AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

HOST RESPONSE AND INFLAMMATION

Triggering Receptor Expressed on Myeloid Cells-1 (TREM-1) Contributes to *Bordetella pertussis* Inflammatory Pathology

Danisha Gallop,[a] Karen M. Scanlon,[a] Jeremy Ardanuy,[a] Alexander B. Sigalov,[b] Nicholas H. Carbonetti,[a] Ciaran Skerry[a]

[a]Department of Microbiology and Immunology, University of Maryland Medical School, Baltimore, Maryland, USA
[b]SignaBlok, Inc., Shrewsbury, Massachusetts, USA

ABSTRACT Whooping cough (pertussis) is a severe pulmonary infectious disease caused by the bacteria *Bordetella pertussis*. Pertussis infects an estimated 24 million people annually, resulting in >150,000 deaths. The NIH placed pertussis on the list of emerging pathogens in 2015. Antibiotics are ineffective unless administered before the onset of the disease characteristic cough. Therefore, there is an urgent need for novel pertussis therapeutics. We have shown that sphingosine-1-phosphate receptor (S1PR) agonists reduce pertussis inflammation without increasing bacterial burden. Transcriptomic studies were performed to identify this mechanism and allow for the development of pertussis therapeutics that specifically target problematic inflammation without sacrificing bacterial control. These data suggested a role for triggering receptor expressed on myeloid cells-1 (TREM-1). TREM-1 cell surface receptor functions as an amplifier of inflammatory responses. Expression of TREM-1 is increased in response to bacterial infection of mucosal surfaces. In mice, *B. pertussis* infection results in Toll-like receptor 9 (TLR9)-dependent increased expression of TREM-1 and its associated cytokines. Interestingly, S1PR agonists dampen pulmonary inflammation and TREM-1 expression. Mice challenged intranasally with *B. pertussis* and treated with ligand-dependent (LP17) and ligand-independent (GF9) TREM-1 inhibitors showed no differences in bacterial burden and significantly reduced tumor necrosis factor-α (TNF-α) and C-C motif chemokine ligand 2 (CCL-2) expression compared to controls. Mice receiving TREM-1 inhibitors showed reduced pulmonary inflammation compared to controls, indicating that TREM-1 promotes inflammatory pathology, but not bacterial control, during pertussis infection. This implicates TREM-1 as a potential therapeutic target for the treatment of pertussis.

KEYWORDS S1P, bordetella, host-directed therapeutics, host-pathogen interactions, pertussis, pulmonary infection, trem-1

Bordetella pertussis is the etiologic agent of whooping cough. Despite widespread vaccination, pertussis is the only vaccine-preventable disease to have been consistently on the rise in the United States since 1976 (1). Antibiotics are ineffective unless administered early during disease (2, 3). Therefore, there is an urgent need for new therapeutics to treat *B. pertussis* (4). Previously, we demonstrated the potential of sphingosine-1-phosphate receptor-1 (S1PR) agonists to dramatically reduce inflammation in the lungs of *B. pertussis*-challenged mice (5, 6). Importantly, despite reducing inflammatory pathology and cytokine expression, S1PR agonists did not impair the ability of mice to control and clear infection (5). However, recent work has shown that chronic administration of S1PR agonists is associated with a reduced ability to clear infection (7). S1PR is present on a wide variety of cell types, regulating a multitude of biological responses (8–11). Understanding the mechanisms by which S1PR agonists protect against disease may inform the development of improved host-directed therapies that promote resolution of inflammation and bacterial clearance.

Citation Gallop D, Scanlon KM, Ardanuy J, Sigalov AB, Carbonetti NH, Skerry C. 2021. Triggering receptor expressed on myeloid cells-1 (TREM-1) contributes to *Bordetella pertussis* inflammatory pathology. Infect Immun 89:e00126-21. https://doi.org/10.1128/IAI.00126-21.

Editor Manuela Raffatellu, University of California San Diego School of Medicine

Address correspondence to Alexander B. Sigalov, sigalov@signablok.com, or Ciaran Skerry, cskerry@som.umaryland.edu.

Received 2 March 2021
Returned for modification 13 April 2021
Accepted 18 May 2021
Accepted manuscript posted online 7 June 2021
Published 16 September 2021

Transcriptomic studies designed to explore the mechanisms of action of S1PR agonists in reducing pertussis inflammation identified differential regulation of multiple genes and pathways. Previously, we demonstrated the ability of peptidoglycan recognition protein 4 (PGLYRP4), a host protein expressed following S1PR agonist treatment, to limit inflammatory responses to tracheal cytotoxin (peptidoglycan [PGN] fragment) following *B. pertussis* infection (12).

Here, we describe the increased expression of triggering receptor expressed on myeloid cells-1 (TREM-1) observed in response to *B. pertussis* infection. Further, we demonstrate the ability of S1PR agonists to decrease TREM-1 expression and the consequences of targeted TREM-1 inhibition during *B. pertussis* infection. TREM-1 is a member of the Ig superfamily expressed on monocytes and neutrophils in response to pattern recognition receptor (PRR) activation (e.g., Toll-like receptors [TLRs]) (13). Here, we report on a possible role for TLR9 in the potentiation of TREM-1 responses to *B. pertussis*. TLR9 is located within endosomes (14) and recognizes unmethylated cytosine- and guanine-rich nucleic acid motifs typical of bacterial DNA (15). TLR9 activation has been associated with enhanced vaccine-mediated protection against *B. pertussis* (16). However, no reports exist detailing its role in *B. pertussis* pathogenesis.

