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Anti-LPS antibodies protect against *Klebsiella pneumoniae* by empowering neutrophil-mediated clearance without neutralizing TLR4

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Initial promising results with immune sera guided early human mAb approaches against Gram-negative sepsis to an LPS neutralization mechanism, but these efforts failed in human clinical trials. Emergence of multidrug resistance has renewed interest in pathogen-specific mAbs. We utilized a pair of antibodies targeting *Klebsiella pneumoniae* LPS, one that both neutralizes LPS/TLR4 signaling and mediates opsonophagocytic killing (OPK) (54H7) and one that only promotes OPK (KPE33), to better understand the contribution of each mechanism to mAb protection in an acutely lethal pneumonia model. Passive immunization 24 hours prior to infection with KPE33 protected against lethal infection significantly better than 54H7, while delivery of either mAb 1 hour after infection resulted in similar levels of protection. These data suggest that early neutralization of LPS-induced signaling limits protection afforded by these mAbs. LPS neutralization prevented increases in the numbers of γδT cells, a major producer of the antimicrobial cytokine IL-17A, the contribution of which was confirmed using *il17a*-knockout mice. We conclude that targeting LPS for OPK without LPS signaling neutralization has potential to combat Gram-negative infection by engaging host immune defenses, rather than inhibiting beneficial innate immune pathways.

Introduction

Antibodies for use against bacterial infection have clear potential benefits in comparison to antibiotics. These include a longer half-life; precision targeting of pathogens, thus, reducing collateral damage to the beneficial microbiome; and the ability to neutralize secreted toxins and engage the immune system directly by promoting opsonophagocytic killing (OPK) and clearance of pathogens by host immune cells. Some of the earliest mAb efforts targeted lipid A endotoxin for the treatment of Gram-negative sepsis based on promising clinical trial results with hyperimmune sera against core glycolipid from the *E. coli* J5 rough (short-chain) LPS mutant and the belief that endotoxin neutralization was a key protective mechanism (1–4). These efforts failed in clinical testing, but multiple retrospective analyses suggest that endotoxin neutralization was never fully achieved and therefore not adequately tested, because the IgM candidate mAbs were insufficiently specific or active, or that lipid A is a poorly accessible target located in the interior of the bacterial outer membrane (5–11). As a result, these early clinical trials dissuaded further efforts to develop antibodies targeting LPS. However, the emergence of antibiotic resistance in Gram-negative bacteria has led some to revisit targeting LPS for treating these serious infections.

Klebsiella pneumoniae, an opportunistic Gram-negative bacterium, is emerging as a serious health concern as resistance to antibiotics spreads (12). *K. pneumoniae* often persists at low levels in the lower gastrointestinal tract, from which it can rapidly expand when the commensal microbiota is disrupted by antibiotic treatment (13). Currently, the ST258 clone, which has the bla_{KPC}-encoded carbapenemase, is spreading globally, due in part to its resistance to antibiotic therapy (14). This clone was also recently reported to avoid clearance by recruited neutrophils, perhaps enabling persistent infection (15). To effectively treat these infections, a mAb might also be required to enhance neutrophil-mediated killing through Fc-mediated mechanisms.

In the current report, we utilize a pair of anti–*K. pneumoniae* LPS-O-antigen mAbs, one that neutralizes LPS signaling and enhances neutrophil-mediated OPK and another that enhances neutrophil OPK but does not neutralize LPS signaling. Surprisingly, LPS neutralization significantly reduced mAb protection when delivered 24 hours prior to infection in a murine *K. pneumoniae* pneumonia model and did not appear to contribute or diminish protection when delivered after challenge. Neutralization of LPS early in infection inhibited the beneficial recruitment of γδT cells. Furthermore, utilizing animal models in which either the TLR4 pathway or the IL-17 pathway is inhibited or neutrophils are depleted, we show that OPK, not LPS neutralization, is the primary mechanism of mAb-based protection. These data suggest that cooperation between host immune pathways and mAb mechanisms of action is required for optimal mAb functionality and that previous antibody therapies might have had unforeseen effects on beneficial components of the immune response. Our data provide a guide for subsequent antibody design by giving mechanistic insight on the synergistic relationship between the immune system and antibody therapy.

Results

The primary aim of earlier efforts to target LPS was to develop antibodies that neutralized the LPS-mediated hyperinflammatory response. However, the importance of LPS neutralization for in vivo protection by mAbs has not been vetted completely. We have developed a large mAb collection targeting several O-types of *K. pneumoniae* LPS. From this collection, antibodies were selected based on their in vitro ability to mediate OPK of *K. pneumoniae*, to increase serum bactericidal activity (SBA), and to neutralize LPS signaling. Two antibodies targeting O1-type LPS that exhibited similar OPK and SBA characteristics, but differed in that one neutralized LPS (54H7) while the other did not (KPE33), were identified (Figure 1A and Supplemental Figure 1, A and B; supplemental material available online with this article; https://doi.org/10.1172/jci.insight.92774DS1). To investigate the protective ability of these mAbs in vivo, each was administered to mice prior to a lethal injection of LPS. As expected 54H7, but not KPE33, protected in this mouse lethal endotoxemia model, confirming that 54H7 neutralizes LPS in vivo (Supplemental Figure 1C).

