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# Human interleukin-1 receptor antagonist is expressed in liver

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Using PCR and Northern blot analysis, an IL-1 receptor antagonist specific transcript was amplified from HepG2- and liver mRNA. cDNA clones coding for IL-1 receptor antagonist were isolated from a liver cDNA library and sequence comparison revealed complete identity with the secreted, monocytic form of IL-1 receptor antagonist.

IL-1 receptor antagonist; Liver; cDNA transcript

## 1. INTRODUCTION

IL-1 receptor antagonist (IL-1ra) is a protein which inhibits IL-1 (IL-1$\alpha$ and IL-1$\beta$) activity, by binding competitively to IL-1 receptors [1]. This naturally occurring inhibitor has been shown to reduce the severity of sepsis, colitis, arthritis and diabetes in animals and it therefore plays an important role in the control of IL-1 mediated inflammatory and autoimmune diseases [2]. IL-1ra is structurally related to IL-1 and shows 18% and 26% identity in amino acid sequence to IL-1$\alpha$ and IL-1$\beta$, respectively.

The cDNA sequence of IL-1ra derived from monocytes has been previously determined [3] and using an intronic polymorphism [4] the localization of the gene on chromosome 2 has been established by linkage analysis [5], as well as by in situ hybridisation [6].

There are two types of IL-1ra, one secreted, soluble form expressed in monocytes and neutrophils and an intracellular form expressed in epithelial cells. This intracellular IL-1ra, which lacks a leader peptide, is derived from the same gene through the use of an alternative transcriptional start site and alternative splicing [7].

In this study we report the expression of the secreted form of the IL-1ra in liver.

## 2. MATERIALS AND METHODS

### 2.1. *cDNA synthesis and PCR amplification*

Total RNA was isolated from the human cell lines HepG2, Chang, U937, Daudi, HL60, Molt4, Raji and T47D as well as from liver, temporal cortex and lung tissue using guanidinium thiocyanate and a CsCl gradient [8]. 10 $\mu$g RNA was used for the first strand cDNA synthesis (cDNA synthesis kit; Amersham, Bucks, UK) and 1/10th of this reaction mixture was used for PCR amplification. The two PCR primer sets described by Haskill et al. [7] were used in order to distinguish between RNA transcripts for he secreted and the intracellular forms of IL-1ra. PCR products were analysed after 30 cycles (94°C, 1 min; 60°C, 30 s, 72°C, 2 min) on a 0.7% (w/v) agarose gel.

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### 2.2. *Northern blot analysis*

A 10 $\mu$g portion of total RNA was electrophoresed in 1.2% (w/v) agarose gel and the RNA transferred to a Hybond-N membrane (Amersham) and hybridised at 65°C with a radiolabelled IL-1ra probe derived from HepG2 mRNA by PCR. The blots were washed at high stringency [8].

### 2.3. *Isolation of IL-1ra clones and cDNA sequencing*

A human liver cDNA library constructed in the eukaryotic expression vector CDM8 [9] was screened with an IL-1ra probe derived from HepG2 mRNA by PCR. The length of the positive cDNA clones was estimated by a *Pst*I-*Hin*dIII restriction digest. The sequence was determined from both strands using the double-strand sequencing kit from Pharmacia (Milton Keynes, UK).

## 3. RESULTS AND DISCUSSION

Using specific PCR primers it was possible to amplify an IL-1ra transcript from the hepatoma cell line HepG2 and from total liver RNA. In liver only the secreted form of the antagonist was present (Table I) and no amplification product was obtained for the intracellular form. Interestingly no amplification product was obtained from the Chang liver cell line which is derived from nonmalignant human liver tissue, indicating a loss of the IL-1ra message in this cell line or need for an external stimulus. As a positive control the monocytic cell line U937 was used. The other cell lines (Molt4, HL60, Daudi, T47D and Raji) and tissues (temporal cortex and lung) were negative for the secreted (Table I) as well as for the intracellular IL-1ra (data not shown).