The engagement of TREM-1 by its ligands amplifies the expression of inflammatory factors, such as tumor necrosis factor-α (TNF-α) and C-C motif chemokine ligand 2 (CCL2) (13). Whether TREM-1 expression is protective in infectious diseases is unclear. TREM-1 activity is protective in models of *Streptococcus suis* infection and bacterial peritonitis (17, 18) and against the dissemination of *Klebsiella pneumoniae* (19). TREM-1-deficient animals, however, were unaffected in their ability to control or clear *Legionella pneumophila* or *Leishmania major* growth and were protected against excessive inflammatory responses to infection (20). These data suggest that TREM-1 blockade may be a therapeutic possibility in certain inflammation-producing bacterial infections while potentially deleterious in others.

Here, we hypothesize that TREM-1 activation serves as a checkpoint between efficient and deleterious inflammatory responses to *B. pertussis*. Additionally, we test the hypothesis that TREM-1 inhibition will reduce pulmonary inflammation without sacrificing bacterial control. The obtained data provide, for the first time, evidence that TREM-1 contributes to the excessive inflammatory response to *B. pertussis* infection and highlight the potential of TREM-1 inhibition for the treatment of pertussis and other hyperinflammatory diseases.

RESULTS

TREM-1 expression is increased in response to *B. pertussis* infection and is reduced by S1PR agonism. Transcriptomic studies comparing RNA isolated from the lungs of animals challenged with *B. pertussis* or a "sham" inoculum of phosphate-buffered saline (PBS) identified TREM-1 and its associated cytokines (TNF-α, CCL-2, C-X-C motif chemokine ligand 3 [CXCL3], and interleukin-1β [IL-1β]) as significantly upregulated during *B. pertussis* infection (Fig. 1A). Additionally, canonical pathway analysis using Ingenuity Pathway Analysis (IPA) software identified the TREM-1 signaling pathway as having the 7th most significant overlap with our data set out of all associated with *B. pertussis* infection (Fig. 1B). Expression of TREM-1-associated genes was confirmed by reverse transcription-quantitative PCR (qRT-PCR) (Fig. 1C). Our previous studies identified S1PR agonists as potential treatments for pertussis disease (5). Interestingly, S1PR agonists reduced the expression of TREM-1 and TREM-1-associated genes in *B. pertussis*-infected mice compared to treatment with a vehicle control (Fig. 1A and B). This is the first time TREM-1 expression has been associated with either *B. pertussis* infection or the S1PR system.

Increased expression of TREM-1 in response to *B. pertussis* infection is TLR9 dependent. TREM-1 is expressed on the surface of macrophages and neutrophils following engagement of TLRs (13). In models of *B. pertussis* pathogenesis, TLR4 has been shown to mediate neutrophil recruitment, bacterial clearance, and cytokine production following *B. pertussis* infection and is required for vaccine-induced protective immunity (21, 22). Our transcriptomic screen identified the expression of DNA-sensing receptor

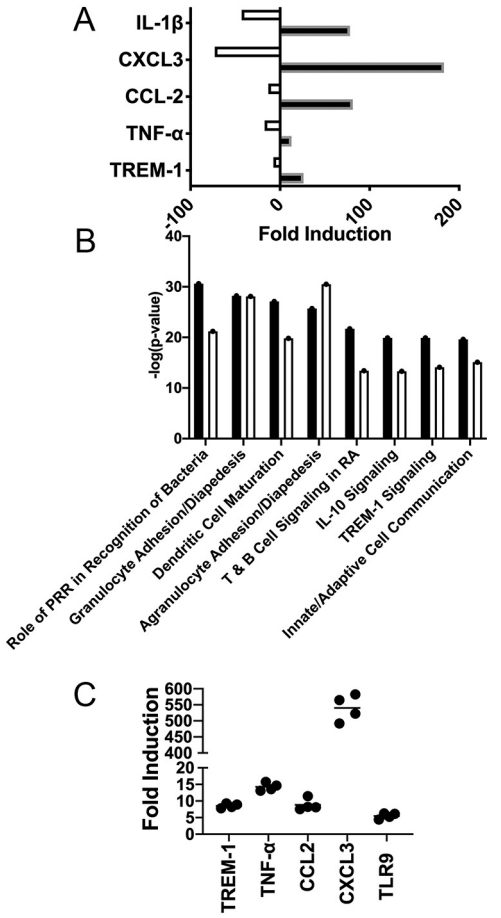

FIG 1 Transcriptomic analysis of TREM-1-associated genes following *B. pertussis* infection. (A) Differential expression of TREM-1-associated genes identified by whole-lung transcriptomics. Gene expression was compared using RNA that had been isolated from whole-lung tissue of C57BL/6 mice 4 days postinfection with *B. pertussis* or PBS sham infection (black bars). White bars represent gene fold induction in infected animals treated with vehicle or S1PR agonist. (B) Canonical pathway analysis performed with the Ingenuity Pathway Analysis software identified TREM-1 signaling as being significantly associated with genes whose expression is increased by *B. pertussis* infection compared to PBS (black bars) and in infected, water control-treated versus S1PR agonist (AAL-R, 0.5 mg/kg)-treated mice (white bars). The expression of TREM-1-associated genes at 4 days postinfection was confirmed by qRT-PCR (C). Fold induction is calculated by normalization to PBS-challenged mice. Each data point represents the mean of triplicate technical replicates from one of four individual mice ($n = 4$).

