IMMUNOMODULATION OF INFLAMMATION IN A MURINE PNEUMOCOCCAL SEPSIS MODEL

By

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SUMMARY OF THE STUDY

Mortality from pneumococcal infections remains high, despite the development of potent antibiotics. Antibiotic resistance among pathogens including *S. pneumoniae* calls for new therapeutics. Immunomodulation represents a novel approach to antimicrobial therapy that depends on bolstering host immunity, rather than direct antimicrobial activity. The use of innate immune stimulation to improve survival has previously been described for Gram negative pathogens.

The effect of TLR4 stimulation on survival in mice during lethal *S. pneumoniae*, serotype 2 infections was examined. C57BL/6, BALB/c, CBA/CaHN-Btk<sup>+</sup>/J, A/J, Rag-1 KO, IL-10 KO, C3H/HeN and C3H/HeJ mice were inoculated intravenously with a lethal dose of 10<sup>9</sup> cfu of *S. pneumoniae* serotype 2 48 hours after treatment with five doses of 10ug highly purified LPS or vehicle (PBS) at 12 hours interval. Another group of LPS or PBS treated C57BL/6 received 25 mg/kg ceftriaxone at 6 hours post infection. Survival was monitored for 5 days. Blood samples were collected at different time points (6h, 12h and 24h) after bacterial challenge for bacteriological examinations, serum cytokine measurements, and biochemical assays for liver function. Spleens were harvested for flow cytometric analysis of splenic lymphocytes or NK activation.

Innate stimulation with LPS reduced systemic bacteremia by at least four logs in LPS-pretreated C57BL/6 (10<sup>4</sup> v.s 10<sup>8</sup> cfu/ml) mice compared with controls during the recorded course of infection. Death in experimental controls occurred within 48 hours. Reduced bacteremia corresponded with improved survival in all 3 strains. Survival for LPS-treated C57BL/6, Balb/c and C3H/HeN was 90% (N=29; p=0.001), 50% (N=14; p = 0.017) and 60% (N=8; p=0.009), respectively, and mortality for controls was 100% for all the strains. Mortality for ceftriaxone-treated C57BL/6 was
Systemic Streptococcus pneumoniae was investigated. Mice were inoculated with LPS, resulting in a lethal meningitis model or sepsis. The effect of LPS pretreatment as adjuvantive therapy in an experimental model of sepsis may be advantageous.

Inflammatory injury associated with sepsis, by simulating early host defense and mucocidal clearance, but not the latter phases of adjuvantive therapies with antibiotics. It is plausible that compounds capable of have implications for prophylactic treatment after an index case is identified or as a strategy to avoid sepsis. These observations provide short-term resistance to infectious challenges such as might occur in the virtual strain of *S. pneumoniae*. IL-4 receptor activity can be potentially exploited to provide evidence that induction of pro-inflammatory cytokines and IL-10, from TLR4 stimulation, prior to infection with *S. pneumoniae*. This study demonstrates a survival benefit from TLR4 stimulation prior to infection with *S. pneumoniae*. LPS pretreatment in CD69 expression of 3.2 vs. 3.8 (p<0.05) than of control inoculated mice, and decreases the adhesion demonstrated by lower mean percentage of CD69 by TLR4 pretreatment. LPS pretreatment restores the splenic NK population in 3.2 vs. 4.5, p<0.025) and ALT [27 vs. 129, p<0.03] to near baseline induced AST [139 vs. 149, p<0.03] and IL-10 [17 vs. 45] and IL-6 levels (reduced the prevented hypoglycemia, glucose level was 149 vs. 45) and IL-10 levels. Pretreatment with a low dose of LPS also reduced AST levels. MCP-1 and TNF-α in CD68 and CD11b were attenuated in C57BL/6/LPS-treated mice 24 hours after infection. The level of TNF-α, IL-12 (p<0.05) and IL-6 in BALB/c mice, 10 hours after infection, suggests that T cells are responsible for protection and B cells are partially involved in survival benefits of LPS. These results suggest that T cells are responsible for protection and B cells are partially involved in survival benefits of LPS. The results in C57BL/6/LPS-treated mice 24 hours after infection. The level of TNF-α, IL-12 (p<0.05) and IL-6 in BALB/c mice, 10 hours after infection, suggests that T cells are responsible for protection and B cells are partially involved in survival benefits of LPS. The results in C57BL/6/LPS-treated mice 24 hours after infection. The level of TNF-α, IL-12 (p<0.05) and IL-6 in BALB/c mice, 10 hours after infection, suggests that T cells are responsible for protection and B cells are partially involved in survival benefits of LPS.
has strong immunosuppressive and anti-inflammatory effects mediated by AhR
such as interleukin-10, peptidoglycan, and bacterial lipopolysaccharide.
Inflammation as a result of the release of proinflammatory bacterial cell components,
bacteria-well, \( P \)-lytes and antibodies can result in the peroxidase enhancement of
bacterial infection - such as meningitis, pneumonia, and
The treatment of pneumococcal infections - such as meningitis, pneumonia, and
Infections and these results warrant further studies.
Intravenous stimulation is an effective therapy in the treatment of pneumococcal
mortality in severe S. pneumoniae infections. These results show the potential for
therapy to the antibiotic Ceftriaxone achieved a survival benefit in the reduction of
In this mouse model, stimulation of TLR4 by high-yield LPS and an additional
Ceftriaxone alone (\( p \leq 0.05 \)).
KC were elevated 12h after bacterial challenge compared to those treated with
and RANTES 12h after bacterial challenge and elevated levels of IL-2, IL-3, IL-4, and
significantly the level of TNF- \( \alpha \)-beta, TNF- \( \alpha \)-gamma, IL-12/20, MIP-1 \( \alpha \), and IL-1 beta.
Injection, compared with animals treated with ceftriaxone alone (AUC, \( p \leq 0.001 \)) after
CFU/ml, \( p \leq 0.002 \) and 12h (0.5 \( \leq 1 \)) and 4.9 \( \leq 4.9 \), for 1.9 \( \leq 1.9 \), 10 \( \leq 10 \), for 1.9 \( \leq 1.9 \), 10 \( \leq 10 \), for 1.9 \( \leq 1.9 \),
resulted in a significant reduction of bacterial levels at 72h, 6h vs. 6h for 1.9 \( \leq 1.9 \), 10 \( \leq 10 \), for 1.9 \( \leq 1.9 \), 10 \( \leq 10 \), for 1.9 \( \leq 1.9 \),
challenge (\( n=5 \) (\( p \leq 0.07 \)) . Administration of LPS in combination with ceftriaxone
mortality were observed when LPS alone was administered in the bacterial
challenge with 50% in C57BL/6 mice (u=7 (\( p \leq 0.03 \)) and no survival benefits (100%
Treatment with a non-lethal dose of LPS beginning 2 hours after infection reduced
receiving ceftriaxone and only (\( n=20 \) ) and 0% of vehicle controls (\( n=10 \) (\( p \leq 0.001 \))).
ceftriaxone and LPS was 80% in C57BL/6 (\( n=20 \)), compared to 40% of mice
injection of ceftriaxone alone. The survival of mice treated with the combination of
with a combination of 25 mg/kg Ceftriaxone (i.p.) and 10mg of LPS (i.v) at 6 hours post
intravenously with a lethal dose of 10^7 cfu of S. pneumoniae serotype 2 and treated

