TREM-1 Promotes Survival during *Klebsiella pneumoniae* Liver Abscess in Mice

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*Klebsiella pneumoniae* liver abscess (KPLA) is prevalent in East Asia. Liver abscess can develop after translocation of *K. pneumoniae* from a patient’s bowel into the liver via the portal circulation. TREM-1 (triggering receptor expressed on myeloid cells 1) amplifies inflammatory signaling during infection, but its role in KPLA is poorly understood. We used an animal study to characterize the role of TREM-1 in KPLA. We compared survival rates, bacterial burdens in tissues, inflammatory cytokine levels, and histology findings between wild-type and Trem-1 knockout (KO) mice after oral inoculation of capsular type K1 *K. pneumoniae*. Translocation of *K. pneumoniae* to mesenteric lymph nodes and liver was examined, and intestinal permeability, antimicrobial peptide expression, and the clearance of *K. pneumoniae* in the small intestine were determined. In the absence of TREM-1, KPLA model mice showed increased *K. pneumoniae* dissemination, enhanced liver and systemic inflammation, and reduced survival. Impaired bacterial clearance in the small intestine causes enhanced *K. pneumoniae* translocation, which renders Trem-1 KO mice more susceptible to *K. pneumoniae* oral infection. In conclusion, TREM-1-mediated bacterial clearance in the small intestine is an important immune response against *K. pneumoniae*. TREM-1 deficiency enhances *K. pneumoniae* translocation in the small intestine and increases mortality rates in mice with KPLA.

*K. pneumoniae* is responsible for nosocomial and community-acquired infection worldwide (1–5). In the past 3 decades, *K. pneumoniae* has become the dominant cause of pyogenic liver abscesses and has contributed to the endemicity of the disease in Taiwan (6–10). *K. pneumoniae* liver abscess (KPLA) is also responsible for the majority of cases of liver abscess in other Asian countries, especially South Korea (11, 12) and Singapore (13). In addition, many researchers have also noted distinctive metastatic complications of KPLA, especially endophthalmitis and meningitis, which cause significant morbidity and mortality (14–16). Even though there is a relatively lower incidence in Western countries, emerging cases in North America (17) and Europe (18) have been identified in recent years. Capsular serotype K1 of *K. pneumoniae* is thought to be the major virulence determinant responsible for KPLA and this invasive syndrome (7).

Current evidence suggests that gastrointestinal colonization by *K. pneumoniae* predisposes individuals to KPLA (6, 19). Liver abscess may develop after translocation of *K. pneumoniae* from a patient’s bowel into the liver via the portal circulation. The high prevalence of virulent *K. pneumoniae* strains colonizing patients of Asian descent might correspond to the prevalence of KPLA in Asian countries (19, 20). Although the intestine is the major reservoir of *K. pneumoniae*, how intestine-colonizing *K. pneumoniae* cells translocate across the intestinal barrier and gain growth advantages in the liver is poorly understood. The mechanisms by which *K. pneumoniae* translocates from the intestine and causes liver abscesses have not been investigated.

TREM (triggering receptor expressed on myeloid cells) proteins are a recently identified family of cell surface receptors broadly expressed on myeloid cells of human and mouse origins (21). TREM-1 is constitutively expressed on monocytes/macrophages and neutrophils and is an important amplifier of inflammation. Engagement of TREM-1 on the cell surface activates a cascade of intracellular events resulting in inflammatory effects, such as cytokine production, degranulation of neutrophils, and phagocytosis. For downstream signal transduction, TREM-1 is coupled to the immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor DAP12 (DNAX activation protein of 12 kDa) (22). Several in vivo studies have corroborated a role for TREM-1 in bacterial infection and sepsis. Initial findings established TREM-1 as an amplifier of the systemic inflammatory response syndrome associated with sepsis. The apparent detrimental effects of overstimulation through TREM-1 and the survival advantage of modulating TREM-1 signaling have been shown in various murine bacterial infection models (23–29). However, recent findings suggested TREM-1 signaling is necessary for successful antimicrobial responses in sepsis and pneumonia murine models (30, 31), indicating that inappropriate modulation of the TREM-1 pathway could have profound effects on septic patients and may be detrimental.

