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Role of complement anaphylatoxin receptors in a mouse model of acute burn-induced pain

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ABSTRACT

The complement system is an essential component of the innate immune response. The anaphylatoxins C3a and C5a are key drivers of the complement system, acting through the receptors C3aR, C5aR1 and C5aR2 to regulate inflammation. While a role for C5a activation of C5aR1 in inflammatory and neuropathic pain has been established, the role of the complement system in burn-induced pain has not been investigated. To address this gap, we assessed the role of complement receptors C3aR, C5aR1 and C5aR2 in a mouse model of acute burn-induced pain. Superficial burn injury was induced in C57BL/6 mice by firm application of left hind paw plantar surface to a hot plate set at 52.5 °C for 25 s. Development of burn-induced mechanical allodynia, thermal allodynia, weight bearing changes and edema was assessed in C3aR$^{-/-}$, C5aR1$^{-/-}$ and C5aR2$^{-/-}$ mice and compared to their wild type controls over three days. Burn-induced mechanical allodynia, thermal allodynia and weight bearing changes developed normally C3aR$^{-/-}$, C5aR1$^{-/-}$ and C5aR2$^{-/-}$ mice. However, burn-induced edema was significantly reduced in C5aR2$^{-/-}$ male mice, but not C5aR2$^{-/-}$ female mice. These results suggest that the complement system has a limited role in the development of acute burn-induced pain.

1. Introduction

The complement system is an essential component of the innate immune system, consisting of over 40 soluble proteins, receptors and regulators (Merle et al., 2015). Activation of the complement system causes a cascade of enzymatic reactions, leading to the production of the anaphylatoxins C3a and C5a. These protein fragments are central regulators of the inflammatory response that exert their specific actions through the receptors C3aR, C5aR1 and C5aR2 (previously known as C5L2) to activate and recruit immune cells to sites of inflammation (Hawksworth et al., 2017; Zipfel and Skerka, 2009). Accordingly, the complement system may be a potential target for the treatment of pain.

Most studies to date have focused on the role of C5a-C5aR1 activation in pain. Intraplantar administration of C5a in mice causes mechanical and heat allodynia, as well as upregulation of other inflammatory mediators, including interleukin-6 (IL-6), tumour necrosis factor (TNF-α), prostaglandin E2 (PGE_2) and nerve growth factor (NGF) (Shutov et al., 2016; Moriconi et al., 2014). In mice deficient of C5aR1, development of mechanical and thermal allodynia is attenuated following administration of Freund's Complete Adjuvant (FCA), and in models of spared nerve injury and post-surgical pain (Shutov et al., 2016; Moriconi et al., 2014; Liang et al., 2012). In addition, the C5aR1 antagonists PMX53 and DF2593A have anti-nociceptive activity in multiple rodent models of inflammatory and neuropathic pain (Shutov et al., 2016; Moriconi et al., 2014; Liang et al., 2012; Ting et al., 2008). This therefore makes C5aR1 a promising novel target for the treatment of pain. While the role of C5aR1 activation in pain is well established, the role of C3aR activation and C5aR2 activation in pain has not been investigated.

Burn injury triggers an acute inflammatory response that results in pain of complex pathology that is difficult to treat (Morgan et al., 2017). In humans and rodents, levels of C3a and C5a are elevated in plasma and in burn blister fluid following partial and full thickness burn injury, suggesting involvement of the complement system in burn-induced pain (Bengtson and Heideman, 1987; Schmid et al., 1997). However, the specific role of C3aR, C5aR1 and C5aR2 activation in burn-induced pain has not been assessed. Therefore, the aim of this study was to use a mouse model of burn injury to assess the development of pain behaviours in C3aR, C5aR1 and C5aR2 knockout mice.

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2. Methods

2.1. Animals

For behavioural assessment we used adult male and female homozygous C3aR deficient (C3aR$^{-/-}$) (Wu et al., 2013), homozygous deficient C5aR1 (C5aR1$^{-/-}$) (Hollmann et al., 2008), and homozygous C5aR2 deficient (C5aR2$^{-/-}$) (Biggins et al., 2017) mice aged 6–8 weeks on a fully congenic C57BL/6J background. Age and sex matched C57BL/6J mice were 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*. Behavioural assessment was performed by a blinded investigator unaware of each animal's genotype.

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 injury model

Anaesthesia was induced and maintained with 2% isoflurane for the duration of the procedure. The plantar surface of the left hind paw of each mouse was held in contact with a hot plate (Hot/Cold Plate, Ugo Basile, Comerio, Italy) set at 52.5 °C for 25 s, producing a superficial burn injury as previously described (Yin et al., 2016). Mice were then immediately returned to their home cages and allowed to recover from anaesthesia. Behavioural assessment was performed daily after burn injury at the time points indicated. The same animals were used for each of the behavioural tests.

2.3. Electronic von Frey

Mechanical allodynia was assessed using electronic von Frey (MouseMet Electronic von Frey, TopCat Metrology Ltd, United Kingdom) as previously described (Deuis and Vetter, 2016). Mice were habituated in individual runs for at least 10 min prior to testing each day. A soft-tipped probe was applied to the plantar surface of the left hind paw and the force was increased continuously at a force rise ramp of ~1 g/s until paw withdrawal occurred. The paw withdrawal force (PWF) was determined by the average of three tests.