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Those treated with ceftriaxone alone (p<0.05).

IL-5 (p=0.014) and Eotaxin (p=0.023) at 12 h after bacterial challenge compared to
12p70 (p=0.001), IL-10 (p=0.027), IL-6 (p=0.010), IFN-gamma (p=0.001), IL-6 (p=0.010), and TNF-alpha (p=0.001). A decrease level of TNF-alpha (p=0.010) in IL-6 (p=0.001), and also
respectively, than those of mice treated with ceftriaxone alone (p=0.05). and also
reduced bacterial by 2.1 log10 and 3.3 log10 lower bacteria at 1=2 h and 24 h
reduced bacterial in C57BL/6 mice (89%, p=0.001). Treatment with ATL313 compared to ATL 313
reduced treatment in Aβ42 AR KO and chimeric mice (77%, N=6/group) compared to ATL 313
reversed after treatment without ATL313 mice compared to ceftriaxone alone treated mice (23%, n=6/group). Survival benefit was
increase of ceftriaxone at 6th after infection. 25ug/kg ATL313 increased survival (69%, n=26) of
without antibiotic ceftriaxone. But when administered in combination with antibiotic
ATL 313 (2.5 25ug/kg), had no survival benefit (100% mortality) when administered
bacterial counts and cytokine measurements.

for 7 days Blood samples were collected at 7 h and 12 h after bacterial challenge for
antibodies IgG or the adenosine A2a receptor (A2aR). Survival was monitored
of C57BL/6 mice were co-infected intraperitoneally with ATL313 and ZM215
of A2aR, 25 mice (5/group) were subjected to intraperitoneal injection of 25ug/kg
CoF (10 CFU) of S. pneumoniae and treated with the A2aR agonist ATL313 (25ug/kg) or
Female C57BL/6 mice were inoculated intravenously with 107 colony forming units
Straphtococcus pneumoniae.

agonist ATL313 as adjuvant therapy in a lethal C57BL/6 mouse model of systemic
receptor (A2aR) expressed on immune cells. We investigated the effect of A2aR
ALT313 or ceftriaxone alone. To assess the importance of NK cells in pneumococcal pneumonia, serotype 2 and treated with a combination of ceftriaxone and ZF/kg treatment. In this study, mice were inoculated intravenously (i/v) with a lethal dose (10^6 CFU) of S. pneumoniae. The ability of an A2a AR agonist to modulate the NK response to improve sepsis has not been tested, although data is scanty and controversial.