The in vivo role of TREM-1 in response to various bacterial infection models can be investigated using Trem-1 knockout (KO) mice. In the present study, we aimed to characterize the role of TREM-1 during KPLA using Trem-1 KO mice in an oral infection model.
model of KPLA and to demonstrate how TREM-1 mediates intestinal immunity.

MATERIALS AND METHODS

Animals. C57BL/6 Trem-1 KO mice were originally established in T. W. Mak’s laboratory (Toronto, Ontario, Canada) and were maintained under specific-pathogen-free conditions in the Laboratory Animal Center at National Yang-Ming University following the guidelines of the IACUC. Six- to 10-week-old, age- and sex-matched wild-type (WT) littermates and Trem-1 KO mice were used for all experiments.

Bacterial isolates of K. pneumoniae. K. pneumoniae strain STR-43 (capsular type K1) was isolated from a patient with liver abscesses, as described previously (32), and was used for all experiments involving the oral infection model of liver abscess. The NTUH-K2044 (capsular type K1) strain with a green fluorescent protein (GFP)-expressing plasmid, which conferred kanamycin resistance, was used for the translocation assay.

Experimental Klebsiella pneumoniae liver abscess model. WT mice and Trem-1 KO littermates were starved of food for 16 h before oral inoculation with 20 μl of bacterial suspension containing 5 × 10⁸ CFU of mid-log-phase K. pneumoniae using a 21-gauge feeding needle. Mice were sacrificed at various time points, and mesenteric lymph nodes (MLNs), small intestine, blood, and liver samples were retrieved. Serial dilutions of tissue homogenates were cultured to enumerate bacterial counts. For histological examination, livers and intestines were fixed in neutral 10% formalin solution and processed for paraffin embedding, and 5-μm-thick sections were prepared and stained with hematoxylin and eosin.

Bacterial burden and pathological examination of the liver and intestine. The degree of liver inflammation was determined by a blinded histopathology score, as described previously (33). A score of 1 indicates that the number of microabscesses on each liver section was <10 and that no necrosis region was found. A score of 2 indicates that the number of microabscesses on each liver section was >10 and ≤20 and that no necrosis region was found. A score of 3 indicates that the number of microabscesses on each liver section was >20 and <30 and that no necrosis region was found. A score of 4 indicates that the number of microabscesses on each liver section was >30 and that no necrosis region was found. A score of 5 indicates that the number of necrosis regions was <5. A score of 6 indicates that the number of necrosis regions was >5 and ≤10. A score of 7 indicates that the number of necrosis regions was >10 and ≤15. A score of 8 indicates that the number of necrosis regions was >15. Three different sections from the largest liver lobule of each mouse were examined. The mean score for each group was generated by examination of liver sections from six mice. Histological assessment for intestines was performed using a scoring system as described previously (34). Heparin-containing blood or liver homogenate in phosphate-buffered saline (PBS) was aseptically collected, serially diluted, and then added (0.1 ml) to Mueller-Hinton agar plates and incubated at 37°C overnight. The number of CFU of K. pneumoniae was then quantified.

Translocation of injected Klebsiella pneumoniae to MLNs, blood, and liver. To assess bacterial translocation from gastrointestinal segments, we used a modified intestinal loop model as previously described (35–38). After anesthesia, a midline laparotomy incision was made. A 10-cm-long segment of the gastrointestinal tract (mid- or distal small intestine or colon) was created with two vascular hemoclips without disrupting the mesenteric vascular arcades. The length of intestine between the two clips was injected with an NTUH-K2044 strain carrying a GFP-expressing plasmid (5 × 10⁸ CFU). After 4 h, mice were sacrificed, blood, MLN, and liver samples were collected, and the prepared homogenates were plated onto Mueller-Hinton agar plates (containing 50 μg/ml kanamycin). GFP-expressing CFU were counted after incubation at 37°C for 20 to 24 h using fluorescence microscopy.

