T$_H$17-Mediated Protection against Pneumococcal Carriage by a Whole-Cell Vaccine Is Dependent on Toll-Like Receptor 2 and Surface Lipoproteins

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A pneumococcal whole-cell vaccine (WCV) confers T$_H$17-mediated immunogenicity and reduces nasopharyngeal (NP) carriage in mice. Activation of Toll-like receptor 2 (TLR2) has been shown to be important for generating T$_H$17 responses, and several lipitated pneumococcal proteins have TLR2-activating properties. Here we investigated the roles of TLR2 and lipoproteins in WCV-induced interleukin-17A (IL-17A) responses and protection against NP carriage. Immunization of Tlr2$^{-/-}$ mice with WCV conferred significantly lower IL-17A levels and reduced protection against NP carriage, compared to wild-type (WT) mice, suggesting that host TLR2 engagement is required for effective immunity and protection elicited by WCV immunization. Using a WCV with deletion of lgt, the gene encoding the enzyme required for lipidation and membrane attachment of prolipoproteins, we show that lipidation and membrane localization of these proteins are critical for the immunogenicity and protective efficacy of the WCV. To evaluate the roles of diacylglycerol transferase (Lgt)-mediated processes in the recall of WCV-induced protective responses, we colonized WCV-immunized animals with a strain in which lgt was deleted. WCV-immunized animals still had significantly reduced colonization burdens, compared to control animals, which suggests that lipidation and membrane localization of pneumococcal prolipoproteins are less critical for the recall of the immune responses elicited by WCV immunization than for the priming of such responses. Elucidation of underlying immune mechanisms and the optimal characteristics of WCV formulations can help guide vaccine development and enhance our understanding of host-pneumococcus interactions.

Disease due to Streptococcus pneumoniae remains a substantial cause of death and morbidity in developing countries (1). Despite the remarkable success of pneumococcal conjugate vaccination programs, less expensive and serotype-independent approaches to pneumococcal immunization are a global health priority (2). One such approach includes the development of a killed nonencapsulated whole-cell vaccine (WCV) that provides serotype-independent protection against invasive or mucosal disease, as well as nasopharyngeal (NP) carriage, the major reservoir for transmission.

Preclinical investigations demonstrated that immunization of mice with WCV conferred serotype-independent T$_H$17-mediated protection against colonization (3). This was corroborated by the finding that interleukin-17A (IL-17A) is critical to clearance of pneumococcal colonization in naive mice and is of particular importance in adaptive immune responses that protect animals against pneumococcal carriage in models of secondary colonization (4). To this end, activation of Toll-like receptor 2 (TLR2) has also been shown to be critical for eliciting pneumococcus-specific memory CD4$^+$ T$_H$17 cell responses (4). Further exploring the role of TLR2 in the generation of antigen-specific T$_H$17 responses, we showed that the IL-17A immunogenicity and protective efficacy of several immunodominant pneumococcal T$_H$17 antigens were dependent on host TLR2 engagement (5). The studied proteins were selected because they elicited the greatest IL-17A responses in screens of the pneumococcal proteome using CD4$^+$ T cells from healthy human adults and WCV-immunized mice (6, 7). The identified proteins are substrate-binding lipoproteins; given their overall representation within the proteome, lipoproteins are overrepresented in the list of top T$_H$17 antigens. We demonstrated that, when they were used as immunogens, posttranslational attachment of lipid moieties to these prolipoproteins was required for optimal antigen-specific IL-17A responses and protection against NP colonization (5).

