS.

Additional FACS analysis of CD44 and CD62L was conducted in this immunization experiment for the detection of memory T cells. To analyze the roles of central memory (CM) and effector memory (EM) $\gamma\delta$ T cells, we will distinguish CD44+CD62L+ cells as CM T cells and CD44+CD62L− cells as EM T cells. Previous studies have indicated that CD44 expression denotes activation, while CD62L is a marker of central memory phenotype [34,38] (Figure S1).

Our analysis focuses on the $\gamma\delta$ T cell inflammatory response. In gating CD44 and CD62L for each memory cell type, CD27+ $\gamma\delta$ T+ cells for IFN- γ production and CD27− $\gamma\delta$ T+ cells for IL-17 production were examined, identifying memory responses among $\gamma\delta$ T cells. Ribot [38] found that CD27− $\gamma\delta$ T cells signaling IL-17 were often CD44^{hi}CD62L^{lo} (EM), highlighting specific $\gamma\delta$ T cell phenotypes associated with cytokine production. In line with this, Misiak [34] noted that CD27−CD44+ $\gamma\delta$ T cells exhibited memory responses in a *Bordetella pertussis* infection model, with a significant increase in CD44+CD62L+CD27− cells post-rechallenge, a phenotype indicative of effector memory function.

2.7. Enzyme Linked Immunosorbent Assays (ELISAs)

Cytokine analysis was completed using BioLegend ELISA MAX™ (BioLegend, Inc., San Diego, CA, USA) for Mouse IFN- γ (Cat# 430804) and Mouse IL-17 (Cat# 432504), using a Molecular Devices SpectaMAX M5 (Molecular Devices, LLC, San Jose, CA, USA) spectrophotometer for the 96-well plate read and SoftMax Pro GxP 5.4.4 software for analysis of the concentration (pg/mL) of cytokines in the initial supernatant capture.

2.8. Statistical Analysis

Statistical differences among groups were analyzed using multiple *t* tests. GraphPad Prism 10 software was used for the statistical analysis (San Diego, CA, USA).

2.9. Ethics Statement

All animals were handled in accordance with good animal practice defined by relevant national and/or local animal welfare bodies. Briefly, mouse euthanasia was performed via CO₂ administration followed by cervical dislocation.

3. Results

3.1. Increased Gamma-Delta T Cells Recruitment and Production of Il-17 and Ifn- γ , in the Lungs of Mice During Primary Lvs Infection

Pulmonary $\gamma\delta$ T cell responses were assessed following intranasal infection of mice with LVS. Intranasal dosing of 10,000 or 20,000 CFU of LVS significantly increased the percentage of $\gamma\delta$ T cells (Figure 3A) by Day 2 and $\alpha\beta$ T cells by Day 5 (Figure 3B), in the mouse lung, compared to PBS control mice. As seen in Figure 3, $\gamma\delta$ and $\alpha\beta$ T cells were both significantly elevated due to infection. Gamma-delta T cells, having tissue-resident

properties within the lung, as well as the ability to recognize antigen independent of MHC restriction, increased significantly by Day 2 and continued to increase by Day 5 post dose, remaining elevated through the end of the study on Day 7 (Figure 3A). The $\alpha\beta$ T cells increased with similar kinetics to the $\gamma\delta$ T cells, albeit at a slower rate, likely due to their need for MHC antigen presentation and subsequent clonal expansion (Figure 3B).

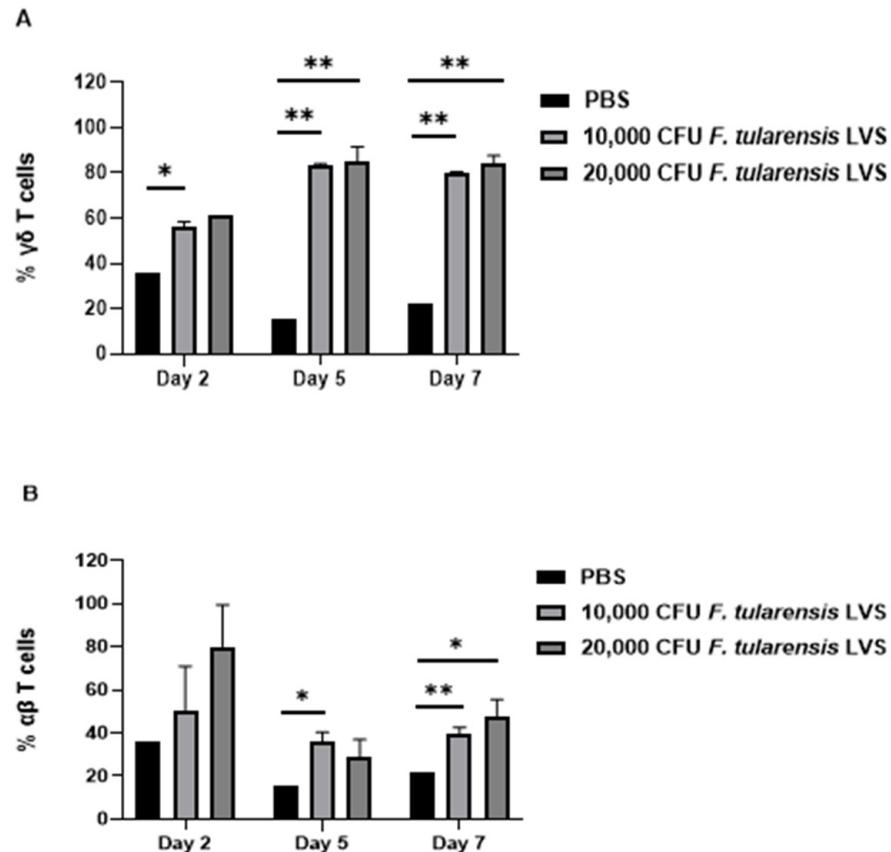


Figure 3. $\gamma\delta$ and $\alpha\beta$ T cells change due to infection. Infection of the mice with either 10,000 or 20,000 CFU *F. tularensis* LVS resulted in an increase in both $\gamma\delta$ and $\alpha\beta$ T cells. A significant increase in $\gamma\delta$ T cells was seen by Day 2 and continued through the close of the study on Day 7 (A). A significant increase was seen in $\alpha\beta$ T cells on Day 5 and continued through the close of the study on Day 7 (B). Lungs were harvested each day, and WBC were analyzed for antibodies for TCR $\gamma\delta$ (GL3) or TCR $\alpha\beta$ (H57.597) through FACS. Due to the variance in the absolute number with sample degradation, the results are presented as a percentage of the parent population (WBC). Three mice were used from each treatment group, each time point; the results are from one of the three experiments. (*) $p < 0.1$; (**) $p < 0.05$; bars represent Standard Deviation (SD).

Phenotypic analysis of $\gamma\delta$ T cells in the lung revealed that the majority of responding cells were CD3+CD4−CD8−. This population significantly increased between Day 5 and Day 7 post-infection compared to PBS controls (Figure 4), confirming the identity of the $\gamma\delta$ T cell population assessed and validating the gating strategy used for downstream analyses.

From the literature, we understand that $\gamma\delta$ T cells predominantly produce IFN- γ and IL-17. As expected, IFN- γ and IL-17 cytokines were significantly elevated in infected mice beginning on Day 5. The significant increase in IFN- γ in the lung (Figure 5A) was correlated to an increase in CD4−CD8−CD27+ cells (possible IFN- γ producing $\gamma\delta$ T cells, Figure 6A), which also commenced on Day 5 through the end of the study on Day 7. Similar observations were made with total IL-17, which increased in the lung tissue (Figure 5B), correlating to a significant increase in possible IL-17 producing $\gamma\delta$ T cells

(CD4–CD8–CD27–), (Figure 6B). These cytokines have been shown to be crucial in the control of respiratory bacterial pathogens [23–27,29].

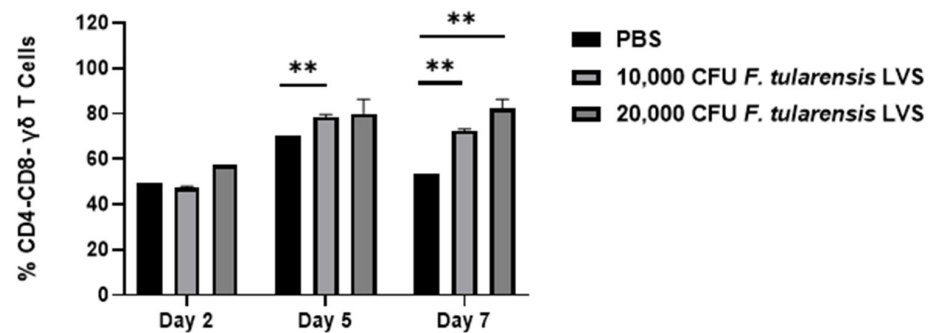


Figure 4. $\gamma\delta$ T cells as a subpopulation of CD4–CD8– and the changes due to infection. Intranasal infection of mice with either 10,000 or 20,000 CFU *F. tularensis* LVS resulted in a significant increase in CD4–CD8– $\gamma\delta$ T cells by Day 5 and continuing through the end of study on Day 7. Lungs were harvested each day, and WBC were analyzed for antibodies for CD4 (RM4-5), CD8a (536.7), and TCR $\gamma\delta$ (GL3) through FACS analysis. Due to the variance in the absolute number with sample degradation, the results are presented as a percentage of the parent population (CD4–CD8–). Three mice were used for each treatment group at each time point; the results are from one of the three experiments. (**) $p < 0.05$; bars represent SD.

