Genes & Diseases (2023) 10, 1214–1216

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RAPID COMMUNICATION

Deletion of G protein-coupled receptor 116 enhances neutrophil function and aggravates lung injury in mice

Acute lung injury (ALI) globally afflicts over 3 million individuals every year. It can eventually develop into acute respiratory distress syndrome (ARDS) with a high mortality of up to 40%. To date, ALI has been undertreated in terms of the feeble efficacy of clinical approaches and the lack of proven pharmacological targets.[1] G protein-coupled receptors (GPCRs), the promising targets of modern medicine, participate intensively in the regulation of human physiology and pathophysiology. Among them, *Gpr116*, which is expressed in alveolar epithelial cells and immune cells, has been reported to play a key role in maintaining alveolar homeostasis and inhibiting inflammation.[2] However, its role in the regulation of neutrophil function and ALI remains to be elucidated. In this study, we demonstrated the inhibitory role of *Gpr116* on neutrophil function, which contributes to nonspecific lung injury in mice induced by lipopolysaccharide (LPS).

In brief, LPS was intratracheally injected at a dose of 10 mg/kg in mice to induce ALI *in vivo*. Neutrophils isolated from the bone marrow of control and myeloid cell-specific *Gpr116* knockout mice were stimulated with phorbol 12-myristate 13-acetate (PMA) (100 ng/mL) *in vitro*. More details are available in the Supplementary materials.

In clinical data, *Gpr*116 mRNA expression in blood samples was lower in patients with ARDS than in non-ARDS patients without statistical significance (Fig. 1A). Furthermore, we detected whether *Gpr116* expression was altered in ALI mice. After administering LPS to C57BL/6J mice, we measured *Gpr116* expression in lung tissue and BALF cells. Compared with that in control mice, the mRNA expression of *Gpr116* in lung tissues and BALF cells of ALI mice decreased (Fig. 1B, C), along with similar changes in the protein expression of G*pr116* in the mouse lungs (Fig. S1). Besides, in the *in vitro* experiment, the mRNA expression of *Gpr116* in bone marrow-derived neutrophils also decreased after PMA stimulation (Fig. S2). More significantly, *Gpr116* expression in the representative immunofluorescence images was apparently downregulated and negatively correlated with the MPO-positive cells in the lungs of ALI mice compared to that of control mice (Fig. S3). These data indicate that *Gpr116* expression is downregulated in lung injury, suggesting that it may play a role in the progression of ALI.

Accumulating evidence has shown that macrophages and neutrophils, the main effector cells of innate immunity, are recruited into the blood and migrate to alveolar capillaries along the gradient of chemoattractant. Both of them can release a variety of bactericidal mediators to induce diffuse damage of the blood-gas barrier and increased respiratory load, resulting in fatal hypoxemia.[3] Therefore, we bred myeloid cell-specific *Gpr116* knockout mice using conditional CRE/LoxP deletion approaches (Fig. S4, S5) to further verify the role of *Gpr116* in immune regulation in ALI. As shown by H&E staining, compared to flox mice, *Gpr116* cKO mice were more sensitive to LPS challenge and had a higher lung injury score (Fig. 1D). In addition, the pathological damage of ALI also involves rupture of alveolar capillary membranes and leakage of protein-rich fluid in the alveoli, leading to pulmonary edema, which could be verified by the protein levels in BALF and lung W/D ratios. Here, we observed a dramatic increase of protein level in the BALF of *Gpr116* cKO mice compared to flox mice (Fig. 1E). Likewise, the W/D ratio of lung tissues was also greater in cKO mice than that in flox mice (Fig. 1F). Furthermore, deletion of *Gpr116* reduced mouse survival rate after LPS stimulation (40 mg/kg, i.t.) (Fig. 1G). In addition, we investigated the effect of *Gpr116* on LPS-induced inflammation. Compared with flox mice, BALF and plasma concentrations of TNF-α and IL-6 in cKO mice increased after LPS injection, indicating that *Gpr116* may inhibit pulmonary and systemic inflammation, although not all these discrepancies were

