International Immunopharmacology 38 (2016) 409–419

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Progranulin protects against endotoxin-induced acute kidney injury by downregulating renal cell death and inflammatory responses in mice

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ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 31 May 2016
Accepted 21 June 2016
Available online xxxx

Keywords:
Progranulin
Endotoxemia
Acute kidney injury
Apoptosis
Inflammation

ABSTRACT

Progranulin (PGRN), a pluripotent secreted growth factor, is involved in various physiologic and disease processes. However, the role of PGRN in endotoxin-induced septic acute kidney injury (AKI) remains unknown. The objective of this study is to investigate the protective effects of PGRN on an endotoxin-induced AKI mouse model by using PGRN-deficient mice and recombinant PGRN (rPGRN) pretreatment. PGRN levels were increased in kidneys of wild-type (WT) mice at 6 and 24 h after lipopolysaccharide (LPS) injection. Renal function detection, hematoxylin and eosin staining, immunohistochemical staining, ELISA and in situ terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick-end labeling were used to reveal tissue injury, inflammatory cell infiltration, production of inflammatory mediators and cell death in mouse kidneys after LPS injection. PGRN deficiency resulted in severe kidney injury and increased apoptotic death, inflammatory cell infiltration, production of pro-inflammatory mediators and the expression and nucleus-to-cytoplasmic translocation of HMGB1 in the kidney. In addition, rPGRN administration before LPS treatment ameliorated the endotoxin-induced AKI in WT mice. PGRN may be a novel biologic agent with therapeutic potential for endotoxin-induced septic AKI possibly by inhibiting LPS-induced renal cell death and inflammatory responses in mice.

1. Introduction

Lipopolysaccharide (LPS), the major component of endotoxin released from the cell wall of Gram-negative bacteria, frequently results in endotoxemia during extremely physiologically stressful conditions, including trauma, burn and infectious diseases, and causes shock and organ damage [1,2]. Sepsis-related endotoxemia is frequently associated with acute kidney injury (AKI), and sepsis is the leading cause of AKI in patients presenting to intensive care units [3,4]. The mortality rate is higher with septic than non-septic AKI [3,4]. However, the underlying mechanisms that lead to AKI during endotoxemia remain incompletely understood [5]. Despite improved nutritional and supportive care, except for renal replacement therapy, effective treatment for this devastating disease is lacking [6–8].

Many inflammatory and hemodynamic consequences are associated with severe sepsis due to endotoxemia [9]. The mechanisms involved in endotoxemia-induced septic AKI are complex and multi-factorial and include uncontrolled systemic inflammatory activation, renal ischemia, coagulopathy, cytokine/chemokine overproduction and deregulated cell apoptosis [10–12]. Among these mechanisms, a robust inflammatory process engaging both innate and adaptive immune responses is believed to cause the initial renal injury and mediate long-term structural changes [13,14]. Exposure of the kidney to high concentrations of LPS increases the production of pro-inflammatory mediators and upregulates adhesion molecules [15,16]. The infiltration of leukocytes induced by early inflammatory responses during kidney injury may lead to further damage [17]. Endotoxemia-mediated multi-factorial pathogenesis can adversely affect renal function and structure and may result in AKI.

Progranulin (PGRN) is a 593-amino-acid autocrine growth factor containing 7.5 repeats of a cysteine-rich GRN motif [18]. PGRN plays a critical role in various physiological processes, including early embryogenesis, wound healing, and host defense responses. PGRN is also widely involved in the pathogenesis of many diseases, including autoimmune disorders, atherosclerosis, and cancer [19–21]. Recent studies have highlighted the

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http://dx.doi.org/10.1016/j.intimp.2016.06.022
1567-5769/

important role of PGRN in inflammatory responses and diseases. PGRN inhibits LPS-induced inflammatory reaction in macrophages [22]. Administration of recombinant human PGRN (rPGRN) effectively alleviates inflammatory responses in rheumatoid arthritis animal models [20]. In a study of infection, PGRN-deficient mice shows limited ability for clearance of *Listeria monocytogenes* infection [22]. In addition, PGRN is reported to bind with Toll-like receptor 9 (TLR-9) and assist in the recruitment of CpG oligodeoxynucleotides in macrophages [23], so PGRN is critical in innate immunity against microorganisms. The involvement of PGRN in kidney injury has been rarely studied. We previously reported the protective role of PGRN in ischemia/reperfusion and cisplatin-induced renal injury in mouse models [24].

The endotoxemic mouse model established by LPS challenge is one of the most commonly used animal models to study the pathogenesis and potential treatment of endotoxemic AKI [9,25]. Therefore, the present study was designed to elucidate the role of PGRN in AKI in an endotoxemic mouse model. The expression of PGRN was increased in the mouse kidney during endotoxemia. PGRN deficiency enhanced the endotoxemia-induced renal function abnormality and injury, and pretreatment of rPGRN ameliorated the endotoxemia-induced renal injury.