Here we evaluate whether host TLR2 and lipoprotein-mediated processes are required for immunogenicity and protective efficacy of WCV. To assess this, we deleted the lgt gene, encoding diacylglycerol transferase (Lgt), from the parent strain in the WCV. Extracellularly secreted pneumococcal prolipoproteins are covalently linked to the cell membrane by Lgt-mediated attachment of a membrane diacylglyceride to a cysteine residue within the lipoprotein signal peptide, via a conserved mechanism for posttranslational lipidation of prolipoproteins (8). Deletion of lgt in S. pneumoniae and other bacteria results in decreases in the lipoprotein contents of cellular membrane extracts and greater prolipoprotein contents in culture supernatants (9–12). Bacterial lipoproteins are potent ligands for TLR2 (13) and are associated with proinflammatory cytokine stimulation (14, 15). The surface expression of pneumococcal lipoproteins has been demonstrated...
to be critical for TLR2-dependent proinflammatory responses from macrophages stimulated in vitro and immune responses in a mouse pneumonia model (16). This requirement for surface localization of lipoproteins for pneumococcal virulence has also been suggested in models of sepsis via intraperitoneal infection and in pneumonia (10). Here we demonstrate a requirement for TLR2 in WCV-mediated immune responses and protection against NP colonization, and we use the WCV with the lgt deletion to evaluate the effects of surface localization of lipoproteins on the proinflammatory, immunogenic, and protective potential of this WCV mutant. Taken together, our findings elucidate host and bacterial factors that are critical for the immunogenicity and efficacy of this WCV candidate, which is currently being evaluated in phase 2 clinical trials (ClinicalTrials.gov registration no. NCT02097472).

MATERIALS AND METHODS

Generation and characterization of wild-type and Δlgt vaccines and challenge strains. The WCV was derived from the RM200 pneumococcal strain, a capsule-negative, autolysin-negative, pneumolysin-protein-expressing strain derived from parent strain Rx1 as described previously (17). To delete the lgt locus from the parent RM200 strain, an overlapping PCR was used to insert a spectinomycin resistance cassette (Spc adenyltransferase) from the pD278 shuttle vector in place of lgt. PCR primer sequences are presented in Table S1 in the supplemental material. Transformed colonies were selected on blood agar plates containing 200 μg/ml spectinomycin (spectinomycin dihydrochloride pentahydrate; Sigma). After sequence confirmation of the Δlgt strain, wild-type (WT) RM200 and Δlgt RM200 were grown in culture and killed in a 1:40 (vol/vol) dilution with chloroform for use as immunogens, as described previously (17), here designated WT or Δlgt whole-cell antigens (WCA) and used as vaccine components and as stimuli in immunogenicity studies. We also constructed an Δlgt deletion strain of a clinical serotype 6B strain (strain 0603) for use in our colonization challenge model in mice, using the same primers and plasmid-encoded spectinomycin resistance cassette as described above. Transformed Δlgt 0603 colonies were also sequence confirmed.

To confirm the loss of membrane-attached lipoproteins in the Δlgt RM200 vaccine strain, lysates were prepared from mid-log-phase bacteria that were lysed in 0.1% deoxycholate at 37°C for 30 min and sonicated. Lysates were incubated overnight in 10% Triton X114 (Sigma) at 4°C. The supernatant from the Triton X114 extract was incubated at 37°C for 30 min and centrifuged to separate the membrane fraction in the detergent phase from the top aqueous phase. The aqueous phase was boiled in lithium dodecyl sulfate (LDS) sample buffer (Invitrogen), and the detergent phase was pelleted, washed in phosphate-buffered saline (PBS), and resuspended in LDS sample buffer without boiling. Lysates and membrane and aqueous phases were separated by SDS-PAGE and transferred to nitrocellulose membranes for Western immunoblotting for detection of specific proteins. For protein-specific detection, recombinant proteins SP_2108 (a pneumococcal surface lipoprotein) and SP_1912 (a control pneumococcal protein of hypothetical function, predicted to be surface expressed) were generated as described previously (3) and injected into rabbits to elicit high-titer protein-specific sera (Cocalico Biologicals, Inc.).