Measurement of intestinal permeability. The assay for intestinal permeability was modified from a method described previously (37). Briefly, after animals were anesthetized, two ends of a 10-cm segment of the gastrointestinal tract were clipped. Fifty microliters of fluorescein isothiocyanate (FITC)-dextran (25 mg/ml, molecular weight [MW] 4,400 [Sigma]) was administered into the clipped intestinal segment. Serum was collected 2 h later, diluted 1:19 in buffer (50 mM Tris [pH 10.3], 150 mM NaCl), and then analyzed for FITC-dextran concentration with a fluorescence spectrophotometer at an excitation wavelength of 480 nm and an emission wavelength of 520 nm.

In vivo K. pneumonia killing. The ligated-small intestine loop experiment was modified as previously described (35). A 10-cm-long segment of the distal small intestine was created and was injected with 250 μl phosphate-buffered saline (PBS) containing 1,000 CFU K. pneumoniae. After 3 h, mice were sacrificed, and the lumens of the isolated segment was flushed with 10 ml of PBS to collect the luminal content for plating.

MPO analysis in the intestine mucosa. A mouse myeloperoxidase (MPO) enzyme-linked immunosorbent assay (ELISA) kit (HyClone Biotechnology, Uden, The Netherlands) was used according to the manufacturer’s guidelines to determine the concentration of MPO in intestine mucosal scrapings (39).

RNA isolation and qRT-PCR. Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, CA). Quantitative real-time PCRs (qRT-PCRs) were set up in triplicate with the Power SYBR green master mix (Roche, Germany) and analyzed with the Stratagene Mx3000P real-time PCR system.

ELISA. The concentrations of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) in the serum were determined by using ELISA sets obtained from R&D Systems according to the vendor’s instructions.

Quantification of NETs, immunofluorescence microscopy, and NET-mediated antibacterial activity. Neutrophils were isolated from bone marrow of 7- to 10-week-old female C57BL/6 or Trem-1 KO mice. Bone marrow was flushed out of the tibia and femur in DPBS (Dulbecco’s PBS without Ca²⁺ and Mg²⁺) and homogenized with a 25-gauge needle. Subsequently, cells were passed through a 70-μm cell sieve, overlaid onto a discontinuous gradient of Histopaque 1083 (Sigma-Aldrich, St. Louis, MO), and spun for 30 min at 2,500 rpm with no brake. The pellets were harvested, and red blood cells were lysed using ACK buffer (155 mM NH₄Cl, 10 mM KHCO₃, 127 mM NaCl, and 10 mM MgCl₂) and washed with DPBS. Cells were resuspended in RPMI culture medium and held at 4°C until use. Neutrophils (2 × 10⁶ well) were stimulated with plate-bound control IgG, agonist anti-TREM-1 (R&D Systems, MN) or phorbol 12-myristate 13-acetate (100 nM) for 18 h. Released extracellular DNA (neutrophil extracellular traps [NET]) was digested by adding 500 μM of micrococcal nuclease (New England BioLabs, Inc., MA) for 1 h at 37°C. Total DNA was isolated from naïve neutrophils and quantified using a Picogreen double-stranded DNA (dsDNA) kit (Invitrogen, Life Technologies) according to the manufacturer’s instructions. The percentage of released NET DNA was calculated by dividing the amount of isolated NET DNA by the mean genomic DNA content. The formation of NETs containing extracellular strands of DNA with granule proteins attached was also detected after staining with Hoechst 33258 and immunofluorescence.

Bacterial activity of neutrophils was ascertained by coincubating neutrophils with K. pneumoniae. Neutrophils (2 × 10⁶ per well) stimulated with plate-bound control IgG, agonist anti-TREM-1 (R&D Systems, MN), or PMNs were incubated with K. pneumoniae (1,000 CFU) for 60 min. Cytotoxins (0.1 μg/ml) was added to each well test well 60 min prior to addition of bacteria to inhibit phagocytosis. After incubation, the contents of each well were scraped thoroughly, serially diluted, and then added (0.1 ml) to Mueller-Hinton agar plates, and the mixture was incubated at 37°C overnight. The number of CFU of K. pneumoniae was then quantified.

