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Research paper

Osteopontin exacerbates *Pseudomonas aeruginosa*-induced bacteremia in mice

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ABSTRACT

Osteopontin (OPN) is a multifunctional protein involved in various pathophysiological processes. However, the role of OPN in *Pseudomonas aeruginosa*-related sepsis is not yet clear. Here, we found that OPN expression was elevated in plasma and spleen samples from *P. aeruginosa*-infected mice. To determine the function of OPN in sepsis, we used wild-type (WT) and OPN-knockout (KO) mice with *P. aeruginosa*-induced bacteremia. We found that OPN-KO mice exhibited reduced mortality compared with WT mice and that OPN exacerbated spleen bleeding and functional impairment. OPN-KO mice exhibited reduced secretion of pro-inflammatory cytokines, such as interferon-γ, interleukin (IL)-1β, IL-12, and tumor necrosis factor-α, whereas levels of anti-inflammatory cytokine IL-10 and the leukocyte trafficking mediator macrophage inflammatory protein (MIP)-2 were not altered. Additionally, the percentages and absolute numbers of B cells were elevated in the spleens of OPN-KO mice. Thus, OPN promoted sepsis in *P. aeruginosa*-infected mice and potentially blocked B cell-dependent immunity.

1. Introduction

Pseudomonas aeruginosa is a multidrug-resistant, gram-negative ubiquitous pathogen [1,2]. In surgical settings, organ transplantation and intravenous drug abuse can easily cause *P. aeruginosa* infections of the bloodstream [3–6]. Bacterial infections play a major role in the development of sepsis, and *P. aeruginosa* is frequently isolated from patients with sepsis [7,8]. Sepsis is a type of severe systemic inflammation; patients suffer from a "cytokine storm" as a result of excessive production of pro-inflammatory cytokines, chemokines, and other inflammatory mediators [9]. Additionally, due to this abnormal host response against invading pathogens, multi-organ failure and death often occur [10]. Unfortunately, early antibiotic therapy for bacterial infections is a challenge because of the increased number of infections caused by multidrug-resistant bacteria, including *P. aeruginosa* [11].

Osteopontin (OPN) is a phosphorylated glycoprotein expressed in many tissues and immune cells during a number of physiological and pathological processes [12,13]. OPN expression is elevated in the context of bacterial infection. For example, OPN is upregulated in *Mycobacterium tuberculosis*-infected mice and is responsible for activating macrophages and increasing pathogen clearance [14]. During *Klebsiella pneumoniae*-induced pneumonia, OPN levels rapidly increase in the bronchoalveolar space, functioning to promote chemotaxis towards neutrophils and thereby facilitating an effective innate immune response [15]. *Helicobacter pylori* infection increases OPN expression in the stomach, but this is correlated with more severe gastric inflammation according to disease progression [16,17]. Moreover, *Streptococcus pneumonia*-induced pneumonia is associated with a rapid increase in pulmonary OPN concentrations in wild-type (WT) mice, whereas OPN-knockout (KO) mice show prolonged survival relative to WT mice [18]. These reports indicate that OPN is closely associated with bacterial infection; however, its specific functions are still unclear. Serum OPN levels are dramatically higher in patients with infection, suggesting that OPN may function to mediate the pathogenesis of systemic inflammatory response syndrome (SIRS) and sepsis [19]. Moreover, persistently elevated OPN serum concentrations are associated with unfavorable outcomes in patients with critical illnesses [20]. Therefore, OPN expression is correlated with experimental markers of the systemic inflammatory response and multi-organ failure.

Despite intensive efforts to elucidate the role of OPN in bacterial infection-related inflammatory pathways, the function of OPN in the

Abbreviations: OPN, osteopontin; WT, wild-type; KO, knockout; SIRS, systemic inflammatory response syndrome

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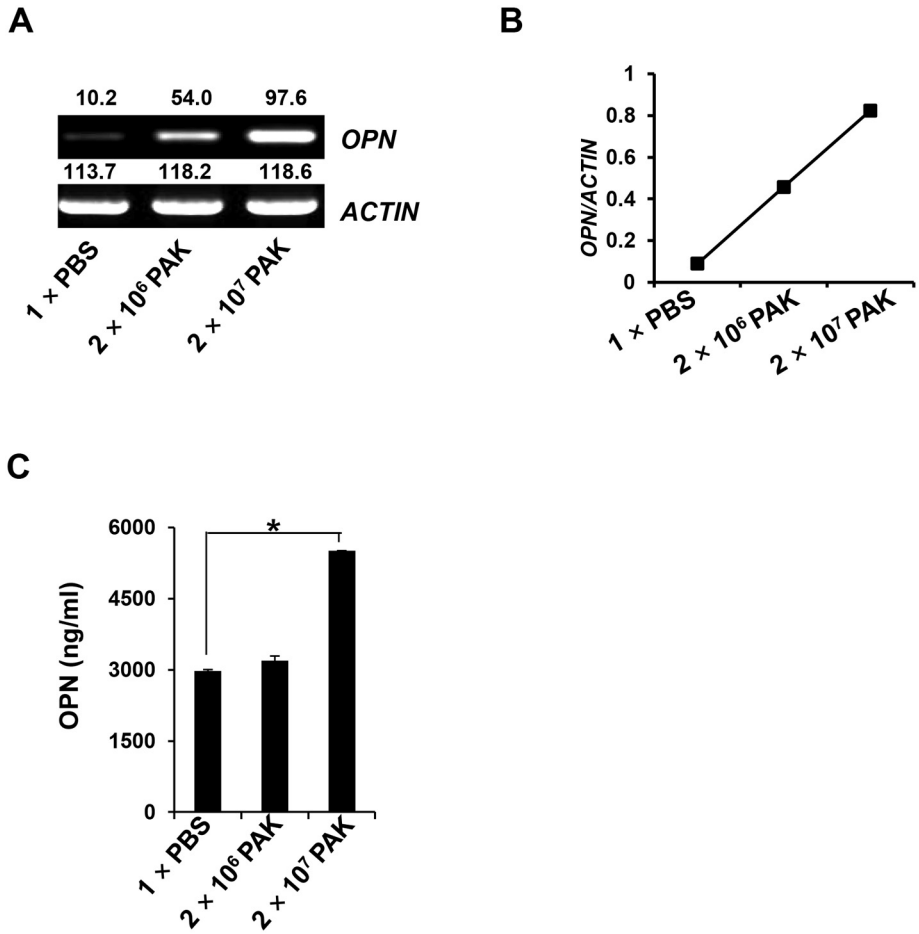