TLR9 as being highly upregulated in the lungs following *B. pertussis* infection. These data were confirmed by qRT-PCR (Fig. 1C). To determine if TLR4 or TLR9 contributes to the induction of TREM-1 expression, we challenged knockout (KO) mice (TLR4 KO, TLR9 KO) with *B. pertussis* and assessed TREM-1 expression by qRT-PCR at 7 days postinfection (dpi). *B. pertussis* infection induces similar TREM-1 expression in wild-type C57BL/6 and TLR4-deficient mice (Fig. 2). This suggests that TLR9 contributes significantly to the induction of TREM-1 expression, and that TLR4 has minimal impact on the expression of TREM-1.

However, the robust upregulation of TREM-1 observed in wild-type and TLR4 KO mice was not evident in TLR9 KO mice (Fig. 2). This result suggests that TLR9, which mediates the recognition of CpG-rich DNA, promotes the expression of TREM-1 following *B. pertussis* infection.

TREM-1 does not contribute to bacterial control in *B. pertussis* infection. Deficiencies in TREM-1 have been linked to increased bacterial burden in animal models of *Streptococcus suis*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* infections (19, 23, 24). In other infectious models, however, TREM-1 promotes inflammation

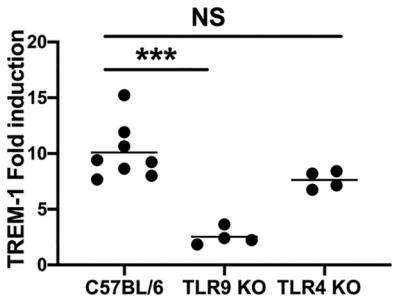

FIG 2 *B. pertussis*-induced expression of TREM-1 is TLR9 dependent. qRT-PCR analysis of lung tissue harvested from C57BL/6, TLR9 KO, or TLR4 KO mice at 7 days postinfection with *B. pertussis*. Data are normalized to PBS-challenged animals of the same genetic background. Each datapoint represents the mean value obtained from triplicate samples from one of four individual mice ($n = 4$); ***, $P < 0.005$; NS, $P > 0.05$.

without contributing to pathogen control or clearance (e.g., *L. pneumophila*, *L. major*) (20). Additionally, inhibiting TREM-1 in combination with antibiotic treatment allowed for the efficient killing of *S. suis* and reduced inflammatory responses (17). To determine if TREM-1 contributes to the control of *B. pertussis* infection, infected mice were treated daily with one of two TREM-1 peptide inhibitors LP17 (5 mg/kg) or GF9 (20 mg/kg) or vehicle, and pulmonary bacterial burden was assessed at 4 and 7 dpi. These inhibitors employ a ligand-dependent (LP17) or ligand-independent (GF9) mechanism of TREM-1 inhibition (Fig. 3A). LP17 is a peptide with sequence homology to the extracellular region of TREM-1 (25). LP17, therefore, functions as a decoy receptor preventing interactions between TREM-1 and its ligands. By contrast, GF9 blocks interactions between the TREM-1 receptor and its signaling partner DNAX-activating protein of 12 kDa (DAP12) in the cell membrane (26). These two different inhibitors were used in our studies to address the uncertain nature of TREM-1 ligands. No differences in bacterial burden were observed between mice treated with vehicle or either TREM-1 inhibitor (Fig. 3B and C) at 4 or 7 dpi. This suggests that TREM-1 upregulation and signaling does not contribute to the control of *B. pertussis* bacterial burden. Additionally, this suggests that TREM-1-targeting therapeutics should not impact the host's ability to control infection.

TREM-1 promotes inflammatory pathology during *B. pertussis* infection. Acute inflammation can limit bacterial growth but does so at the risk of damaging the host. For this reason, anti-inflammatories have been widely applied in diseases of sterile inflammation, but less so in infectious diseases. Delineating protective and beneficial elements of the inflammatory response to infectious diseases will benefit the design of host-directed therapeutics. To determine if TREM-1 contributes to the inflammation observed during murine *B. pertussis* infection, mice were challenged with *B. pertussis* before treatment with LP17 (5 mg/kg), GF9 (20 mg/kg), or vehicle control. Lungs were harvested at 4 dpi, and expression of CCL-2, CXCL3, and TNF-α was measured by qRT-PCR. Expression of each analyte was found to be increased upon infection with *B. pertussis*, and that increase was significantly lower following TREM-1 inhibition than following treatment with vehicle control (Fig. 4A to C). TNF-α protein production was assessed by enzyme-linked immunosorbent assay (ELISA) at 4 and 7 dpi. At both time points, TNF-α levels were increased in response to *B. pertussis* infection and were significantly decreased by GF9-mediated TREM-1 inhibition (Fig. 4D). These data suggest that TREM-1 contributes to the expression of inflammatory cytokines observed following *B. pertussis* infection of C57BL/6 mice.

Pulmonary histopathology was assessed at 7 dpi to determine the contribution of TREM-1 to lung inflammation. Lungs taken at 7 dpi from *B. pertussis*-infected mice receiving LP17 (5 mg/kg) or GF9 (20 mg/kg) demonstrated reduced bronchovascular inflammation and consolidation of alveolar spaces compared to lungs from vehicle controls (Fig. 5), although only GF9 treatment reached statistical significance. Further studies are needed to determine if this effect is dose related or associated with a ligand-independent