To confirm the PCR analysis, Northern blots were performed with RNA extracted from liver tissue and from the cell lines HepG2, Chang and Raji. As shown


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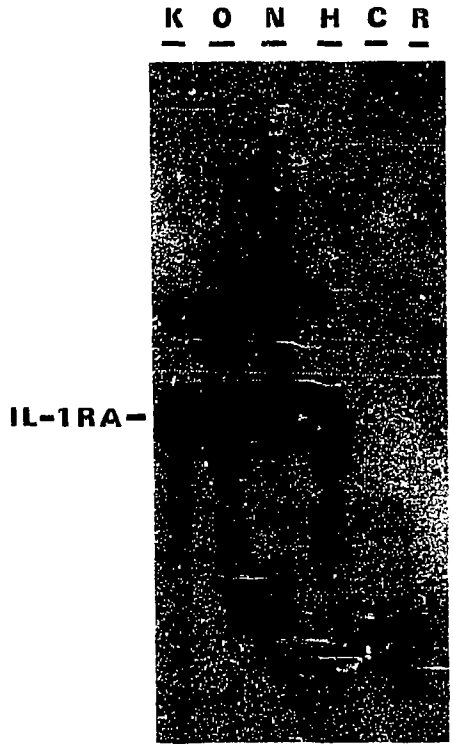


Fig. 1. Northern blot analysis of total RNA isolated from liver tissue (lanes 1–3; individuals: K, O, N,) and the cell lines HepG2 (H), Chang (C) and Raji (R) probed with an IL-1ra cDNA probe. The additional fainter bands are probably due to background-hybridization or not totally spliced mRNA.

in Fig. 1 all three liver RNAs (individuals: K, O, N) contained the IL-1ra transcript (lanes 1–3), as did the HepG2 cell line (lane 4). No IL-1ra transcript was detected in the cell lines Chang and Raji (lanes 5 and 6), in agreement with the findings obtained by PCR analysis.

To establish the sequence of this liver IL-1ra transcript a liver cDNA library subcloned into the CDM8 expression vector was screened with an IL-1ra probe. Three positive clones were isolated and a 1.65 kb insert from clone pIL1ra-L was sequenced. This clone contains a short 5'-untranslated region (8 bp) followed by the leader sequence and the coding region for the mature secreted protein. The 3'-untranslated region is 1108 bp long. Sequence comparison revealed that this sequence is identical to the cDNA corresponding to the secreted form of the IL-1ra from monocytes [3].

Table I

PCR analysis of cell line and tissue cDNA transcripts

| | | sec. IL-1ra |
|---|---|---|
| HepG2 | (hepatoma) | + |
| Chang | (hepatocyte) | – |
| Liver | (tissue) | + |
| U937 | (monocytic) | + |
| Daudi | (B-lymphoblastoid) | – |
| HL60 | (neutrphilic) | – |
| Molt4 | (T-lymphoblastoid) | – |
| Raji | (B-lymphoblastoid) | – |
| T47D | (breast carcinoma) | – |
| Cortex | (tissue) | – |
| Lung | (tissue) | – |

Since the IL-ra transcript was detected (by PCR and Northern blot) in liver tissue as well as in the hepatoma cell line HepG2 the transcript in the tissue is likely to be derived from hepatocytes and therefore represents a true IL-1ra transcript in human liver.

Zahedi et al. demonstrated the induction of the IL-1ra gene during inflammatory and acute phase reactions in the mouse liver, suggesting a possible involvement in the control of inflammatory mediators [10]. Many proteins involved in immune and inflammatory responses are expressed and/or controlled in the liver by an IL-1 stimulus mainly derived from macrophages. This includes several complement proteins such as C3, C5 and Factor B as well as acute phase proteins such as serum amyloid P component [11] and serum amyloid A [12]. It is necessary to control this IL-1 transcriptional activation in the liver in order to prevent an overexpression which could lead to pathophysiological effects. Therefore, the existence of a natural IL-1ra expressed in the liver is essential and the knowledge of the cDNA sequence of this transcript opens the possibility to investigate the specific control and the interaction with other proteins in the liver environment. The expression of the secreted form of the IL-1ra in liver is consistent with the fact that this protein competes in the extracellular environment with IL-1 for the binding to IL-1 receptors.

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