K. pneumoniae is a major cause of severe pneumonia. Therefore, the antibodies were tested in an acutely lethal *K. pneumoniae* pneumonia model, with a very narrow therapeutic window for successful treatment. mAbs were delivered either 24 hours prior to bacterial challenge or 1 hour after bacterial challenge, a time by which we expected LPS to have activated the TLR4 pathway, which is not so late that even standard-of-care antibiotics cannot rescue the animals (16, 17). Administration of the nonneutralizing mAb KPE33 24 hours prior to infection significantly increased survival as compared with prophylaxis with either 54H7 ($P < 0.0001$) or control IgG (c-IgG) ($P < 0.0001$), while both KPE33 and 54H7 protected to a similar degree when delivered 1 hour after infection (Figure 1, B and D). Reduced prophylactic efficacy of 54H7 as compared with KPE33 was also observed in C3H/HeOuJ mice or with another *K. pneumoniae* strain (Figure 1, C and G, and Supplemental Figure 2, A and B). Together, these data suggest that neutralization of LPS at the onset of infection may reduce the efficacy of an OPK-mediating mAb. As opposed to mAb effects in WT mice, prophylaxis with either KPE33 or 54H7 failed to protect against *K. pneumoniae* infection of mice with an impaired TLR4 signaling pathway (C3H/HeJ), confirming the importance of this signaling pathway for optimal efficacy of these mAbs (Figure 1E and Supplemental Figure 2C).

Counter to our initial predictions, the ability to neutralize LPS did not influence early bacterial clearance. Administration of both 54H7 and KPE33 24 hours prior to infection significantly reduced *K. pneumoniae* CFU ($P < 0.01$) 24 and 48 hours after infection compared with c-IgG; the 48-hour time point was the only point at which CFU were significantly lower in KPE33-treated mice as compared with 54H7-treated mice ($P = 0.0434$) (Figure 1, F and G). Prophylaxis of C3H/HeJ mice with either mAb did not reduce CFU below levels observed in c-IgG–treated mice (Figure 1H). Similarly, in WT C57BL/6 mice, both mAbs reduced levels of IL-1β, KC/GRO, TNF, IL-6, IL-10, and IFN-γ compared with c-IgG (Figure 2, A and B, and Supplemental Figure 3, A–D). Histological analysis of the lung revealed a steady increase (8–48 hours after infection) in lung damage in mice prophylactically treated with c-IgG or 54H7, while little damage was observed in KPE33-treated animals (Figure 2C and Supplemental Figure 4). Therefore, the increased mortality and lung inflammation associated with use of 54H7 as compared with KPE33 was not due to alterations in the acute proinflammatory response to the pathogen or differences in early (24 hours) bacterial clearance.