Statistical analysis. Statistical significance was evaluated by the non-parametric, two-tailed Mann-Whitney U test for analysis of variables not normally distributed. Statistical significance was defined as P < 0.05.
RESULTS

TREM-1 expression is upregulated in liver and intestine and is important for host survival after oral administration of *K. pneumoniae*. To exploit the constitutive and infection-induced expression of TREM-1 within liver and intestine in KPLA *in vivo*, tissue samples were collected from WT mice following oral infection with *K. pneumoniae* and assayed for TREM-1 mRNA levels. TREM-1 expression is markedly enhanced in livers and intestines following oral infection with *K. pneumoniae* (Fig. 1).

We next examined the function of TREM-1 in bacterial liver abscess by inoculating WT and Trem-1 KO mice with $5 \times 10^8$ CFU of *K. pneumoniae*. The survival rate was assessed up to 14 days postinfection. Trem-1 KO mice died significantly earlier, with an overall survival of 28.6% compared with 61.9% of WT mice ($P = 0.026$) (Fig. 2A). KPLA mice develop liver inflammation and injury. To obtain an insight into the role of TREM-1 in KPLA, semi-quantitative analyses were performed on liver sections prepared from WT and Trem-1 KO mice 48 h after oral *K. pneumoniae* administration. Significantly more liver damage (assessed by a higher pathology score) was observed in Trem-1 KO sections than in WT sections (Fig. 2B to D).

**Bacterial translocation is enhanced in Trem-1 KO mice after oral administration of *K. pneumoniae*.** To determine whether reduced survival of Trem-1 KO mice is associated with changes in bacterial loads, *K. pneumoniae* CFU counts were quantified from MLN, liver, and blood samples collected 48 h after oral inoculation while all animals from both groups were alive to avoid survivor bias. The bacterial loads in MLN, liver, and blood were significantly higher in Trem-1 KO mice than those in WT mice (Fig. 3A to C), demonstrating that the increased mortality of KO mice in KPLA is associated with the enhanced translocation of *K. pneumoniae* from the intestine. Of note, few inflammatory cells infiltrate the liver abscesses in WT mice (Fig. 3D and E).

**FIG 1** TREM-1 gene expression in WT mice with KPLA. Upregulation of *Trem-1* mRNA levels in liver at 24 and 48 h ($n = 6$, independent experiment) (A) and in distal small intestine at 48 h ($n = 9$, independent experiment) (B) after *K. pneumoniae* oral infection compared with that in the sham control by qRT-PCR. Data are expressed as means ± standard deviations (SD) for each group. Statistical significance was defined as $P < 0.05$ versus the sham control.

**FIG 2** TREM-1 is important for host survival after oral administration of *K. pneumoniae*. (A) Kaplan-Meier survival plot of Trem-1 KO and WT mice ($n = 21$ per group, 4 independent experiments) following oral administration with *K. pneumoniae* ($P = 0.026$). (B) Trem-1 KO mice have increased histologic evidence of liver abscess and necrosis, as shown by pathological scores following *K. pneumoniae* challenge ($n = 15$ per group, 4 independent experiments; $P = 0.013$). Liver abscess and necrosis were examined by hematoxylin and eosin staining and observed under a microscope (magnification, ×200) in Trem-1 KO (C) and WT (D) mice 48 h after oral *K. pneumoniae* administration.
trating the lamina propria were observed in either group, and there was no significant difference in levels of intestinal cellular infiltration between WT and Trem-1 KO mice (data not shown).

**Trem-1 KO mice show increased local and systemic cytokine production following K. pneumoniae oral infection.** To obtain further insight into the impact of TREM-1 on liver inflammation during KPLA, the hepatic expression of proinflammatory cytokines TNF-α, IL-1β, and IL-6 was measured by qRT-PCR. Unexpectedly, the expression of all examined cytokines in the liver was significantly higher in Trem-1 KO mice than in WT mice (Fig. 4A to C). Inflammatory cytokine protein levels in serum during infection were analyzed 48 h after bacterial challenge, and significantly higher levels of IL-6 were observed in Trem-1 KO sera compared with WT samples ($P = 0.026$) (Fig. 4D). In addition, a trend toward increased TNF-α and IL-1β levels in Trem-1 KO mice sera can also be found, although the increase was not statistically significant (Fig. 4E and F).