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needed. To investigate the role of decreased NK cell activation by A2AAR treatment is not known and an improved understanding of NK cell function during sepsis is molecular and functional mechanism by which the negative impact of NK cells occurs compartment during the course of severe bacterial infections. However, the precise interactions that occur between NK cells and the monocytes of macrophages analysis of NK cell activation during sepsis may yield insights into the A2L313 on NK cell function during sepsis is needed. These results suggest that cell activation. A further study to improve understanding on the negative impact of cytokine levels more substantially by targeting an upstream event in the cascade (NK cells) produces in the treatment of pneumococcal infection. It is possible to reduce promise in the treatment of pneumococcal infection. It is possible to reduce therapeutic agents that inhibit the activity of NK and NKT cells may therefore hold early even in the inflammatory cascade is inhibited by A2AAR activation. regulation by A2AAR activation. Furthermore, profound protection is imparted when this study implicates NK cells as one of the mediator of inflammation sensitive to certificates alone (p=0.01) in pneumococcal animal models. The results of the antibiotic resulted in 80% survival compared with 40% of mice that received antibiotics (p=0.02). Blockade of NK cell activation with the treatment with Pk136 and reduced the level of interleukin and plasma IFN-γ (0.5% compared to 12.1%) (p=0.011). Reduced the release of perforin (3.4% vs 0.21%) (p<0.05) and also regulated C669 expression, mean percentage 32.7 ± 6.227 vs 70.03 ± 8.163 (p=0.027). showed a high level of NK cell population (2.64% ± 0.277). certificates (n=4) showed a high level of NK cell population (2.64% ± 0.277). monitored for seven days. Splenocytes were harvested and processed to assess NK cell activation (expression of markers CD69 and NK1.1) perforin and interleukin gamma (using flow cytometry). Treatment with the combination of A2L and NK-1 (PK136) antibodies 2 days before bacterial challenge. Survival was anti-NK-1 (PK136) antibodies 2 days before bacterial challenge. Survival was CK7BL/6 and jackson mice were treated with a single i.p. injection of 200 µg.
current wave of enthusiasm regarding the treatment of patients with anti-TNF.

alpha provides survival benefits when combined with cetuximab. Moreover, the
antibacterial host defense and late neutrophilization of the same endogenous TNF-
In conclusion, this study indicates that early TNF-alpha is a critical component of
compared with cetuximab-treated mice (p<0.05).

interleukin-6 (IL-6) (255 v/2806 pg/ml), were reduced in Enercept-treated mice
cytokines, TNF-alpha (78 v/5801 pg/ml), IFN-gamma (604 v/2642 pg/ml),
25% (n=120) survival of cetuximab alone treated mice (p=0.01). Enercept therapy
significantly improved bacterial clearance and survival (70%, n=10) compared with
after bacterial challenge. Survival was monitored for several days. Serum samples
from mice were analyzed for immunoreactivity cytokines with bead-based multi-analyte
after bacterial challenge. Survival was monitored for several days. Serum samples
then treated with 25mg/kg cetuximab with/without 100ug of Enercept (i.v.) at 4 hours
inoculated intravenously with a lethal dose (100µl) of S. pneumoniae serotype 2 and
larger since it appears early and is related to disease severity. Bacterial mice were
adjunctive therapy against systemic S. pneumoniae. TNF is a major therapeutic
our study of Enercept (a tumor necrosis factor alpha neutralizing agent) as an
the release of proinflammatory cytokine components after antibacterial treatment prompted
Growing antibiotic resistance and periocular enhancement of lethality as a result of

in the control immune system when it runs away in sepsis.

understanding gained in this process will potentially provide tools with which we may
whether organ damage corresponds to areas where NK cells traffic. Furthermore, the
sepsis and where and to what extent they proliferate. This will permit clarity on
pathophysiology of sepsis and determine where NK cells traffic during experimental
A 5% AR against on NK cell trafficking during sepsis. The study how NK cells impact the
in the improved outcomes from experimental sepsis. We need to study the impact of

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Immunomodulation and antiinflammatory agents in infections.

model of systemic S. pneumoniae infection in order to assess therapeutic benefits of would affect progression of pneumococcal infection, making use of a lethal murine infections. Thus, it is important to study how immunomodulation of inflammation an important adjuvant role in the management of patients with severe bacterial manipulation of the immune system with cytokine, anti-cytokine strategies may play immunosuppressant regimens and the emergence of resistant bacterial strains. As the treatment of infections becomes increasingly complicated by aggressive pneumococcal infections in a clinical set-up.

benefit of enercept as an adjuvant agent to antibiotics in the treatment or Therefore, more investigations are warranted to further assess the therapeutic pneumococcal infection and underlines the need for further research in the field. outlines the potential usefulness of enercept as an adjuvantive therapy for Infections complications that may occur as a result of this specific therapy. The study antibodies or soluble receptors must be tempered by the awareness of potential