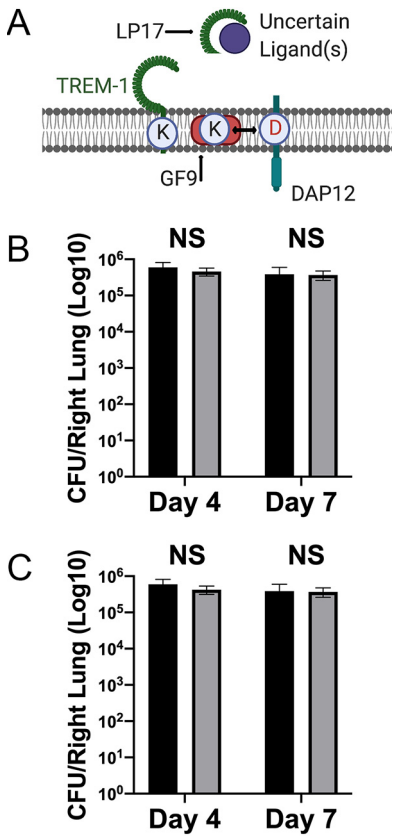

FIG 3 TREM-1 does not contribute to bacterial control in *B. pertussis*. We used ligand-dependent (LP17) and ligand-independent (GF9) mechanisms to inhibit TREM-1 activity. LP17 binds potential TREM-1 ligands, preventing receptor binding. GF9 prevents transmembrane interactions between TREM-1 and its binding partner DAP12 (A). Bacterial burden was assessed at 4 and 7 dpi by plating the lungs of *B. pertussis*-challenged C57BL/6 mice receiving TREM-1 inhibitors (gray bars) LP17 (5 mg/kg) (B) or GF9 (20 mg/kg) (C) or vehicle control (black bars) on Bordet-Gengou agar plates. Vehicle controls consisted of 5 mg/kg scrambled peptide (LP17 studies) or PBS (GF9 studies). Each data point represents the average of three technical replicates obtained from one of four individual mice ($n = 4$). Data are shown as mean with standard deviation. *P* values were determined by two-way ANOVA using Sidak's multiple-comparison test; NS, $P > 0.05$.

mechanism of TREM-1 inhibition. In summary, these data demonstrate that TREM-1 promotes inflammation and lung pathology in *B. pertussis* infection without inhibiting the antibacterial response. They also highlight the therapeutic potential of inhibiting TREM-1 for the treatment of pertussis.

DISCUSSION

The development of next-generation therapeutics for pertussis that improve clinical disease, reduce long-term lung damage, and retain the ability of antimicrobials to reduce dissemination is essential. *B. pertussis* infection elicits potent inflammatory responses, resulting in severe lung damage (27–29). Further, the infection is associated with long-term pulmonary sequelae (30). We hypothesize that reducing the inflammatory response to *B. pertussis* will improve the long-term outcomes of mild to severe disease and potentially rescue fatal infection. Previously, our group identified that short-term intranasal treatment with an S1PR agonist reduced inflammatory responses without impacting bacterial control in adult mice and prolonged survival in a neonatal model of lethal disease (5). However, long-term treatment of *B. pertussis*-challenged mice with oral S1PR agonists has been associated with a reduced ability to clear the infection (7). Transcriptomic studies attempting to discern the beneficial components of S1PR agonist treatment identified TREM-1 expression as increased during *B. pertussis* infection and decreased by treatment with S1PR agonists (Fig. 1).

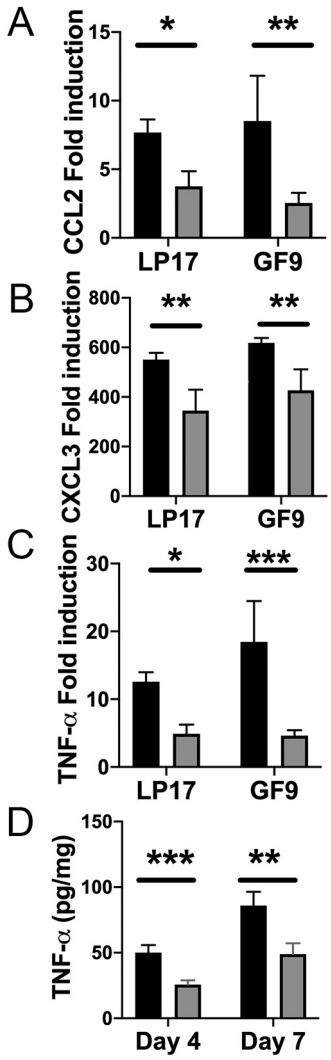

FIG 4 TREM-1 promotes inflammatory responses to *B. pertussis*. Lungs were isolated from *B. pertussis*-challenged mice treated with TREM-1 inhibitors LP17 (5 mg/kg) or GF9 (20 mg/kg) at 4 dpi to assess expression of CCL2 (A), CXCL3 (B), and TNF-α (C) by qRT-PCR. qRT-PCR data are normalized to uninfected animals. Black bars represent *B. pertussis*-challenged, vehicle-treated animals. Gray bars represent *B. pertussis*-challenged animals treated with TREM-1 inhibitors LP17 (5 mg/kg) or GF9 (20 mg/kg). TNF-α protein levels were increased in response to *B. pertussis* infection (black bars) and decreased in response to treatment with GF9 (gray bars) (D). Data represent mean and standard deviation values obtained from triplicate technical replicates and four biological replicates ($n = 4$). *P* values were determined by two-way ANOVA using Sidak's multiple-comparison test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

TREM-1 is known to contribute to the inflammatory response to many pathogens. However, its role in bacterial control appears to be pathogen specific. TREM-1 inhibition using recombinant extracellular TREM-1 during *S. suis* infection reduced bacterial clearance despite promoting the resolution of inflammatory responses in mice (17). However, when combined with antibiotic treatment, TREM-1 inhibition not only dampened inflammatory responses but also promoted clearance of *S. suis* (17). Future studies will assess the potential of combining antibiotics with TREM-1 inhibitor treatment. In mouse models of bacterial (*L. pneumophila*), fungal (*L. major*), and viral (influenza) infections, the deletion of murine TREM-1 reduced inflammation without impacting pathogen control (20). Studies using TREM-1 inhibitors demonstrate its importance in sepsis, cancer, and collagen-induced arthritis (18, 26, 31).

