IL-17 mediated macrophage polarization increased inflammatory damage in SWI-ALI models

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Research

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Abstract

Background

Seawater inhalation induced acute lung injury (SWI-ALI) is the common accident in daily naval training. To investigate the mechanism of SWI-ALI will help to improve the treatment effect. Alveolar macrophages (AM) is the majority of alveolar, also paly the key role in SWI-ALI repair. IL-17 also paly the key role in the innate immunity process.

Method

In this study, we used seawater induced the ALI in mouse model. And the lungs and serum were exacted at D1, D3, D7 and D14. The AM polarization were tested by flow cytometry. The IL-17 concentration were tested by ELISA. Then the IL-17 function were confirmed by in vitro test. The mouse alveolar epithelial cell and mouse AM were co-cultured. The test compared the wound healing effect of MAE with and without IL-17.

Result

The AM switch into M1 and IL-17A increased were found after seawater dosing. And the IL-17a supplement attenuated wound healing of alveolar epithelial cells through improve the polarization of AM were confirmed in vitro model.

Conclusion

The high IL-I7 micro-environment will increased the inflammatory damage through induced macrophage polarization in acute lung injury. The IL-17 antagonists have the potential to increase clinical effect in SWI-ALI treatment.

Background.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are characterized by an acute inflammatory process in the airspaces and lung parenchyma. Seawater inhalation induced acute lung injury (SWI-ALI) is a common accident in daily naval training. The alveolar epithelial and pulmonary capillary endothelial impaired caused by seawater inhalation injury resulting in the significant hypoxemia [1–2]. Due to these patients are critically ill and frequently have coexisting conditions including sepsis and multiple organ failure, the patients are at higher risk of respiratory failure [3–5]. There is considerable experimental and clinical evidence that the changes of cytokines and immunity process play the key role in SWI-ALI development. But the conventional therapies such as mechanical ventilation are not adequate for all SWI-ALI treatments. So, to investigate the mechanism of SWI-ALI inflammation will help to find novel therapy [6].
Macrophages play key roles during mammalian development and tissue injury repair [7–8]. The resident tissue macrophages cloud recruited macrophages or monocytes from bone marrow after organ injury induced by infection, autoimmune disorders, mechanical or toxic injuries, and various other causes [9]. Alveolar macrophages (AM) are the most important component of the alveoli, accounting for 80–90% of the alveolar cells. The AM polarized and cytokine released is the first trigger of SWI-ALI induced Inflammatory process [10]. The activated macrophage can recruit the monocytes and neutrophils to improve the lung injury repair [11]. So, we propose the macrophage polarization and the relevant cytokines change will affect the SWI-ALI repair.

In this study we established the SWI-ALI model. The significant change of IL-17 and macrophage polarization were found at D3 and D7 after seawater injected. The IL-17 and macrophage relationship were confirmed in vitro.

Material And Methods

Animal and Ethics Statement

60 Female BALB/c nude mice (6-8 weeks old) were maintained in a light and temperature- controlled room. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Bioduro (China) Co., Ltd under SPF protocol. The ethic number was HRJ-FFS-ON-20200127-01.

Mouse primary cells isolation and culture condition

Mouse primary alveolar epithelial cells (MAE) and AM were isolated from health BALB/C mice. Lung were excised and stored at cold PBS before used. The lung single cell suspensions (SCS) were prepared by Lung Dissociation Kit, mouse (130-095-927, Miltenyi). The AM were selected by CD45 dynabeads (11153D, Thermo) and puried by maintained in RPMI-1640 (22400-097, Thermo) with M-CSF (10ng/mL) for two weeks. And MAE were negative selected by CD45 dynabeads. MAE was maintained in DMEM (11965-175, Thermo) medium. 10% fetal bovine serum (16000-044, Gibco) and with 10,000 units penicillin and 10 mg streptomycin/mL (V900929, Sigma-Aldrich) were supplemented into all above medium. The cells were incubated in humidified atmosphere containing 5% CO₂ at 37°C.

SWI-ALI model

SWI-ALI models were established via intratracheal instillation 100 mL seawater to each mouse. The seawater was configured by added sea salt (S9883, Sigma-Aldrich) into sterile ddH₂O. Control group were received intratracheal instillation PBS with the same volume. Five mice were sacrificed at D1, D3, D7 and D14.

