**RESEARCH LETTER**

**Interleukin-34: an important modifier in the pathogenesis of influenza pneumonia**

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Influenza is an acute respiratory virus infection of worldwide health importance [1, 2]. Interleukin-34 (IL-34) is an important inflammatory cytokine [3, 4]. We tested here whether IL-34 contributes to the immunopathology of influenza virus infection.

Twenty-two H1N1-infected patients were enrolled, and seven H1N1 patients were diagnosed with severe pneumonia. There was a dramatic increase in serum IL-34 levels in H1N1 patients at initial diagnosis (Fig. 1a). Influenza patients with severe disease displayed significantly higher serum IL-34 levels compared with those with mild disease (Fig. 1b). Besides, serum IL-34 concentrations were significantly decreased after these patients had recovered from acute infection (Fig. 1c). Furthermore, female C57BL/6j mice (8–10 weeks of age) were intranasally infected with 20 TCID50 of influenza virus strain A/PR/8/34 (H1N1), and we found that IL-34 levels were significantly increased in the lung and blood after H1N1 infection (Fig. 1d).

Next, a lethal murine model was established by intranasally infecting female C57BL/6j mice with 90 tissue culture infectious dose 50 (TCID50) of influenza virus strain A/PR/8/34, and body weight and survival change was assessed out to 14 day. IL-34 blockade was performed by tail vein injection with 10 μg of sheep anti-mouse IL-34 antibody (R&D systems, AF5195) on day of influenza infection, followed by booster doses of 5 μg on day 2 and 4. We found that mice treated with anti-IL-34 antibodies suffered significantly less weight loss than mice treated with IgG control and started to regain body weight by day 8 after viral infection, while mice treated with IgG control continued to lose body weight until death (Fig. 1e). Furthermore, all of mice treated with IgG control were dead within 11 days post infection, whereas 50% of mice treated with anti-IL-34 antibodies survived beyond day 14 post infection (Fig. 1f). Virus titers were also measured from lung tissues 5 days post infection. Interestingly, viral titers were similar between IgG control and anti-IL-34-treated mice (Fig. 1g), suggesting that IL-34 up-regulation did not affect influenza virus replication in the lung. We assessed the histological changes of lung injury using a standardized lung injury scoring system [5], and the lung injury score was significantly lower in mice treated with anti-IL-34 antibodies as compared to mice treated with IgG control (Fig. 1h).

We further investigated the direct influence of IL-34 on non-lethal influenza virus infection, C57BL/6j mice were intranasally infected with influenza virus strain PR8 at a dose of 20 TCID50 and then 2 μg of recombinant mouse IL-34 protein (R&D systems, 5195-ML) was inoculated into mice through tail vein injection. In mice treated with recombinant IL-34 protein, we observed a more severe decrease in weight following infection and a delayed recovery compared to mice treated with saline control (Fig. 1i). Moreover, all of mice treated with saline control survived beyond day 14 post non-lethal influenza virus infection, whereas 40% mice treated with recombinant IL-34 protein were dead within 5 days post infection (Fig. 1j). We next examined viral clearance by quantitating lung viral titers in mice treated with or without IL-34 on day 5 after non-lethal influenza virus infection, and there was no significant difference in viral titers (Fig. 1k).

Histological evaluation revealed that the lung injury...
score was significantly higher in IL-34-treated mice as compared to saline-treated mice after non-lethal influenza virus infection (Fig. 1).

Collectively, our data demonstrated a detrimental role of IL-34 in the immunopathology of influenza virus infection. We therefore speculate that excessive IL-34 amounts may have important implications in the development of influenza virus-induced lung injury.
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Authors’ contributions
BX, XL, YG, XL, LR, JC conceived and designed the experiments. BX, XL, YG, XL, LR performed the laboratory experiments. BX, XL, YG, XL, LR, JC analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data sharing is offered under the format of collaborative projects. Proposals can be directed to the corresponding author.

Declarations

Ethical approval
This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University and written consent was obtained from participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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