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Short communication

Extracellular adenosine 5′-triphosphate and lipopolysaccharide induce interleukin-1β release in canine blood

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ABSTRACT

Binding of extracellular adenosine 5′-triphosphate (ATP) or lipopolysaccharide (LPS) to the damage-associated molecular pattern receptor P2X7 or the pathogen-associated molecular pattern receptor Toll-like receptor (TLR)4, respectively, can induce the release of the pleiotropic cytokine interleukin (IL)-1β in humans and mice. However, the release of IL-1β in dogs remains poorly defined. Using a canine IL-1β enzyme-linked immunosorbent assay, this study investigated whether ATP or LPS could induce IL-1β release in a canine blood-based assay. Short-term incubations (30 min) with ATP induced IL-1β release in LPS-primed canine blood, and this process could be near-completely impaired by the P2X7 antagonist, A438079. In contrast, ATP failed to induce IL-1β release from blood not primed with LPS. ATP-induced IL-1β release was observed with LPS-primed blood from eight different pedigrees or cross breeds. Long-term incubations (24 h) with LPS induced IL-1β release in canine blood in a concentration-dependent manner. This process was not altered by co-incubation with A438079. LPS-induced IL-1β release was observed with blood from 10 different pedigrees or cross breeds. These results demonstrate that both extracellular ATP and LPS can induce IL-1β release in dogs, and that ATP- but not LPS-induced IL-1β release in blood is dependent on P2X7 activation. These findings support the role of both P2X7 and TLR4 in IL-1β release in dogs.

1. Introduction

Interleukin (IL)-1β (or IL-IF2) is a pleiotropic cytokine predominately produced by monocytes and macrophages, and has key roles in immunity, inflammation, haematopoiesis and metabolism (Dinarello, 2011). As a result, IL-1β has emerged as a promising therapeutic target for treating various local and systemic inflammatory conditions in humans (Dinarello et al., 2012). IL-1β is also expressed in dogs (Jalilian et al., 2012b); however, knowledge of IL-1β in canine biology is largely limited to a small number of mRNA expression studies, where IL-1β has been reported to be up-regulated in either infectious, musculoskeletal, cardiac or respiratory disorders (Chen et al., 2012; Kiczak et al., 2008; Maccoux et al., 2007; Unver et al., 2006). More recently, increased IL-1β release has been observed in canine inflammatory disorders of the bowel and eye (Maeda et al., 2012; Wichayacoop et al., 2009). Nevertheless very little is known about the mechanisms involved in IL-1β release in dogs. Thus, further information regarding canine IL-1β and its release

Abbreviations: ATP, adenosine 5′-triphosphate; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; LPS, lipopolysaccharide; PBMCs, peripheral blood mononuclear cells; TLR, Toll-like receptor.

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is required to understand the role of this cytokine in canine biology and to translate any potential therapeutic benefits of targeting IL-1β in humans to dogs.

IL-1β is not constitutively expressed in monocytes, but is upregulated following exposure of cells to pro-inflammatory mediators including lipopolysaccharide (LPS) (Dinarello, 1996), a process referred to as priming. In humans and mice, LPS binds to the pathogen-associated molecular pattern receptor Toll-like receptor (TLR)4 to induce IL-1β expression and synthesis in monocytes (Hoshino et al., 1999; Medzhitov et al., 1997). Incubation with LPS also induces the expression of NALP3 (or NLRP3) (Bauernfeind et al., 2009), which assembles with caspase-1 to form the NALP3 inflammasome to mediate IL-1β maturation and release (Agostini et al., 2004). Although LPS appears sufficient to induce the slow (24 h) release of IL-1β (Lepe-Zuniga and Gery, 1984), a second signal, such as activation of the damage-associated molecular pattern receptor P2X7 by its ligand extracellular adenosine 5′-triphosphate (ATP), is required for the rapid (30 min) release of IL-1β following its synthesis (Grahames et al., 1999; Solle et al., 2001). P2X7 activation induces IL-1β release from LPS-primed canine monocytes (Jalilian et al., 2012a) and in LPS-primed canine blood (Roman et al., 2009), however, it remains unknown if LPS priming is required for P2X7-induced IL-1β release in dogs. In human monocytes, LPS can induce the slow release of IL-1β by both P2X7-dependent (Netea et al., 2009; Piccini et al., 2008) and P2X7-independent mechanisms (Ward et al., 2010). LPS can also induce IL-1β release from canine peripheral blood mononuclear cells (PBMCs) (Baggio et al., 2005), but whether this process is dependent on P2X7 activation remains to be established. Blood-based assays are commonly used to study cytokine release *ex vivo*, as they require fewer manipulations compared to assays that use purified blood leukocytes, and thereby limit the inadvertent activation of cells. Therefore using a blood-based assay the current study investigated first, if canine P2X7-induced IL-1β release requires LPS priming and second, if LPS is sufficient to induce canine IL-1β release and if so, whether this process involves P2X7 activation.