Macrophage stimulations and cytokine evaluation. Murine macrophages from the RAW 264.7 cell line were cultured in Dulbecco’s modified Eagle’s medium (DMEM)/F-12 medium with 10% fetal bovine serum (FBS) and ciprofloxacin, as adherent cells in 24-well plates. Peritoneal macrophages were harvested from naïve C57BL/6J mice 3 to 4 days following intraperitoneal injection of 3 ml of thioglycollate medium (Remel; Thermo Scientific). Using either cell source, approximately 1 × 10⁶ cells, in a volume of 500 μl per well, were stimulated with various concentrations of whole-cell antigens. Supernatants from stimulated cells were harvested 1, 2, 4, 8, 18, 24, 48, and 72 h later and were evaluated for concentrations of proinflammatory cytokines, including IL-6 and tumor necrosis factor alpha (TNF-α) (mouse cytokine enzyme-linked immunosorbent assay [ELISA] DuoSets; R&D Systems).

Mice. For experiments in wild-type mice, 4- to 6-week-old female C57BL/6J mice (strain 000664; The Jackson Laboratory, Bar Harbor, ME) were used. For experiments with TLR2-deficient animals, C57BL/6 Tlr2−/− female mice (C57B6.129-Tlr2−/−H9004, strain 0046501; The Jackson Laboratory) were used. Ten animals were included in each immunization and challenge group. All animal studies were conducted in accordance with institutional guidelines approved by the IACUC of Boston Children’s Hospital and Harvard Medical School.

Immunization and immunogenicity studies. Animals were injected with one to three doses of the WT RM200 or Δlgt RM200 whole-cell vaccines (i.e., WT WCV or Δlgt WCV, respectively). Vaccines were prepared at least several hours prior to use, as 100 μg of whole-cell antigens adsorbed to 250 μg of aluminum (as aluminum hydroxide, Alhydrogel; Brentntag) per dose. For immunization with a synthetic TLR2 agonist, Pam3CSK4 (Pam3CSK4 VacciGrade; InvivoGen) was added to the indicated vaccine to provide 20 μg Pam3CSK4 per dose. All immunizations, in 200-μl volumes in sterile saline solution, were administered subcutaneously in the dorsum of animals. Multiple-dose schedules were administered using 2-week intervals. Two to 3 weeks following the last immunization, animals were bled by retro-orbital venipuncture under anesthesia. Blood was stimulated with either WT WCA or Δlgt WCA, as described previously (3). After 6 days of incubation, IL-17A levels were measured in the supernatant of each stimulation by enzyme-linked immunosorbent assay (IL-17A ELISA; R&D Systems).

Colonization. Animals were challenged with 2 × 10⁹ CFU of WT strain 0603, a serotype 6B clinical strain, or the Δlgt 0603 strain. Colonization inocula were administered in 20-μl volumes in sterile PBS, applied in drops to the anterior nares of gently restrained, nonanesthetized animals. Seven days following infection with Δlgt 0603 or 10 days following infection with WT 0603, animals were euthanized by CO₂ inhalation, and the density of pneumococcal colonization was determined by plating of tracheal wash samples, as described previously (18).

Statistical analyses. PRISM (version 5.0d; GraphPad Software, Inc.) was used for analysis and graphing of all data. The Mann-Whitney test was used to determine significance; P values of <0.05 were considered significant.

RESULTS

WCV-induced Th17 responses and protection against NP colonization require host TLR2 signaling. Prior work demonstrated a critical role for CD4+ T cells and IL-17A in mediating the protective effects of WCV immunization in mouse models of NP colonization (3). The role of TLR2 in eliciting pneumococcal protein-specific IL-17A responses was subsequently demonstrated in mice using vaccines composed of candidate pneumococcal Tlr2−/− antigens (5). Others have more broadly demonstrated a role for TLR2 engagement during immunization to enhance the generation of antigen-specific memory CD4+ T cell responses (19). We hypothesized that the immunogenicity and protective efficacy of WCV also might depend on intact host TLR2 activity.