**TREM-1 is essential for preventing translocation of K. pneumoniae in the intestine.** To directly assess the translocation of *K. pneumoniae* across the intestinal barrier, we measured the CFU of GFP-expressing bacteria in the MLNs, liver, and blood (indicative of transmigrated bacteria) after injecting GFP-expressing *K. pneumoniae* cells into the ligated intestinal loop in vivo. The highest *K. pneumoniae* translocation was previously observed in the distal small intestine in WT mice in our pilot examination. Therefore, we injected GFP-expressing *K. pneumoniae* cells into the loops of the distal small intestine of WT and Trem-1 KO mice and found that the numbers of transmigrated bacteria from intestine to MLNs, blood and liver are significantly higher in Trem-1 KO mice than those in WT mice (Fig. 5), implicating that TREM-1 is crucially involved in the prevention of *K. pneumoniae* intestinal translocation.

**Diminished clearance of K. pneumoniae in the small intestine of Trem-1 KO mice.** Several mechanisms (such as compromised antimicrobial host defense, increased intestinal permeability, or reduced intestinal inflammation) could lead to the enhancement of *K. pneumoniae* translocation in KPLA Trem-1 KO mice. First, we investigated in vivo luminal killing of *K. pneumoniae* in the intestinal loops of WT and Trem-1 KO mice and found that *K. pneumoniae* cells were killed more effectively in WT intestinal loops than in Trem-1 KO ones ($P = 0.038$) (Fig. 6). However, the levels of expression of antimicrobial peptides in the collected intestinal loops 4 h after *K. pneumoniae* injection were comparable in the intestinal loops of WT and Trem-1 KO mice.
(Fig. 7A), excluding the possibility that TREM-1 contributes to the production of antimicrobial peptides that limit K. pneumoniae growth in KPLA.

We further examined the role of TREM-1 in intestinal barrier regulation. Intestinal permeability was assessed by injecting FITC-dextran into K. pneumoniae-injected intestinal loops in mice. No significant difference was observed between WT and Trem-1 KO mice (70 ± 17 and 56 ± 20 fluorescence units per microliter of serum, respectively; n = 3 per group; P = 0.406) 2 h after FITC-dextran injection. Furthermore, a similar conclusion can be made from the analyses of mRNA expression of tight junction proteins, such as occludin, claudin-1, claudin-4 and ZO-1, in the intestine loops. In accordance with our previous cytokine analyses in oral infection models, no differences in TNF-α, IL-6, IL-1β, IL-22, and IL-23 mRNA levels in the small intestines from WT and Trem-1 KO mice were determined (data not shown).

TREM-1 triggers NET formation in neutrophils. Neutrophils, with high levels of TREM-1, play critical bactericidal roles in the first line of immune protection. Whether the reduction of K. pneumoniae clearance in Trem-1 KO mice is associated with a decrease of neutrophil infiltration was further investigated by MPO measurements of small intestinal mucosal scrapings. The levels of MPO in Trem-1 KO intestinal mucosa (7,731 ± 2,574 pg/mg protein) and WT samples (6,384 ± 2,863 pg/mg protein) (P = 0.7) were comparable, indicating that Trem-1 plays only a minor role in neutrophil recruitment into intestinal mucosa.

It was recently demonstrated that neutrophil extracellular trap (NET) formation is mechanistically linked to improved K. pneumoniae killing in a murine pneumonia model (40). WT neutrophils produce NET in response to agonist TREM-1 antibody stimulation in vitro, which is abolished in Trem-1 KO cells (Fig. 8A), suggesting a positive role of TREM-1 in mediating NET formation, which is important for local trapping of invading K. pneumoniae in the intestines of KPLA mice. The TREM-1-mediated NET DNA bactericidal effect in vitro is shown in Fig. 8B. The percentage of K. pneumoniae survival after incubation with PMA-stimulated WT neutrophils was 72.4% (P = 0.036), and it was 85.7% in anti-TREM-1 antibody-pretreated group (P = 0.036).

