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Short communication

Interleukin-17 in experimental *Klebsiella* sepsis

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Abstract

Background and Purpose: *Interleukin-17 (IL-17) is a cytokine that was described to be important in pathogenesis of several inflammatory conditions. Literature data suggest that modulation of IL-17 production or its biological effects may be beneficial in order to control the level of inflammation. However, data regarding its possible role in the pathogenesis of systemic infections are sparse.*

Materials and Methods: *Therefore, we decided to examine the kinetics of systemic IL-17 production in our model of experimental murine Klebsiella sepsis. We also examined IL-17 production in mice treated with anti-lipopolysaccharide (anti-LPS) antibody prior to infection in order to see whether this pre-treatment, that was previously shown to significantly enhance the survival, is associated with changes in IL-17 production pattern.*

Results: *Our results show that experimental Klebsiella sepsis is associated with an increased systemic IL-17 production. The increase in production is much greater in saline pre-treated group compared with anti-LPS pre-treated animals.*

Conclusions: *We concluded that high levels of IL-17 in the blood may contribute the mortality in mice with systemic Klebsiella infection. We also concluded that diminished IL-17 production in anti-LPS pre-treated animals is, at least partially, responsible for greater survival in this group.*

INTRODUCTION

IL-17 was declared to be proinflammatory cytokine. It is produced by activated T cells, mainly the Th17 subset of $CD4^+$ T cells. It was described to be important in pathogenesis of different chronic inflammatory conditions and a promising therapeutic target for the control of these conditions *(1, 2)*. In addition, IL-17 was also described to be important in the pathogenesis of different bacterial infections including pneumonia, gastric, intestinal and intraperitoneal infections *(3–7)*, as well as in protection against several fungal infections including *Candida albicans* and *Cryptococcus neoformans* systemic infections *(8–10)*. However, only few literature data have reported the involvement of IL-17 in bacterial sepsis with its possible role as a regulator of the host response to bacterial sepsis *(11–14)*.

We have previously described antilipopolysaccharide (anti-LPS) monoclonal antibody Ru-O1 (MAb Ru-O1) that expressed protective properties in a model of lethal *Klebsiella* sepsis *(15)*. In order to elucidate possible mechanisms of this protective effect we have previously investigated the kinetics of the production of several pro- and antiinflammatory cytokines that were described to be important mediators of inflammation during systemic infection *(16, 17)*. We found that the

survival of animals pre-treated with MAb Ru-O1 was associated with decreased production of proinflammatory cytokines (IL-1β, IL-6, IL-12, TNF-α and IFN-γ) and increased production of antiinflammatory cytokine IL-10, twenty four hours after the infection compared to non pre-treated infected mice. Since IL-17 was recently described to be important mediator of inflammatory response during several systemic infections, we hypothesized that this could also be the case in our model of severe *Klebsiella* sepsis in mice. We decided to monitor the kinetics of IL-17 production in pre-treated and non pre-treated mice during the first 24 hours after the infection, since non pre-treated mice begun to succumb the infection after that time point.

MATERIALS AND METHODS

Animals

Male BALB/c mice eight to ten weeks old weighing 20 to 25 g each, were obtained from the Facility for Laboratory Animals of the School of Medicine, University of Rijeka. Mice were housed in specific pathogen-free conditions, kept in plastic cages and given standard laboratory food (Standard pellets, Faculty of Biotechnology, Domžale, Slovenia) and water ad libitum.

Bacteria and experimental infection

Highly virulent strain of *K. pneumoniae* Caroli (O1:K2) was used in our experiments. Experimental infection was performed according to the procedures that were described previously *(15)*. Mice were injected intraperitoneally with 50 CFU of bacteria. They were separated in 2 groups (N=6 in each group). The first group received 1 mg of Ru-O1 monoclonal antibody per mouse (pre-treated group) and the second group received saline 4 hours prior to infection (non pre-treated group). Animals were sacrificed at different time points and the blood was collected. Plasma samples were separated and stored at –20°C until testing.

ELISA

ELISA kit for mouse IL-17 determination was purchased from Bender MedSystems (Austria). Assay was preformed following the manufacturer's protocol. The overall intrassay reproducibility, expressed by coefficient of variation was declared to be less than 10%. The results are presented as median values with associated interquartile range.

Statistical analysis

Statistical significance of the difference between cytokine concentrations of non pre-treated, pre-treated, and uninfected control groups were determined by Mann-Whitney test using MedCalc Statistical Software, version 9.2.1.0 (MedCalc Software, Belgium). Differences were considered statistically significant at $p<0,05$.