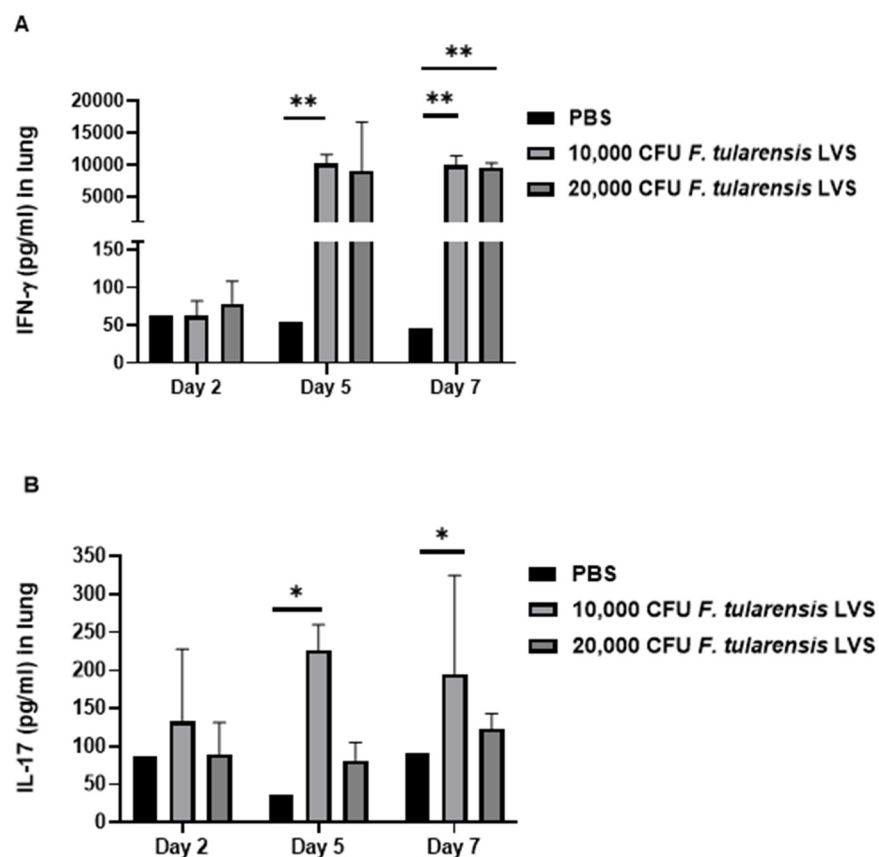


Figure 5. Changes in cytokine response due to infection. Infection of the mice with either 10,000 or 20,000 CFU *F. tularensis* LVS resulted in an increase in IFN- γ (A) and IL-17 (B) beginning on Day 5 and continuing through the close of the study on Day 7. Lungs were harvested each day and the supernatant from the first wash of dissociated lung cells was analyzed by ELISA for IFN- γ or IL-17 concentrations. Note: 20,000 CFU was determined to be too high of a dose for mice, causing the immune system to proceed in shutting down thus a possible reason for the decrease seen in the 20,000 CFU dosage when assaying IL-17. Three mice were used from each treatment group on each day; the results are from one of the three experiments. (*) $p < 0.1$; (**) $p < 0.05$; bars represent SD.

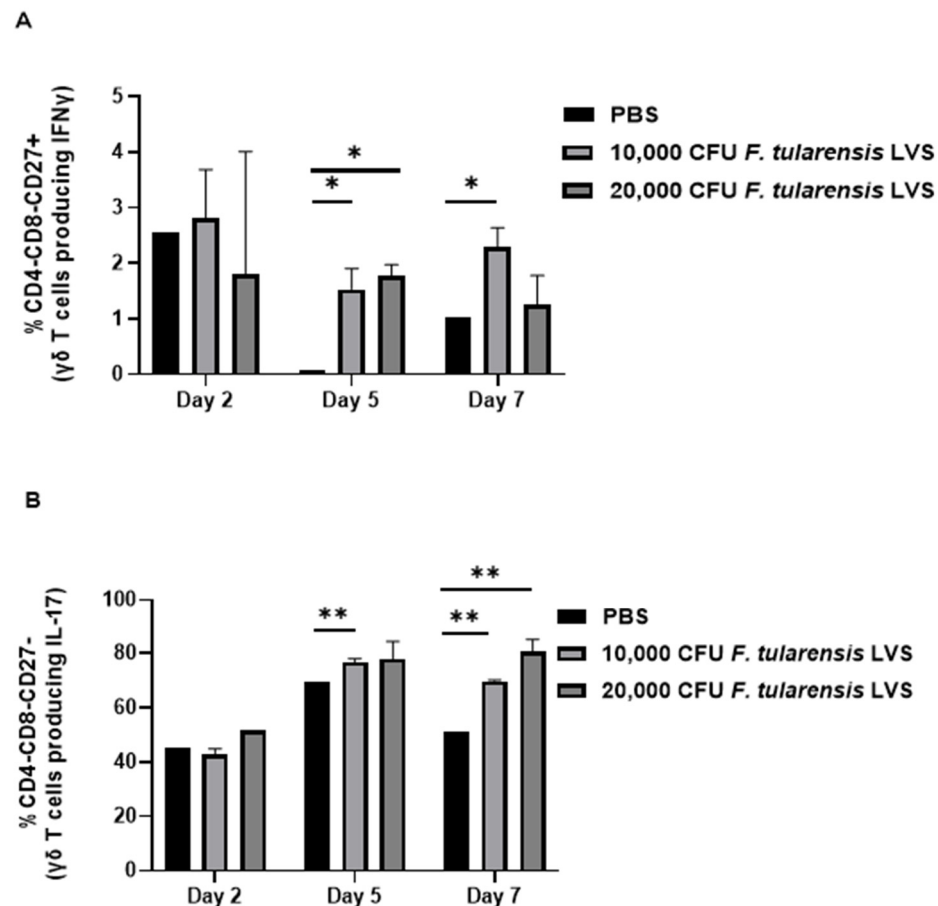


Figure 6. Changes in $\gamma\delta$ T cells producing IFN- γ or IL-17 due to infection. Intranasal dosing in mice with either 10,000 or 20,000 CFU *F. tularensis* LVS resulted in a significant increase in the percentage of IFN- γ or IL-17 signaling through $\gamma\delta$ T cells beginning on Day 5 through the end of study on Day 7 (A). Lungs were harvested on each day, perfused, and the cells manually dissociated and filtered. Lung cells were stained with CD4 (RM4-5), CD8a (53-6.7), and CD27 (LG.3A10). Those cells that were CD4-CD8-CD27+ are considered $\gamma\delta$ T cells producing IFN- γ . Cells that were CD4-CD8-CD27- are considered $\gamma\delta$ T cells producing IL-17. The percentage presented is a percentage of CD45+ (white blood cell) as the parent population (B). Three mice were used from each treatment group on each day; the results are from one of the three experiments. (*) $p < 0.1$; (**) $p < 0.05$; bars represent SD.

Thus far, we have demonstrated an inflammatory response during LVS infection through recruitment of both $\alpha\beta$ T cells and $\gamma\delta$ T cells, and an increased concentration of IFN- γ and IL-17 within the lung tissue of infected mice. As seen in Figure 6, the $\gamma\delta$ T cells that possibly produce IFN- γ are significantly increased on Day 5, and levels of IFN- γ production are still elevated through the end of the study on Day 7 (Figure 6A) with a similar trend observed with IL-17 producing $\gamma\delta$ T cells (Figure 6B).

For the rest of the study, we proceeded to utilize the 10,000 CFU *F. tularensis* lethal dose as it was deemed sufficient to cause mouse morbidity without compromising the cellular architecture of the lung tissue, sample quality, or subsequent sample analysis.

3.2. Recruitment of IL-17 Producing $\gamma\delta$ T Cells and Early IL-17 Production During Lvs Infec-Tion in the Lungs of Ift Immunized Mice

Studies have demonstrated that $\gamma\delta$ T cells rapidly accumulate in the lungs following bacterial exposure and produce the cytokines IFN- γ and IL-17, which aid in bacterial clearance. Similar responses have been observed in mice, cattle, humans, chickens, and swine, highlighting $\gamma\delta$ T cells' role in early immune defense and pathogen clearance [18–22].