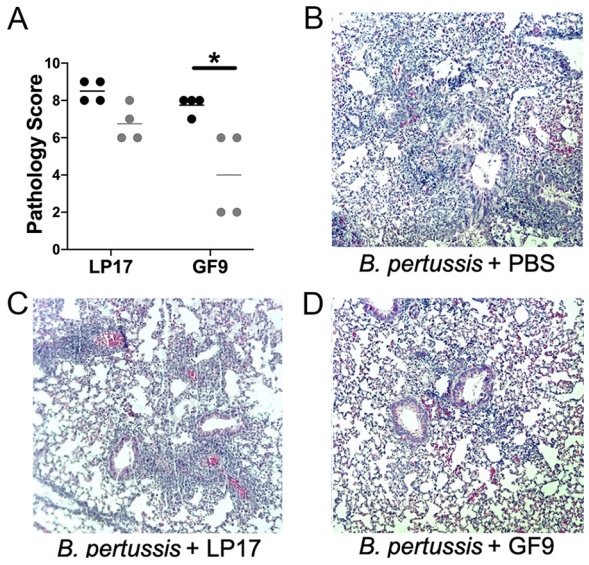

FIG 5 TREM-1 pathogenesis in *B. pertussis* infection. Lungs were isolated from *B. pertussis*-challenged mice treated with TREM-1 inhibitors LP17 (5 mg/kg, gray dots) or GF9 (20 mg/kg, gray dots) or vehicle (black dots; 5 mg/kg scrambled peptide for LP17 studies and PBS for GF9 studies) at 7 dpi. Lung tissues were stained with hematoxylin and eosin for assessment of histopathology. Three blinded investigators scored slides on a scale of 1 to 9 using our established semiquantitative system (A). Representative images are shown for infected C57BL/6 mice treated with vehicle (B), LP17 (C), or GF9 (D). Each data point represents one biological replicate ($n = 4$); *, $P < 0.05$.

To determine if TREM-1 could be a candidate for host-directed therapies in *B. pertussis*, we challenged mice with *B. pertussis* before treatment with one of two TREM-1 inhibitors, LP17 or GF9, or vehicle control. Mice treated with TREM-1 inhibitors showed no signs of altered bacterial control (Fig. 3). However, TREM-1 inhibitor-treated mice demonstrated reduced expression of inflammatory markers (Fig. 4). Further, TREM-1 inhibition was associated with reduced inflammatory pathology in the lungs of inhibitor-treated mice compared to in vehicle-treated control mice (Fig. 5). These data demonstrate for the first time a role for TREM-1 in pertussis pathogenesis. The ability to reduce inflammation without impacting bacterial control highlights the potential of TREM-1 inhibition as a therapy for pertussis.

Due to the uncertain nature of TREM-1 ligand(s), two classes of TREM-1 inhibitors were used in these studies: ligand-dependent and ligand-independent inhibitors LP17 and GF9, respectively. LP17 functions as a decoy receptor and competes with TREM-1 recognition of its endogenous ligand(s) (32) (Fig. 3A). By contrast, GF9 functions within the cell membrane by interfering with the interaction between TREM-1 and its signaling partner DAP12, thereby allowing receptor-ligand interactions but preventing downstream signaling (26) (Fig. 3A). In these studies, both GF9 and LP17 significantly reduced *B. pertussis*-induced expression of TREM-1 and its associated cytokines. Further, histopathology revealed that TREM-1 inhibition reduced inflammation in the lungs of infected mice (Fig. 5). The ability to reduce inflammation without inhibiting bacterial killing makes TREM-1 inhibitors attractive candidates for host-directed therapeutics for pertussis. TREM-1 may function as an inflammatory checkpoint in the response to infectious diseases. We hypothesize that initial macrophage activation results in sufficient antibacterial responses to resolve infection, but repeated or secondary activation via TREM-1 promotes a hyperinflammatory response that may be deleterious to the host. This is supported by the significant, but not complete, disruption to inflammatory responses observed in TREM-1 inhibitor-treated mice.

TREM-1 is expressed primarily on monocytes and neutrophils. GF9 formulated into macrophage-specific high-density lipoprotein (HDL)-mimicking particles (GF9-HDL) targets monocyte TREM-1 (26). In this situation, GF9 released by GF9-HDL in the cell reaches

its intramembrane site of action from inside the cell. Our previous data demonstrated an essential role for monocyte S1P1 in S1PR agonist-mediated disease attenuation (5). Therefore, we hypothesize that the efficacy of TREM-1 inhibitors for the treatment of *B. pertussis* can be further improved by formulation with HDL-mimicking particles.

TREM-1 expression is induced by the action of pathogen-associated molecular patterns (PAMPs) on PRRs (13). Our transcriptomic studies identified TLR9 as highly expressed in response to *B. pertussis* infection. Studies in TLR9 KO mice demonstrated that DNA sensing is a major driver of TREM-1 expression in response to *B. pertussis* infection (Fig. 2). Future studies will seek to determine the potential of TLR9-targeting therapies for the treatment of pertussis disease.

TREM-1 ligands include peptidoglycan complexed with host peptidoglycan recognition protein-1 (PGLYRP1) (33). It is possible that *B. pertussis* PGN, tracheal cytotoxin (TCT), may be contributing to TREM-1 activation. In addition to dampening TREM-1 expression, S1PR agonists induce expression of PGLYRP4 but not PGLYRP1 (12). PGN, when bound to PGLYRP4, fails to induce the inflammatory responses induced by PGLYRP1-bound PGN (34). Future work will explore the hypothesis that PGLYRP4 competes with PGLYRP1 for PGN, influencing TREM-1 activation.