Based on the lack of LPS neutralization activity of KPE33, we hypothesized that the primary mechanisms through which KPE33 promotes clearance of *K. pneumoniae* is OPK by recruited neutrophils or complement-mediated SBA. We therefore tested the ability of KPE33 to reduce bacterial bur-
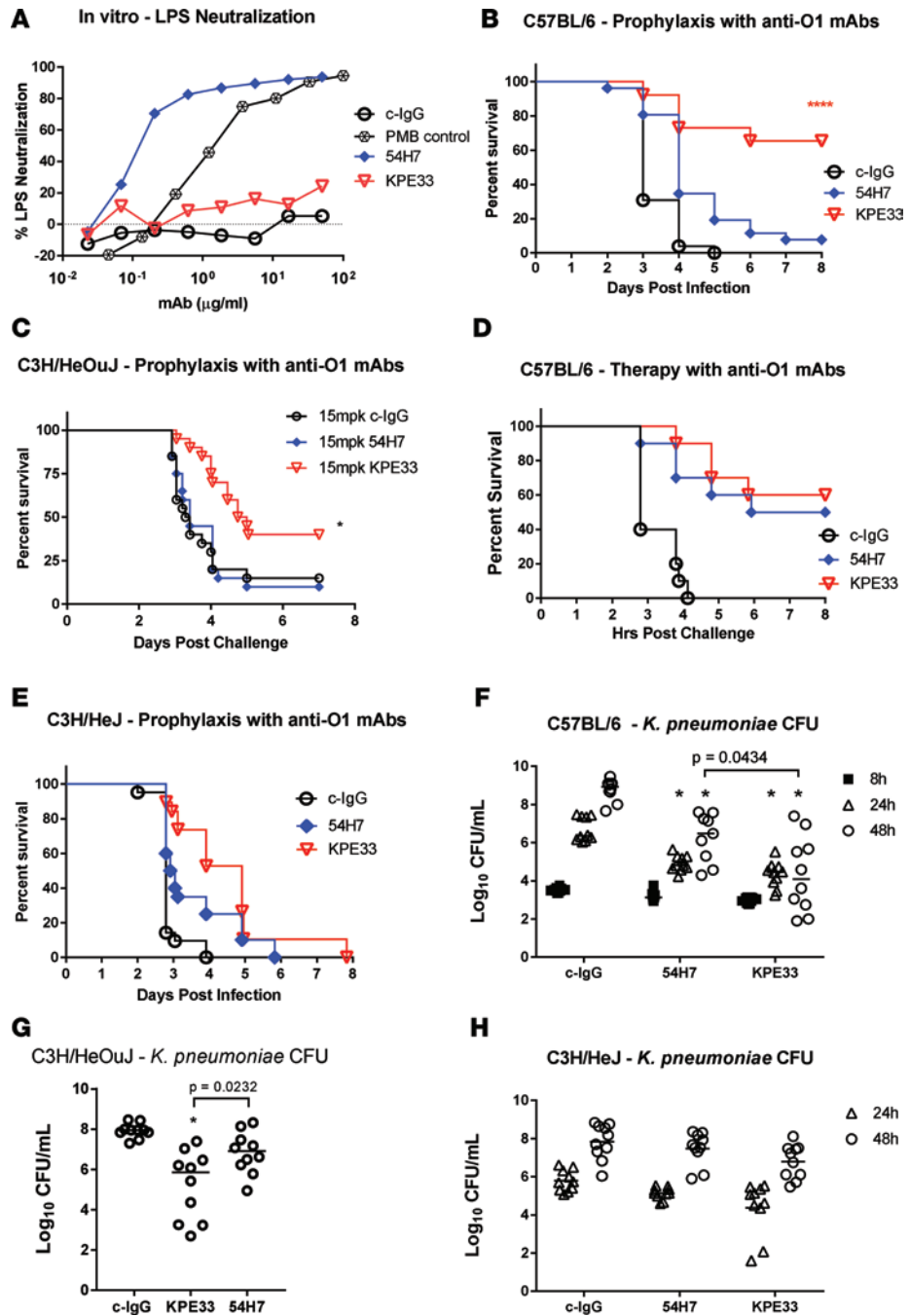
Figure 1. Anti-LPS mAbs protect against *K. pneumoniae* pneumonia. (A) In vitro neutralization of LPS at various concentrations of mAb or polymixin b (PMB) control. **(B)** Survival of C57BL/6 mice prophylactically immunized with control or anti-LPS mAbs and infected 24 hours later with *K. pneumoniae* (1×10^4 CFU KP8045) (****$P < 0.0001$ KPE33 vs. 54H7) ($n = 26$ per group). **(C)** Survival of C3H/HeOuJ mice prophylactically immunized 24 hours prior to infection (1×10^4 CFU KP8045) ($n = 20$, *$P = 0.0042$). mpk, mg/kg. **(D)** Survival of C57BL/6 mice treated with control or anti-LPS mAbs 1 hour after infection with *K. pneumoniae* (1×10^4 CFU KP8045) ($n = 10$ per group). **(E)** Survival of C3H/HeJ mice prophylactically immunized with control or anti-LPS mAbs and infected 24 hours later with *K. pneumoniae* (1×10^4 CFU KP8045) ($n = 20$ per group). **(F)** *K. pneumoniae* CFU recovered from the lungs of C57BL/6 mice prophylactically immunized with control or anti-LPS mAbs 24 hours prior to infection (1×10^4 CFU KP8045) (*$P < 0.05$ vs. c-IgG). **(G)** *K. pneumoniae* CFU recovered from the lungs of C3H/HeOuJ mice at 48 hours after infection. Mice were prophylactically immunized with either control or anti-LPS mAbs 24 hours prior to infection or similar (1×10^4 CFU KP8045) (*$P < 0.0001$ vs. c-IgG). **(H)** *K. pneumoniae* CFU recovered from the lungs of C3H/HeJ mice prophylactically immunized with control or anti-LPS mAbs 24 hours prior to infection (1×10^4 CFU KP8045). Statistical significance was determined by ANOVA followed by Dunn's test (bacterial CFU) or log-rank test (survival). Data are representative of at least 2 independent experiments (**A** and **F–H**) or the compilation of at least 2 independent experiments (**B–E**). (**F–H**) Bars represent medians.