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.08.017

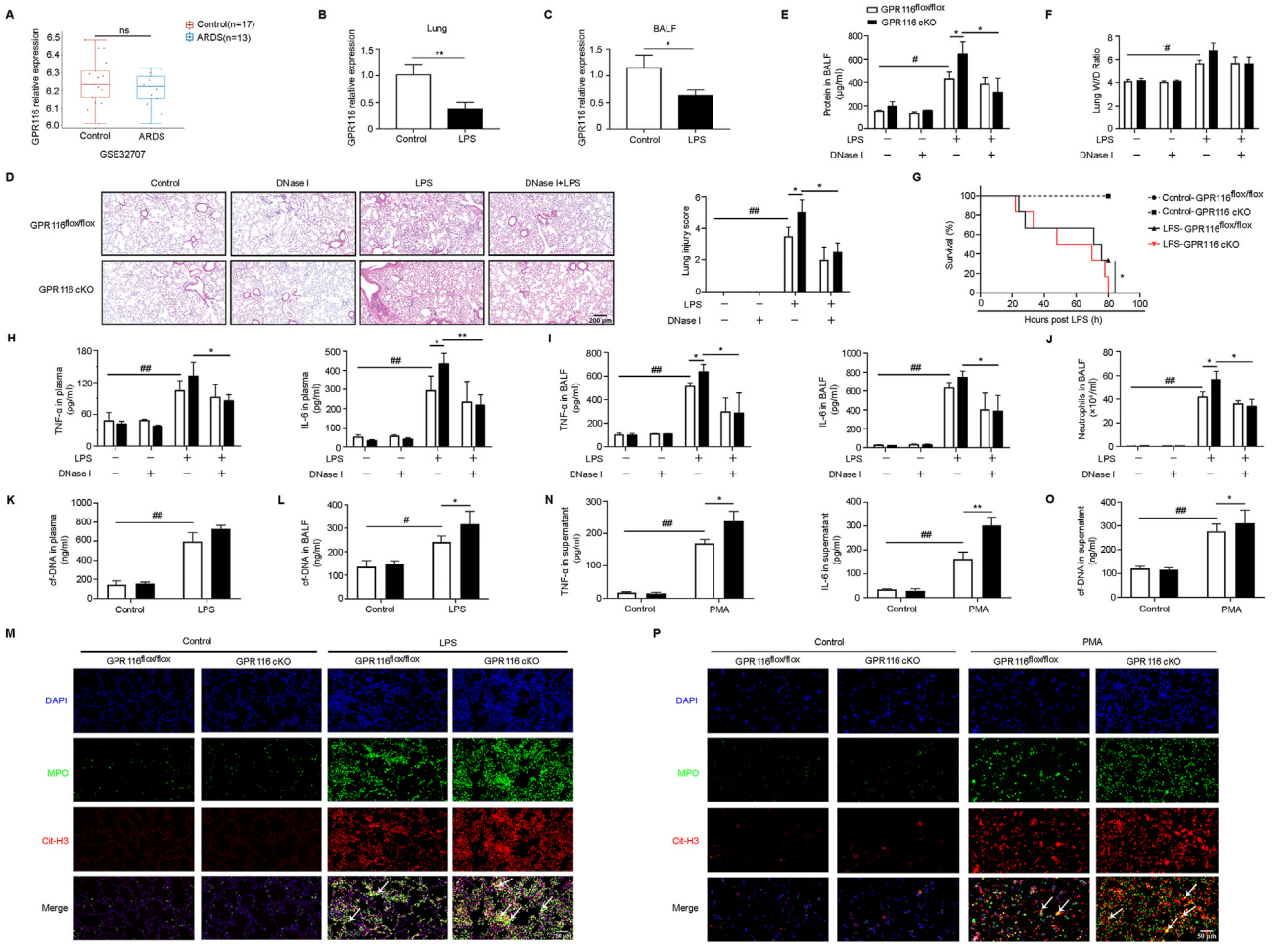

Figure 1 The pathological effect of *Gpr116* deletion in ALI mice and primary neutrophils. **(A)** *Gpr116* mRNA levels in human blood samples based on the available GEO dataset (GSE 32707; *t*-tests). **(B)** *Gpr116* mRNA levels in mouse lungs ($n = 5$; Student's *t*-test). **(C)** *Gpr116* mRNA levels in BALF cells of mice ($n = 5$; Student's *t*-test). **(D)** Representative histopathological images (scale bars: 200 μm) and injury scores of mouse lungs ($n = 5$; one-way analysis of variance). **(E)** Total protein concentrations in mouse BALF ($n = 3$ for control and DNase I mice; $n = 5$ for LPS and DNase I + LPS mice; one-way analysis of variance). **(F)** Lung wet-to-dry (W/D) weight ratios ($n = 5$; one-way analysis of variance). **(G)** Survival curves of mice challenged with LPS (40 mg/kg, i.t.) ($n = 5$ for control mice; $n = 8$ for LPS mice; log-rank test). **(H)** Plasma concentrations of TNF-α and IL-6 ($n = 5$; one-way analysis of variance). **(I)** BALF concentrations of TNF-α and IL-6 ($n = 5$; one-way analysis of variance). **(J)** BALF concentrations of neutrophils quantified by flow cytometry ($n = 5$; one-way analysis of variance). **(K)** Plasma levels of cf-DNA ($n = 5$; one-way analysis of variance). **(L)** BALF levels of cf-DNA ($n = 5$; one-way analysis of variance). **(M)** Representative immunofluorescence images of NETs formation in mouse lungs (DAPI: blue; Cit-H3: red; MPO: green; $DAPI^+$ $Cit\text{-}H3^+$ MPO^+ NETs are indicated by white arrowheads; scale bars: 50 μm). **(N)** TNF-α and IL-6 concentrations in the supernatants of bone marrow neutrophils from mice ($n = 5$; one-way