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The vagus nerve alters the pulmonary dendritic cell response to injury

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ABSTRACT

Background: We have shown previously that vagal nerve stimulation (VNS) protects against burn-induced acute lung injury (ALI). Although the mobilization and activation of immune cells is central to tissue injury caused by the systemic inflammatory response, the specific inflammatory cell populations that are modulated by VNS have yet to be fully defined. The purpose of this study was to assess whether VNS alters inflammatory cell recruitment to the lung after severe burn injury.

Materials and methods: Male C57BL/6 mice were subjected to 30% total body surface area steam burn with and without electrical stimulation of the right cervical vagus nerve. The relative levels of pulmonary dendritic cells (DC) and macrophages were compared at 4 h *versus* 24 h after burn injury. Lung tissue injury was characterized by histology to assess changes in lung architecture, and measure the protein levels of interleukin 6 and transforming growth factor-β1.

Results: Severe burn caused an increase in pulmonary DC recruitment at 4 h after injury that persisted at 24 h after severe burn, whereas there was no change in the number of pulmonary macrophages. In contrast, VNS limited the burn-induced recruitment of pulmonary DC. VNS prevented histologic lung injury and attenuated the release of interleukin 6 and transforming growth factor-β1 in the lung after burn injury.

Conclusions: VNS is an effective method to limit pulmonary DC recruitment to the lung and prevent ALI after burn injury. Identifying strategies to limit inflammatory cell recruitment to the lung may have clinical utility in preventing ALI in severely burned patients.

1. Introduction

Severe injury such as trauma or burn is often associated with a systemic inflammatory response that results in tissue injury and multiorgan dysfunction. Acute lung injury (ALI) is a common site of distant organ injury that affects severely injured patients and can progress to acute respiratory distress syndrome (ARDS). ARDS develops in 6.5%–14.3% of severely burned patients leading to prolonged mechanical ventilation, longer intensive care unit, and hospital stays,

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and is associated with increased morbidity and mortality [1,2]. This is independent of inhalation injury, and is defined as indirect ALI. The immune mechanisms that cause ALI are still not well understood and there are currently no therapeutic measures or medications to prevent or directly treat ALI.

There is mounting evidence that dysregulation of the innate immune system contributes to the inititation and progression of ALI and ARDS [3,4]. It has been shown that the antigen presenting cells of the immune system play a role in the lung immune response to injury that causes ALI [5]. Dendritic cells (DC) are potent antigen-presenting cells that respond to sites of injury and are crucial for the priming phase of the immune response [6]. ALI is more severe in mice lacking DC, suggesting that it plays a crucial role in mediating lung injury [5]. On activation, pulmonary DC produce a range of inflammatory mediators [7,8] including the cytokines interleukin (IL)-6 and transforming growth factor (TGF)-beta [9,10]. TGF-beta has been shown to be an early critical mediator of ALI [11]. Pulmonary macrophages likely play a role in the resolution of ALI as the increase in number of macrophages in ALI is delayed [4].

Vagal nerve stimulation (VNS) has been shown to exert potent anti-inflammatory effects in multiple models of injury by altering the response of myeloid and lymphoid cells [12,13]. We have previously shown that VNS prevents ALI after burn injury with decreased recruitment of neutrophils and decreased activation of the nuclear factor-kappa B signaling pathway [14], as well as a reduction in vascular permeability of lung capillaries [15]. The protective effects of VNS on the lung were lost in animals that underwent surgical abdominal vagotomy, suggesting that VNS alters the systemic inflammatory response to injury via the gut-lung axis [14,16]. The ability of VNS to modulate the recruitment of inflammatory cells to the lung after injury is unknown. We postulated that VNS would limit lung injury by preventing recruitment of mature DC to the lung in a mouse model of severe burn injury.

2. Materials and methods

2.1. Animal model of severe burn injury

Male C57Bl/6 mice 8–10 wk old (Jackson Laboratories, Sacramento, CA) were placed under general anesthesia using inhaled isoflurane. The dorsal fur was removed using an electronic clipper. While under general anesthesia, mice were placed in a template estimating 30% total body surface area and subjected to a steam burn for 7 s as we have previously described [17,18]. Immediately after burn, animals received a subcutaneous injection of 1.0 mL of 0.9% normal saline containing 0.1 mL of buprenorphine (12 μg/mL) for analgesia and fluid resuscitation. This model of burn injury is associated with a mortality of ~5%. Sham animals were placed under anesthesia, underwent dorsal fur removal, and received an injection of buprenorphine, but were not burned. All animal experiments were approved by the University of California Animal Subjects Committee and were conducted in accordance with accepted guidelines for animal studies.

2.2. Vagal nerve stimulation

Based on previous studies suggesting a role for VNS in regulating the inflammatory response in the lung, animals were treated with cervical VNS at 30 min after injury to determine whether VNS alters inflammatory cell recruitment to the lung. Thirty minutes after burn injury, a right cervical neck incision was performed and the right cervical vagal nerve exposed. Electrical VNS was performed using a square wave generator at 5 V, with a frequency of 5 Hz for 10 min. The incision was closed with skin glue. The burn animals who did not receive VNS underwent right cervical incision and exposure of the vagus nerve, but did not receive electrical stimulation. Mice were selected for VNS with no formal randomization.

2.3. Histologic evaluation of the lung

Lungs were harvested 4 h after injury for hematoxylin and eosin staining. The lungs were collected after first an intracardiac flush with 10 mL of phosphate buffered saline, then the lungs were injected with intratracheal 4% Paraformaldehyde, and then fixed in a 10% formalin solution before being embedded in paraffin. Hematoxylin and eosin staining of the lung was performed by the University California, San Diego Histology Core Services ($n = 3$ mice per experimental condition). An experienced investigator blinded to experimental groups analyzed multiple fields from sections of the lung images at ×20 and ×60 with a light microscope. Sections were scored according to the pulmonary injury scoring system previously used by our laboratory [19]. Sections were rated on a scale from 0–3 ranging from normal to severe. Sections were analyzed based on the amount of intra-alveolar hemorrhage, pulmonary congestion, edema, and infiltration of inflammatory cells to yield a maximum possible score of 12. Lung injury scores were averaged for each experimental condition.

2.4. Enzymatic lung digestion and flow cytometry

Lung tissue cells were isolated from normal saline perfused whole lung collected at 4 h and 24 h after burn. A total of five mice were used for each group and each time point. The lung tissue was then minced and placed in the enzyme solution collagenase A/dispase II and incubated at 37°C for 20 min. The lung tissue was then passed through a 70 μm filter and digestion was quenched with 5% fetal bovine serum solution. The cells were stained with anti-mouse monoclonal antibodies including PE Cy7-labeled anti-CD11c (HL3; BD Biosciences, San Jose, CA), APC Cy7-labeled anti-CD11 b (M1/70; BD Biosciences), and APC-labeled anti-MHC II (M5/114.15.2; eBioscience, San Diego, CA). Vermaelen and Pauwels [20] have shown that mouse and human pulmonary DC can be distinguished in dissociated lung tissue on the basis of autofluorescence. Cells were analyzed by gating for CD11c + cells before identifying cells with low autofluorescence (pulmonary DC) or high autofluorescence (pulmonary macrophages) [20]. Each cell population was then characterized for the presence of cell surface markers CD11 b or MHCII to assess a DC phenotype. Cells were acquired and analyzed using BD Accuri C6 Flow Cytometer.