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Short communication

Deficiency of protease-activated receptor-1 limits bacterial dissemination during severe Gram-negative sepsis (melioidosis)

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Abstract

Protease-activated receptor-1 (PAR-1) is a G-coupled transmembrane receptor expressed by multiple cell types present in the lungs that can be activated by various proteases generated during acute inflammation. In this study we aimed to investigate the role of PAR-1 during melioidosis, a common cause of (pneumo)sepsis in Southeast Asia in a murine model of intranasal inoculation of the causative pathogen *Burkholderia* (*B.*) *pseudomallei*. Our results show that endogenous PAR-1 facilitates bacterial growth and dissemination during murine melioidosis, which is associated with increased cell influxes. However, these observations have no impact on survival.

Keywords: Protease-activated receptor-1; Pneumonia; Sepsis; Lung inflammation; Melioidosis

1. Introduction

Melioidosis, caused by the Gram-negative bacterium *Burkholderia* (*B.*) *pseudomallei*, is a severe septic disease, characterized by pneumonia and rapid bacterial dissemination to distant body sites, with many possible disease manifestations, septic shock being the most severe [1–3]. In Southeast-Asia and Northern-Australia melioidosis is an important cause of community-acquired sepsis with a mortality rate of up to 40% despite appropriate antibiotic therapy [1,2]. Additionally, recently *B. pseudomallei* was classified as a 'Tier 1' disease agent considered to be an exceptional threat to security [4]. Protease-activated receptors (PARs) are G-protein-coupled receptors that carry their own ligand thereby converting an extracellular proteolytic cleavage event into an intracellular signal [5,6]. PARs, of which four subtypes have been described (PAR-1 to −4), are widely distributed throughout the airways and can be activated by a variety of proteases, including pro-inflammatory factors and proteases involved in the coagulation system [5–7]. Regulation of PAR activity by proteases is important under pathological circumstances during which these proteases are released or activated. PAR activation can play a paradoxical role as it can result in both beneficial and deleterious effects depending on the PAR subtype that is activated and on the nature of the activating trigger [5–7]. When cleaved by activated protein C (APC), PAR-1 has anti-inflammatory and barrier protective effects [7,8], while PAR-1 exerts barrier disruptive effects when it is activated by high levels of thrombin [7,9,10]. Data on PAR-1 during inflammatory conditions are limited. The host response during melioidosis is characterized by upregulation of a large number of pro-inflammatory and procoagulant factors with protease activity

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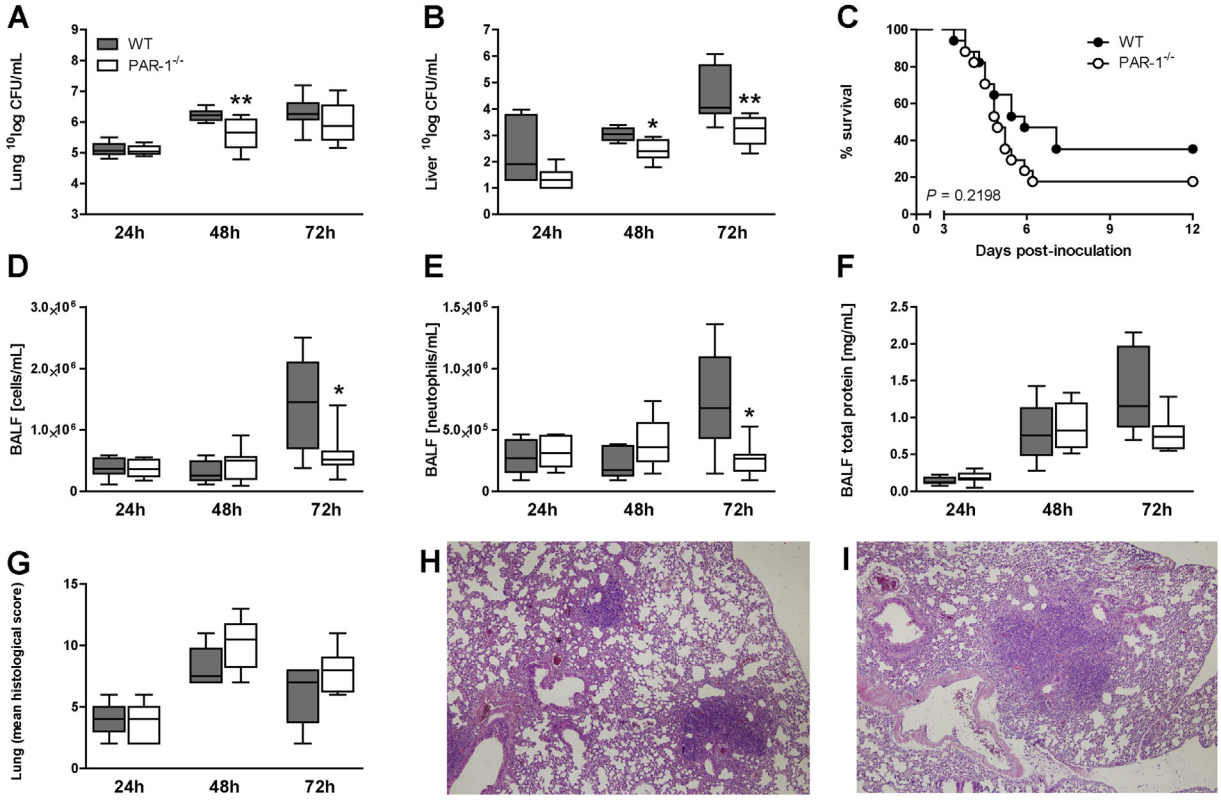