Immune cell responses within the lung were assessed following intranasal *F. tularensis* LVS infection in naïve and *iFt*-immunized mice. During primary LVS infection, a significant increase in total white blood cells (WBCs) was observed in the lung by Day 5 post-infection and remained elevated through Day 7 compared to PBS controls (Figure 7A). Neutrophil numbers were significantly increased over the same time frame (Figure 7B), with additional increases observed in monocytes, eosinophils, and basophils.

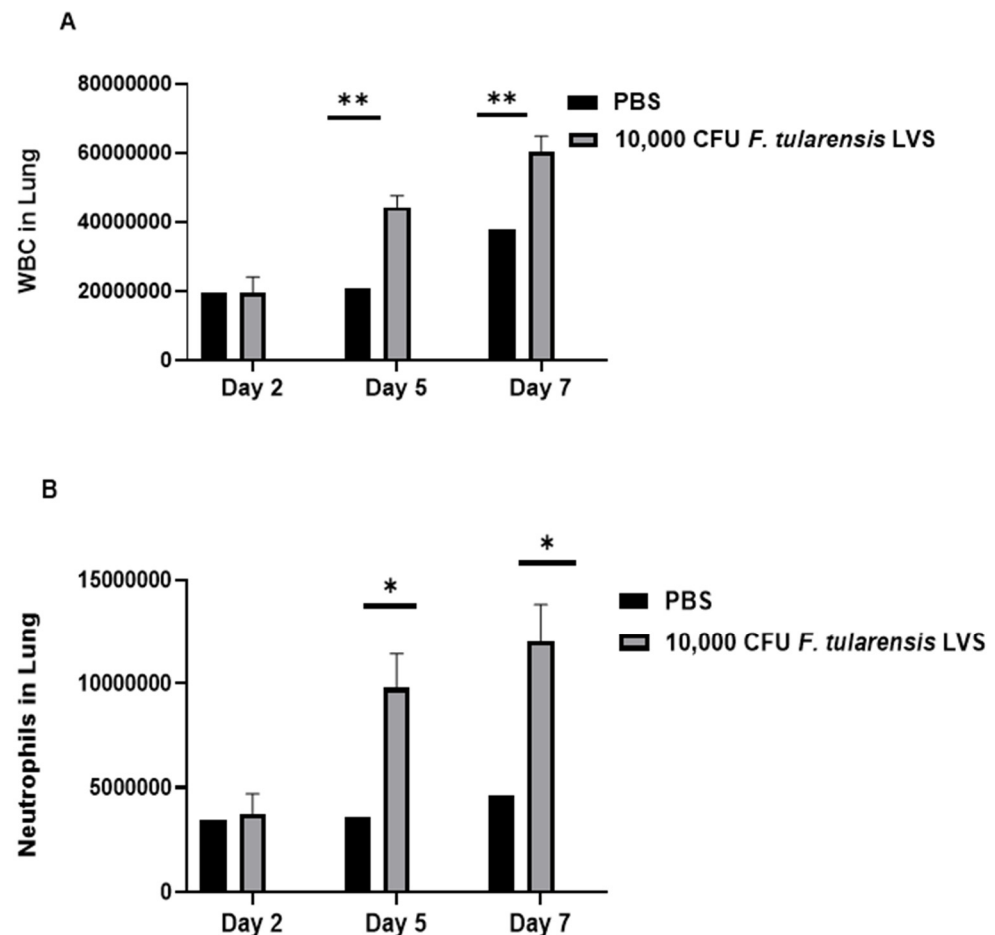


Figure 7. Primary infection with *F. tularensis* causes an increase in WBC and neutrophils. Inflammation due to intranasal dosing of 10,000 CFU of *F. tularensis* LVS caused WBC's (A) including neutrophils (B) to increase significantly beginning on Day 5 post infection. Lungs were harvested and perfused from uninfected mice (PBS) as a baseline control. The WBC and neutrophil cell counts were obtained by analysis using an automated cell counter validated for use on mouse lung tissue. Three mice were used from each treatment group at each timepoint; the results are from one of the three experiments. (*) $p < 0.1$; (**) $p < 0.05$; bars represent SD.

To assess the effect of immunization, WBCs, $\gamma\delta$ T cells, and neutrophils were evaluated in mice immunized intranasally with *iFt* and subsequently challenged with lethal LVS. On Day 7 post-challenge, total WBCs, $\gamma\delta$ T cells, and neutrophils were significantly increased in both immunized and control mice; however, all three populations were reduced in *iFt*-immunized mice compared to PBS controls (Figure 8A–C, respectively). All *iFt*-immunized mice survived with no physical signs of infection to the end of the study, whereas PBS control mice began showing signs of morbidity due to infection by Day 7.

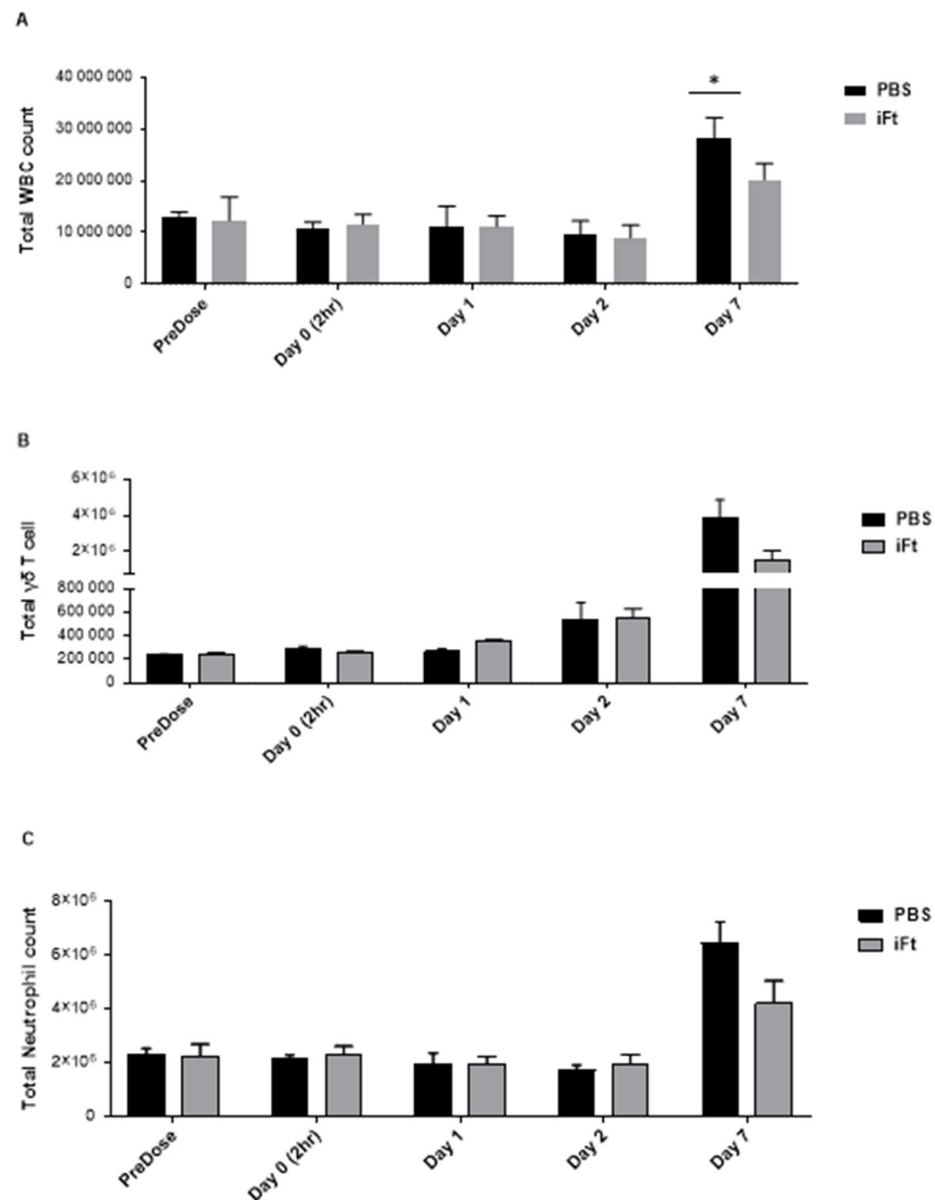


Figure 8. Immunized mice show decreases in WBC, $\gamma\delta$ T cells, and neutrophils on Day 7. Mice immunized with *iFt* has a decrease in WBCs (A), $\gamma\delta$ T cells (B), and neutrophils (C) on Day 7 compared to the PBS group indicating less inflammation due to infection from *F. tularensis* LVS. Mice in the *iFt* group were intranasally immunized with *iFt* on Day 0, followed by a booster of the same immunization on Day 21. Challenge of 10,000 CFU *F. tularensis* LVS was then administered intranasally on Day 34 of the study. White blood cells and neutrophil counts were determined by an automated cell counter (Hemavet) validated for use with mouse tissue. Gamma-delta T cell counts were determined through flow cytometry and provided as an absolute count. Three mice were used for each group at each timepoint; the results are from one of the two experiments. (*) $p < 0.1$; bars represent SD.