An impressive aspect of S1PR-mediated attenuation of pertussis disease is the ability to delay treatment without affecting outcomes (5). It will be important to assess the impact of delaying TREM-1 inhibitor administration to more therapeutically relevant time points on anti-inflammatory capability. The data presented here demonstrate a role for TREM-1 in the inflammatory response to *B. pertussis* despite not contributing to bacterial control. These data suggest that TREM-1 may be a future target for host-directed therapies in pertussis.

MATERIALS AND METHODS

Bacterial strains. A streptomycin- and nalidixic acid-resistant derivative of the *B. pertussis* Tohama I strain, developed by Carbonetti et al. (35), was used for all experiments described. *B. pertussis* was grown on Bordet-Gengou (BG) agar plates supplemented with 10% defibrinated sheep blood and 200 μg/ml streptomycin.

Mouse infections. Six-week-old C57BL/6 mice (Charles River) and TLR4 and TLR9 knockout mice (Jackson Laboratory) were used in accordance with the University of Maryland, Baltimore (UMB), Institutional Animal Care and Use Committee. For infections, bacterial inocula were prepared in sterile phosphate-buffered saline (PBS) following a 48-h incubation on BG agar. Anesthetized mice (isoflurane) received 2×10^6 CFU *B. pertussis* intranasally in a total volume of 50 μl.

TREM-1 inhibitor studies. Ligand-dependent TREM-1 inhibitory peptide LP17 (LQVTDSGLYRCVIYHPP, C-terminal amidated; Thermo Fisher) is based on the complementarity-determining region 3 (CDR3) region of TREM-1 (32). Based on previous publications (32), a solution of a scrambled peptide (TDSRCVIGLYHPPLQVY, C-terminal amidated; Thermo Fisher) in sterile PBS was used as a vehicle in LP17 studies. Ligand-independent TREM-1 inhibitory peptide GF9 (GFLSKSLVF; SignaBlok) is based on the transmembrane domain of TREM-1 (26). LP17 (5 mg/kg), GF9 (20 mg/kg), or vehicle controls (scrambled peptide and PBS, respectively) were administered daily by intraperitoneal injection beginning on day 0 based on previous studies (26, 32). Lungs were harvested at 4 and 7 days postinfection (dpi) for assessment of bacterial burden (superior, middle, and inferior lobes), inflammatory cytokine expression (postcaval lobe), and pulmonary histopathology (left lobe).

RNA isolation and processing. Lung tissue was harvested directly into RNAlater (Sigma-Aldrich) and stored overnight at 4°C before RNA extraction using RNA Stat60 (TelTest, Inc.) as per the manufacturer's instructions. In brief, samples were homogenized using an Omni TH mixer (Omni, Inc.), phase separated with the addition of chloroform, and precipitated with isopropanol. RNA was quantified, and 1 μg was reverse transcribed using a reverse transcription system (Promega). Quantitative real-time PCR was performed in a BIORAD CFX96 real-time PCR instrument. The hypoxanthine phosphoribosyltransferase (*HPRT*) gene was used as an internal housekeeping control gene, with all genes normalized to the *HPRT* gene and expression calculated as fold change compared with PBS-inoculated control animals (calculated by the threshold cycle $2^{-\Delta\Delta CT}$ method).

Pathology. Lungs were perfused with PBS before removal into 10% (wt/vol) neutral buffered formalin. Hematoxylin-eosin staining was performed by the Pathology, Electron Microscopy and Histology Laboratory at the University of Maryland. A semiquantitative scoring system was employed based on the degree of infiltrate in the bronchovascular region, and the degree of tissue consolidation was observed. Lung sections were given a score between 0 and 9 based on the degree of bronchovascular inflammation (0 to 3), the percentage of bronchovascular bundles involved (0 to 3), and the amount of consolidation or infiltration of cells in the alveolar spaces (0 to 3).

RNA sequencing. RNA sequencing analysis was performed by the Informatics Resource Center, Institute for Genome Sciences, University of Maryland School of Medicine (UMSOM). Paired-end Illumina libraries were mapped to the mouse reference, Ensembl release GRCm38.74, using TopHat v1.4.0 with

the default mismatch parameters. Read counts for each annotated gene were calculated using HTSeq. The DESeq Bioconductor package (v1.5.24) was used to estimate dispersion, normalize read counts by library size to generate the counts per million for each gene, and determine differentially expressed genes between two conditions. Differentially expressed transcripts with a false discovery rate of <0.05 and a $\log_2$ fold change in expression were used for downstream analysis. The list of differentially expressed genes was used to compute the enrichment of biological pathways using Ingenuity Pathway Analysis (IPA). IPA was used to identify canonical pathways that were most significantly associated with the data sets analyzed.

Enzyme-linked immunosorbent assay. Protein levels of TNF-α were assessed by enzyme-linked immunosorbent assay (ELISA) (BioLegend) using whole-lung tissue homogenate at 4 and 7 dpi from mice challenged with *B. pertussis* and treated with GF9 (20 mg/kg) or PBS. Lungs were harvested into 2 ml of PBS containing 20 μl of proteinase inhibitor cocktail (Sigma). Lungs were then homogenized as described above and centrifuged at 300 $\times$ *g* for 10 min at 4°C, and the supernatant was harvested for protein detection.

