Progranulin aggravates pulmonary immunopathology during influenza virus infection

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ABSTRACT

Progranulin (PGRN) exerts multiple functions in various inflammatory diseases. However, the role of PGRN in the pathogenesis of virus infection is unknown. Here, we demonstrated that PGRN production was up-regulated in clinical and experimental influenza, which contributed to the deleterious inflammatory response after influenza virus infection in mice. PGRN-deficient mice were protected from influenza virus-induced lung injury and mortality. Decreased mortality was associated with significantly reduced influx of neutrophils and monocytes/macrophages, release of cytokines and chemokines, and permeability of the alveolar–epithelial barrier without affecting viral clearance. Our findings suggest that PGRN exacerbates pulmonary immunopathology during influenza virus infection.

INTRODUCTION

Influenza is an acute respiratory virus infection of global importance.[1] Inflammation triggered by influenza is important for the control of virus proliferation, but a disproportionate inflammatory response is coupled with lung damage and often lethal lung injury syndromes.[2 3]

Progranulin (PGRN) is an autocrine growth factor, and it is widely expressed in epithelia, bone marrow and immune cells, etc.[4] We recently reported that PGRN was up-regulated during bacterial infection, and it contributed to host defence against bacterial pneumonia and sepsis.[5 6] The role of PGRN in the immunopathology of influenza infection, however, remains unknown. Herein, we first aimed to determine the extent of PGRN production during influenza. In addition, we investigated whether PGRN could modulate immunopathology during influenza.

METHODS

See online supplement for additional methods.

RESULTS

As shown in figure 1A, in the 26 paediatric patients infected with influenza (online supplementary table 1), the serum levels of PGRN were significantly elevated (176.73 ng/mL, 78.47–386.11) with median sera concentrations approximately two-fold higher than in healthy controls (88.69 ng/mL, 50.71–149.30). Influenza-infected patients were further divided into two different groups on the basis of clinical severity, and no variation in age and gender between the groups were observed and no patients had underlying diseases. Interestingly, patients with severe disease displayed significantly higher PGRN levels compared with those with mild disease (figure 1B). Furthermore, there was a significant decrease in the PGRN concentration of influenza patients with severe disease after they had recovered from acute infection (figure 1C). Additionally, we correlated the serum PGRN concentrations and the serum cytokine/chemokine levels in influenza patients, and PGRN showed a significant and positive correlation with IL-6 and CCL2, but not TNF-α and IFN-γ (online supplementary figure 1). However, there was no apparent correlation between serum PGRN and initial influenza virus concentrations in nasopharyngeal aspirate samples (data not shown).

In wild-type (WT) mice after infection with PR8, PGRN levels were significantly increased in the lung and blood (figure 1D). Both TLR2$^{-/-}$ and TLR4$^{-/-}$ mice had similar PGRN levels when compared with WT mice after influenza, while IFNAR$^{-/-}$ mice had significantly lower PGRN levels in the lung and blood compared with WT mice (figure 1E), suggesting that influenza promoted PGRN production in an IFNAR-dependent manner. To examine whether PGRN production is involved in the host response during influenza, WT and PGRN$^{-/-}$ mice were infected intranasally with PR8. Compared with WT mice, PGRN$^{-/-}$ mice had significantly less weight loss on virus infection from day 5 to day 8post-infection (figure 2A), and they displayed significantly decreased mortality following infection (figure 2B). Remarkably, the decreased mortality in PGRN$^{-/-}$ mice was not due to an improved viral elimination (figure 2C), and addition of recombinant PGRN had no effects on the virus titer in culture supernatants of A549 cells (online supplementary figure 2). In line with the high mortality rate, WT mice presented with lethargy and piloerection on day 4 and day 7 after influenza infection, and histological examination of the lungs of WT mice infected with influenza virus revealed more severe lung inflammation and more cell infiltration when compared with that of PGRN$^{-/-}$ mice on day 4 and day 7 (figure 2D and F). Moreover, the wet-to-dry lung tissue ratio, a measure of lung oedema, was markedly lower in PGRN$^{-/-}$ mice after 4 or 7 days of infection, and PGRN$^{-/-}$ mice had decreased capillary leakage in the respiratory tract, leading to significantly lower albumin concentrations in the bronchoalveolar lavage fluid (BALF) of these mice (figure 2E and G).