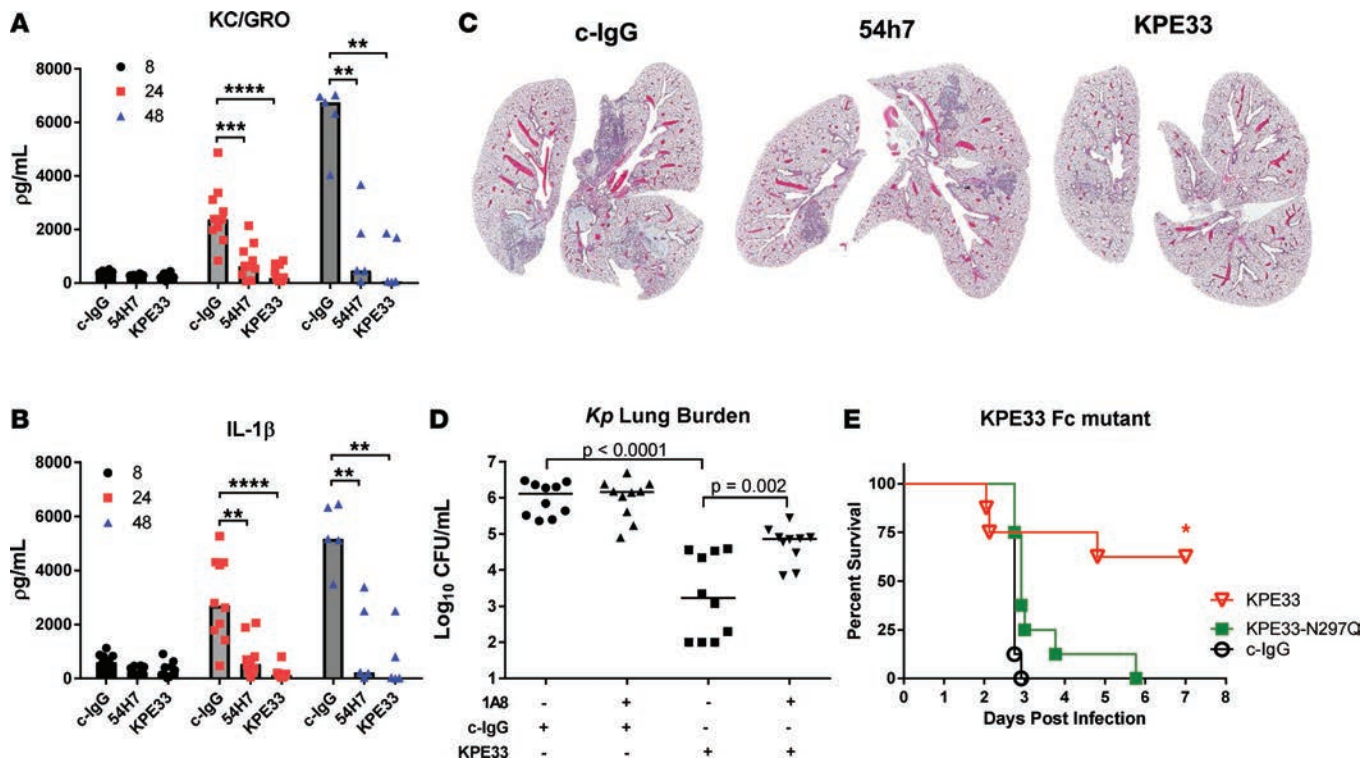
Figure 2. Protection against respiratory infection with *K. pneumonia* is primarily due to opsonophagocytic killing. (**A**) ELISA measurement of KC/GRO expression 8, 24, and 48 hours following infection of C57BL/6 mice with *K. pneumoniae* (1×10^4 CFU KP8045). mAbs were delivered 24 hours prior to infection. (**B**) ELISA measurement of IL-1β expression 8, 24, and 48 hours following infection of mice with *K. pneumoniae* (1×10^4 CFU KP8045). mAbs were delivered 24 hours prior to infection. (**C**) Hematoxylin and eosin–stained lung sections from prophylactically immunized C57BL/6 mice (54H7, KPE33, or c-IgG, 24 hours prior to infection). Lungs were harvested 48 hours after infection with *K. pneumoniae* (1×10^4 CFU KP8045). Original magnification, ×20. (**D**) *K. pneumoniae* CFU recovered from the lungs of C57BL/6 mice 24 hours after infection with 1×10^4 CFU KP8045. Mice were prophylactically immunized with c-IgG or KPE33 as well as the neutrophil-depleting mAb 1A8 24 hours prior to infection. (**E**) Survival of C57BL/6 mice prophylactically immunized with c-IgG, KPE33, or KPE33-N297Q and infected 24 hours later with *K. pneumoniae* (1×10^4 CFU KP8045) (*n* = 8 per group). Statistical significance was determined by ANOVA followed by Dunn's test (**A**, **B**, and **D**) (*$P < 0.05$, **$P < 0.01$. ***$P < 0.001$, ****$P < 0.0001$ mAb vs. c-IgG) or log-rank test (**E**)(*$P < 0.05$ KPE33 vs. KPE33-N297Q). Data are representative of at least 2 independent experiments. (**A**, **B**, and **D**) Bars represent medians.