Serum cytokine detection

Serum sample were exacted from jaws vein of mice. The serum sample were stored at -80 °C before used. IL-17a detection kit were purchased from Biolegend (432504). ELISA procedure were tested as
standard protocol. Each sample were tested three replicates.

**SWI-ALI in vitro model**

SWI-ALI *in vitro* model was established by MAE and AM co-culture. 20000 MAE were seeded into each well and allowed to growth over night. The wound was created in the center of each well using a scratcher. AM were added into macrophage group and macrophage+IL-17a group after wound made at 1:10 ratio of effect: target (E:T). IL-17a 10ng/mL were added into IL-17a group and macrophage+IL-17a group and control group. The wounds were photographed and measured every three hours for two days. After two days the supernatants were collected and detected by fluorescence-activated cell sorting (FACS).

**Macrophage polarization detection**

The SCS from the lung of SWI-ALI model at D1, D3, D7, D14 and supernatants form *in vitro* SWI-ALI model were detected by BD Celesta. M1 were defined by F4/80 + (565411, BD) and Ly6C + (560593, BD).

**Statistical analysis**

Statistical significant analysis of the data was using the analysis of variance and the Student’s t-test. And all data were presented as the mean ± standard deviation (SD). ELISA was calculated by 4 parameters logistic regression. The tests were conducted by using the GraphPad Prism 7.0 software (GraphPad software, USA).

**Result**

**Serum concentration of IL17 increased significantly after SWI-ALI.**

The serum concentration of SWI-ALI group and control group were showed in figure 1. The concentration of IL-17a in SWI-ALI group at D1, D3, D7 and D14 were 55.21±1.91, 186.04±8.01, 172.33±7.92 and 15.64±0.36. In compassion, the concentration of IL-17a in control group at D1, D3, D7 and D14 were 15.88±0.60, 15.47±0.47, 15.83±0.23 and 15.62±0.15. The concentration of IL-17a in SWI-ALI group at D1, D3, D7 were higher than the control group at the same time (P<0.001).

**The percent of M1 was increasing during SWI-ALI process**

The AM typing of SWI-ALI group and control group were showed in figure 2. The percent of M1 in SWI-ALI group at D1, D3, D7 and D14 were 38.8±10.99, 45.75±7.02, 17.45±5.55 and 21.13±2.79. The percent of M1 in control group at D1, D3, D7 and D14 were 18.16±2.76, 13.28±1.96, 19.03±1.76, 19.38±2.39. The M1 percent of SWI-ALI group at D1, D3 and D7 were higher than control group (P<0.05). The M1 percent of SWI-ALI group and control group at D1, D7 and D14 had no significantly different (P>0.05).

**IL-17 attenuated the wound healing at SWI-ALI in vitro model.**
The wound width changing of each group were show in figure 3. The wounds of control group, macrophage group and IL-17a group were completed healed before 39h, 33h and 36h. The wounds of macrophage and IL-17a group were not completed healed after 2d. The final wound width of macrophage+ IL-17a group was 751.79±180.42mm2.

**IL-17 can induce AM polarization**

The AM typing from the co-culture supernatants of each group were showed in figure 4. The M1 percent of macrophage group and macrophage+IL-17a group were 0.85±0.47 and 16.77±2.60. The M1 percent of macrophage group was higher than the macrophage+IL-17a group ($P<0.01$).

**Discussion**

IL-17 is a typical Inflammatory cytokines family which played the key role in various inflammation induced by direct injury or pathogen invasion [12–14]. IL-17 family’s cytokines were secreted by T helper 17(Th17) cells during inflammation [15]. The secreted IL-17 further activates a complex network of cytokine interactions to help repair the injury. But the IL-17 is a double-edged sword. The high IL-17 concentration also distributed to autoimmune disease like Systemic Lupus Erythematosus [14]. IL-17a is a member of IL-17 family which can bind IL-17Ra to perform the biological effect. IL-17Ra were higher expression at fibroblast and lung [16]. As the reports, high IL-17 were found at patient’s bronchoalveolar lavage fluid (BALF) after SWI-ALI [17–18].