Statistical analysis. Data were analyzed and graphs prepared in GraphPad Prism statistical software. Graphs represent mean values with standard deviation. Student's *t* tests were used to determine significance between two data sets, and two-way ANOVA was used to determine significance between multiple groups. For canonical pathway analysis, the Fisher exact test was used to calculate a *P* value, determining the probability that the association between the genes in the data set and the canonical pathway was significant.

Data availability. Data discussed in the manuscript have been deposited to the NCBI Sequence Read Archive under BioProject PRJNA493118 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA493118).

ACKNOWLEDGMENTS

This work was supported by funding from the National Institute for Health, National Institute for Allergy and Infectious Diseases grants AI-140035 (C.S.), AI-095190 and AI-136377 (J.A.), AI-141372 (N.H.C.), the National Cancer Institute grant R44CA217400 (A.B.S.), and the National Institute of General Medicine Sciences grant R43GM128369 (A.B.S.).

REFERENCES

1. van Panhuis WG, Grefenstette J, Jung SY, Chok NS, Cross A, Eng H, Lee BY, Zadorozhny V, Brown S, Cummings D, Burke DS. 2013. Contagious diseases in the United States from 1888 to the present. N Engl J Med 369:2152–2158. https://doi.org/10.1056/NEJMms1215400.
2. Altunaiji S, Kukuruzovic R, Curtis N, Massie J. 2007. Antibiotics for whooping cough (pertussis). Cochrane Database Syst Rev https://doi.org/10.1002/14651858.CD004404.pub3:CD004404.
3. Tiwari T, Murphy TV, Moran J, National Immunization Program CDC. 2005. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. MMWR Recomm Rep 54:1–16.
4. Scanlon KM, Skerry C, Carbonetti NH. 2015. Novel therapies for the treatment of pertussis disease. Pathog Dis 73:ftv074. https://doi.org/10.1093/femspd/ftv074.
5. Skerry C, Scanlon K, Ardanuy J, Roberts D, Zhang L, Rosen H, Carbonetti NH. 2017. Reduction of pertussis inflammatory pathology by therapeutic treatment with sphingosine-1-phosphate receptor ligands by a pertussis toxin-insensitive mechanism. J Infect Dis 215:278–286. https://doi.org/10.1093/infdis/jiw536.
6. Skerry C, Scanlon K, Rosen H, Carbonetti NH. 2015. Sphingosine-1-phosphate receptor agonism reduces *Bordetella pertussis*-mediated lung pathology. J Infect Dis 211:1883–1886. https://doi.org/10.1093/infdis/jiu823.
7. Wilk MM, Misiak A, McManus RM, Allen AC, Lynch MA, Mills KHG. 2017. Lung CD4 tissue-resident memory T cells mediate adaptive immunity induced by previous infection of mice with *Bordetella pertussis*. J Immunol 199:233–243. https://doi.org/10.4049/jimmunol.1602051.
8. Maceyka M, Harikumar KB, Milstien S, Spiegel S. 2012. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 22:50–60. https://doi.org/10.1016/j.tcb.2011.09.003.
9. Blaho VA, Hla T. 2014. An update on the biology of sphingosine 1-phosphate receptors. J Lipid Res 55:1596–1608. https://doi.org/10.1194/jlr.R046300.
10. Blaho VA, Hla T. 2011. Regulation of mammalian physiology, development, and disease by the sphingosine 1-phosphate and lysophosphatidic acid receptors. Chem Rev 111:6299–6320. https://doi.org/10.1021/cr200273u.
11. Obinata H, Hla T. 2012. Sphingosine 1-phosphate in coagulation and inflammation. Semin Immunopathol 34:73–91. https://doi.org/10.1007/s00281-011-0287-3.
12. Skerry C, Goldman WE, Carbonetti NH. 2018. Peptidoglycan recognition protein 4 suppresses early inflammatory responses to *Bordetella pertussis* and contributes to sphingosine-1-phosphate receptor agonist-mediated disease attenuation. Infect Immun 87:e00601-18. https://doi.org/10.1128/IAI.00601-18.
13. Bouchon A, Dietrich J, Colonna M. 2000. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 164:4991–4995. https://doi.org/10.4049/jimmunol.164.10.4991.
14. Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T, Golenbock DT. 2004. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat Immunol 5:190–198. https://doi.org/10.1038/ni1028.
15. Latz E, Visintin A, Espevik T, Golenbock DT. 2004. Mechanisms of TLR9 activation. J Endotoxin Res 10:406–412. https://doi.org/10.1179/096805104225006525.
16. Auderset F, Ballester M, Mastelic-Gavillet B, Fontannaz P, Chabaud-Riou M, Reveneau N, Garinot M, Mistretta N, Liu Y, Lambert PH, Ochs M, Siegrist CA. 2019. Reactivating immunity primed by acellular pertussis vaccines in the absence of circulating antibodies: enhanced bacterial control by TLR9 rather than TLR4 agonist-including formulation. Front Immunol 10:1520. https://doi.org/10.3389/fimmu.2019.01520.
17. Yang C, Zhao J, Lin L, Pan S, Fu L, Han L, Jin M, Zhou R, Zhang A. 2015. Targeting TREM-1 signaling in the presence of antibiotics is effective against streptococcal toxic-shock-like syndrome (STSLS) caused by *Streptococcus suis*. Front Cell Infect Microbiol 5:79. https://doi.org/10.3389/fcimb.2015.00079.
18. Bouchon A, Facchetti F, Weigand MA, Colonna M. 2001. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 410:1103–1107. https://doi.org/10.1038/35074114.
19. Lin YT, Tseng KY, Yeh YC, Yang FC, Fung CP, Chen NJ. 2014. TREM-1 promotes survival during *Klebsiella pneumoniae* liver abscess in mice. Infect Immun 82:1335–1342. https://doi.org/10.1128/IAI.01347-13.
20. Weber B, Schuster S, Zysset D, Rihs S, Dickgreber N, Schurch C, Riether C, Siegrist M, Schneider C, Pawelski H, Gurzeler U, Ziltener P, Genitsch V, Tacchini-Cottier F, Ochsenbein A, Hofstetter W, Kopf M, Kaufmann T, Oxenius A, Reith W, Saurer L, Mueller C. 2014. TREM-1 deficiency can attenuate disease severity without affecting pathogen clearance. PLoS Pathog 10:e1003900. https://doi.org/10.1371/journal.ppat.1003900.