**DISCUSSION**

KPLA is thought to develop after translocation of K. pneumoniae from a patient’s bowel into the liver via the portal circulation. In the present study, we demonstrate that TREM-1 plays a novel role in modulating host mucosal immunity in the small intestine in the KPLA model. Mice deficient for TREM-1 show increased K. pneumoniae dissemination, enhanced liver and systemic inflammation, and reduced survival. The diminished capacity to kill K. pneumoniae in the lumen of the distal small intestine in Trem-1 KO mice suggests an ineffective bacterial clearance resulting in increased bacterial translocation.

Bacterial translocation describes a phenomenon in which live bacteria or their products cross the intestinal barrier, which normally plays a pivotal role in protection against systemic dispersion of luminal bacteria (commensals or pathogens) and antigenic molecules (41). Previously, the lymphatic route was suggested to be the principal pathway for bacterial compounds to access the systemic circulation (41). Therefore, the identification of intestinal bacteria in normally sterile MLNs is considered direct evidence of bacterial translocation. The culture techniques used in previous studies could not directly demonstrate that the transmigrated bacteria actually derive from intestinal flora (41). In order to confirm that capsular type K1 K. pneumoniae can translocate to extraintestinal tissues, we inoculated mice with a GFP-expressing K. pneu-

**FIG 5** TREM-1 is essential to prevent K. pneumoniae translocation from the intestine. Translocation of GFP-expressing K. pneumoniae from intestinal loops to MLNs (A), liver (B), and blood (C) is significantly increased in Trem-1 mice compared with WT KO mice (n = 9 each, 2 independent experiments).

**FIG 6** Diminished clearance of K. pneumoniae in the small intestine of Trem-1 KO mice. K. pneumoniae cells (1,000 CFU/mouse) were injected into intestinal loops of WT and Trem-1 KO mice. Sections of the intestine were harvested 2 h after injection, and CFU were determined (n = 5 each, 3 independent experiments).
moniae strain in the small intestine loop. Bacterial CFU were easily visualized and counted using fluorescence microscopy. The translocation of GFP-expressing K. pneumoniae from the gut to MLNs, liver and blood, represents a pathophysiological pathway for the development of liver abscess.

We demonstrate that the increased susceptibility of Trem-1 KO mice to K. pneumoniae oral infection is due to enhanced K. pneumoniae translocation in the small intestine, which is further supported by the intestinal loop K. pneumoniae injection results. It has been suggested that intestinal inflammation mediated by inflamed cells in the lamina propria is involved in upregulation of intestinal permeability leading to enhanced translocation of bacteria or their products (38). Besides, compromised antimicrobial peptide production has also been reported to predispose hosts to bacterial translocation in experimental cirrhosis (42). In addition, epithelial openings exploited by invasive pathogens could also facilitate their migration across the epithelium to initiate disease (43). However, our data show that the overflow of K. pneumoniae from the intestine in Trem-1 KO mice is not simply a result of compromised antimicrobial peptide production or imbalanced intestinal permeability and inflammation. The intestinal bacterial trapping/killing contributed by TREM-1 might be the most crucial step to control K. pneumoniae translocation. NETs are composed of chromatin and antimicrobial cytoplasmic and granular proteins, such as elastase and catalase. Exuded NETs form a fibrillar matrix that entraps invading pathogens and brings them into the proximity of antimicrobial proteins, thus facilitating killing. It has recently been demonstrated that NETosis is an important aspect of innate immunity against K. pneumoniae (40). Our study further suggests that the production of NETs induced by infection can be modulated by TREM-1.