den in the lungs of mice systemically depleted of neutrophils. As previously reported, bacterial burden in the lungs of mice 24 hours after infection was the same in control mice and in mice treated with an anti-Ly6G antibody (1A8) to deplete neutrophils (500 μg/mouse) (Figure 2D and Supplemental Figure 5A) (18). Prophylaxis with KPE33 significantly ($P < 0.0001$) reduced bacterial CFU recovered from the lungs 24 hours after infection in control mice but not in neutrophil-depleted mice, suggesting that the protective activity of KPE33 was mediated to a substantial degree by neutrophil engagement. To test the role of the KPE33 Fc region on its protective activity, we generated a mutant version of the mAb with a single amino acid substation (KPE33-N297Q), such that its complement and Fcγ receptor–binding function was removed, and tested its ability to protect against lethal *K. pneumoniae* infection (19, 20). Bacterial CFU in the lungs were significantly greater in mice prophylaxed with KPE33-N297Q as compared with KPE33-treated animals ($P = 0.0005$); all mice receiving KPE33-N297Q succumbed to infection (Figure 2E and Supplemental Figure 5B). The data confirm that neutrophil OPK, via Fc engagement, significantly contributes to protection associated with KPE33.

TLR4 signaling has been implicated in the recruitment and activation of γδT cells, a key cell population in the innate immune defense of the mucosa (21, 22). Not only do γδT cells expand in response to LPS, but mice lacking γδT cells have been shown to be more susceptible to *K. pneumoniae* respiratory infection (23, 24). In response to *K. pneumoniae*, the γδT cell population was found to be increased 8 and 24 hours following infection. However, increases in γδT cell numbers were inhibited by prophylaxis with the LPS-neutralizing mAb 54H7 (8 hours $P = 0.0071$, 24 hours $P = 0.0044$) but not inhibited with the nonneutralizing mAb KPE33, consistent with previous observations of impaired recruitment of γδT cells in TLR4-null mice (Figure 3A). Treatment of mice with either 54H7 or KPE33 1 hour

after infection did not reduce the numbers of γδT cells 24 hours after infection, supporting the hypothesis that it is neutralization of LPS signaling very early in infection, as would happen with 54H7 prophylaxis, that prevents recruitment of these beneficial cells (Figure 3B).

γδT cells are major producers of IL-17, which activates key antimicrobial pathways and phagocytic function of neutrophils (25–27). Analysis of the number of IL-17+ γδT cells in the lungs 8 and 24 hours following infection demonstrated a significant decrease in the number of IL-17+ γδT cells 24 hours after infection in mice prophylactically treated with 54H7 as compared with mice treated with c-IgG (P = 0.135) or KPE33 (P = 0.0338) (Supplemental Figure 6A). Expression of the cytokine IL-22, which is produced by Th17 cells, was significantly increased 24 hours after infection in the airways of KPE33-treated mice as compared with c-IgG– or 54H7-treated mice (Supplemental Figure 6B). We utilized IL-17A-/- mice to demonstrate the contribution of IL-17 signaling to the protection afforded by KPE33. WT and IL-17A-/- mice were prophylactically treated with c-IgG, 54H7, or KPE33 and infected with a lethal dose of *K. pneumoniae*. No difference in survival was observed between WT and IL-17A-/- mice prophylactically immunized with 54H7 (Figure 3C); however, a significantly (P < 0.0001) lower level of survival was observed in IL-17A-/- mice compared with WT mice passively immunized with KPE33 (Figure 3D). Therefore, signaling induced by IL-17, a major product γδT cells, is required for optimal protection provided by KPE33.

Discussion

mAbs hold significant promise for the treatment and/or prevention of major bacterial infections (28). Currently, mAbs have been approved or are in development for numerous infectious diseases, including RSV, influenza, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (18, 20, 29–32). These mAbs target secreted virulence factors, surface/structural proteins, or both, resulting in neutralization of pathogen-induced host damage and improved pathogen clearance. There has, however, been a high rate of clinical failure in mAbs against bacterial targets, specifically LPS, perhaps due to unintended effects on the innate immune system.