In this study, we used seawater to established SWI-ALI model. The IL-17a were increasing rapidly after SWI-ALI and existed more than a week. The high serum IL-17a level shows the innate immunity triggered immediately. Activated Th17 cells recruit neutrophil though the secreted IL-17 to heal the impaired epithelium and airway [19]. But the persist secreting IL-17 also remain the increasing inflamed damage. So, we want to confirm whether the IL-17 improve or attenuated SWI-ALI repair.

AM is the majority part of alveolar and the macrophage is classical organ damage restorer. The activated macrophage is the main source of IL-17 [20]. Plasticity is the characteristics of macrophages. Macrophages show different phenotype at different micro-environments. Macrophage can polar into the inflammatory M1 or anti-inflammatory M2 [21–22]. The FACS show the AM polarization were increased during SWI-ALI process. The M1 percent were highest at the D3 after SWI-ALI. And the polarization of macrophages rapidly disappeared after D7. Zhou and his colleagues report that the M1 macrophages can impair the keratinocyte migration via TNF-α [23]. So, the increased M1 percent antagonize epithelial repair.

The relationship of IL-17 and macrophage polarization were confirmed by SWI-ALI *in vitro* model. The supplement of IL-17a increased M1 percent and attenuated the wound healing of PAE. But the macrophage alone show improves the wound healing effect. And the IL-17a alone not influent the wound healing. It’s seem that the IL-17 via induce the macrophage polarization to increase the SWI-ALI damage.
during inflammation. This show a potential that the IL-17 antagonist could help the clinical treatment in SWI-ALI.

Conclusion

This study found that the IL-17 and M1 percent of AM increased during the SWI-ALI inflammation. And confirm that the IL-17 can affect the macrophage switch to M1 to increase the inflammatory damage. This may help to improve the clinical effect of SWI-ALI treatment.

Abbreviations

ALI: Acute lung injury; ARDS: acute respiratory distress syndrome; AM: Alveolar macrophages; MAE: Mouse primary alveolar epithelial cells; SCS: single cell suspensions; FACS: fluorescence-activated cell sorting; SD: standard deviation; Th17: T helper 17; BALF: bronchoalveolar lavage fluid.

Declarations

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Authors’ contributions

JYZ conducted the experiments. JP, RZ, JF and YPH collected and analyzed the data. JYZ, JP, RZ, JF and YPH wrote the manuscript. JWX and YS supervised the project and revised the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Bioduro (China) Co., Ltd under SPF protocol. The ethic number was HRJ-FFS-ON-20200127-01.

Consent for publication
Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Chen L, Chen J, Xie G, Zhu L. MiR-222 inhibition alleviates Staphylococcal Enterotoxin B-induced inflammatory acute lung injury by targeting Foxo3. J Biosci. 2020;45:65.

2. Fanelli V, Ranieri VM. Mechanisms and clinical consequences of acute lung injury. Ann Am Thorac Soc. 2015;12(Suppl 1):3–8.

3. Butt Y, Kurdowska A, Allen TC. Acute Lung Injury: A Clinical and Molecular Review. Arch Pathol Lab Med. 2016;140(4):345–50.

4. Dias-Freitas F, Metelo-Coimbra C, Roncon-Albuquerque R Jr. Molecular mechanisms underlying hyperoxia acute lung injury. Respir Med. 2016;119:23–8.

5. Kim J, Na S. Transfusion-related acute lung injury; clinical perspectives. Korean J Anesthesiol. 2015;68(2):101–5.

6. Mokra D, Mikolka P, Kosutova P, Mokry J. Corticosteroids in Acute Lung Injury: The Dilemma Continues. Int J Mol Sci. 2019;20(19):4765.

7. Mao R, Wang C, Zhang F, Zhao M, Liu SY, Liao GN, et al. Peritoneal M2 macrophage transplantation as a potential cell therapy for enhancing renal repair in acute kidney injury. J Cell Mol Med. 2020;24(6):3314–27.

8. Myose Y, Hikiji H, Tsujisawa T, Sonoki K, Yada N, Inoue H. Macrophage-mediated repair of intraepithelial damage in rat oral mucosa. Arch Oral Biol. 2019;97:185–90.