To evaluate the requirement for intact TLR2 signaling, we immunized WT and Tlr2−/− C57BL/6 mice with the WT WCV. Animals were subcutaneously immunized three times, at 2-week intervals, with either alum-adsorbed WCV or alum alone. Two weeks following the last immunization, whole blood from immunized animals was stimulated with the pneumococcal WCA in vitro. WCV immunization was associated with significantly greater stimulation of IL-17A responses from whole blood from WT animals than from Tlr2−/− animals (P = 0.005) (Fig. 1A).
Accordingly, pneumococcal colonization burdens in WCV-immunized WT mice were also significantly smaller than those in WCV-immunized Tlr2−/− mice (P = 0.0008) (Fig. 1B), and the protection conferred by WCV vaccination was significantly abrogated in Tlr2−/− animals. Taken together, these findings suggest a critical role for intact host TLR2 signaling in elicitation of IL-17A and protective efficacy against NP colonization following WCV immunization.

Deletion of lgt from the WCA-derived pneumococcal strain results in loss of membrane attachment of prolipoproteins. We previously confirmed that the immunogenicity and protective efficacy of several dominant pneumococcal T317 antigens are attributable to the posttranslational attachment of lipid moieties to these proteins and were dependent on host TLR2 engagement (5). After secretion, these candidate prolipoproteins are among the dozens of pneumococcal proteins that are programmed for lipidation and covalent attachment to the cell membrane via diacylglycerol transferase (Lgt) (8, 20). For several Gram-positive bacteria, others demonstrated that, in the absence of functional Lgt, prolipoproteins were in decreased abundance in the cell membrane but in greater abundance in culture supernatants (11, 21). This was also confirmed for a S. pneumoniae lgt deletion mutant (10). We constructed an lgt deletion mutant in our vaccine background strain, strain RM200, by insertional deletion of a spectinomycin resistance cassette in place of lgt. There was no difference in the growth phenotypes of the Δlgt strain versus the wild-type strain in Todd-Hewitt yeast broth. To confirm the lipoprotein compartment phenotype of the deletion mutant, we fractionated the WT and Δlgt strains into membrane and aqueous phases and immunoblotted these fractions with sera specific for SP_2108, a pneumococcal substrate-binding lipoprotein encoded with a signal sequence that programs the lipoprotein for Lgt-mediated lipidation and membrane attachment, or SP_1912, a nonlipoprotein used as a control (5). SP_1912 was present at similar levels in whole lysates and membrane and aqueous phases of WT and Δlgt cultures (Fig. 2A).

FIG 1 WCV-elicited IL-17A responses and pneumococcal colonization burdens in WT and Tlr2−/− mice. (A) WT and Tlr2−/− mice in the C57BL/6 background (10 animals/group) were immunized subcutaneously 3 times with either alum alone or WCV adsorbed on alum. Several weeks after the last immunization, whole blood was stimulated with whole-cell antigens. IL-17A levels measured in supernatants of whole-blood cultures are shown. (B) Two weeks later, animals were colonized intranasally with 2 × 10^5 CFU of type 6B pneumococci in a 20-μl volume. Pneumococcal density was enumerated in nasal washes 10 days later. Bars represent medians. P values were calculated with the Mann-Whitney test.

In contrast, SP_2108 was detected in abundance in the membrane phase of the WT strain but was almost exclusively detectable only in the aqueous phase of the Δlgt strain, thereby confirming the loss of Lgt function anchoring SP_2108 to the cell membrane (Fig. 2A).

