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INTERLEUKIN-33 MEDIATES FORMALIN-INDUCED INFLAMMATORY PAIN IN MICE

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Abstract—Interleukin-33 (IL-33), a member of the IL-1 family, has attracted growing interest since its discovery in 2003. IL-33 has been implicated in many diseases, including arthritis, asthma, allergies, and cardiovascular and infectious diseases. However, few studies have investigated its role in the transmission and modulation of pain. The present study was designed to explore the possible roles of IL-33 and its receptor, ST2, in formalin-induced inflammatory pain in mice. We found that both subcutaneous (s.c., 300 ng) and intrathecal injection (i.t., 3 ng) of recombinant IL-33 (rIL-33) increased paw lifting and licking time not only in normal mice but also in formalin models. Administration of ST2 antibody, which blocked the IL-33/ST2 signaling, alleviated the formalin-induced spontaneous pain behavior. Moreover, the ST2$^{-/-}$ mice showed significantly decreased pain behavior, as well as reduced ultrasonic vocalization induced by formalin, compared with the wild-type group. Additionally, ST2 antibody alleviated the potentiating effects of rIL-33 on pain behavior in the formalin mice, indicating that IL-33 plays a role in pain modulation through its ST2 receptor. These data suggest IL-33 and its ST2 receptor mediate formalin-induced inflammatory pain, and as a result this cytokine and its receptor may be new targets for the development of analgesics.

Key words: interleukin-33, ST2, pain, formalin test.

INTRODUCTION

Interleukin-33 (IL-33) is a novel member of IL-1 family, which also includes IL-1 and IL-18. It was first identified as a nuclear factor in high endothelial venules (Baekkevold et al., 2003) and later recognized as a specific extracellular ligand for ST2 (Schmitz et al., 2005) based on its β-trefoil structure at the carboxyl terminus, which is a conserved structure for IL-1 cytokines. In the cell nucleus, IL-33 may act as a transcriptional repressor with a DNA-binding domain (Carriere et al., 2007; Moussion et al., 2008), and it is known to regulate the transcription of nuclear factor-κBp65 (Choi et al., 2012). Similar to high-mobility group box 1 (HMGB1), known as damage-associated molecular patterns (DAMPs or alarmins), IL-33 is released from necrotic cells when tissue is injured (Moussion et al., 2008; Cayrol and Girard, 2009; Haraldsen et al., 2009).

ST2 has two major isoforms: the transmembrane ST2 (ST2 or ST2L) and the soluble ST2 (sST2) (Iwahana et al., 1999), and the latter is considered a decoy receptor for IL-33 (Xu et al., 1998; Sanada et al., 2007; Haraldsen et al., 2009). IL-33 exerts its biological activity by interacting with its heteromeric receptor composed of ST2 and the IL-1 receptor accessory protein (IL-1RAcp).

The IL-33/ST2 signaling pathway has attracted much attention and has been shown to mediate a broad range of diseases (Sanada et al., 2007; Xu et al., 2008; Prefontaine et al., 2009; Willart et al., 2012), especially inflammatory and autoimmune diseases (Walzl et al., 2001; Kurowska-Stolarska et al., 2008; Jovanovic et al., 2012). It is also been reported that IL-33, like other IL-1 cytokines, induces inflammatory pain in the peripheral nervous system and mediates antigen-induced cutaneous and articular hypernociception in mice via the IL-33 → tumor necrosis factor-α (TNF-α) → IL-1β → interferon-γ (IFN-γ) → endothelin-1 (ET-1) → prostaglandin E2 (PGE2) signaling cascade (Verri et al., 2008), suggesting that IL-33 plays an important role in the modulation of arthritic pain in the peripheral nervous system.

The role of IL-33 in pain modulation in the central nervous system (CNS) is less well known, although many studies have shown that it is expressed at high levels in the CNS and localized in astrocytes, which contributes to central sensitization, suggesting that IL-33 may have a close relationship with pain modulation in the CNS. Therefore, using the classic mouse-model of

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Abbreviations: CNS, central nervous system; ERK, extracellular-signal-regulated kinases; IL-33, interleukin-33; MAPK, mitogen-activated protein kinases; rIL, recombinant IL-33; SEM, standard error; SPSS, Statistical Package for the Social Sciences; PBS, phosphate buffer solution; USVs, ultrasound vocalizations; WT, wild-type.