Previously developed antibodies against LPS have stressed the need to neutralize LPS to provide the greatest likelihood for clinical success (33). Therefore, we utilized a pair of mAbs targeting the O-antigen of *K. pneumoniae* LPS to better understand the contribution of LPS neutralization to antibody defense in a pneumonia model. In vitro, both antibodies promoted comparable neutrophil-mediated killing of *K. pneumoniae*; however, in vivo prophylaxis with the LPS-neutralizing antibody (54H7) afforded significantly less protection than the non-LPS-neutralizing antibody (KPE33). This observation correlated with reduced numbers of γδT cells in 54H7-treated animals as compared with KPE33-treated mice. Use of either mAb 1 hour after infection increased survival and did not alter the numbers of γδT cells. These data suggest that more successful mAbs work synergistically with the host response to the pathogen and antibodies that impair the host's ability to acutely respond to the major Gram-negative pathogen-associated molecular pattern, LPS, early in infection inadvertently prevent beneficial components of the host response.

LPS, via TLR4-dependent signaling, triggers numerous immune pathways, leading to neutrophil recruitment, antimicrobial peptide production, and adaptive immune cell activation. These pathways are required for the host to sense a bacterial pathogen and to mount a protective immune response (34–36). In fact, TLR4-knockout mice, which lack the ability to respond to LPS, exhibit a defect in clearing multiple Gram-negative pathogens, including *K. pneumoniae* (37–39). Unlike the knockout mice, neutralization of LPS with 54H7 did not reduce animal survival or increase bacterial burden as compared with c-IgG, despite reducing levels of proinflammatory cytokines in the lung. However, despite the ability of 54H7 to promote OPK, it did not improve either bacterial clearance or survival. In contrast, prophylaxis with KPE33 (which does not neutralize LPS) improved both murine survival and bacterial clearance in WT mice. The protective effect was, however, lost in mice with an impaired TLR4 pathway or in WT mice lacking neutrophils, indicating that some LPS signaling through TLR4 is essential for KPE33 efficacy. These findings are further supported by the inverted dose response observed in studies involving 54H7. As mAb concentrations increase, presumably neutralizing a greater amount of shed LPS, protection afforded by the mAb is reduced. In response to LPS, the number of γδT cells increases rapidly at the site of infection where they secrete cytokines, including IL-17, a central mediator in host defense at mucosal surfaces (40). Prophylaxis with 54H7 prevented increases in the numbers of these cells in the lung, correlating with reduced protection by this mAb. The importance of LPS-stimulated IL-17 signaling in anti–O-antigen mAb-mediated protection was confirmed using *il17a*-knockout mice. In these mice, prophylaxis with KPE33 was no longer protective against *K. pneumoniae* infection, similar to the lack of protection seen with 54H7 in WT mice.
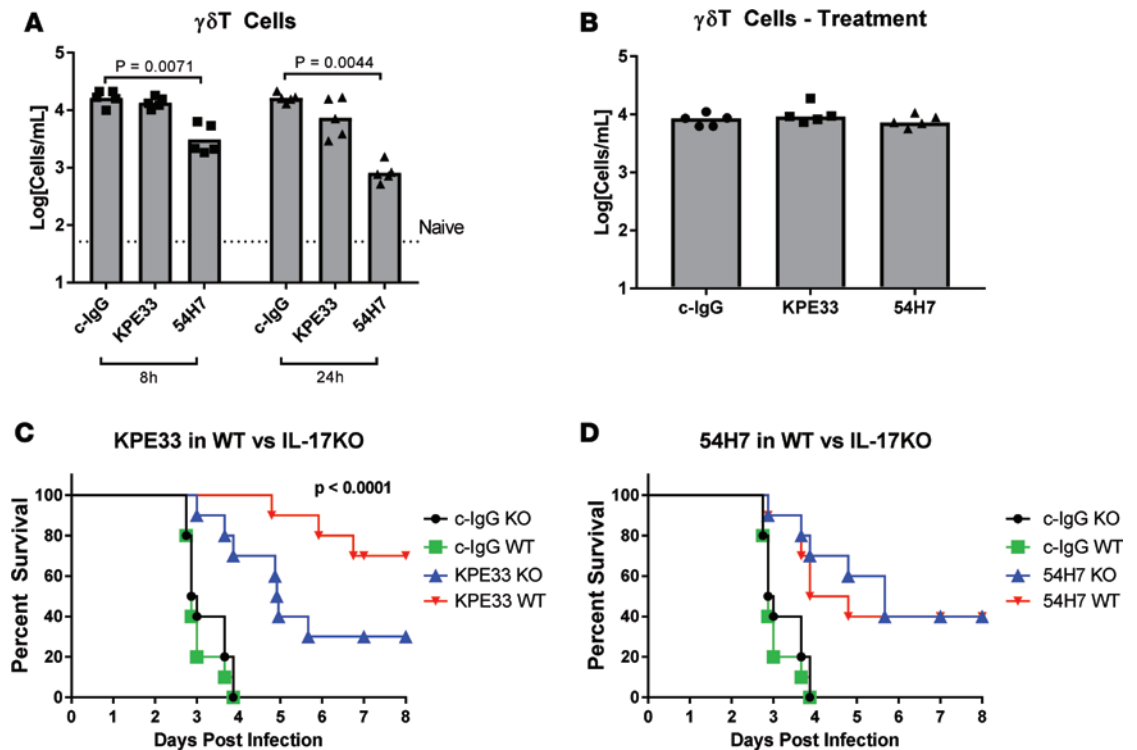
Figure 3. γδT cell recruitment and IL-17 signaling correlate with mAb protection. (A) The number of γδTCR+ T cells in the lungs of untreated C57BL/6 mice (naive) and of mice prophylactically treated with c-IgG or anti-LPS mAbs and then infected with *K. pneumoniae* (1×10^4 CFU KP8045) for 8 and 24 hours. **(B)** The number of γδTCR+ T cells in the lungs of mice treated (1 hour after infection) with anti-LPS mAbs and infected for 8 hours with *K. pneumoniae* (1×10^4 CFU KP8045). **(C)** Survival of C57BL/6 (WT) and *il17a* (KO) mice prophylactically immunized with c-IgG or KPE33 and infected 24 hours later with *K. pneumoniae* (1×10^4 CFU KP8045) (the *P* value indicates significance between KPE33 WT and KPE33 KO) (*n* = 10 per group). **(D)** Survival of C57BL/6 (WT) and *il17a* (KO) mice prophylactically immunized with c-IgG or 54H7 and infected 24 hours later with *K. pneumoniae* (1×10^4 CFU KP8045) (*n* = 10 per group). Statistical significance ($P < 0.05$) was determined by ANOVA followed by Dunn's test (**A**) or log-rank test (**C**). Data are representative of at least 3 independent experiments (**A** and **B**) or the compilation of at least 2 independent experiments (**C** and **D**). Note that the c-IgC groups are presented in both **C** and **D** for reference. (**A** and **B**) Bars represent median.