IFN- γ levels in the lung were significantly increased on Day 7 post-challenge in *iFt*-immunized mice (Figure 9A). Analysis of $\gamma\delta$ T cells and CD27+ cells (possible IFN- γ producing $\gamma\delta$ T cells) (Figure 9B) revealed that the IFN- γ response to infection post-immunization was not from $\gamma\delta$ T cells.

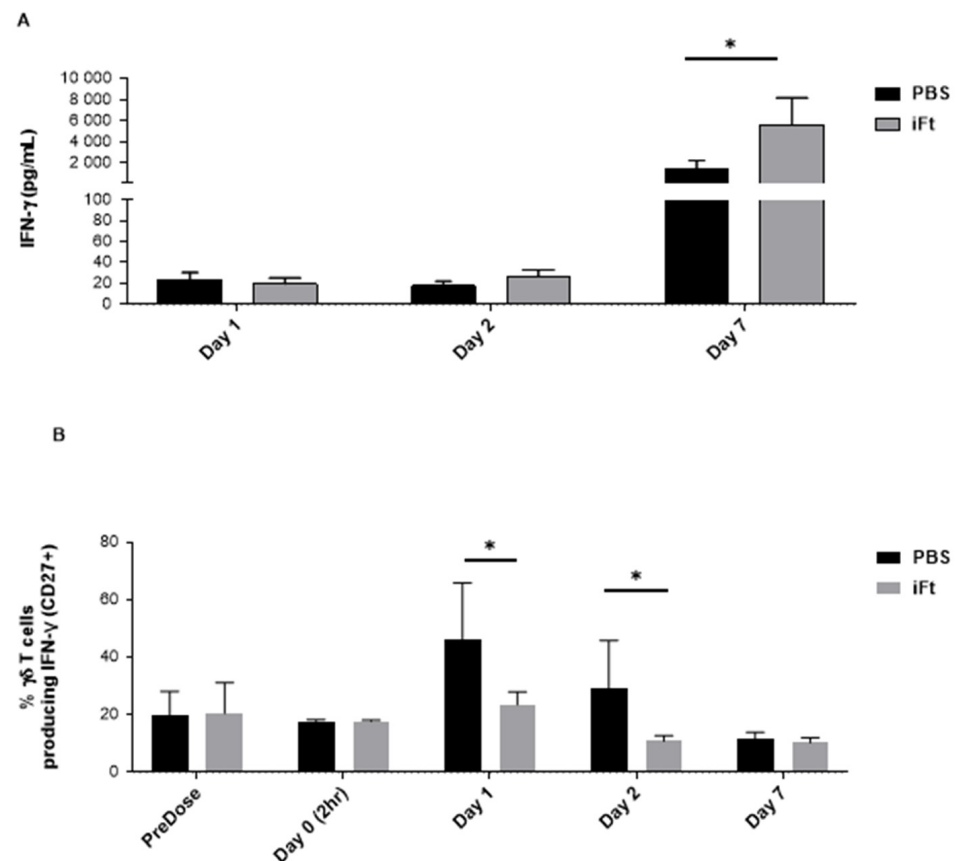


Figure 9. $\gamma\delta$ T cells producing IFN- γ are not a primary source of IFN- γ in immunized mice. IFN- γ (A) was elevated in mice immunized with iFt on Day 7 of the experiment. The concentration of IFN- γ in the lung was determined by ELISA. Cells positively labeled for $\gamma\delta$ T and CD27 (B) in mice immunized with iFt showed no correlation with IFN- γ . Mice were intranasally immunized with iFt on Day 0, followed by a booster of iFt on Day 21, challenge of 10,000 CFU of *F. tularensis* LVS was then administered intranasally on Day 34 of the study. Mice that were not immunized nor boosted but did receive the challenge administration of *F. tularensis* LVS are considered naïve (PBS) and serve as a baseline as to how infection progresses without immunization. Three mice were used for each group at each timepoint; the results are from one of the two experiments. Repeat analysis verified results. Analysis was determined by flow cytometry where $\gamma\delta$ T cells (TCR- $\gamma\delta$ GL-3) were labeled with CD27 (LG.3A10) for positive or negative cell receptor engagement. Cells TCR- $\gamma\delta$ +CD27+ were analyzed as producing IFN- γ . (*) $p < 0.1$; bars represent SD.

However, early cytokine responses following LVS challenge revealed a significant increase in total pulmonary IL-17 in iFt-immunized mice as early as Day 2 post-challenge compared to PBS controls (Figure 10A). This increase in IL-17 was also noted in a significant rise in CD27- $\gamma\delta$ T cells (possibly IL-17 producing $\gamma\delta$ T cells) on Day 1 post-challenge (Figure 10B). By Day 7, the percentage of CD27- $\gamma\delta$ T cells was comparable between immunized and control groups, although total IL-17 levels remained significantly elevated in immunized mice.

This data shows that iFt immunization with a decrease in WBCs, $\gamma\delta$ T cells, and neutrophils provides protection following LVS challenge, accompanied by early increases in CD27- $\gamma\delta$ T cells and sustained pulmonary IFN- γ and IL-17 levels.

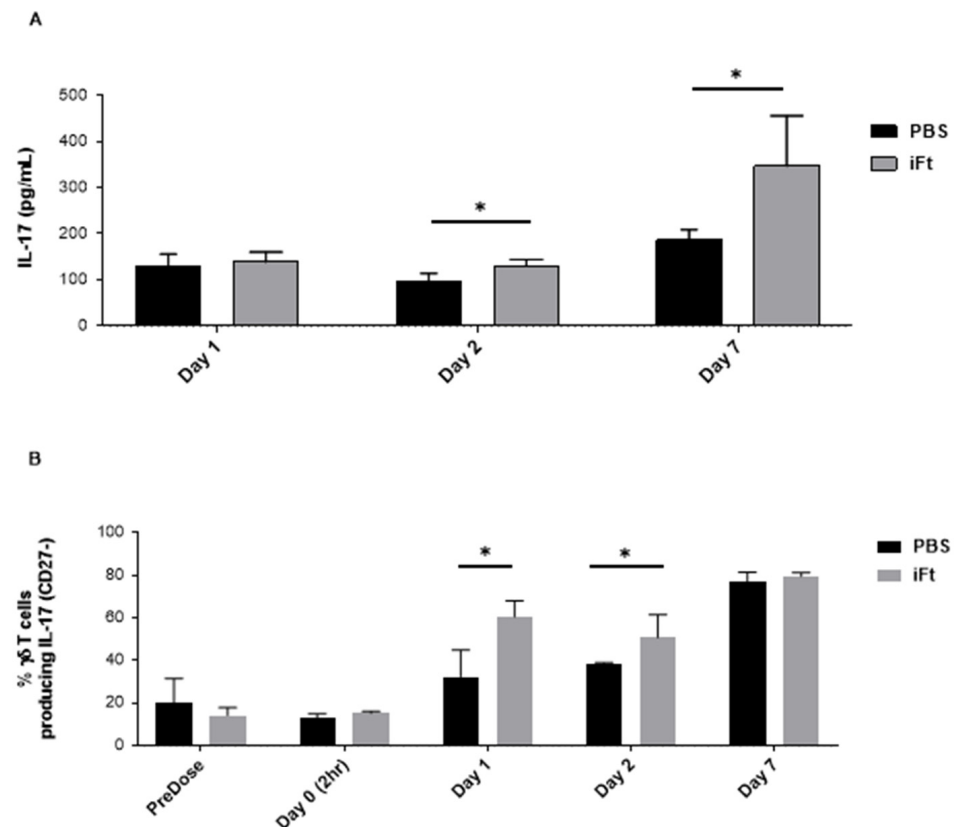


Figure 10. $\gamma\delta$ T cells producing IL-17 increase in immunized mice. IL-17 concentration in mouse lungs is greater in mice immunized with iFt on Day 7 showing a robust response to antigen challenge (A). The $\gamma\delta$ T cells producing IL-17 also provide a robust response to antigen challenge as early as Day 1 and Day 2 (B). By Day 7 the $\gamma\delta$ T cells producing IL-17 are the same as those mice not immunized (B). Mice in the iFt group were intranasally immunized with iFt on Day 0, followed by a booster of iFt on Day 21. Challenge of 10,000 CFU *F. tularensis* LVS was then administered intranasally on Day 34 of the study (iFt group). Three mice were used for each group at each timepoint; the results are from one of the two experiments. Repeat analysis verified results. IL-17 concentration in mouse lung tissue was obtained from ELISA analysis. Analysis was determined by flow cytometry where $\gamma\delta$ T cells (TCR- $\gamma\delta$ GL-3) were labeled with CD27 (LG.3A10) for positive or negative cell receptor engagement. Cells TCR- $\gamma\delta$ +CD27– were analyzed as producing IL-17. (*) $p < 0.1$; bars represent SD.