Fig. 1. Mice were inoculated intranasally with 500 CFU of *B. pseudomallei* and sacrificed after 24, 48 and 72 h. Bacterial loads were determined in lung homogenates (A), and liver homogenates (B). Mortality did not differ between WT mice ($n = 17$) and PAR-1$^{-/-}$ mice ($n = 17$; C). Cellular influx (D), numbers of neutrophils (E) and total protein content (F) in broncholaveolar lavage fluid (BALF) are shown for infected WT and PAR-1$^{-/-}$ mice. Lung histopathology scores did not differ between WT and PAR-1$^{-/-}$ mice (G): both WT (H) and PAR-1$^{-/-}$ mice (I) showed comparative inflammatory infiltrates in the lungs characterized by interstitial inflammation together with necrosis, endothelialitis, bronchitis, oedema, thrombi and pleuritis 48 h after inoculation (H&E staining, original magnification ×40). Data are expressed as box and whisker plots showing the smallest observation, lower quartile, median, upper quartile and largest observation. Grey boxes/black dots represent WT mice, white boxes/white dots represent PAR-1$^{-/-}$ mice ($n = 7$–8 mice per group for each time point, in survival studies $n = 17$–18 mice per group). *$P < 0.05$ and **$P < 0.01$ for WT versus PAR-1$^{-/-}$ mice(Mann–Whitney U test). For survival studies mortality was assessed every 6 h. Comparison between groups was done by Kaplan–Meier analysis followed by log rank tests.

[3,11]. In this study we investigated the role of PAR-1 in the host response during melioidosis.

2. Methods

Ten weeks-old male wild-type (WT) C57BL/6 mice were compared with mice deficient for PAR-1 (PAR-1$^{-/-}$) on a C57BL/6 background.PAR-1$^{-/-}$ mice, on a C57BL/6 background (backcrossed > 10×), were obtained from The Jackson Laboratory (Bar Harbour, ME) and generated as described [12]. The number of mice per group used in each experiment are provided in the Figure Legend. For each experiment all mice were infected at the same time point to avoid variance in the bacterial inoculum. All experiments were approved by the Animal Care and Use Committee of the Academic Medical Center. Melioidosis was induced by intranasal inoculation of 500 CFU of *B. pseudomallei* [13,14]. Mice were sacrificed after 24, 48 and 72 h and survival studies were performed, during which mice were checked every 6 h until death occurred. Samples were harvested and processed as described [13,14]. Bilateral bronchoalveolar lavage fluid (BALF) was obtained by exposing the trachea through a midline incision followed by cannulation with a sterile T-catheter and instilling and retrieving of two 0.5 mL aliquots of sterile phosphate buffered saline. Tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, IL-12p70, interferon (IFN)-γ and monocyte chemotactic protein (MCP)-1 were measured by cytometric bead array multiplex assay (BD Biosciences, San Jose, CA). Paraffin-embedded lung sections were stained with haematoxylin and eosin and semi-quantitatively analysed for inflammation and tissue damage as described [13,14].

3. Results

Deficiency of PAR-1 protected mice from bacterial growth and dissemination as reflected by decreased bacterial loads in lung and liver homogenates of PAR-1$^{-/-}$ mice 48 h after infection ($P < 0.01$ and 0.05 versus WT mice respectively; Fig. 1A, B). Moreover, 72 h after infection bacterial loads in livers and BALF of PAR-1$^{-/-}$ mice still were lower in comparison with WT mice (for liver $P < 0.01$; Fig. 1B, for BALF $P < 0.05$, not shown). In addition, our results demonstrate that decreased bacterial burdens as were observed in PAR-1$^{-/-}$ mice did not protect from lethality ($P = 0.22$; Fig. 1C). Next,