Consistent with the decrease in pulmonary immunopathology and mortality in PGRN$^{-/-}$ mice, influenza-infected PGRN$^{-/-}$ mice exhibited significantly decreased levels of IL-6, CCL2, CXCL1 and myeloperoxidase (MPO), but not TNF-α and IFN-γ, in lung homogenate (figure 3A). Additionally, the number of total leukocytes, neutrophils and

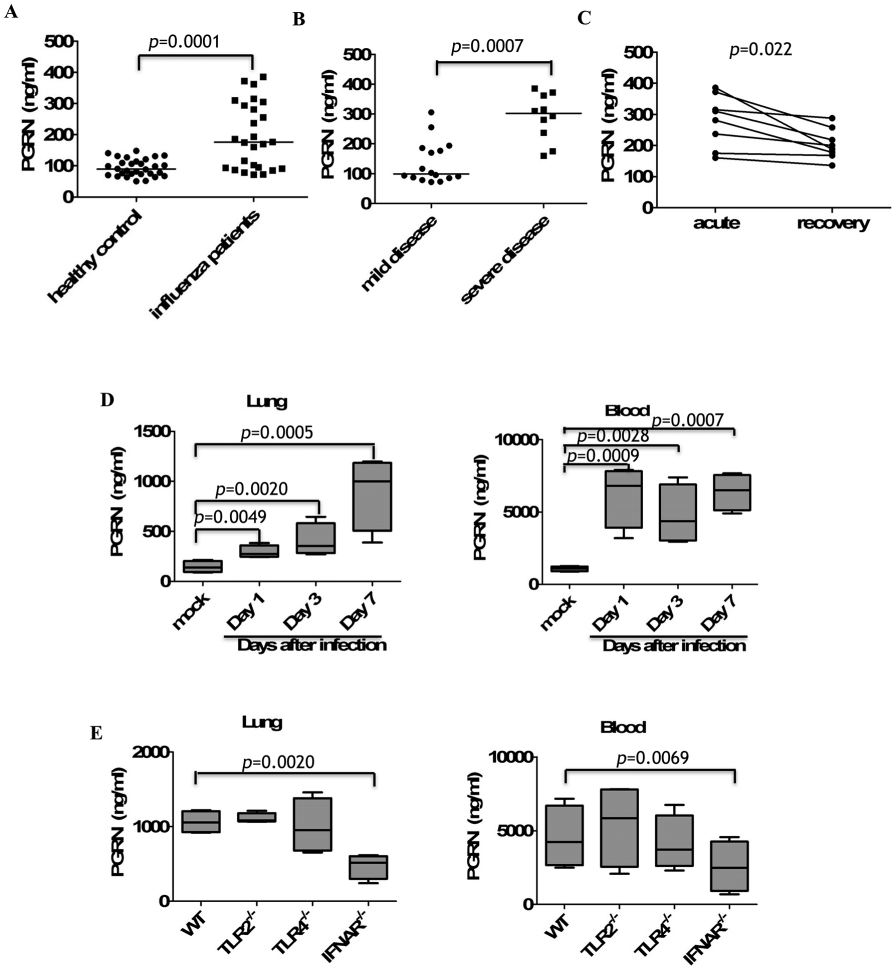

Figure 1 PGRN levels were elevated in clinical and experimental influenza. (A) PGRN concentrations were measured by ELISA in serum samples collected from 26 paediatric patients infected with 2009 pandemic H1N1 and from 30 healthy age- and gender-matched control participants. Horizontal bars represent median values, and dots represent individual participants. P value was determined by Mann–Whitney *U* test. (B) Serum PGRN levels in influenza patients with severe disease (n=10) and patients with mild disease (n=16). P value was determined by Mann–Whitney *U* test. (C) Serum concentrations of PGRN in influenza patients (n=8) in the acute and recovery phases. P value was determined by Wilcoxon signed-rank test. (D) C57BL/6 mice (n=6 per group) were intranasally infected with PR8 (50 pfu), and lungs and blood were collected at the indicated times. Samples were assayed for PGRN content by ELISA. P values were determined by Kruskal–Wallis test followed by Dunn's multiple comparisons post test. (E) PGRN concentrations in the lung and blood isolated from TLR2$^{-/-}$, TLR4$^{-/-}$, IFNAR$^{-/-}$ and WT mice (n=6 per group) on day 7 after intranasal PR8 infection. P values were determined by Kruskal–Wallis test followed by Dunn's multiple comparisons post test. IFNAR=type I IFN-α/β receptor; TLR2 and TLR4=Toll like receptor types 2 and 4, respectively; WT=wild type.

monocytes/macrophages in the BALF was significantly reduced in PGRN$^{-/-}$ mice (figure 3B).