3.3. Lung IL-17 Producing $\gamma\delta$ T Cells Exhibit Both an Effector and Central Memory Phenotype in iFt-Immunized Mice

To assess whether intranasal immunization with iFt induces $\gamma\delta$ T cell memory responses that may contribute to protection against lethal LVS challenge, $\gamma\delta$ T cell memory phenotypes were evaluated in the lung following infection. Since immunization with iFt did not recruit significant numbers of CD27+ $\gamma\delta$ T cells (possibly producing IFN- γ) in the lungs of immunized mice, memory cell analysis in this $\gamma\delta$ T cell subpopulation was not conducted. However, iFt immunized mice exhibited an early (Day 1) increase in IL-17 producing $\gamma\delta$ T cells, coinciding with elevated lung IL-17 levels, suggesting a potential early role in protection due to memory formation.

$\gamma\delta$ T cell memory populations were defined based on surface expression of CD44 and CD62L. Effector memory (EM) $\gamma\delta$ T cells were identified as CD44+CD62L–, while central memory (CM) $\gamma\delta$ T cells were identified as CD44+CD62L+. CD44 expression was used as a marker of antigen-experienced T cells, whereas CD62L expression was used to distinguish central memory populations. Additional characterization of IL-17 producing $\gamma\delta$ T cells was performed based on CD27 expression.

In Figure 11 we show a correlation between the $\gamma\delta$ T cell effector memory population and the $\gamma\delta$ T cell production of IL-17. As can be seen in Figure 11A, EM $\gamma\delta$ T cells in mice immunized with *iFt* are significantly elevated on Day 1 and 2 post challenge (Figure 11A). EM $\gamma\delta$ T cells producing IL-17 (CD44+CD62L–TCR $\gamma\delta$ +CD27–) (Figure 11B) are significantly increased on Day 1 through Day 2 in mice immunized with *iFt*, correlating to the increase seen in the EM $\gamma\delta$ T cells in Figure 11A and Supplementary Figure S2. These findings align with the increase in IL-17 production by $\gamma\delta$ T cells observed on Day 1 post challenge seen in Figure 7, confirming that this early IL-17 response from $\gamma\delta$ T cells likely originates from an effector memory $\gamma\delta$ T cell population. There is no difference on Day 7, but the early reaction of $\gamma\delta$ T cells producing IL-17 could be seen as possible protection in mice immunized with *iFt*.

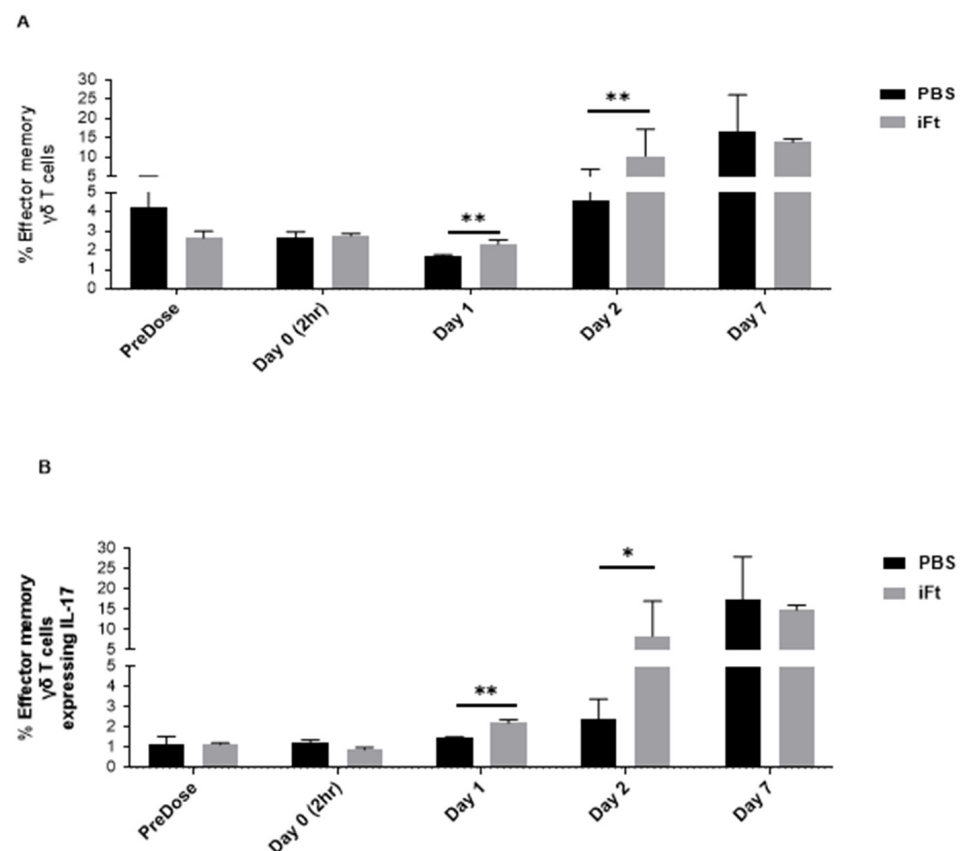


Figure 11. $\gamma\delta$ effector memory T cells producing IL-17 (CD44+CD62L–TCR $\gamma\delta$ +CD27–) increase in immunized mice. In the $\gamma\delta$ effector memory T cells (A) there is a significant increase on Day 1 and a continued increase on Day 2 in mice immunized with *iFt*. The $\gamma\delta$ effector memory T cells in mice immunized with *iFt*, shows a correlation with $\gamma\delta$ T cells producing more IL-17 on both Day 1 and Day 2 (B). Mice were intranasally immunized with *iFt* on Day 0, followed by a booster of *iFt* on Day 21, challenge of 10,000 CFU of *F. tularensis* LVS was then administered intranasally on Day 34 of the study. Mice that were not immunized nor boosted but did receive the challenge administration of *F. tularensis* LVS are considered naïve (PBS) and serve as a baseline as to how infection progresses without immunization. Three mice were used for each group; the results are from one of the two experiments. Repeat analysis verified results. (*) $p < 0.1$; (**) $p < 0.05$; bars represent SD. Supplementary Figure S2 shows contour plot of Day 2, PBS compared to *iFt* mice.

Figure 12 compares the central memory (CM) $\gamma\delta$ T cell population with IL-17 producing CM $\gamma\delta$ T cells. As shown in Figure 12A, CM $\gamma\delta$ T cells were significantly increased in *iFt*-immunized mice by Day 2 post-challenge. Correspondingly, IL-17 production by CM $\gamma\delta$ T cells (CD44+CD62L+TCR $\gamma\delta$ +CD27–) (Figure 12B) was also significantly elevated at this time point, demonstrating a strong association between CM $\gamma\delta$ T cell expansion and IL-17

production. By Day 7 post-challenge, CM $\gamma\delta$ T cells remained elevated (Figure 12A); however, IL-17 production by these cells significantly decreased (Figure 12B and Supplementary Figure S3) in *iFt*-immunized mice. Together, these findings suggest an early, coordinated increase in CM $\gamma\delta$ T cells and IL-17 production following LVS challenge, followed by a decline in IL-17 output despite sustained CM $\gamma\delta$ T cell levels.