RESULTS

IL-17 concentration two hours after the infection in both groups (non pre-treated and pre-treated) remained within the level of uninfected control animals (Figure 1). After 6 hours the plasma concentration in non pre-treated mice increases to the level of 766 pg/mL while in pre-treated animals remained almost unchanged. The concentration in non pre-treated animals slightly decreases 12 hours post infection and finally increases again and reaches final value of 755 pg/mL after 24 hours. In the pre-treated group the IL-17 concentration remained almost unchanged until 24 hours post infection when it increases to the level of 535 pg/mL. IL-17 level in this group reaches its maximum 48 hours after the infection and than slightly begun to decrease (data not shown). The difference in IL-17 concentration between two experimental groups was greatest 6 hours post infection when the concentration in non pre-treated group was more then three times higher compared to the pre-treated group. Differences in plasma IL-17 concentrations between non pre-treated and pre-treated mice were statistically significant for time points 6, 12 and 24 hours post infection ($p=0.0022$; 0.0152; and 0.0152 respectively). Differences between non pretreated and uninfected animals were statistically significant at 6, 12 and 24 hours ($p=0.0022$ for all time points). Statistically significant difference was also noticed between pre-treated group and uninfected animals 24 hours after the infection ($p=0.0043$).

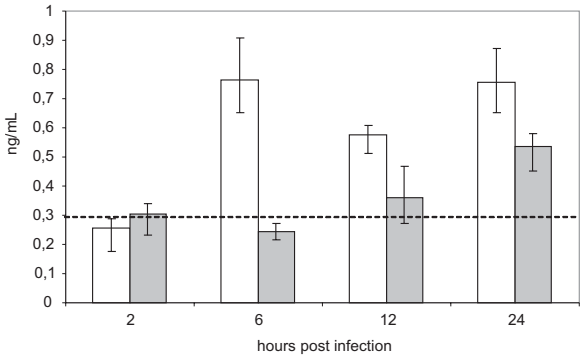

Figure 1. *Kinetics of IL-17 production in non pre-treated (white bars) and pre-treated mice (grey bars). Data were presented as plasma IL-17 concentration in ng/mL. Error bars represent the interquartile range. Dashed line represents median value of IL-17 concentration in uninfected control mice (0,291 ng/mL).*

DISCUSSION

Morbidity and mortality due to bacterial sepsis still remains important medical problem, especially in hospital environments. Diagnostic and therapeutic options are usually limited since the condition is often rapidly evolving and is frequently caused by resistant bacterial strains. Therefore, studies of pathogenesis are valuable source of information that can help us to get better insight into the natural course of infection. These information may be

useful in order to enable earlier recognition of the developing, life threatening, pathologic condition and/or to create alternative immunomodulatory protocols for the prevention and treatment. IL-17 was described to be important in various inflammatory processes. Different diseases like rheumatoid arthritis, asthma and some other inflammatory disorders are accompanied with changes in IL-17 production *(18, 19)*. On the other hand, several recent reports described its involvement in the pathogenesis of different infections *(3–10)*. Although IL-17 was first described in 1993 *(20),* only few recent articles reported its possibly important role in the pathogenesis of sepsis. We found that surprising because of its described proinflammatory and immunomodulatory properties. Besides that, IL-17 receptors (IL-17R) are ubiquitously expressed in a variety of tissues and organs suggesting the likelihood of its systemic effects *(21)*. Since the sepsis is a condition characterized by a systemic inflammation and multi-organ involvement we reasonably hypothesized that IL-17 should be considered important in pathogenetic events during sepsis. Our results have shown to be concordant with our hypothesis since the production of IL-17 started to increase soon after the intraperitoneal infection. It acted as other pro-inflammatory cytokines we studied before in our experimental model, with significant increase in the production during the first 24 hours of infection *(16, 17)*. Interestingly, the pattern of its dynamics resembles the kinetic of TNF-α. Both cytokines expressed biphasic pattern of production with the first peak after 6 and the second after 24 hours of infection according to our experimental design. We believe that this synchronized mode of the production between two cytokines may be important feature of the developing systemic inflammation during sepsis. Namely, several recent articles described close functional and synergistic cooperation between these cytokines. One of these studies reported that this synergy resulted in increased production of IL-6 *(22)*. This finding is comparable with results of our previous study in a model of mouse *Klebsiella* sepsis. We found a dramatic increase in the production of IL-6 24 hours after the infection. Results of another study reported that this synergism resulted in tissue destruction *(23)*. Such tissue destruction in multiple organs may be, at least partially, responsible for multiple organ failure that appears during late stages of sepsis.

LPS is known to be important pathogenetic factor in the development of sepsis. Anti-LPS monoclonal antibody administration results in decreased pro-inflammatory cytokines and increased IL-10 production *(16, 17)*. We concluded that such cytokine production pattern in pre-treated animals contributed their survival after systemic infection. LPS was also described to be a strong inducer of IL-17 production *(24, 25)*. The administration of anti-LPS MAb, in our experimental model may also exert its beneficial effect by diminishing LPS stimulation of IL-17 production, consequently resulting in the survival of anti-LPS pre-treated animals.

Taken together, we believe that our results demonstrate the important role of IL-17 in a model of severe *Klebsiella* sepsis. Since our results are descriptive it is necessary to conduct much more detailed future research using IL-17 receptor antagonist or IL-17 knock out mice to clarify exact mechanisms of our findings. We believe that this could give contribution to better management of these severe infections.

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