DISCUSSION

Our findings have identified a potential role for PGRN in the immunopathology of influenza infection. The production of PGRN was up-regulated in H1N1-infected patients. Importantly, patients with more severe clinical manifestations had significantly higher PGRN concentrations than patients with mild clinical manifestations, and there was a significant decrease in the PGRN concentration of influenza patients after they had recovered from acute infection, suggesting that an increase of PGRN may be a potential predictor of prognosis in influenza. Using a mouse model of fatal H1N1 respiratory infection, we first showed that loss of PGRN decreased weight loss and mortality without compromising virus clearance, suggesting that PGRN plays a pathogenic role during influenza.

PGRN deficiency attenuated lung inflammation and immunopathology following influenza infection and resulted in less lung injury and oedema. Experimental evidence has shown that PGRN had proinflammatory effects in obesity and insulin-resistant diabetes mellitus, which was associated with macrophage infiltration and cytokine (IL-6) production regulated by PGRN in adipose tissue.[7–9] Besides, direct PGRN treatment could increase the accumulation of neutrophils and macrophages in the wound.[10] Our results are also consistent with our previous finding of decreased pulmonary inflammatory responses in PGRN$^{-/-}$ mice during bacterial pneumonia.[6] However, the current report differs from this study, because bacterial clearance was impaired by the generation of a decreased inflammatory response during acute bacterial pneumonia, whereas ablated inflammation during influenza pneumonia is beneficial to host outcome. The decrease in pulmonary immunopathology in PGRN$^{-/-}$ mice was not due to an improved viral elimination, but it was associated with a significant decrease of pulmonary IL-6, CCL2, CXCL1 and MPO. PGRN deficiency did not affect the levels of pulmonary TNF-α and IFN-γ