2.4. Thermal probe test

Heat allodynia was assessed using the thermal probe test (MouseMet Thermal, TopCat Metrology Ltd, United Kingdom) as previously described (Deuis and Vetter, 2016). Mice remained in the same individual runs used for electronic von Frey. A 2.5 mm diameter metal probe pre-heated to 37 °C was applied to the plantar surface of the left hind paw and heated at a rate of 2.5 °C/s until paw withdrawal occurred. The paw withdrawal temperature (PWT) was determined by a single test. A cut off temperature of 60 °C was used to prevent tissue damage.

2.5. Weight bearing

Changes in weight-bearing of the hind limbs was assessed using the Catwalk XT (Noldus Information Technology, The Netherlands) as previously described (Yin et al., 2016). Mice were allowed to freely traverse across an elevated enclosed glass walkway with internally reflected light. Illuminated paw prints were recorded below by a high-speed camera and analysed using the Catwalk XT software. For a run to be successful, mice had to walk across the glass walkway in less than 10 s with a speed variance of less than 100%. Three successful runs were recorded for each mouse at each time point. The parameter 'mean intensity of the 15 most intense pixels' was used as a surrogate measure of weight bearing for the left hind paw, and this was normalised to the right hind paw of each animal as previously described (Yin et al., 2016).

2.6. Paw thickness

Paw thickness was measured along the distal-proximal axis at the metatarsal level using a digital vernier caliper (Kincrome, Vic, Australia) at the completion of daily behavioural tests. The paw thickness of the left hind paw was normalised to the right hind paw for each individual animal.

2.7. Data analysis

Data were plotted and analysed by GraphPad Prism, version 6.0. Statistical significance was defined as $P < .05$ and was determined by one-way ANOVA with Dunnett's post-test or two-way ANOVA with Sidak's post-test compared to wild type animals, as indicated. Data are expressed as the mean ± standard error of the mean (SEM).

3. Results

3.1. Basal mechanical and heat sensitivity is normal in C3aR$^{-/-}$, C5aR1$^{-/-}$ and C5aR2$^{-/-}$ mice

To determine if complement receptors contribute to basal mechanical and thermal sensitivity, we assessed the mechanical and heat thresholds of C3aR$^{-/-}$, C5aR1$^{-/-}$ and C5aR2$^{-/-}$ mice prior to burn injury. While this has previously been reported in C5aR1$^{-/-}$ mice (Shutov et al., 2016; Liang et al., 2012), this is the first study to report basal sensitivity of C3aR$^{-/-}$ and C5aR2$^{-/-}$ mice. No significant differences were found in basal mechanical thresholds in males (Fig. 1A; PWF: wild type, 3.5 ± 0.1 g; C3aR$^{-/-}$, 3.4 ± 0.2 g; C5aR1$^{-/-}$, 4.0 ± 0.3 g; C5aR2$^{-/-}$, 3.9 ± 0.2 g;) or females (Fig. 1B PWF: wild type, 3.3 ± 0.1 g; C3aR$^{-/-}$, 3.5 ± 0.2 g; C5aR1$^{-/-}$, 3.3 ± 0.1 g; C5aR2$^{-/-}$, 3.6 ± 0.2 g;) compared to wild type controls. Similarly, no significant differences were found in basal heat thresholds of males (Fig. 1C; PWT: wild type, 50.8 ± 0.2 °C; C3aR$^{-/-}$, 51.1 ± 0.4 °C; C5aR1$^{-/-}$, 51.1 ± 0.5 °C; C5aR2$^{-/-}$, 51.4 ± 0.9 °C) or females (Fig. 1D; PWT: wild type, 50.8 ± 0.3 °C; C3aR$^{-/-}$, 51.1 ± 0.8 °C; C5aR1$^{-/-}$, 51.2 ± 0.4 °C; C5aR2$^{-/-}$, 51.7 ± 0.7 °C) compared to wild type controls.

3.2. Acute burn-induced mechanical allodynia, thermal allodynia, reduced weight bearing and edema develop normally in C3aR$^{-/-}$ mice

To determine the functional contribution of C3aR to the development of burn-induced pain, we assessed the mechanical thresholds, heat thresholds, and changes in weight bearing behaviour of male and female C3aR$^{-/-}$ mice following a burn injury to the hind paw. Burn-induced mechanical allodynia developed normally in both male and female C3aR$^{-/-}$ mice, with no significant differences compared to wild type controls at any of the time points tested (Fig. 2A,B). Similarly, burn-induced heat allodynia developed normally in both male and female C3aR$^{-/-}$ mice, with no significant differences compared to wild type controls at any of the time points tested (Fig. 2C,D). Burn injury led to a reduction in weight bearing of the injured hind paw, and this was not significantly different at any of the time points tested in male or female C3aR$^{-/-}$ mice compared to wild type controls (Fig. 2E,F). To determine the role of C3aR in the development of burn-induced edema, we also assessed the paw thickness of male and female C3aR$^{-/-}$ mice following burn injury. No significant differences in paw thickness were found in male or female C3aR$^{-/-}$ mice at any of the time points tested compared to wild type controls (Fig. 2G, H).

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