As mentioned, activation of key immune pathways is critical for protection against bacterial infection; however, how these pathways contribute to, or synergize with, antimicrobial mAb functionality has been underappreciated. The mAbs being developed for use against *K. pneumoniae* function primarily through an OPK modality, enabling neutrophils to recognize and kill mAb-opsonized bacteria. IL-17, via the epithelium, recruits neutrophils to the lung and polarizes them toward a PMN-I phenotype, making cells more capable of antimicrobial function than the alternative PMN-II cells, and increases the antimicrobial capacity of interstitial macrophages (26, 41, 42). This is made more relevant during infection with a pathogen, such as *K. pneumoniae*, some strains of which, including clinically relevant ST258 isolates, have been reported to exhibit enhanced evasion of neutrophil-mediated clearance (15, 43). In the context of a *K. pneumoniae* infection, the OPK functionality of LPS-specific mAbs synergizes with the antimicrobial effects of IL-17 on neutrophil function.

The first antibodies against these bacteria that progressed into clinical trials were directed toward neutralization of LPS. New approaches for treatment of MDR pathogens are required; however, design of next-generation treatments must take care not to impair host immune function. We utilized two antibodies with similar binding characteristics to *K. pneumoniae* LPS O-antigen to demonstrate that neutralization of LPS early in the course of infection impairs an essential component of the immune response. We also demonstrate that Fc receptor engagement is essential for the optimal function of these antibodies. While our data do not suggest that neutralizing LPS is detrimental to the host, we have found that neutralizing LPS can limit the protective potential of antibodies for use against Gram-negative infections. These findings could influence design, and use, of antibodies targeting Gram-negative pathogens. Treatment of disease, as opposed to prophylactic administration, as well as targeting LPS without inhibiting immune responses to this essential PAMP would reduce unintended effects on beneficial components of the innate immune system.

Methods

Strain information. *K. pneumoniae* strains were purchased from ATCC (KP8045) or IHMA (KP1131115). Single colonies were selected from overnight growth on Luria-Bertani (LB) agar, suspended in LB broth with 10% glycerol, and stored at –80°C. Swabs of *K. pneumoniae* strains were taken from frozen stocks and grown to a lawn overnight on trypticase soy agar plates. Bacteria were removed from the plates, suspended in PBS, pH 7.2, to an $OD_{650\,nm}$ of 0.5, and diluted to the appropriate challenge concentration. All challenge CFU were confirmed by serial dilution.

Pneumonia model. Specific pathogen–free 7- to 8-week-old female C57BL/6J, C3H/HeJ, C3H/HeOuJ, and C57BL/6J IL-17A$^{-/-}$ mice were obtained from Jackson Laboratories. Anti–*K. pneumoniae* LPS antibodies were purified as previously described (44) and prepared fresh from stocks for each experiment. KPE33, KPE33-N297Q, 54H7, or c-IgG were administered (15 mg/kg) in 0.5 ml PBS i.p. at the time described in the text (44). Animals were briefly anesthetized and maintained in 3% isoflurane (Butler Schein Animal Health) with oxygen at 3 l/min and infected intranasally with *K. pneumoniae* in 50 μl PBS. Animals were euthanized with CO_2 at the indicated time points, and lung tissue was collected for analysis. Histopathology was performed as previously described (45). Bacterial load in the lung was determined by plating serial dilutions of lung homogenate on trypticase soy agar. In select experiments, neutrophils were depleted with anti-Ly6G antibody (1A8) delivered i.p. (500 μg/mouse) 24 hours prior to infection as previously described (18). Control mice were given rat IgG2a at the same concentration. Depletion antibodies and control IgG were from Bio X Cell.