Deletion of lgt diminishes the IL-6 and TNF-α stimulatory activities of WCA in murine macrophages in vitro. Deletion of lgt from S. pneumoniae attenuates the TLR2 activity and proinflammatory properties of pneumococcal lysates used to stimulate human reporter cell lines, macrophages, or human peripheral blood mononuclear cells (PBMCs) in vitro (16). We saw no differences in the activation of human embryonic kidney cells transfected with TLR2 (HEK-TLR2) following stimulation with a range of doses of WT or Δlgt WCA (data not shown). However, we did identify significantly higher levels of IL-6 secretion from RAW murine macrophages at 18 and 24 hours following stimulation with WT WCA, compared to Δlgt WCA (Fig. 2B), and from peritoneal macrophages from C57BL/6 mice at 24 and 48 h only (Fig. 2C). Similar evaluation of TNF-α release demonstrated no significant difference for WT versus Δlgt WCA stimulation of RAW macrophages (data not shown), but significantly higher levels of TNF-α were measured from peritoneal macrophages following 24 h of stimulation with WT WCA, compared to Δlgt WCA (Fig. 2D).

Immunogenicity and protective efficacy of the WCV require membrane attachment of lipoproteins. To test the hypothesis that the membrane attachment of lipoproteins is critical for the immunogenicity and protective efficacy of the WCV, we immunized C57BL/6 mice with a single dose of alum alone or either WT or Δlgt WCV (both adsorbed on alum). Pneumococcal WCA stimulation of whole blood from animals immunized with the Δlgt WCV elicited significantly less IL-17A than did that of whole blood from animals immunized with the WT WCV (P = 0.03) (Fig. 3A). Immunized animals were then challenged intranasally with live serotype 6B pneumococci, and the burden of pneumococci in nasal washes was determined 10 days after infection.
While a single dose of the WT WCV significantly reduced the burden of bacteria, compared to alum-immunized animals ($P = 0.0005$), protection against nasopharyngeal carriage was significantly abrogated in the animals immunized with $\Delta lgt$ WCV ($P < 0.0005$) (Fig. 3B).

We next tried to restore the protective efficacy of the $\Delta lgt$ WCV by coadministration with a TLR2 agonist. As shown in Fig. S1 in the supplemental material, while the colonization burden of animals immunized with $\Delta lgt$ WCV with Pam3CSK4, a synthetic TLR2 agonist, was significantly lower than that of mice immunized with $\Delta lgt$ WCV alone ($P = 0.0435$), the colonization burden of mice immunized with WT WCV was still significantly lower than that of the mice immunized with $\Delta lgt$ WCV with Pam3CSK4 ($P = 0.0171$).

Membrane localization of prolipoproteins does not appear to play a major role in recall of WCV-induced IL-17A responses. The data presented above strongly suggest that priming of $T_{h17}$ responses by the WCV is highly dependent on TLR2 and the membrane localization of lipoproteins. What is less clear, however, is whether $lgt$ and subsequent lipidation and membrane localization of prolipoproteins are also critical for the recall of WCV-induced immune responses in previously primed animals. To evaluate this issue, we immunized mice with WT or $\Delta lgt$ WCV, as described previously, and bled them following a single immunization. We stimulated whole blood from immunized animals and again we noted significant decreases in IL-17A responses in mice immunized with $\Delta lgt$ WCV, compared to mice immunized with WT WCV ($P < 0.0001$) (Fig. 4A), regardless of the $lgt$ genotype of the in vitro stimulus. While the median IL-17A responses of mice immunized with WT WCV were slightly lower following $\Delta lgt$ WCA stimulation than after WCA stimulation, the difference was not significant (the median IL-17A levels for WCV-immunized mice were 542 pg/ml after $\Delta lgt$ WCA stimulation and 925 pg/ml after WT WCA stimulation; $P = 0.8287$) (Fig. 4A). This suggests that, unlike during immunization, the lipidation and membrane localization of prolipoproteins are less critical during recall of the IL-17A responses following initial priming.
Membrane localization of lipoproteins in the challenge strain is not required for protective efficacy of the WCV against colonization. We next evaluated whether the presence of lipoproteins on the membranes of the challenge strain is a requirement for successful eradication of colonization in WCV-immunized mice. To address this question, we generated an lgt deletion mutant in our colonization strain, clinical serotype 6B pneumococcus 0603. Deletion of lgt in other pneumococcal strains has been shown to attenuate the ability of the bacteria to colonize, as evidenced by earlier clearance of Δlgt pneumococci from nasal washes from mice following intranasal infection (10). To evaluate whether our mutant 0603 strain could successfully colonize mice, we intranasally challenged naïve C57BL/6 mice with the live Δlgt 0603 mutant strain. Nasal washes performed on days 5, 7, and 10 showed decreasing burdens of pneumococci recovered from the nasopharynx by day 10 but a high colonizing burden on day 7 (see Fig. S2 in the supplemental material). Therefore, we colonized WCV-immunized animals with either WT or Δlgt 0603 and performed nasal washes 7 days following colonization, for enumeration of pneumococcal burdens.

