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Veterinary Immunology and Immunopathology 109 (2006) 219–231
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Molecular characterization and functional expression of equine interleukin-1 type I and type II receptor cDNAs

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Received 4 April 2005; received in revised form 5 July 2005; accepted 12 August 2005

Abstract

cDNA generated from lipopolysaccharide-stimulated equine peripheral blood mononuclear cells was used to amplify and clone type I and type II equine interleukin-1 receptors (IL-1RI and IL-1RII) using primers derived from semi-conserved regions between human and mouse IL-1RI and IL-1RII sequences, respectively. 5′ and 3′ terminal sequences of equine IL-1RI and IL-1RII were amplified by 5′ and 3′ rapid amplification of cDNA ends. The deduced amino acid sequence of equine IL-1RI demonstrated 77, 64 and 63% similarity with human, mouse and rat sequences, respectively. The predicted amino acid sequence of equine IL-1RII demonstrated 70, 60 and 58% similarity with human, mouse and rat sequences, respectively. Recombinant equine soluble IL-1RI and IL-1RII produced in insect cells bound recombinant equine IL-1α and IL-1β. Furthermore, both receptors suppressed the growth inhibitory activities of equine IL-1α and IL-1β toward A375 cells in a dose-dependent manner, indicating that the present equine IL-1RI and IL-1RII cDNA encodes biologically active proteins.

Keywords: Equine; Interleukin-1 receptor; Sequence; Expression; IL-1RI; IL-1RII

1. Introduction

Interleukin-1 (IL-1) is a proinflammatory cytokine that plays a central role in the regulation of inflammatory reactions, including fever, acute phase response, and connective tissue degradation and remodeling (Dinarello, 1996). The IL-1 family currently consists of two agonists, IL-1α and IL-1β, and one antagonist, IL-1 receptor antagonist (Ra). There are two distinct IL-1 receptors (IL-1Rs). The 80 kDa type I receptor (IL-1RI), which is found in small numbers on nearly all cells, is the predominant

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0165-2427/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2005.08.018

IL-1R expressed on T cells and fibroblasts (Mantovani et al., 2001) and transduces signals by binding of IL-1α and IL-1β but not IL-1ra (Sims et al., 1988, 1993). The 68 kDa type II IL-1R (IL-1RII) is found predominantly on B cells and neutrophils (Mantovani et al., 2001) and functions as a decoy receptor without participating in IL-1 signaling (McMahan et al., 1991; Colotta et al., 1993). The extracellular domains for human IL-1RI and IL-1RII are 319 and 332 amino acids, respectively, and both receptors contain a single transmembrane domain. IL-1RI possesses a cytoplasmic domain of 213 amino acids, whereas IL-1RII has only 29 residues in its cytoplasmic domain. Signal transduction by IL-1RI is only possible in concert with the IL-1 receptor accessory protein (IL-1RacP) (Greenfeder et al., 1995; Wesche et al., 1996). IL-1-binding IL-1RII also interacts with IL-1RacP and may contribute to anti-IL-1 action (Lang et al., 1998; Malinowsky et al., 1998).

Soluble IL-1Ra and IL-1Rs are naturally occurring regulatory proteins that influence the biological activities of IL-1 (Dinarello, 1996; Fernandez-Botran et al., 1996). Blockade of membrane IL-1Rs with IL-1Ra in experimental models of lethal endotoxemia results in protection of the animal (Ohlsson et al., 1990; Alexander et al., 1991). However, this requires large amounts of IL-1Ra, since only 1% of IL-1Rs need to be occupied to induce a signal (Dinarello, 1996). In contrast, neutralizing circulating IL-1 is easier, since IL-1 concentrations during diseases are relatively low and may be in the picomolar range. Soluble recombinant forms of IL-1RI have been developed, and their IL-1 binding characteristics are similar to those of membrane IL-1RI (Dower et al., 1989). Administration of soluble IL-1RI in vivo reduces the severity of active arthritis and experimental autoimmune encephalomyelitis and is able to reduce some of the inflammatory effects of lipopolysaccharide (LPS) (Drevlow et al., 1996; Jacobs et al., 1991; Preas et al., 1996). Rapid shedding of soluble IL-1RII occurs upon exposure of cells to TNF-α, chemoattractants and metalloproteases (Colotta et al., 1995; Orlando et al., 1997a, 1997b). This release of soluble receptors has been thought to be an early event in the inflammatory cascade, which acts to limit its severity. These findings indicate that inhibition of IL-1-mediated pathways by soluble IL-1Rs is a potential therapeutic approach in severe inflammatory diseases.

Recombinant equine soluble IL-1RI and IL-1RII would be of benefit in studies on the roles of IL-1α, IL-1β and IL-1ra in the pathogenesis of equine inflammatory diseases such as joint disease and in potential uses of soluble IL-1RI and IL-1RII in the treatment of inflammatory diseases. However, there has been no report on the sequences of equine IL-1RI and IL-1RII. Here, we describe the cloning and functional expression of equine IL-1RI and IL-1RII to establish a basis for research on inflammatory responses in horses.

2. Materials and methods

2.1. Preparation of cDNA

Equine peripheral blood mononuclear cells (PBMC) were isolated from blood of a healthy normal thoroughbred by centrifugation using the Ficoll-Conray method (specific gravity, 1.077). The PBMC (2×10^6 ml^{-1}) were cultivated in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, USA) and LPS (*Escherichia coli* 0111:B4, Sigma, St. Louis, USA) (5 μg/ml) at 37 °C under 5% CO_2 in air. After cultivation for 18 h, the PBMC were collected by centrifugation. Total RNA was extracted from the cells with TRIzol (Invitrogen) according to the operation manual for the reagent. Reverse transcription of the RNA was performed with a superscript preamplification system (Invitrogen).

2.2. Polymerase chain reaction (PCR) amplification

PCR primers were prepared on the basis of sequences of human and mouse IL-1RI (Sims et al., 1988, 1989) and IL-1RII (McMahan et al., 1991) by comparing the sequences. The primer sequences used for amplification of equine IL-1RI cDNA were 5′-GGCAAGCAATATCCTATTACCCG-3′ (hum-T1-F; 610–632 nt in human IL-1RI coding sequence) and 5′-CCAGAACCTTGTCTTTGCAGACTG-3′ (hum-T1-R; 1570–1593 nt in human IL-1RI sequence). The primer sequences used for amplification of equine IL-1RII cDNA were 5′-TCTGGCACCTACGTCTG-CAC-3′ (hum-T2-F; 307–326 nt in human IL-1RII