Fig. 1. OPN expression is promoted following *P. aeruginosa* infection in mice (A) WT mice were infected with the PAK strain of *P. aeruginosa* via the tail vein. At 3 h post-infection, RNA was extracted from the spleen for analysis of OPN expression by RT-PCR (n = 2 for each group). (B) Plasma samples were collected at 6 h post-infection, and OPN protein levels were quantified by ELISA (n = 3 for each group). All data are representative of at least three independent experiments and are expressed as means ± SDs. $^{*}p < 0.05$.

pathogenesis of sepsis in patients with *P. aeruginosa* infection is unclear. Therefore, in this study, we investigated the functions of OPN in mice intravascularly infected with *P. aeruginosa*. *P. aeruginosa* infection increased mortality rates and spleen tissue damage and upregulated interferon (IFN)-γ, interleukin (IL)-1β, IL-12, and tumor necrosis factor (TNF)-α. In OPN-KO mice, the percentages and absolute numbers of B cells ($B220^{+}/CD19^{+}$) increased unexpectedly. These findings demonstrate that OPN, a pro-inflammatory inducer in *P. aeruginosa*-infected mice, promotes mortality in mice with bacteremia and possibly inhibits B cell-dependent immunity.

2. Materials and methods

2.1. Mice and bacterial infections

Age- (7–10 weeks) and sex-matched C57BL/6 WT and OPN-KO mice were obtained from Hangzhou Normal University Experimental Animal Center (Hangzhou, China). All mice were maintained under specific pathogen-free standard conditions. The PAK strain of *P. aeruginosa* was cultured in Bacto Tryptic Soy Broth (cat. No. 6292241; BD Biosciences). Bacterial numbers were calculated by measuring the absorbance at a wavelength of 600 nm, and an absorbance of 0.5 was assumed to represent a bacterial concentration of 3×10^{8} CFU/mL [21]. WT and OPN-KO mice were injected with the PAK strain of *P. aeruginosa* in 100 μL of 1 × PBS via the tail vein to construct a blood infection model. All animal experiments were approved by the Animal Ethics Committee of Hangzhou Normal University.

2.2. Bacterial clearance assay

Spleens from WT and OPN-KO mice infected with 5×10^{7} CFU/mouse *P. aeruginosa* were homogenized on a cell strainer (cat. No. 352340; BD Falcon), serially diluted with 1 × PBS, and plated on tryptic soy agar plates. The plates were incubated at 37 °C overnight to allow colony formation, and viable counts were then determined.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Plasma samples from PAK-infected WT and OPN-KO mice were subjected to ELISAs for the detection of inflammatory mediators. IFN-γ (cat. No. DY485), IL-10 (cat. No. DY417), and MIP-2 (cat. No. DY452) ELISA kits were purchased from R & D Systems. The OPN (rodent) ELISA kit (cat. No. ADI-900-090A) was purchased from Enzo Life Science.

2.4. Flow cytometric analysis of immune cells from mouse spleens

Spleens from infected WT and OPN-KO mice (5×10^{7} CFU/mouse of PAK, intravenous injection) were homogenized, filtered through a cell strainer (cat. No. 352340; BD Falcon), harvested, and washed once with 1 × PBS prior to the lysis of red blood cells using RBC lysis buffer (cat. No. C3702; Beyotime). Isolated splenocytes were stained with antibodies for fluorescence-assisted cell sorting (FACS) analysis of the percentages of immune cells. The following antibodies (purchased from BD Biosciences) were used: NK1.1-PE (cat. No. 553165), CD3e-FITC (cat. No. 553062), CD11b-PE (cat. No. 557397), Ly-6G/Ly6C-FITC (cat. No. 553126), CD45R/B220-PE (cat. No. 553090), and CD19-FITC (cat. No. 553785).

2.5. Statistical analysis

All experiments were repeated at least three times. Results are