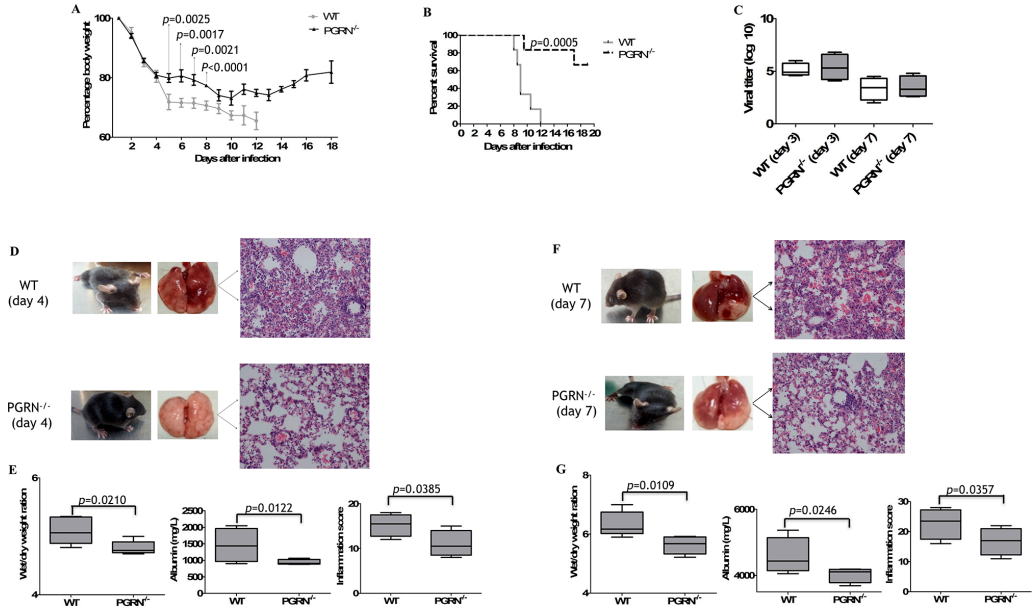

Figure 2 Loss of PGRN attenuated influenza-induced lung injury and mortality. WT and PGRN$^{-/-}$ mice were infected intranasally with 500 pfu of PR8. (A) Weight changes of mice (n=20 per group) after influenza infection in WT and PGRN$^{-/-}$ mice. P value was determined by Mann–Whitney *U* test. (B) Kaplan–Meier survival curves were shown and significance was determined using the log-rank test (n=20 per group). P value was determined by log-rank survival test. (C) Viral titers in the lung tissues from infected WT and PGRN$^{-/-}$ mice (n=6 per group), and no significant difference was found between WT and PGRN$^{-/-}$ mice after influenza virus infection (Mann–Whitney *U* test). (D) Representative clinical appearance and histopathological analysis of lungs in WT and PGRN$^{-/-}$ mice on day f4 after PR8 infection (n=6 per group). (E) Wet to dry lung weight ratios, albumin concentrations in BALF, and inflammation scores of pulmonary tissues from WT and PGRN$^{-/-}$ mice (n=6 per group) on day 4 after PR8 infection. Pvalue was determined by Mann–Whitney *U* test. (F) Representative clinical appearance and histopathological analysis of lungs in WT and PGRN$^{-/-}$ mice on day 7 after PR8 infection (n=6 per group). (G) Wet to dry lung weight ratios, albumin concentrations in BALF, and inflammation scores of pulmonary tissues from WT and PGRN$^{-/-}$ mice (n=6 per group) on day 7 after PR8 infection. P value was determined by Mann–Whitney *U* test.

during influenza pneumonia, which is consistent with our previous findings in PGRN$^{-/-}$ mice during bacterial pneumonia,[6] suggesting that PGRN did not regulate the production of TNF-α and IFN-γ on microbial infection. The exact mechanisms implicated in the role of PGRN in regulating the expression of inflammatory mediators require further studies. Taken together, these results suggested that PGRN induced by influenza led to exuberant pulmonary inflammation through augmenting accumulation of neutrophils

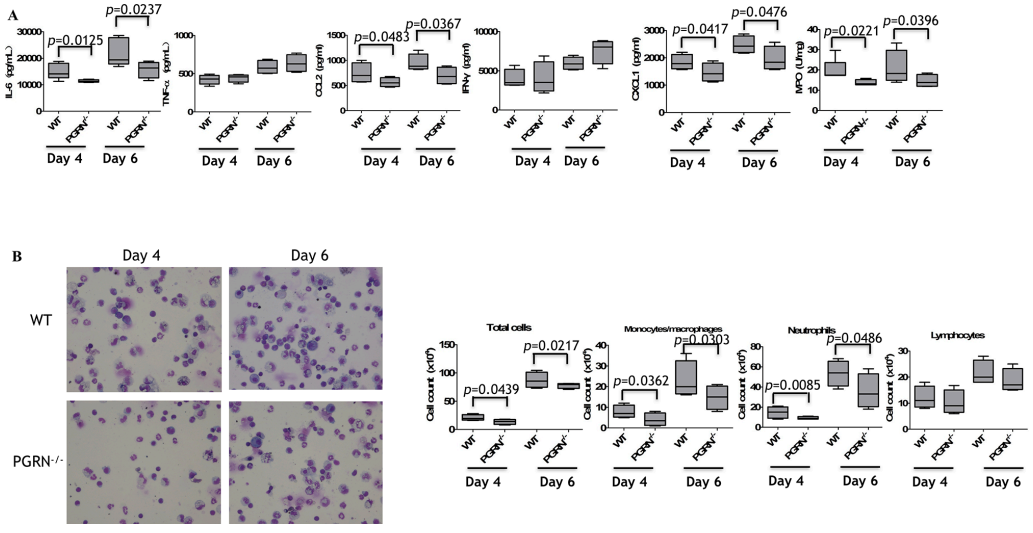

Figure 3 Loss of PGRN decreased pulmonary inflammation during influenza virus infection. WT and PGRN$^{-/-}$ mice were infected intranasally with 500 pfu of PR8. (A) Cytokines/chemokines and MPO in the lungs of WT and PGRN$^{-/-}$ mice (n=6 per group) infected with influenza virus. Samples were collected at day 4 and day 6 after infection.P values were determined by Kruskal–Wallis test followed by Dunn's multiple comparisons post-test. (B) Effects of PGRN deficiency on lung leucocyte influx during influenza virus infection. BALF samples were collected for Cytospin centrifugation and Diff-Quik staining at day 4 and day 6 in WT and PGRN$^{-/-}$ mice (n=6 per group) after influenza virus infection. The total number of leukocytes in the BALF was counted, and the total number of macrophages, neutrophils and lymphocytes was determined. P values were determined by Kruskal– Wallis test followed by Dunn's multiple comparisons post test.

and monocytes/macrophages, as well as enhancing production of cytokines and chemokines, which finally resulted in severe lung injury and substantially reduced survival during influenza infection.

Contributors Conception hypothesis and design: JC and YY; data acquisition and analysis: QL, XY, HT, JC and YY; manuscript preparation: JC and QL.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Clinical Research Ethics Committee of The First Affiliated Hospital of Chongqing Medical University.

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