analysis of variance). **(O)** cf-DNA concentrations in the supernatants of bone marrow neutrophils from mice ($n = 5$; one-way analysis of variance). **(P)** Representative immunofluorescence images of NETs formation in bone marrow-derived neutrophils (DAPI: blue; Cit-H3: red; MPO: green; the white arrow indicates $DAPI^+$ $Cit\text{-}H3^+$ MPO^+ NETs; scale bars: 50 μm). Data are expressed as mean ± SEM of three repeated trials; $^*P < 0.05$, $^{**}P < 0.01$, $\#P < 0.05$, $\#\#P < 0.01$.

statistically significant (Fig. 1I, H). These results are consistent with the reported role of *Gpr116* in immune regulation, for example, *Gpr116* suppresses inflammatory responses of alveolar macrophages via NF-κB signaling.[2]

In fact, lung is thought to be main reservoir of neutrophils, and neutrophil functions involving recruitment, reactive oxygen species (ROS) production, and NETs release are closely associated with the pathogenesis of ALI. Nevertheless, the role of *Gpr116* in neutrophil function remained to be illuminated. Here, we observed a dramatic increase in neutrophil lung infiltration in *Gpr116* cKO mice compared to flox mice (Fig. 1J; Fig. S7). It has been reported that NETs, the new mechanisms of neutrophil immunity, are large, extracellular, web-like structures composed of cytosolic and granular proteins that are assembled on a scaffold of decondensed chromatin. Excessive NETs formation, not only a pathological result but also a pathogenic factor, plays an important role in ALI.[4,5] Therefore, we speculated that the protective effect of *Gpr116* on ALI may be achieved by regulating the release of NETs. Subsequently, PicoGreen assays, Western blotting and immunofluorescence staining were performed to

