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Chromosomal mapping of the human interleukin-1 receptor antagonist gene (IL-1RN) and isolation of specific YAC clones

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Abstract

Using a panel of somatic rodent–human cell hybrids, we show that the interleukin-1 receptor antagonist gene (IL-1RN) maps to the long arm of human chromosome 2. Linkage studies permitted the regional localization of this gene to band q14-21. This is the same region in which the IL-1α and IL-1β genes are localized. Three yeast artificial chromosome (YAC) clones containing the IL-1RN gene were isolated, and these will be used for further characterization of this chromosome 2 region.

Introduction

The IL-1 receptor antagonist is a protein which inhibits the binding of IL-1α and IL-1β to the IL-1 receptors, neutralizing the biological activity of these cytokines [1]. Possible control of IL-1 expression level and/or inhibition of the binding of IL-1 species to the receptor could play an important role in the treatment of many immune and inflammatory diseases, such as rheumatoid arthritis and septic shock. The loci for human IL-1α and IL-1β and for IL-1 receptor type I are all localized on the long arm of chromosome 2. Therefore, we investigated whether the gene for the IL-1 receptor antagonist (IL-1RN) is also localized on the same chromosome. In addition, a YAC library was screened to obtain IL-1RN-specific clones to further characterize this region of chromosome 2.

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Materials and methods

A triallelic IL-1RN length variation polymorphism within intron 2 of the IL-1RN gene, which was used for genetic linkage, has been previously described [2]. Further use of cell hybrids and linkage analysis permitted the chromosomal localization of this gene to chromosome 2q14-21 [3].

Isolation of IL-1RN-specific YAC clones: DNA pools from the Imperial Cancer Research Fund YAC library were kindly provided by Dr. Tony Monaco (Institute of Molecular Medicine, Oxford). These pools were screened by PCR using an IL-1RN-specific primer set (5′ oligo 5′CAG CTC TCA CCT GCC CAT CTT TTG3′ and 3′ oligo 5′CTC GTC CTC CTG GAA GTA GAA TTT GG3′). A 244 bp fragment was amplified from genomic DNA and from YAC clones coding for the IL-1RN gene. DNA from positive YAC clones was separated using pulsed-field gel electrophoresis (PFGE), transferred on to nitrocellulose and hybridized with

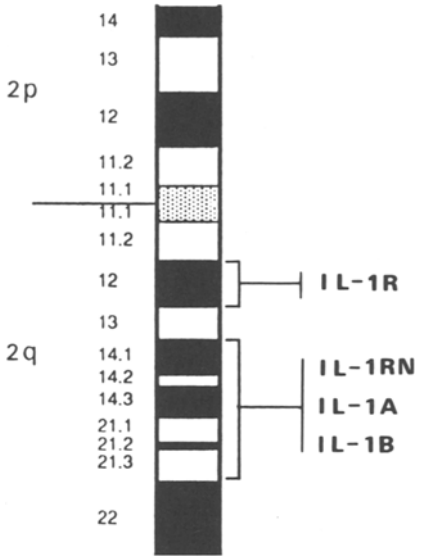

Figure 1a
Partial genetic map of chromosome 2, showing the IL-1 receptor (IL-1R), the IL-1 receptor antagonist (IL-1RN), the IL-1α (IL-1A) and the IL-1β (IL-1B) loci.

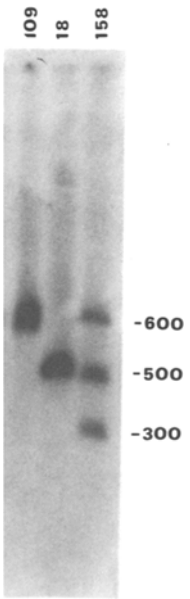

Figure 1b
The three YAC clones which contain the IL-1RN gene are shown. Track 1, clone 109 (620 kb); track 2, clone 18 (500 kb); track 3, clone 158 (620, 500 and 300 kb). DNA from YAC clones was electrophoresed by PFGE, the DNA transferred to nitrocellulose and hybridized with an IL-1RN specific probe.

an IL-1RN-specific genomic probe. This confirmed the presence of the IL-1RN gene in the selected clones and was used to estimate the length of the genomic YAC inserts.

Results and discussion

Somatic cell and deletion hybrids confirmed the presence of the IL-1RN gene on the long arm of chromosome 2. Linkage analysis permitted the regional localization to band q14-21 (Fig. 1). This is the region where IL-1α and IL-1β also map (Fig. 1a). This result was an additional confirmation that an early gene duplication event resulted in the creation of an IL-1 gene family.

To characterize further this IL-1 gene family, a YAC library was screened and three positive IL-1RN clones were obtained. The YAC-DNA was separated by PFGE and the filter-blotted DNA was hybridized with a genomic IL-1RN probe. Clone 109 had an insert of approximately 620 kb whereas clone 18 had one of 500 kb. Clone 158 showed three hybridizing bands (620, 500 and 300 kb), which are probably due to fragmentation of the YAC (Fig. 1b). These clones will now be used to characterize further this region of chromosome 2 and to search for possible additional genes of this IL-1 gene family.

Acknowledgements

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