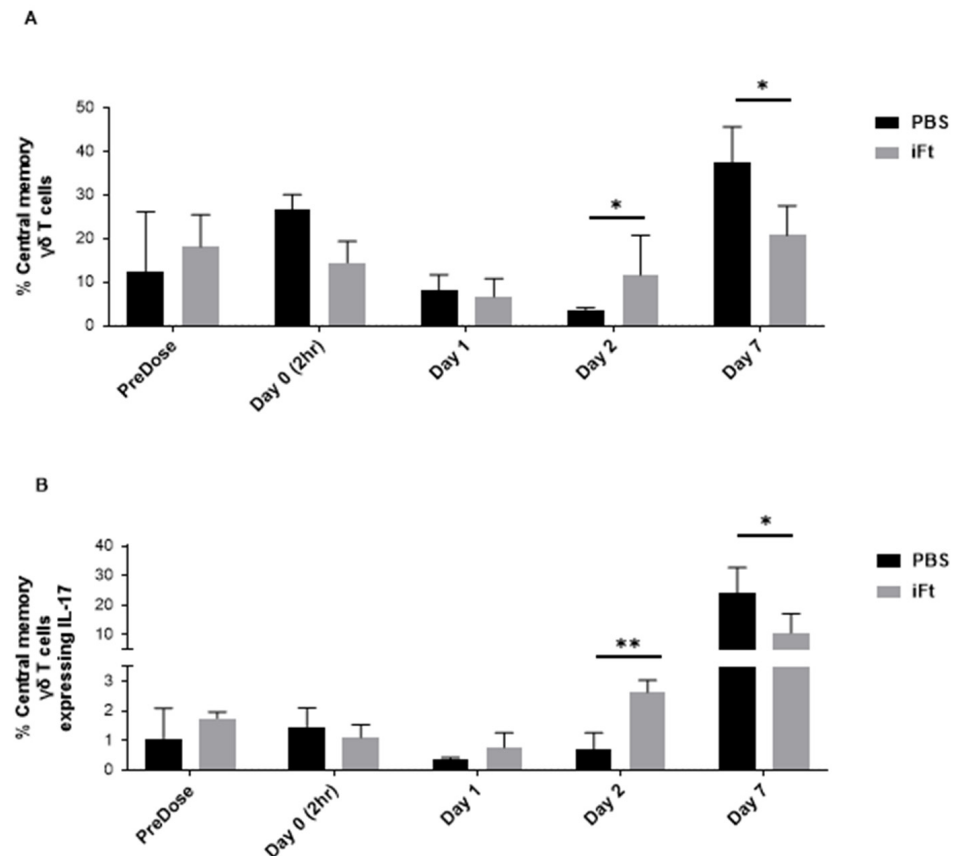


Figure 12. $\gamma\delta$ central memory T cells producing IL-17 (CD44+CD62L+TCR $\gamma\delta$ +CD27 $^{-}$) changes in immunized mice. In the $\gamma\delta$ central memory T cells (A) there is a significant increase on Day 2 and a decrease on Day 7 in mice immunized with *iFt*. The $\gamma\delta$ central memory T cells in mice immunized with *iFt*, shows a correlation with $\gamma\delta$ T cells producing more IL-17 on both Day 2 and less on Day 7 (B). Mice were intranasally immunized with *iFt* on Day 0, followed by a booster of *iFt* on Day 21, challenge of 10,000 CFU of *F. tularensis* LVS was then administered intranasally on Day 34 of the study. Mice that were not immunized nor boosted but did receive the challenge administration of *F. tularensis* LVS are considered naïve (PBS) and serve as a baseline as to how infection progresses without immunization. Three mice were used for each group; the results are from one of the two experiments. Repeat analysis verified results. (*) $p < 0.1$; (**) $p < 0.05$; bars represent SD. Supplementary Figure S3 shows contour plot of Day 2, PBS compared to *iFt* mice.

Collectively, these data demonstrate that *iFt* immunization induces both EM and CM $\gamma\delta$ T cell populations in the lung that respond rapidly following LVS challenge. Effector memory $\gamma\delta$ T cells producing IL-17 were elevated as early as Day 1 post-challenge, while IL-17 producing CM $\gamma\delta$ T cells peaked by Day 2 post-challenge. The association between these $\gamma\delta$ T cell memory subsets and IL-17 production supports a role for lung-resident $\gamma\delta$ T cell memory responses in the early immune response to LVS infection in *iFt*-immunized mice.

4. Discussion

The specific aim of this research was to investigate and characterize the immune response of $\gamma\delta$ T cells upon intranasal immunization with *iFt* followed by a LVS challenge.

$\gamma\delta$ T cells are known to bridge the innate and adaptive immune system's response in a protective role against viruses, bacteria, wound healing, cancer therapy, and autoimmune disease in humans. A deeper understanding of how $\gamma\delta$ T cells are recruited to LVS infection, both in primary infection and in response to immunization with *iFt*, provides valuable insights into the role of this cell type.

Initial experiments showed that $\gamma\delta$ T cells were rapidly and significantly recruited in the lungs of LVS-infected mice as early as Day 2 post-infection and remained elevated through Day 7. This response preceded the accumulation of conventional $\alpha\beta$ T cells, which did not increase significantly until Day 7, suggesting that lung-resident $\gamma\delta$ T cells may contribute to early containment of LVS. Analysis of cytokine responses revealed an increase in IFN- γ within lung tissue correlating with IFN- γ production by $\gamma\delta$ T cells, with significant increases beginning on Day 5 through Day 7. Additionally, IL-17 levels in the lung increased beginning on Day 5 and remained elevated through the end of the study, closely correlating with the expansion of IL-17 producing $\gamma\delta$ T cells.

The initial objective of the immunization study was to examine the relationship between $\gamma\delta$ T cells producing IFN- γ or IL-17 and the recruitment of neutrophils, thereby assessing their potential contribution to early innate immune responses in the lungs of immunized mice. During primary LVS infection, a significant increase in total white blood cells and neutrophils was observed beginning on Day 5 post-infection, consistent with an inflammatory response. Following intranasal immunization with *iFt* and subsequent LVS challenge, immunized mice exhibited reduced numbers of white blood cells, $\gamma\delta$ T cells, and neutrophils in the lungs by Day 7 compared to control mice. This reduction likely reflects improved bacterial control and resolution of inflammation.

To determine whether early $\gamma\delta$ T cell cytokine responses were associated with later reductions in inflammatory cells, we examined the recruitment of $\gamma\delta$ T cells producing IFN- γ and IL-17 following challenge. Notably, IL-17 levels in the lungs of immunized mice were significantly elevated as early as Day 2 post-challenge compared to PBS controls. Further analysis demonstrated a significant increase in IL-17 producing $\gamma\delta$ T cells on Day 1 post-challenge, indicating that $\gamma\delta$ T cells contribute, at least in part, to this early IL-17 response. Given their localization at mucosal sites within the lung, these IL-17-producing $\gamma\delta$ T cells are well positioned to respond rapidly to infection.

Contrary to our initial expectation, immunization did not elicit a marked increase in white blood cells, $\gamma\delta$ T cells, or neutrophils before Day 7, suggesting that *F. tularensis* may initially evade immune detection following *iFt* immunization or that protective immunity in this model relies on early cytokine signaling rather than overt cellular recruitment. It is also possible that subsequent activation of adaptive immune mechanisms, including CD4+ IFN- γ producing T cells, contributes to protection at later time points [4,42].

IFN- γ production in immunized mice was significantly increased at later stages of infection, particularly by Day 7 post-challenge. These findings are consistent with previous studies in our lab demonstrating that IFN- γ mediated protection following intranasal immunization targeting Fc γ receptors is largely driven by CD4+ effector T cells producing IFN- γ [4]. Thus, while IFN- γ remains essential for protection in our lab's vaccine model, $\gamma\delta$ T cells appear to play a limited role in IFN- γ production through *iFt* immunization. Notably, $\gamma\delta$ T cells exhibited minimal IFN- γ expression, indicating that $\gamma\delta$ T cells preferentially adopt an IL-17 producing phenotype rather than an IFN- γ -producing one. This finding is consistent with findings from pulmonary infection models using *B. thailandensis*, further supporting a pivotal role for IL-17 producing $\gamma\delta$ T cells in early mucosal immunity [16].

The contribution of IL-17 producing $\gamma\delta$ T cells was most evident during the early phase of infection (Days 1–2), whereas by Day 7, IL-17 levels were comparable between groups, coinciding with the emergence of adaptive immune responses. While $\gamma\delta$ T cells

represent a key early source of IL-17, other innate-like T lymphocytes, including natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells, may also contribute to this response [43]. Future studies employing IL-17 neutralization in *iFt*-immunized mice, followed by LVS challenge, will be critical for defining the precise contribution of IL-17 and its cellular sources in protective immunity against *F. tularensis*.

Importantly, IL-17 levels remained significantly higher in immunized mice, possibly correlating with survival through the end of the study, supporting a protective role for IL-17 in this immunization model. However, IL-17 likely contributes to protection through multiple mechanisms beyond neutrophil recruitment alone. Previous work using the Fcγ receptor targeted vaccine model in our lab demonstrated reduced IL-10 production following immunization, a cytokine produced by *F. tularensis*-infected macrophages to evade immune detection, thereby permitting a more effective inflammatory response and bacterial clearance [3]. IL-10 is a critical regulator of IL-17, restraining neutrophil recruitment to limit lung pathology [44,45]. At the same time, IL-17 plays an essential role in promoting IL-12 driven Th1 immunity and inducing IFN-γ production, both of which are required for effective bacterial killing during LVS infection [45,46]. Together, these studies suggest that the reduced IL-10 environment induced by our current vaccine model may permit a transient, regulated IL-17 response that supports protection without progressing to pathological inflammation.