The bacterial burdens in alum-immunized animals colonized with the Δlgt 0603 strain were significantly lower than those in alum-immunized animals colonized with the WT 0603 strain (P = 0.0015) (Fig. 4B), which suggests that there is some attenuation of the Δlgt 0603 strain with respect to NP colonization. Despite this, immunization with the WT WCV conferred a statistically significant reduction in carriage, compared to alum immunization, in animals colonized with Δlgt 0603 (P = 0.0140) (Fig. 4B).

**DISCUSSION**

Progress in the development of serotype-independent pneumococcal vaccines has been enhanced by our broadened understanding of the niche-specific mechanisms of adaptive immunity to pneumococcal infection. As correlates of protection against vaccine-type invasive pneumococcal disease, capsule-specific antibody titers have been the benchmark by which polysaccharide conjugate vaccines have been evaluated. The correlation of capsular antibodies with protection from mucosal phases of disease, such as carriage, sinusitis, and otitis media, has been less clear, however (reviewed in reference 22). Furthermore, evolving evidence in support of an important role for cellular immunity, particularly CD4+ T_{H17} cell-mediated immunity, in the clearance of pneumococcal NP carriage, the major reservoir for pneumococcal transmission, has bolstered the objective of developing a vaccine that elicits both humoral immunity and cellular immunity to conserved pneumococcal antigens (reviewed in references 23 and 24). To this end, a nonencapsulated whole-cell vaccine should present a multiplicity of such antigens, and the WCV candidate has indeed been shown to confer protection in varied mouse models of carriage, aspiration pneumonia, and sepsis, using several different pneumococcal challenge serotypes (17, 25). Additional ex vivo characterization of the serotype coverage elicited by WCV immunization demonstrated broad capsular antibody and IL-17A immunogenicity across a wide range of clinical and laboratory strains (26). The precise antigenic specificity of the WCV has remained unclear, however, and attempts to attenuate its efficacy, including treatment with periodate to damage teichoic acid and elution of surface choline-binding proteins through growth of the WCA in excess choline, have failed to affect its potency (R. Malley, unpublished data). Given the very low concentrations of WCA that are sufficient to confer significant protection against pneumococcal NP colonization (as low as 1 μg of total protein per immunization, as described in reference 27), it is reasonable to assume that no single antigen (or even group of antigens) is uniquely responsible but, rather, protection is the result of eliciting immunity to a large number of antigens.

Based on the overrepresentation of lipoproteins among the top tier of T_{H17} antigens identified from proteomic screens (7), we hypothesized that the lipidation and surface localization of pneumococcal prolipoproteins might be critical for WCV immunogenicity and efficacy. The findings described here confirm a critical role of Lgt-mediated processes in WCV-elicited immunogenicity and protection. At the outset, we hypothesized that such an effect could be seen on the basis of the loss of immunoprotective antigens from the WCV and/or reductions in the inflammatory potential of the WCV (a reduced adjuvant effect). To test the latter, we tried to restore the protective efficacy of the Δlgt WCV through coadministration with a synthetic TLR2 agonist, Pam3CSK4. The finding of only partial restoration of the protective efficacy of the Δlgt WCV with the addition of a synthetic TLR2 agonist may be explained in several ways. The reduced adjuvant effect may only partially explain the decreased protective efficacy of the Δlgt WCV; the loss of surface localization of critical T_{H17} antigens also