2. Materials and methods

2.1. Materials

RPMI-1640 medium and penicillin–streptomycin–glutamine were from Invitrogen (Grand Island, NJ). LPS (*Escherichia coli* serotype 055:B5), ATP (BioXtra) and bovine serum albumin (fatty acid and globulin free) were from Sigma Chemical Co (St Louis, MO). A438079 was from Tocris Bioscience (Ellisville, MO).

2.2. Blood-based IL-1β release assay

Peripheral blood was collected into VACUETTE® lithium heparin tubes (Greiner Bio-One, Frickenheisen, Germany) from either 11 pedigree or four cross breed dogs with informed, signed consent of pet owners, and with the approval of the University of Wollongong Ethics Committee (Wollongong, Australia). Eleven of the animals were healthy at the time of blood collection, while four presented with minor health problems (an Alaskan Malamute with a fractured carnassial tooth, a Maltese and Shih Tzu cross with dental disease, a Labrador Retriever with otitis externa and a Shar Pei with bilateral otitis externa). All animals had no evidence of fever at the time of blood collection.

The release of IL-1β from cells in blood was performed as described (Perregaux et al., 2000), with minor modification. Briefly, 100 μL of canine blood and 100 μL of RPMI-1640 medium (containing 10 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine) were combined into flat-bottomed 96-well plates (Greiner Bio-One). In the first series of experiments, diluted blood was incubated in the absence or presence of 0.1 μg/mL LPS for 2 h at 37 °C/5% CO_2, and then in the absence or presence of 6 mM ATP for a further 30 min. In some experiments, 50 μM A438079 was added during the final 15 min of the 2 h LPS incubation before ATP addition. In the second series of experiments, diluted blood was incubated with 0–10 μg/mL LPS in the absence or presence of 50 μM A438079 for 24 h at 37 °C/5% CO_2. Samples from each series were centrifuged at 700 × *g* for 10 min, and the cell-free supernatants stored at −80 °C until required. The amount of IL-1β in cell-free supernatants was quantified using a Canine IL-1β VetSet™ enzyme-linked immunosorbent assay (ELISA) Development Kit and ELISA Accessory Pack (both Kingfisher Biotech, St. Paul, MN) with 4% bovine serum albumin in Dulbecco's phosphate-buffered saline used as the Assay Diluent as per the manufacturer's instructions.

2.3. Statistical analysis

Differences between groups were compared using a one-way analysis of variance (using Tukey's post test).

3. Results and discussion

3.1. P2X7 activation induces IL-1β release in canine blood and requires priming with LPS

Our group and one other have previously demonstrated that P2X7 activation induces canine IL-1β release from LPS-primed monocytes (Jalilian et al., 2012a) or in LPS-primed blood (Roman et al., 2009), however, it remains unknown if LPS priming is required for P2X7-induced IL-1β release in dogs. Therefore, to confirm that P2X7 activation induces IL-1β release in blood, LPS-primed blood was incubated in the absence or presence of the P2X7 antagonist A438079 (Nelson et al., 2006) before 30 min incubation in the absence or presence of ATP, and measurement of IL-1β in cell-free supernatants by ELISA. Consistent with previous findings (Roman et al., 2009), ATP in the absence of P2X7 antagonist induced IL-1β release in LPS-primed blood, which was on average four-fold greater than that of LPS-primed blood incubated in the absence of ATP, which was minimal (Fig. 1A). In LPS-primed blood, pre-incubation with A438079 impaired ATP-induced IL-1β release by 97 ± 9% (mean ± standard deviation) compared to ATP-induced IL-1β release in the absence of A438079