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Treatment with a neutralising anti-rat interleukin-17 antibody after multiple-trauma reduces lung inflammation

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ABSTRACT

Background: It has been well recognised that a deficit of numbers and function of $CD4^+CD25^+Foxp3^+$ cells (Treg) is attributed to the development of autoimmune diseases and inflammatory diseases; additionally, IL-17-producing cells (Th17) have a pro-inflammatory role. The balance between Th17 and Treg may be essential for maintaining immune homeostasis and has long been thought as one of the important factors in the development/prevention of autoimmune diseases and inflammatory diseases. In our previous research, we explored that cytokines (IL-17) and the balance of Treg/Th17 had a significant relevance with tissue (lung) inflammation and injury in acute-phase after multiple-trauma.
Objective: To more verify whether an imbalance of Treg/Th17 is characteristic of rats suffering from multiple trauma.
Methods and subjective: Using IL-17 monoclonal antibody (IL-17mAb)-treated multiple-trauma rat, we tested the pathogenic role of IL-17 in the development of multiple-trauma. Rat models were treated respectively with IL-17mAb or rat IgG 2A isotype control or phosphate-buffered solution after model was established. Normal rats only received anaesthesia and cannulation were taken as sham. Rats in each group were killed respectively at the end of 1 h, 4 h, 8 h after injection. Collected serum and lung samples for assessment dynamically of MPO, IL-17, IL-6, and TGF-β-mRNA, and cytokine (IL-17, IL-6, TGF-β) and lung tissue for pulmonary histological analysis.
Results: Neutralisation of IL-17 with anti-IL-17 can decrease serum IL-17 level and the IL-17-mRNA transcript level in lung, and ameliorate tissue inflammatory, defer disease course.
Conclusion: Our data suggest that IL-17 is crucially involved in the pathogenesis of multiple-trauma in rat, IL-17 inhibition might ameliorate the lung inflammation in acute-phase after multiple-trauma.

Introduction

Recently, numerous studies [1,2] focus on trauma have shown that multiple trauma (for example: burn, high energy trauma, low energy trauma in elders, and so on) is associated with the development of systemic inflammatory response syndrome and strongly correlated with Multiple Organ Dysfunction Syndrome (MODS). Lung is the first and primary target organ to be affected in the post-injury period, and acute lung injury increases the incidence and mortality from multiple-organ failure [3–5]. In our previous research [6], we explored that cytokines (IL-17) and the imbalance of Treg/Th17 had a significant relevance with tissue (lung and intestine) inflammation and injury in acute-phase (within 8 h) after multiple-trauma. This study is designed to more verify whether an imbalance of Treg/Th17 is characteristic of rats suffering from multiple trauma by blockage of IL-17 with IL-17mAb.

Materials and methods

Animals

Female SD (Sprague-Dawley) rats (300–350 g) were purchased from Beijing Haidian Thriving Experimental Animal Center and were housed in a climate-controlled barrier facility with 12 h light/dark cycles at (24 ± 2)°C and free access to food and water for a period of at least 1 wk prior to experimental procedures and maintained in Beijing Military Generational Hospital, the Institute

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of Traumatic Orthopedics. Institutional approval was obtained and institutional guidelines regarding animal experimentation were followed which was approved by the institutional ethics committee.

Grouping of animals and IL-17 neutralisation

80 rats were randomly divided into four groups. The sham group: only received anaesthesia, cannulation, and observation. While the models of the bilateral femoral shaft fractures with haemorrhagic shock were established, the rats were injected intraperitoneally with 100 μg IL-17 monoclonal antibody ($n = 20$, ab118869, abcam, IL-17mAb group), or 100 μg isotype control immunoglobulin (Ig) G2A Ab ($n = 20$, R&D, Systems, Inc., Minneapolis, MN. MAB006, isotype control group), or PBS ($n = 20$, PBS group). Rats in all groups were killed respectively at the end of 1 h, 4 h, 8 h after injection, six to seven rats per group per time point.

Experimental model of multiple trauma

Eighty rats were weighed and anaesthetised respectively with 10%Chloral-hydrate (3.5 mL/kg, i.p.) and then placed in a supine position on a warming pad (25 °C).

A standard closed bilateral mid-femoral fracture was produced in sixty rats. The specific method is as follows: reference to the Einhorn methods [7], we self-designed fracture combat device which composed of the bracket, pillar on the central axis, blunt bone knife and cylindrical weight (500 g). Rats were fixed at the bottom of the device in supine position with the femoral shaft fixed in the anvil groove of the bracket (pitch 15 mm) and the bone knife located in the mid-femoral shaft. The 500 g weight was lift to 15 cm (a moderate change according to rat) height and free fall along axis, through the impact of the central steel plate by weight, the hydraulic conductivity to blunt bone knife caused femoral shaft fractures. With the law, the femoral shaft fractures on the other side was caused. After the model was established. The fracture was immediately viewed by digital radiography (LX-60, Faxitron X-ray Corporation, Wheeling, IL), and rats with a proximal or distal fracture or with excessive comminution were excluded from the study. Observed local skin integrity with or without bleeding. The rat models of bilateral femoral shaft fractures were placed for half an hour at room temperature.