21. Higgins SC, Jarnicki AG, Lavelle EC, Mills KH. 2006. TLR4 mediates vaccine-induced protective cellular immunity to *Bordetella pertussis*: role of IL-17-producing T cells. J Immunol 177:7980–7989. https://doi.org/10.4049/jimmunol.177.11.7980.
22. Moreno G, Errea A, Van Maele L, Roberts R, Leger H, Sirard JC, Benecke A, Rumbo M, Hozbor D. 2013. Toll-like receptor 4 orchestrates neutrophil recruitment into airways during the first hours of *Bordetella pertussis* infection. Microbes Infect 15:708–718. https://doi.org/10.1016/j.micinf.2013.06.010.
23. Hommes TJ, Hoogendijk AJ, Dessing MC, Van't Veer C, Florquin S, Colonna M, de Vos AF, van der Poll T. 2014. Triggering receptor expressed on myeloid cells-1 (TREM-1) improves host defence in pneumococcal pneumonia. J Pathol 233:357–367. https://doi.org/10.1002/path.4361.
24. Yang C, Chen B, Zhao J, Lin L, Han L, Pan S, Fu L, Jin M, Chen H, Zhang A. 2015. TREM-1 signaling promotes host defense during the early stage of infection with highly pathogenic *Streptococcus suis*. Infect Immun 83:3293–3301. https://doi.org/10.1128/IAI.00440-15.
25. Gibot S, Cravoisy A. 2004. Soluble form of the triggering receptor expressed on myeloid cells-1 as a marker of microbial infection. Clin Med Res 2:181–187. https://doi.org/10.3121/cmr.2.3.181.
26. Sigalov AB. 2014. A novel ligand-independent peptide inhibitor of TREM-1 suppresses tumor growth in human lung cancer xenografts and prolongs survival of mice with lipopolysaccharide-induced septic shock. Int Immunopharmacol 21:208–219. https://doi.org/10.1016/j.intimp.2014.05.001.
27. Paddock CD, Sanden GN, Cherry JD, Gal AA, Langston C, Tatti KM, Wu KH, Goldsmith CS, Greer PW, Montague JL, Eliason MT, Holman RC, Guarner J, Shieh WJ, Zaki SR. 2008. Pathology and pathogenesis of fatal *Bordetella pertussis* infection in infants. Clin Infect Dis 47:328–338. https://doi.org/10.1086/589753.
28. Zimmerman LI, Papin JF, Warfel J, Wolf RF, Kosanke SD, Merkel TJ. 2018. Histopathology of *Bordetella pertussis* in the baboon model. Infect Immun 86:e00511-18. https://doi.org/10.1128/IAI.00511-18.
29. Mahon BP, Sheahan BJ, Griffin F, Murphy G, Mills KH. 1997. Atypical disease after *Bordetella pertussis* respiratory infection of mice with targeted disruptions of interferon-gamma receptor or immunoglobulin mu chain genes. J Exp Med 186:1843–1851. https://doi.org/10.1084/jem.186.11.1843.
30. Britten N, Wadsworth J. 1986. Long term respiratory sequelae of whooping cough in a nationally representative sample. Br Med J (Clin Res Ed) 292:441–444. https://doi.org/10.1136/bmj.292.6518.441.
31. Shen ZT, Sigalov AB. 2017. Rationally designed ligand-independent peptide inhibitors of TREM-1 ameliorate collagen-induced arthritis. J Cell Mol Med 21:2524–2534. https://doi.org/10.1111/jcmm.13173.
32. Gibot S, Buonsanti C, Massin F, Romano M, Kolopp-Sarda MN, Benigni F, Faure GC, Bene MC, Panina-Bordignon P, Passini N, Levy B. 2006. Modulation of the triggering receptor expressed on the myeloid cell type 1 pathway in murine septic shock. Infect Immun 74:2823–2830. https://doi.org/10.1128/IAI.74.5.2823-2830.2006.
33. Read CB, Kuijper JL, Hjorth SA, Heipel MD, Tang X, Fleetwood AJ, Dantzler JL, Grell SN, Kastrup J, Wang C, Brandt CS, Hansen AJ, Wagtmann NR, Xu W, Stennicke VW. 2015. Cutting edge: identification of neutrophil PGLYRP1 as a ligand for TREM-1. J Immunol 194:1417–1421. https://doi.org/10.4049/jimmunol.1402303.
34. De Marzi MC, Todone M, Ganem MB, Wang Q, Mariuzza RA, Fernandez MM, Malchiodi EL. 2015. Peptidoglycan recognition protein-peptidoglycan complexes increase monocyte/macrophage activation and enhance the inflammatory response. Immunology 145:429–442. https://doi.org/10.1111/imm.12460.
35. Carbonetti NH, Artamonova GV, Mays RM, Worthington ZE. 2003. Pertussis toxin plays an early role in respiratory colonization by *Bordetella pertussis*. Infect Immun 71:6358–6366. https://doi.org/10.1128/IAI.71.11.6358-6366.2003.