![FIG 3 IL-17A responses and colonization burdens following immunization with either WT WCV or Δlgt WCV. WT mice were immunized subcutaneously once with alum alone or with either WT WCV or Δlgt WCV (both adsorbed on alum). (A) Two weeks later, whole blood was stimulated with WCA, and IL-17A levels were measured in culture supernatants. (B) Two weeks after bleeding, animals were colonized intranasally with type 6B pneumococci. Pneumococcal density was determined in nasal washes 10 days after colonization. Bars represent medians. P values were calculated using the Mann-Whitney test. NS, not significant.](image-url)
may play a role. Alternatively, the delivery of the Δlgt WCV mixed with Pam3CSK4 may not fully recapitulate the more physiological TLR2 agonists that are attached to the WT WCV, as it has been shown that antigen presentation by dendritic cells is most efficient when TLR ligands are delivered with phagocytosed antigenic cargo (28). Thus, given our findings, it appears that the reduced immunogenicity and protection observed with the Δlgt WCV are at least in part consequences of the reduced adjuvant effect, which requires TLR2 and lipoproteins for effective priming. With that being said, even the Δlgt WCV can confer protection with additional doses (as shown with two doses) (see Fig. S3 in the supplemental material), suggesting that, with repeated doses, the reduced immunogenicity of the mutant WCA can be overcome. Furthermore, our finding that Δlgt WCA stimulation activated HEK-TLR2 cells to a similar level, compared with WT WCA stimulation, implies that TLR2 ligands other than Lgt-dependent lipoproteins contribute to the inflammatory responses to the WCA preparation. One such ligand is likely pneumococcal lipoteichoic acid (LTA), as purified LTA has been demonstrated to elicit robust TLR2-dependent inflammation in vivo (29).

This work provides further evidence for the role of host TLR2 engagement in the generation of IL-17A-mediated mechanisms of adaptive immunity against pneumococcal colonization. This corroborates the findings of others regarding the roles of TLR2 and subsequently IL-17A in the immunity to recurrent colonization that is elicited by primary colonization in mice (4). Furthermore, work with other pathogens in models of enteric infection has suggested a critical role for TLR2 activation in eliciting protective mucosal IL-17A responses (30, 31). While we demonstrate significant deficits in the IL-17A responses to WCV immunization in Thr2<sup>-/-</sup> mice (Fig. 1A), these mice do generate modest IL-17A responses, which supports a possible role for non-TLR2-dependent mechanisms of eliciting IL-17A responses.

Beyond pneumococcal infections, researchers have demonstrated the role of IL-17A-mediated mechanisms of adaptive immunity against mucosal phases of infections with a range of pathogens, such as Salmonella enterica, Mycobacterium tuberculosis, Bordetella pertussis, and Staphylococcus aureus (32–34). Our work with several formulations of protein subunit vaccines composed of pneumococcal lipoproteins that generate protective IL-17A responses (5, 7), taken together with the findings presented here, lend support to the consideration of lipoproteins as candidate vaccine antigens. This may be particularly relevant for pathogens that meet the criteria of requiring both IL-17A-mediated

![FIG 4](cvi.asm.org)
adaptive immune responses for protection and lipoprotein lipidation and surface localization for proinflammatory capacity and virulence. There is evidence of overlap of these two conditions for a number of pathogens, including S. aureus (11, 35) and M. tuberculosis (36, 37). In addition to supporting such a rationale for vaccine approaches to other pathogens, the findings described here highlight host and bacterial factors critical for the immune responses elicited by a pneumococcal whole-cell vaccine candidate that is currently being investigated in a phase 2 clinical trial.

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