The right carotid artery of twenty normal rats and twenty rat models of bilateral femoral shaft fractures was isolated and cannulated with a polyethylene catheter through a neck incision. The arterial catheter was used for blood withdrawal and was connected to a multi-channel biologic recorder (model MP150, USA) for continuous haemodynamic monitoring (mean arterial pressure and heart rate). In the same way, the left carotid vein was cannulated for fluid infusion and reinfusion of the shed blood. The arterial catheter was heparinised (500 U/kg). Blood losses during the procedure were measured by absorbing all blood from the incision with preweighed gauze sponges, which were then reweighed. The formula 1 g = 0.9 ml of blood was used. Using the method of Capone et al. [8], shock was induced by controlled haemorrhage to a MAP (mean arterial pressure) of 35–40 mmHg which was sustained for 45 min by the withdrawal or return of shed blood (SB). Hypotension was maintained until the base deficit was >20 meq/L. Resuscitation was performed by infusing twice the SB volume in NS over 30 min, half the volume of the shed blood over 30 min, and then twice the SB volume in NS over 60 min via the femoral vein. The rat model of bilateral femoral shaft fractures with haemorrhagic shock and single haemorrhagic shock was established. Body temperature was maintained at 37 ± 0.5 °C with a rectal probe and a servo-controlled heating pad during procedures.

Tissue harvest and cytokine determination

Rats were sacrificed at the end of 1 h, 4 h, 8 h after trauma (anaesthesia). The blood samples were collected into collection tubes containing 0.2 ml sodium heparin for assessment of Treg cell and Th17 cell. Plasma was obtained after centrifugation and stored at −80 °C for the measurement of cytokines (IL-17, IL-6, TGF-β). The bronchoalveolar lavage fluid (BALF) of right lung was collected as above. The recovered BALF was centrifuged at 3000 rpm for 10 min, and the supernatant was frozen at −80 °C until further analysis. Tissue samples from the middle lobe of the right lung was quickly removed and flushed with 0.01 mol/L cold phosphate buffer solution (PBS, pH 7.4) and immersed for 24 h in 10% buffered formalin for assessment of lung histological change [9–11].

Semi-quantitative RT-PCR detection of IL-17, IL-6, TGF-β mRNA

Total mRNA was extracted from homogenised heart tissues by using of the TRIZOL Reagent® (Invitrogen, USA), and then used to synthesise cDNA with an RT Kit (Ferma, USA). Reverse-transcription PCR (RT-PCR) was performed with first-strand cDNA synthesised with 1 μg of total RNA and oligo d (T)18 primers according to the manufacturer's instructions. The primers for the RT-PCR assays for IL-17A, IL-6 and TGF-β were designed by Primer Premier 5.0 (Table 1). Rat Gapdh, a reference gene, was used to normalise each sample and each gene. Prepared cDNA was used for PCR amplification with the above primers under the following conditions: pre-heating at 94 °C for 3 min, denaturing at 94 °C for 30 s, annealing at 64.9 °C (IL-17) or 62 °C (IL-6) or 62.5 °C (TGF-β) for 30 s, and extension at 72 °C for 60 s. The reaction repeated for 35 cycles followed by incubation at 72 °C for 10 min. PCR products were analysed by electrophoresis on a 2% agarose gel containing 0.5 mg/ml ethidium bromide. The resulting bands were observed and photographed under ultraviolet light, and then measured using the Digital Gel Imaging Analyst (Nikon 990-Doc 1000, USA). Density was determined for each sample PCR product, including the positive control. Background density was subtracted from each band and the relative values of IL-17, IL-6 and TGF-β mRNA were calculated using Gapdh mRNA as a standard. PCR products was sequenced by Songon Biotech Co., Ltd. (Shanghai, China), and blasted in the NCBI Blast bank.

Assay of cytokine in the serum

IL-17, IL-6 and TGF-β levels were determined by the ABC enzyme-linked immunosorbent assay system (R&D Systems, USA) according to manufacturer's instructions. All experiments are performed at least in triplicate samples. A standard curve using recombinant cytokine was generated for each assay.

Lung myeloperoxidase activity

MPO (myeloperoxidase) activity was detected as a marker of polymorphonuclear (PMN) infiltration in pulmonary. Enhanced

Table 1
Sequences of primers for RT-PCR.

Molecule	Sequence (5′–3′)
IL-6	Sense: 5′ CACAGAAGGAGTGGCTAAGGACCA 3′ Antisense: 5′ ACGCACTAGGTTTGCCGAGTAGA 3′
IL-17	Sense: 5′ AGTTGGACCACCACATGAATTCT 3′ Antisense: 5′ ACGCATGGCGGACAATAGAG 3′
TGF-β	Sense: 5′ CACTGGAGCCTCGAATGTC 3′ Antisense: 5′ CAGGGAAGAATCTGGAAAGGT 3′
Gapdh	Sense: 5′ ACTCTACCCACGGCAAGTTCA 3′ Antisense: 5′ GACGCCAGTAGACTCCACGAC 3′