detect the marker levels of NETs involving cf-DNA, citrullinated histone H3 (Cit-H3), and MPO. As expected, we found that *Gpr116* deletion dramatically increased the release of NETs in ALI mice (Fig. 1K—M; Fig. S8—9). ROS production in mouse lungs was also detected to further explore the regulatory effect of *Gpr116* on neutrophil function. As Figure S6 shows, *Gpr116* deficiency dramatically increased the production of ROS in ALI mouse lungs. DNase I is a specific inhibitor of NETs and can reduce NETs-associated citrullinated histones and minimize immune cell recruitment. To further confirm the inhibitory effects of *Gpr116* on NETs release, we eliminated the formation of NETs by DNase I pretreatment before LPS stimulation. It was observed that specific inhibition of NETs reversed the aggravation of lung injury induced by *Gpr116* deletion, as indicated by the reduced lung injury score (Fig. 1D), as well as the decreased protein levels in BALF and lung W/D ratios (Fig. 1E, F). Furthermore, the ELISA assays illustrated that the exacerbation of pulmonary and systemic inflammatory responses following *Gpr116* deletion in ALI mice was abrogated by DNase I preintervention, as evidenced by the decreased TNF-α and IL-6 levels in BALF (Fig. 1I) and plasma (Fig. 1H), respectively. Taken together, these data suggest that *Gpr116* may protect mice against lung injury by inhibiting neutrophil recruitment, NETs release, ROS production and inflammatory response.

Rather, we cannot exclude the pathogenic role of macrophages during the progression of ALI. Therefore, we carried out magnetic isolation (Fig. S10) of neutrophils from bone marrow of control and conditional CRE/LoxP deletion of *Gpr116* mice to further investigate the molecular mechanism of protective role of *Gpr116*. Consistent with the *in vivo* data, the NETs release of neutrophils from *Gpr116* cKO mice increased notably after PMA challenge (Fig. 1O, P). Besides, the elevation of TNF-α and IL-6 levels in neutrophil supernatant induced by *Gpr116* deletion was remarkable (Fig. 1N). These results elucidate the suppressive role of *Gpr116* in neutrophil function, which potentially contributes to diffuse damage of blood-air barrier in an ALI mouse model.

In conclusion, our data delineate that *GPR116* can inhibit neutrophilic lung infiltration, NETs release, ROS production and inflammatory response to alleviate lung injury and mortality in ALI mice, which extends the therapeutic potential of *GPR116*. Further studies are needed to clarify the underlying mechanism of the regulation of *GPR116* in neutrophil function to provide compelling evidence that *GPR116* is a promising therapeutic target in alleviating ALI.

Author contributions

T Wang and JJ Bian designed the research; T Wang, Y Wang, and Q Xiang performed the research and analyzed the data; SW Lin, PP Jin, J Wang, and N Li offered technical support; T Wang, JF Wang and JJ Bian drafted and revised the manuscript.

Conflict of interests

The authors declare no conflict of interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81871579) and the Military Medical Science and Technology Youth Cultivate Program (China) (No. 19QNP018).

Ethics declaration

All experimental procedures were approved by the Laboratory Animal Ethics Committee of the Naval Medical University and were performed according to the relevant guidelines.

Availability of data and materials

The datasets used in this study are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.08.017.

References

1. Spinelli E, Mauri T, Beitler JR, Pesenti A, Brodie D. Respiratory drive in the acute respiratory distress syndrome: pathophysiology, monitoring, and therapeutic interventions. *Intensive Care Med*. 2020;46(4):606—618.
2. Kubo F, Ariestanti DM, Oki S, et al. Loss of the adhesion G-protein coupled receptor ADGRF5 in mice induces airway inflammation and the expression of CCL2 in lung endothelial cells. *Respir Res*. 2019;20(1):11.
3. Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. *N Engl J Med*. 2017;377(6):562—572.
4. Lefrançais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. *JCI Insight*. 2018;3(3):98178.
5. Shi Y, Liu T, Nieman DC, et al. Aerobic exercise attenuates acute lung injury through NET inhibition. *Front Immunol*. 2020;11:409.

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27 March 2022
Available online 24 September 2022

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