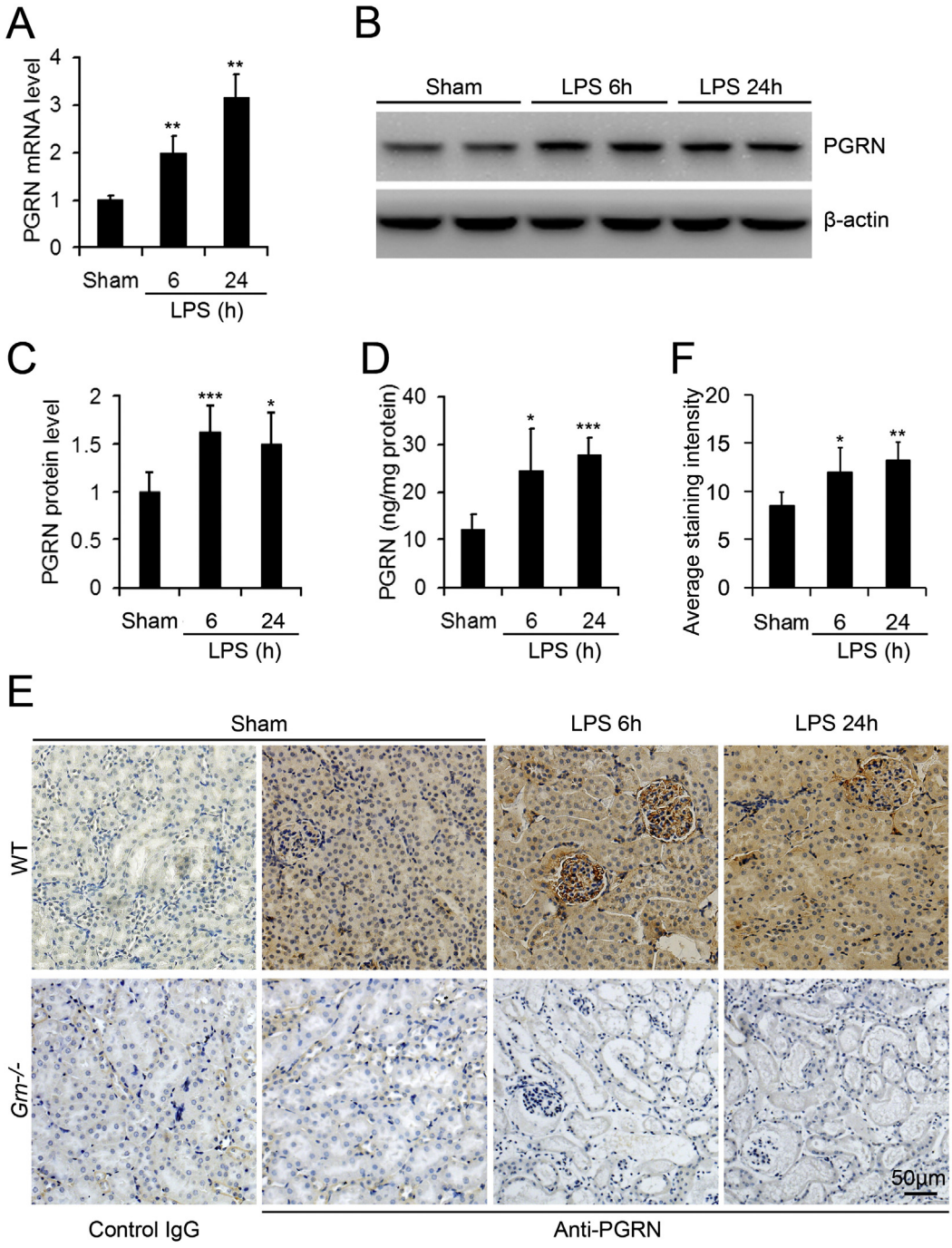

Fig. 1. Progranulin (PGRN) expression was enhanced in the kidney of an endotoxemic mouse model induced by lipopolysaccharide (LPS) injection at 25 mg/kg. (A) Relative mRNA level of progranulin (PGRN) in the kidney. (B, C) Representative western blot gel documents and summarized data showing the protein levels PGRN in kidney. β-actin was a loading control. (D) ELISA of PGRN secretion in kidney homogenates. (E) Representative immunohistochemical staining of wild-type (WT) and PGRN deficient ($Grn^{-/-}$) mouse kidney for PGRN or negative control IgG. (F) Quantification of staining intensity. Data are mean ± SD.*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with wild-type (WT) mice without LPS injection (Sham) ($n = 6$ mice/group).