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Role of the NLRP3 inflammasome in a model of acute burn-induced pain

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ABSTRACT

The NLRP3 inflammasome is a multi-protein complex that assembles in response to tissue damage or infection, triggering activation of caspase-1, an enzyme that converts interleukin (IL)-1β into its active form. A role for the NLRP3 inflammasome is emerging in inflammatory pain, but its influence in other pain types is largely unexamined. Therefore the aim of this study was to assess the role of the NLRP3 inflammasome and its downstream product caspase-1 in a model of acute burn-induced pain in male mice. A superficial burn was induced on the plantar surface of the left hind paw using a hot plate set at 52.5°C for 25 s. Development of burn-induced mechanical allodynia, thermal allodynia, edema and weight bearing changes was assessed in *Nlrp3*$^{-/-}$ and caspase-1-deficient (*Ice*$^{-/-}$) mice, and in mice administered the selective NLRP3 inflammasome inhibitor MCC950. Burn-induced mechanical and thermal allodynia developed normally in *Nlrp3*$^{-/-}$ and *Ice*$^{-/-}$ mice and mice administered MCC950. Burn-induced edema was significantly reduced in *Ice*$^{-/-}$ mice only. Burn-induced weight bearing changes were attenuated in *Nlrp3*$^{-/-}$ mice and mice administered MCC950 72 h after burn only. This study suggests that NLRP3 and its downstream product caspase-1 have a limited role in the development of burn-induced pain.

1. Introduction

Inflammasomes, which include NLRP1, NLRP3, NLRC4 and AIM2, are multi-protein complexes that assemble in response to tissue damage or infection. The NLRP3 inflammasome is of particular interest, as it is implicated in a range of autoimmune, inflammatory and metabolic diseases, including gout, diabetes and multiple sclerosis [1,2]. Once activated, the NLRP3 inflammasome triggers activation of caspase-1 (formally known as interleukin-1 converting enzyme; ICE), an enzyme that cleaves interleukins (IL)-1β and -18 into their active forms

Abbreviations: BL, baseline; ICE, interleukin-1 converting enzyme; IL, interleukin; PWF, paw withdrawal force; SEM, standard error of the mean; wt, wild type.

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[1,3]. IL-1β is an important mediator of the inflammatory response, promoting fever, vasodilation and infiltration of immune cells into inflamed tissue [4]. In addition, IL-1β causes mechanical and thermal hypersensitivity when administered peripherally, through both direct activation of nociceptors and through upregulation of other pro-nociceptive mediators, such nerve growth factor, cyclooxygenase type 2 and substance P [3–8].

Burn triggers an acute inflammatory response, which can be restricted to the site of injury or involve systemic inflammatory processes, resulting in pain of a complex pathology that can be difficult to manage. IL-1β is reported to be present in the blister fluid of burns patients and elevated in plasma from humans with a burn, suggesting involvement of IL-1β in burn-induced pain [9–12]. In addition, transcripts of NLRP3 have been shown to be upregulated in the adipose tissue of burns patients, suggesting that the generation of IL-1β, which was also upregulated, may be a consequence of NLRP3/caspase-1-dependent pathways following burn [12]. However, the specific role of the NLRP3 inflammasome and its downstream products in burn-induced pain has not been assessed, and was the focus of the present study. Using a previously established mouse model of acute burn [13], we assessed burn-induced mechanical allodynia, thermal allodynia, edema and weight bearing changes in *Nlrp3* and *Ice* knockout mice, and in mice administered the selective NLRP3 inflammasome inhibitor MCC950 [14].

2. Methods

2.1. Animals

For behavioural assessment we used adult male *Nlrp3*$^{-/-}$ [15] and *caspase-1*$^{-/-}$/*caspase-11*^{null} (*Ice*$^{-/-}$) [16] mice aged 6–8 weeks, backcrossed on C57BL/6J background at least ten times, with age matched male C57BL/6J mice used as controls. Animals were housed in groups of 3 or 4 per cage, under 12 h light–dark cycles and had standard rodent chow and water ad libitum. All behavioural assessment was performed by a blinded investigator who was unaware of the genotype and/or treatments received.

Ethical approval for in vivo experiments in animals was obtained from the University of Queensland animal ethics committee. Experiments involving animals were conducted in accordance with the Animal Care and Protection Regulation Qld (2012), the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, 8th edition (2013) and the *International Association for the Study of Pain Guidelines for the Use of Animals in Research*.

2.2. Burn model

To induce a mild superficial burn, the entire plantar surface of the left hind paw of mice was applied with firm pressure to a Peltier plate (Hot/Cold Plate, Ugo Basile, Comerio, Italy) set at 52.5 °C for 25 s under light isoflurane (3%) anaesthesia as previously described [13]. The resulting burn caused localised erythema and edema, without the presence of blisters. Behavioural assessment was performed daily for three days after the burn. The same animals were used for each of the behavioural tests. Wild type animals were randomized to receive either MCC950 (50 mg/kg) [14] or vehicle control (saline) administered by intraperitoneal injection (10 μL/g) at the time of burn, then once daily for three days.

2.3. Electronic von Frey

Mechanical allodynia was assessed using an electronic von Frey apparatus (MouseMet Electronic von Frey, TopCat Metrology) as previously described [13]. Mice were habituated in individual mouse runs for at least 5 min prior to testing. A soft-tipped probe was applied to the plantar surface of each hind paw with pressured applied at a force rise rate of ~1 g/s. The force that elicited a paw withdrawal was determined by the MouseMet Software. The paw withdrawal force (PWF) was determined from the average of three tests, separated by at least 2 min.

2.4. Hargreaves test

Mice were habituated individually in polyvinyl boxes (10 × 10 × 10 cm) placed on heated glass maintained at 25 °C for at least 30 min prior to behavioural assessment using the Hargreaves test (Plantar Analgesia Meter, IITC, CA, USA). The radiant heat light source (intensity 15%) was focused on the plantar surface of each hind paw and the time taken for the mouse to withdraw the paw was recorded, with a cut-off of 20 s to prevent tissue damage. The mean time to withdrawal was determined from the average of three tests, separated by at least 1 min.

2.5. Paw thickness

Paw thickness was measured along the distal-proximal axis at the metatarsal level using a digital vernier caliper (Kincrome, Vic, Australia) whilst under light isoflurane (2%) anaesthesia. The paw thickness of the ipsilateral hind paw was normalised to the contralateral hind paw for each individual animal and measured 6 h following burn, then daily for 3 days.

2.6. Weight bearing

The Catwalk XT (Noldus Information Technology, The Netherlands) was used to assess weight-bearing behaviour. Mice were placed individually at one of elevated enclosed glass walkway and allowed to walk freely to the other end, with a high-speed camera located below to record illuminated paw prints. Criteria for a successful run were a completion time of less than 10 s and a speed variance of less than 100%. Three successful runs were recorded for each animal at each time point. Recordings were analysed using the Catwalk XT software using the paw pressure parameter 'mean intensity of the 15 most intense pixels' for the left hind paw as a measure of weight bearing.

2.7. Data analysis

Data were plotted and analysed by GraphPad Prism, version 6.0. Statistical significance was defined as $P < 0.05$ and was

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