Further analysis in the immunization study provided evidence of a memory function in γδ T cells; memory being a key correlate of protection in vaccine development. Since we showed that γδ T cells are not the main producers of the IFN-γ observed in our immunization model, we focused on the memory function of IL-17-producing γδ T cells. γδ T cells producing IL-17 had EM as well as CM cell phenotypes. EM γδ T cells producing IL-17 were significantly elevated in immunized mice beginning on Day 1 and continued to be elevated on Day 2. By Day 7, the EM γδ T cells producing IL-17 in immunized mice were comparable to the control PBS mice. The CM γδ T cells producing IL-17 show a significant correlation to the CM γδ T cell response in immunized mice. By Day 7, there was a significant decrease in CM γδ T cells producing IL-17 in immunized mice, which correlated with reductions in CM γδ T cells and neutrophils. Collectively, these results demonstrate that *iFt* immunization generates both EM and CM γδ T-cell memory subsets in the lung that respond rapidly to LVS challenge. The early and significant IL-17 production by EM γδ T cells on Day 1 and CM γδ T cells on Day 2 likely promotes timely bacterial clearance, providing a mechanistic basis for the enhanced protection observed in immunized mice. Thus, IL-17 producing lung-resident γδ T-cell memory populations appear to play a possible pivotal role in mediating immunity against LVS infection.

5. Conclusions

In this study, we provide a comprehensive in vivo characterization of γδ T cell responses following intranasal immunization with inactivated *F. tularensis* (*iFt*) and subsequent lethal challenge with LVS. Our findings demonstrate enhanced recruitment of γδ T cells to the lung in immunized mice, accompanied by increased IL-17 production and the expansion of both central and effector memory γδ T cell populations. These results support a previously underappreciated role for γδ T cells in shaping protective mucosal immunity in pulmonary tularemia.

Mechanistically, our data suggest that γδ T cells contribute to early control of bacterial progression primarily through IL-17 mediated pathways. The elevated IL-17 response observed in immunized mice is consistent with enhanced recruitment and activation of neutrophils and other innate effector populations, which are critical for early bacterial containment in the lungs. Although γδ T cells were not the dominant producers of IFN-γ

in this model, their rapid IL-17 driven activity highlights their importance in coordinating early inflammatory responses and bridging innate and adaptive immunity. These findings align with emerging evidence that lung-resident $\gamma\delta$ T cells function as early sentinels that shape downstream immune responses during respiratory infection.

Importantly, we observed an increase in IL-17 producing $\gamma\delta$ T cells exhibiting both effector and central memory phenotypes following *iFt* immunization. This supports the concept that $\gamma\delta$ T cells possess adaptive-like features and may contribute to durable protection upon pathogen re-exposure. The identification of memory-associated $\gamma\delta$ T cell subsets in this model provides new insights into their potential role in vaccine-induced mucosal immunity and highlights their relevance as targets for next-generation vaccination strategies.

Collectively, this work advances the current understanding of $\gamma\delta$ T cell function in pulmonary tularemia and identifies IL-17 producing $\gamma\delta$ T cells as key contributors to the protective mechanisms elicited by intranasal immunization. These findings extend previous observations of $\gamma\delta$ T cell involvement in early host defense and suggest that modulation of this population may enhance protective responses against intracellular respiratory pathogens.

Future studies will be required to define the precise mechanisms underlying $\gamma\delta$ T cell mediated protection. Targeted depletion of $\gamma\delta$ T cells and $\alpha\beta$ T cells will be essential to delineate their relative contributions to vaccine-induced immunity. Further investigation of $\gamma\delta$ T cell subsets, including V δ 1 and V δ 2 populations, will help distinguish tissue-resident from recruited cells and clarify their respective roles in pulmonary defense. In addition, *in vivo* neutralization of IL-17 will establish its functional importance in this model, while detailed characterization of neutrophil and macrophage activation will provide insight into downstream effector mechanisms. Finally, evaluating the balance between pro-inflammatory and regulatory pathways, including IL-17 and IL-10 signaling, may further reveal the immunological framework that governs protection following mucosal vaccination.

Together, these studies will refine our understanding of $\gamma\delta$ T cell driven immunity and support the development of improved mucosal vaccines against *F. tularensis* and other respiratory pathogens.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/vaccines14070590/s1>: Figure S1: Contour plot analysis of memory $\gamma\delta$ T cells; Figure S2: Contour plot of effector memory $\gamma\delta$ T cells; Figure S3: Contour plot of central memory $\gamma\delta$ T cells on Day 7.

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References

1. Degabriel, M.; Valeve, S.; Biosset, S.; Henry, T. Pathogenicity and virulence of *Francisella tularensis*. *Virulence* **2023**, *14*, 2274638. [CrossRef] [PubMed]
2. Putzova, D.; Senitkova, I.; Stulik, J. Tularemia vaccines. *Folia Microbiol.* **2016**, *61*, 495–504. [CrossRef] [PubMed]
3. Babadjanova, Z.; Wiedinger, K.; Gosselin, E.J.; Bitsaktsis, C. Targeting of a Fixed Bacterial Immunogen to Fc Receptors Reverses the Anti-Inflammatory Properties of the Gram-Negative Bacterium, *Francisella tularensis*, during the Early Stages of Infection. *PLoS ONE* **2015**, *6*, e0129981. [CrossRef] [PubMed]
4. Bitsaktsis, C.; Babadjanova, Z.; Gosselin, E.J. In vivo mechanisms involved in enhanced protection utilizing an Fc receptor-targeted mucosal vaccine platform in a bacterial vaccine and challenge model. *Infect. Immun.* **2015**, *83*, 77–89. [CrossRef] [PubMed]
5. Krocova, Z.; Macela, A.; Kubelkova, K. Innate Immune Recognition: Innate Immune Recognition: Implications for the Interaction of *Francisella tularensis* with the Host Immune System. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 446. [CrossRef] [PubMed]
6. Shepherd, F.R.; McLaren, J.E. T Cell Immunity to Bacterial Pathogens: Mechanisms of Immune Control and Bacterial Evasion. *Int. J. Mol. Sci.* **2020**, *21*, 6144. [CrossRef] [PubMed]
7. Cruz-Adalia, A.; Veiga, E. Close Encounters of Lymphoid Cells and Bacteria. *Front. Immunol.* **2016**, *7*, 405. [CrossRef] [PubMed]
8. Silva-Santos, B.; Serre, K.; Norell, H. $\gamma\delta$ T cells in cancer. *Nat. Rev. Immunol.* **2015**, *15*, 683–691. [CrossRef] [PubMed]
9. Lança, T.; Correia, D.V.; Silva-Santos, B. Role of $\gamma\delta$ T Lymphocytes in Cancer and Immunosurveillance and Immunotherapy. In *Cancer Immunology: Bench to Bedside Immunotherapy of Cancers*; Rezaei, N., Ed.; Springer: Cham, Switzerland, 2015; pp. 231–262. [CrossRef]
10. Biały, S.; Bogunia-Kubik, K. Uncovering the mysteries of human gamma delta T cells: From origins to novel therapeutics. *Front. Immunol.* **2025**, *16*, 1543454. [CrossRef] [PubMed]
11. Kabelitz, D.; Déchanet-Merville, J. Editorial: “Recent advances in gamma/delta T cell biology: New ligands, new functions, and new translational perspectives. *Front. Immunol.* **2015**, *6*, 371. [CrossRef] [PubMed]
12. Paul, S.; Shilpi Lal, G. Role of gamma-delta ($\gamma\delta$) T cells in autoimmunity. *J. Leukoc. Biol.* **2015**, *97*, 259–271. [CrossRef] [PubMed]
13. Tyler, C.J.; Doherty, D.G.; Moser, B.; Eberl, M. Human V γ 9/V δ 2 T cells: Innate adaptors of the immune system. *Cell. Immunol.* **2015**, *296*, 10–21. [CrossRef] [PubMed]
14. Kroca, M.; Tärnvik, A.; Sjöstedt, A. The proportion of circulating $\gamma\delta$ T cells increases after the first week of onset of tularemia and remains elevated for more than a year. *Clin. Exp. Immunol.* **2000**, *120*, 280–284. [CrossRef] [PubMed]
15. Sumida, T.; Maeda, T.; Takahashi, H.; Yoshida, S.; Yonaha, F.; Sakamoto, A.; Tomioka, H.; Koike, T.; Yoshida, S. Predominant Expansion of Vgamma9/Vdelta2 T cells in a Tularemia Patient. *Infect. Immun.* **1992**, *60*, 2254–2558. [CrossRef] [PubMed]
16. Wright, S.W.; Sengyee, S.; Ekchariyawat, P.; Phunpang, R.; Dulsuk, A.; Rerolle, G.; Bashmail, A.; Chantratita, N.; Gharib, S.A.; West, T.E. $\gamma\delta$ T Cells Mediate Protection against Neutrophil-associated Lung Inflammation in Pulmonary Melioidosis. *Am. J. Respir. Cell Mol. Biol.* **2024**, *71*, 546–558. [CrossRef] [PubMed]
17. King, D.P.; Hyde, D.M.; Jackson, K.A.; Novosad, D.M.; Ellis, T.N.; Putney, L.; Stoval, M.Y.; Van Winkle, L.S.; Beaman, B.L.; Ferrick, D.A. Cutting edge: Protective response to pulmonary injury requires gamma delta T lymphocytes. *J. Immunol.* **1999**, *162*, 5033–5036. [PubMed]
18. Blumberman, S.L.; Herzog, C.T.A.; Baldwin, C.L. WC1+ $\gamma\delta$ T cell memory population is induced by killed bacterial vaccine. *Eur. J. Immunol.* **2007**, *37*, 1204–1216. [CrossRef] [PubMed]
19. Chen, Z.W.; Letvin, N.L. Adaptive immune response of V γ 2V δ 2 T cells: A new paradigm. *Trends Immunol.* **2003**, *24*, 213–219. [CrossRef] [PubMed]
20. Hoft, D.F.; Brown, R.M.; Roodman, S.T. Bacille Calmette-Guérin Vaccination Enhances Human gamma delta T Cell Responsiveness to Mycobacteria Suggestive of a Memory-Like Phenotype. *J. Immunol.* **1998**, *161*, 1045–1054. [CrossRef] [PubMed]
21. Murphy, A.G.; O’Keeffe, K.M.; Lalor, S.J.; Maher, B.M.; Mills, K.H.G.; McLoughlin, R.M. *Staphylococcus aureus* Infection of Mice Expands a Population of Memory $\gamma\delta$ T Cells That Are Protective against Subsequent Infection. *J. Immunol.* **2014**, *192*, 3697–3708. [CrossRef] [PubMed]
22. Okamura, M.; Lillehoj, H.S.; Raybourne, R.B.; Babu, U.S.; Heckert, R.A. Cell-mediated immune responses to a killed *Salmonella enteritidis* vaccine: Lymphocyte proliferation, T-cell changes and interleukin-6 (IL-6), IL-1, IL-2, and IFN- γ production. *Comp. Immunol. Microbiol. Infect. Dis.* **2004**, *27*, 255–272. [CrossRef] [PubMed]
23. Velikkakam, T.; Gollob, K.J.; Dutra, W.O. Double -negative T cells: Setting the stage for disease control or progression. *Immunology* **2021**, *165*, 371–385. [CrossRef] [PubMed]
24. Elkins, K.L.; Cowley, S.C.; Bosio, C.M. Innate and adaptive immune responses to an intracellular bacterium, *Francisella tularensis* live vaccine strain. *Microbes Infect.* **2003**, *5*, 135–142. [CrossRef] [PubMed]
25. Leiby, D.A.; Fortier, A.H.; Crawford, R.M.; Schreiber, R.D.; Nacy, C.A. In Vivo Modulation of the Murine Immune Response to *Francisella tularensis* LVS by Administration of Anticytokine Antibodies. *Infect. Immun.* **1992**, *60*, 84–89. [CrossRef] [PubMed]