Previous animal studies of TREM-1 blockade suggested that modulation of the TREM-1 pathway can decrease inflammation without sacrificing bacterial control upon infection (23–29). We
initially hypothesized that TREM-1 deficiency would decrease inflammation and improve survival in a murine KPLA model. However, we found that inflammatory cytokine production is not impaired during infection, despite the loss of the TREM-1-amplifying pathway. The explanation is as follows. It is notable that WT mice have approximately 100-fold fewer bacteria in the liver in KPLA, providing a less potent proinflammatory stimulus. Therefore, while high bacterial burdens originating from bacterial translocation are present, the bacterial load drives the extent of mediator production in the livers, overruling the possible amplification of inflammation by TREM-1. Consequently, depletion of TREM-1 results in increased mortality due to the marked bacterial translocation. Correspondingly, Klesney-Tait et al. recently developed a Trem-1 KO mouse model in an intestinal infection to demonstrate that KO mice more susceptible to KPLA. This is the first study to demonstrate TREM-1-mediated intestinal immunity in which the absence of TREM-1 and Trem-3 markedly increased mortality following Pseudomonas aeruginosa challenge (44). Their results suggested that impaired neutrophil transepithelial migration is the mechanism underlying the failure to clear lung bacteria, resulting in increased mortality in the absence of TREM-1. In contrast, our study is the first to use a Trem-1 single KO mouse model in an intestinal infection to demonstrate that TREM-1 signaling is essential for host defense against bacterial infection. Thus, when developing potential therapeutic agents to modulate TREM-1 signaling, such as TREM-1 inhibitors, it is important to achieve a balance between pathogen control and host tissue damage.

Tu et al. first described the oral infection model of KPLA and demonstrated how the intestine-colonizing K. pneumoniae bacteria could acquire an ability to translocate across the intestinal barrier, disseminate systematically, and finally gain growth advantages within specific niches in the liver (45). The oral infection model of KPLA was regarded as the most suitable animal model in the subsequent studies (33, 46, 47). Of note, a high dose of K. pneumoniae has to be used to establish the colonization and subsequent bacterial translocation in the mouse model (45). In contrast, humans can be colonized asymptomatically with K. pneumoniae, and many other factors can predispose to the overgrowth of colonized K. pneumoniae, such as diabetes and antibiotic use (33). Despite the fact that this model may not fully recapitulate the human situation, by using the Trem-1 KO mice, we provide evidence demonstrating the involvement of Trem-1 in host defense against the translocation of K. pneumoniae in the intestine.

The most critical molecular mechanism contributed by TREM-1 remains to be clarified. It will be of great significance to further identify the missing pieces in the mystery of TREM-1-mediated signaling, such as the recent finding that Btk as a positive regulator in the ITAM-mediated Trem-1/DAP12 pathway (48). Further study of the isolated inflammatory cells from the affected small intestine will be informative to elucidate TREM-1 signaling in the small intestine.

In conclusion, our results implicate Trem-1 as a novel player in effective host mucosal immunity in the small intestine in KPLA. In the absence of Trem-1, KPLA mice demonstrate increased bacterial dissemination, enhanced liver and systemic inflammation, and reduced survival. Impaired bacterial clearance in the small intestine leading to enhanced K. pneumoniae translocation renders Trem-1 KO mice more susceptible to KPLA. This is the first study to demonstrate TREM-1-mediated intestinal immunity in an infectious disease model. Further study of the role of TREM-1 molecules in intestinal immunity may provide important information for future development of therapeutic approaches to enhance mucosal resistance to bacterial pathogens.

ACKNOWLEDGMENTS

This work was supported by grants to C.-P. F. from National Science Council (NSC 100-2314-B-010-017-MY3), grants to N.-J. C. from the National Science Council (NSC97-2320-B-010-032-MY2 and NSC99-2320-B-103-MY3), Taipei Veterans General Hospital (V97S5-001, V98S5-008, V99S5-002, and V100E4-003), and the Yen Tjing Ling Medical Foundation (CI-100-116) and a grant (98A-C-D117) from the Ministry of Education Aim for the Top University Plan in Taiwan.

We are indebted to Jin-Town Wang, National Taiwan University College of Medicine, Taiwan, for kind help in providing NTUH-K2044 with a GFP-expressing plasmid strain. We are also deeply grateful to Tak W. Mak for providing Trem-1 KO mice.

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