9. Boniakowski AM, denDekker AD, Davis FM, Amrita Joshi AS, Kimball M, Schaller, et al. SIRT3 Regulates Macrophage-Mediated Inflammation in Diabetic Wound Repair. J Invest Dermatol. 2019;139(12):2528–37.

10. Bone NB, Liu Z, Pittet JF, Zmijewski JW. Frontline Science: D1 dopaminergic receptor signaling activates the AMPK-bioenergetic pathway in macrophages and alveolar epithelial cells and reduces endotoxin-induced ALI. J Leukoc Biol. 2017;101(2):357–65.
11. Wang F, Fu X, Wu X, Zhang JH, Zhu JL, Zou Y, et al. Bone marrow derived M2 macrophages protected against lipopolysaccharide-induced acute lung injury through inhibiting oxidative stress and inflammation by modulating neutrophils and T lymphocytes responses. Int Immunopharmacol. 2018;61:162–8.

12. Gurczynski SJ, Moore BB. IL-17 in the lung: the good, the bad, and the ugly. Am J Physiol Lung Cell Mol Physiol. 2018;314(1):6–16.

13. Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol. 2009;123(5):986–96.

14. Tan HL, Rosenthal M. IL-17 in lung disease: friend or foe? Thorax. 2013;68(8):788–90.

15. Shilling RA, Wilkes DS. Role of Th17 cells and IL-17 in lung transplant rejection. Semin Immunopathol. 2011;33(2):129–34.

16. Wu Z, He D, Zhao S, Wang H. IL-17A/IL-17RA promotes invasion and activates MMP-2 and MMP-9 expression via p38 MAPK signaling pathway in non-small cell lung cancer. Mol Cell Biochem. 2019;455(1–2):195–206.

17. Ding Q, Liu GQ, Zeng YY, Zhu JJ, Liu ZY, Zhang XG, et al. Role of IL-17 in LPS-induced acute lung injury: an in vivo study. Oncotarget. 2017;8(55):93704–11.

18. Li C, Yang P, Sun Y, Li T, Wang C, Wang Z, et al. IL-17 response mediates acute lung injury induced by the 2009 pandemic influenza A (H1N1) virus. Cell Res. 2012;22(3):528–38.

19. Lee HC, Liu FC, Tsai CN, Chou AH, Liao CC, Yu HP. Esculetin Ameliorates Lipopolysaccharide-Induced Acute Lung Injury in Mice Via Modulation of the AKT/ERK/NF-κB and RORγt/IL-17 Pathways. Inflammation. 2020;43(3):962–74.

20. Kim HK, Garcia AB, Siu E, Tilstam P, Das R, Roberts S, et al. Macrophage migration inhibitory factor regulates innate γδ T-cell responses via IL-17 expression. FASEB J. 2019;33(6):6919–32.

21. Zhang J, Qiao Q, Liu M, He T, Shi J, Bai X, et al. IL-17 Promotes Scar Formation by Inducing Macrophage Infiltration. Am J Pathol. 2018;188(7):1693–702.

22. Zhang Q, Atsuta I, Liu S, Chen C, Shi S, Shi S, et al. IL-17-mediated M1/M2 macrophage alteration contributes to pathogenesis of bisphosphonate-related osteonecrosis of the jaws. Clin Cancer Res. 2013;19(12):3176–88.

23. Huang SM, Wu CS, Chiu MH, Wu CH, Chang YT, Chen GS, et al. High glucose environment induces M1 macrophage polarization that impairs keratinocyte migration via TNF-α: An important mechanism to delay the diabetic wound healing. J Dermatol Sci. 2019;96(3):159–67.

Figures
Figure 1

The serum concentration of SWI-ALI group and control group. The significant difference (P<0.001) between control group and seawater group were showed as ***.
Figure 2

AM typing of each group. The significant different (P<0.05) between control group and seawater group were showed as *. The significant different (P<0.001) between control group and seawater group were showed as ***.

![Figure 2 Image]

Figure 3

![Figure 3 Image]
The wound healing assay of AM and ALE co-culture system. A. the image of each group at D0 and D2. B. The wound width change curve of each group.

Figure 4

The AM typing from the co-culture supernatants of each group. A. The histogram of each group. B. The M1 percent of each group. The significant different (P<0.01) between control group and seawater group were showed as *. 