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acute inflammatory pain by intra-plantar injection of formalin, the present study assessed the possible roles of peripheral and spinal IL-33 in acute inflammatory pain.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on adult male BALB/c mice aged 7–9 weeks and weighing 20–25 g; mice were supplied by the Experimental Animal Center, Chinese Academy of Sciences, Shanghai. $ST2^{-/-}$ BALB/c mice were generated, as previously described, and provided by Dr. Andrew McKenzie at the MRC Laboratory of Molecular Biology, Cambridge (Brint et al., 2004). The $ST2^{-/-}$ BALB/c mice were healthy and did not display overt phenotypic abnormalities (Townsend et al., 2000). The gene-targeted mice were backcrossed to the respective background for 10 generations. $ST2^{-/-}$ and the wild-type (WT) littermates of the same background were kept in the same animal facility for an extended period. Prior to experimental manipulation, mice were allowed to acclimate for 1 week in groups of four mice per cage, and maintained under controlled conditions (22 ± 1 °C, 6 a.m.–6 p.m. alternate light–dark cycles) with food and water *ad libitum*. All experiments were conducted strictly in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the International Association for the Study of Pain (M. Zimmermann, 1983). All efforts were made to minimize the number of animals used and their suffering.

Formalin test

To measure the spontaneous pain behavior induced by formalin, mice were acclimated in Plexiglas chambers (15 cm × 12 cm × 10 cm) for at least 30 min before the experiments. Mice were injected with 20 μl of 5% formalin (in normal saline) into the right hind paw plantar and then immediately returned to the behavioral chamber.

A mirror was positioned below the behavioral chamber at a 45° angle for the unobstructed observation of the injected paw. The pain behavior induced by formalin was monitored by measuring the lifting and licking duration of the injected paw every 5 min during the 45-min observation period.

Drug administration

The mouse ST2 antibody and normal goat IgG were dissolved in sterile phosphate buffer solution (PBS), and mouse recombinant IL-33 (rIL-33) was dissolved in sterile PBS containing 1% bovine serum albumin. All drugs were purchased from American R&D Systems. ST2 antibody and/or rIL-33 were/was administered, either subcutaneously into the right hind paw or intrathecally, 30 min before formalin injection. The control groups were administered normal goat IgG or sterile PBS, respectively.

The intrathecal injection was conducted via lumbar puncture, as previously described (Hylden and Wilcox, 1980). Briefly, the mice were anesthetized with isoflurane. Then, drugs (5 μl in volume) were injected into the subarachnoid space of lumbar vertebrae L5 and L6 with a 5-μl syringe (Hamilton, 33-gauge needle). A tail flick indicated that the needle had pierced the dura.

Ultrasonic vocalizations

Ultrasonic vocalizations were recorded and analyzed by a Mini-3 Bat detector (Ultra Vox 4-channel system; Noldus Information Technology, Leesburg, VA, USA) and data acquisition software (Ultra Vox 2.0; Noldus Information Technology) as described previously (Han et al., 2005; Han and Neugebauer, 2005; Neugebauer et al., 2007). The mice were placed in a recording chamber made of Plexiglas (15 cm × 12 cm × 10 cm). Mice were acclimated to the chamber at least 30 min every time for 2 days prior to the experiments. On the experimental day, mice were also acclimated for 30 min. Immediately, after formalin or normal saline injection, the ultrasonic vocalizations were recorded for every 5-min period during the 45-min recording. The detector was positioned above the chamber, with the frequency filter set at 30 kHz and the amplitude filter set at 4 to minimize background noise (Han et al., 2005). Environmental noise levels were standardized to minimize their influence on ultrasound recordings.

Statistical analysis

Data were presented as the mean ± standard error (S.E.M.), and all statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 17.0 statistical software (SPSS Inc., Chicago, IL, USA). For multiple comparisons, a one-way analysis of variance (ANOVA) was used, which was followed by the least-significant difference test to compare different treatment groups. Student's *t*-tests were used when two treatment groups were compared. In all statistical analyses, $P < 0.05$ as considered the criteria for significance.

RESULTS

Recombinant IL-33 potentiated the formalin-induced inflammatory pain response in mice

Recombinant IL-33 induced mild spontaneous pain behavior in normal mice. Subcutaneous injection (s.c.) of rIL-33 (300 ng) induced significant increases in paw lifting and licking time during the 45 min following injection, compared with the PBS control group ($P < 0.05$). (Fig. 1A–D). IL-33 induced mild spontaneous pain behavior continuously without intermission, which differed from the formalin-induced two-phase pain behavior. Similarly, intrathecal injection of rIL-33 (3 ng) also induced a remarkable increase in paw lifting and licking time, compared with the PBS group ($P < 0.05$) (Fig. 1A–D).

Recombinant IL-33 exacerbated formalin-induced pain behavior in mice. To observe the effect of IL-33 on the formalin-induced pain behavior, rIL-33 was given subcutaneously (30, 100 and 300 ng) or intrathecally (0.33 and 1 ng) 30 min before formalin injection. Subcutaneous administration of 300 ng rIL-33 markedly increased paw lifting and licking time in both two phases in formalin mice compared with the PBS group (Fig. 2A, B), indicating that IL-33 potentiates formalin-induced pain behavior. Additionally, IL-33 (s.c., 30 ng, 100 ng) prolonged paw licking time in the late phase ($P < 0.05$) (Fig. 2A, B). Intrathecal injection of rIL-33 significantly increased paw lifting time in the two phases, but showed no significant influence on the licking time ($P < 0.05$) (Fig. 2C, D).