Endotoxemia model. Prophylactic i.p. administration of LPS mAbs was performed as described above. Highly purified in-house *Kp* O1 cps- 43816 LPS was diluted in IR Saline (Baxter International) to a final concentration of 200 ng/ml. D-galactosamine (D-gal; Sigma-Aldrich) was prepared by resuspending in IR saline to obtain a final concentration of 120 mg/ml. The reagents were combined in 1:1 ratio and mixed thoroughly, and 200 μl of the D-gal/LPS solution was administered by the i.p. route, resulting in 20 ng of O1 LPS/12 mg D-gal delivered for i.p. challenge. Survival was monitored for 7 days.

OPK and serum bactericidal assay. Assays were performed as described previously, with modifications (20). Log-phase cultures of luminescent *K. pneumoniae* (measured in lux) were combined in 384-well plates with diluted baby rabbit serum (1:4 SBA, 1:40 OPK), DMSO-differentiated HL-60 (for a neutrophil model), and mAbs at concentrations indicated. The ratio of bacteria to HL-60 was held constant at 1:10. Plates were incubated at 37°C for 2 hours with shaking (250 rpm), at which time the RLUs were measured using an Envision Multilabel plate reader (Perkin Elmer). The percentage of killing was determined by comparing RLUs derived from assays with no mAb to RLUs obtained from assays with mAb or c-IgG.

LPS neutralization assays. The RAW264.7 murine macrophage cell line was transfected with a firefly luciferase reporter gene under the control of an NF-κB promoter (RAW264.7-lux). mAbs at the indicated concentrations were mixed with LPS and incubated at 4°C for 1 hour, at with time they were diluted 1:10 into 96-well plates containing preseeded RAW264.7-lux cells. Following a 2.5-hour incubation, Steady Glo solution (Promega) was added to each well, and the RLUs were measured 20 minutes later using an Envision Multilabel plate reader. The percentage of inhibition was determined by comparing RLUs derived from assays without mAb to RLUs from assays with LPS-specific mAbs or c-IgG.

Flow cytometry. Lungs were digested in RPMI-containing liberase (42.4 μg/ml) and DNAse (10 U/ml) for 45 minutes at 37°C, homogenized through a 40-μm filter (Corning Inc.), pelleted (500 *g*, 5 minutes), and washed twice in FACS buffer (PBS with 5% fetal bovine serum, and 0.1% sodium azide). Red blood cells were removed with ACK Lysing Buffer (Life Technologies), Fc receptors were blocked with anti-mouse CD16/CD32 (eBioscience), and cells were stained with antibodies against CD45 (BV711, clone 30-F11), CD3 (APC/Cy6, clone 17A2), TCRγδ (PE/Cy7, clone GL3), and IL-17A (PE, clone TC11-18H10.1) from BioLegend. Cells were imaged using the LSR II Flow Cytometer (BD Biosciences) and analyzed with FlowJo (as shown in Supplemental Figure 7).

Cytokine measurements. Lungs were homogenized and cellular debris was pelleted by centrifugation (500 *g*, 5 minutes). Supernatants were aliquoted and stored at –80°C until assayed. Cytokines were quantified using the V-PLEX Proinflammatory mouse cytokine multiplex kit (Mesoscale Diagnostics) according to the manufacturer's instructions. IL-22 was measured using Luminex beads, and expression was measured using a Luminex 100 multiplex bead reader (Millipore).

Statistics. Data were analyzed using 2-tailed *t* tests, Mann-Whitney tests, ANOVA followed by Dunnett's test, or Kruskal-Wallis followed by Dunn's test. All statistical analyses were performed using Graph-

Pad Prism version 6.0. Histopathological analyses were performed by a pathologist who was blinded to group allocation. $P \leq 0.05$ was considered statistically significant.

Study approval. All animal studies were approved by the MedImmune Institutional Animal Care and Use Committee and were conducted in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility in compliance with US regulations governing the housing and use of animals.

Author contributions

TSC, MP, QW, and CKS designed experiments, analyzed data, and wrote the manuscript. LC performed histological analysis and wrote the manuscript. CC, XX, EC, and DC produced the antibodies. MEP, JB, RC, ES, and PW designed experiments and analyzed data. DC, BRS, and JS wrote the manuscript.

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