26. Conlan, J.W.; Sjöstedt, A.; North, R.J. CD4+ and CD8+ T-cell-dependent and -independent host defense mechanisms can operate to control and resolve primary and secondary *Francisella tularensis* LVS infection in mice. *Infect. Immun.* **1994**, *62*, 5603–5607. [\[PubMed\]](#)
27. Cowley, S.C.; Hamilton, E.; Frelinger, J.A.; Su, J.; Forman, J.; Elkins, K.L. CD4–CD8– T cells control intracellular bacterial infections both in vitro and in vivo. *J. Exp. Med.* **2005**, *202*, 309–319. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Jones, J.W.; Broz, P.; Monack, D.M. Innate immune recognition of *Francisella tularensis*: Activation of type-I interferons and the inflammasome. *Front. Microbiol.* **2011**, *2*, 16. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Khader, S.A.; Gopal, R. IL-17 in protective immunity to intracellular pathogens. *Virulence* **2010**, *1*, 423–427. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Bekiaris, V.; Šedý, J.R.; Ware, C.F. Mixing signals: Molecular turn ons and turn offs for innate $\gamma\delta$ T-cells. *Front. Immunol.* **2014**, *5*, 654. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Bonneville, M.; O'Brien, R.L.; Born, W.K. $\gamma\delta$ T cell effector functions: A blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* **2010**, *10*, 467–478. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Serre, K.; Silva-Santos, B. Molecular Mechanisms of Differentiation of Murine Pro-Inflammatory $\gamma\delta$ T Cell Subsets. *Front. Immunol.* **2013**, *4*, 431. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Sjöstedt, A.; Conlan, J.W.; North, R.J. Neutrophils are critical for host defense against primary infection with the facultative intracellular bacterium *Francisella tularensis* in mice and participate in defense against reinfection. *Infect. Immun.* **1994**, *62*, 2779–2783. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Misiak, A.; Wilk, M.M.; Raverdeau, M.; Mills, K.H.G. IL-17-Producing Innate and Pathogen Specific Tissue Resident Memory $\gamma\delta$ T Cells Expand in the Lungs of *Bordetella pertussis*-Infected Mice. *J. Immunol.* **2017**, *198*, 363–374. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Comeau, K.; Paradis, P.; Schifffrin, E.L. Human and murine memory $\gamma\delta$ T cells: Evidence for acquired immune memory in bacterial and viral infections and autoimmunity. *Cell. Immunol.* **2020**, *357*, 104217. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Lalor, S.J.; McLoughlin, R.M. Memory $\gamma\delta$ T cells—Newly Appreciated Protagonists in Infection and Immunity. *Trends Immunol.* **2016**, *37*, 690–702. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Ribot, J.C.; deBarros, A.; Pang, D.J.; Neves, J.F.; Peperzak, V.; Roberts, S.J.; Girardi, M.; Borst, J.; Hayday, A.C.; Pennington, D.J.; et al. CD27 is a thymic determinant of the balance between interferon- γ and interleukin 17-producing $\gamma\delta$ T cell subsets. *Nat. Immunol.* **2009**, *10*, 427–436. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Wei, Y.-L.; Han, A.; Glanville, J.; Fang, F.; Zuniga, L.A.; Lee, J.S.; Cua, D.J.; Chien, Y.-H. A highly focused antigen receptor repertoire characterizes $\gamma\delta$ T cells that are poised to make IL-17 rapidly in naïve animals. *Front. Immunol.* **2015**, *6*, 118. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Lee, J.; Choi, K.; Olin, M.R.; Cho, S.-N.; Molitor, T.W. $\gamma\delta$ T Cells in Immunity Induced by *Mycobacterium bovis* Bacillus Calmette-Guérin Vaccination. *Infect. Immun.* **2004**, *72*, 1504–1511. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Rawool, D.B.; Bitsakis, C.; Li, Y.; Gosselin, D.R.; Lin, Y.; Kurkure, N.V.; Metzger, D.W.; Gosselin, E.J. Utilization of Fc Receptors as a Mucosal Vaccine Strategy against an Intracellular Bacterium, *Francisella tularensis*. *J. Immunol.* **2008**, *180*, 5548–5557. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Ojeda, S.S.; Wang, Z.J.; Mares, C.A.; Chang, T.A.; Li, Q.; Morris, E.G.; Jerabek, P.A.; Teale, J.M. Rapid dissemination of *Francisella tularensis* and the effect of route of infection. *BMC Microbiol.* **2008**, *8*, 215. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Barthels, D.A.; House, R.V.; Gelhaus, H.C. The immune response to *Francisella tularensis*. *Front. Microbiol.* **2025**, *16*, 1549343. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Okoye, G.D.; Kumar, A.; Ghanbari, F.; Chowdhury, N.U.; Wu, L.; Newcomb, D.C.; Kaer, L.V.; Algood, H.M.S.; Joyce, S. Single-cell map of innate-like lymphocyte response to *Francisella tularensis* infection reveals interleukin-17 dependent protection by MAIT cells. *iScience* **2025**, *28*, 111810. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Metzger, D.W.; Salmon, S.L.; Kirimanjeswara, G. Differing Effects of Interleukin-10 on Cutaneous and Pulmonary *Francisella tularensis* Live Vaccine Strain Infection. *Infect. Immun.* **2013**, *81*, 6. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Slight, S.R.; Monin, L.; Gopal, R.; Avery, L.; Davis, M.; Cleveland, H.; Oury, T.D.; Rangel-Moreno, J.; Khader, S.A. IL-10 Restrains IL-17 to Limit Lung Pathology Characteristics following Pulmonary Infection with *Francisella tularensis* Live Vaccine Strain. *Am. J. Pathol.* **2013**, *183*, 1397–1404. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Li, Y.; Wei, C.; Xu, H.; Jia, J.; Wei, Z.; Guo, R.; Jia, Y.; Wu, Y.; Li, Y.; Qi, X.; et al. The immunoregulation of Th17 in host against intracellular bacterial infection. *Mediat. Inflamm.* **2018**, *2018*, 6587296. [\[CrossRef\]](#) [\[PubMed\]](#)

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