

A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types

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cDNA clones corresponding to an $M_r \sim 80\,000$ receptor (type I receptor) for interleukin-1 (IL-1) have been isolated previously by mammalian expression. Here, we report the use of an improved expression cloning method to isolate human and murine cDNA clones encoding a second type ($M_r \sim 60\,000$) of IL-1 receptor (type II receptor). The mature type II IL-1 receptor consists of (i) a ligand binding portion comprised of three immunoglobulin-like domains; (ii) a single transmembrane region; and (iii) a short cytoplasmic domain of 29 amino acids. This last contrasts with the ~ 215 amino acid cytoplasmic domain of the type I receptor, and suggests that the two IL-1 receptors may interact with different signal transduction pathways. The type II receptor is expressed in a number of different tissues, including both B and T lymphocytes, and can be induced in several cell types by treatment with phorbol ester. Both IL-1 receptors appear to be well conserved in evolution, and map to the same chromosomal location. Like the type I receptor, the human type II IL-1 receptor can bind all three forms of IL-1 (IL-1 α , IL-1 β and IL-1 γ). Vaccinia virus contains an open reading frame bearing strong resemblance to the type II IL-1 receptor.

Key words: B cell/chromosome mapping/expression cloning/interleukin-1/receptor

Introduction

The cytokines collectively termed IL-1 (IL-1 α , IL-1 β and IL-1 γ) together effect and/or modulate a number of host defense processes, including immune responses, inflammation and hematopoiesis. These effects are exerted via specific receptors for IL-1, which are present on a wide variety of different cell types (Dower and Urdal, 1987). One species

of receptor, of $M_r \sim 80\,000$, which has been thought to be the predominant form found on T cells and fibroblasts, has been well characterized (Dower *et al.*, 1985, 1986; Bird and Saklatvala, 1986; Kilian *et al.*, 1986; Chin *et al.*, 1987; Bird *et al.*, 1988; Urdal *et al.*, 1988), and cDNA clones isolated (Sims *et al.*, 1988, 1989). However, IL-1 receptors on cells or cell lines representative of B cells, monocytes, neutrophils, bone marrow cells and hepatoma cells differ in size and/or antigenicity from the receptors found on T cells and fibroblasts (Matsushima *et al.*, 1986; Horuk *et al.*, 1987; Rhyne *et al.*, 1988; Bensimon *et al.*, 1989; Bomsztyk *et al.*, 1989; Chizzonite *et al.*, 1989; Mizuno *et al.*, 1989; Scapigliati *et al.*, 1989; Benjamin and Dower, 1990; Solari, 1990; Spriggs *et al.*, 1990). In B cells and monocytes, this clearly is not a consequence of post-translational modification; rather, the IL-1 receptor on these cells is the product of a different gene (Bomsztyk *et al.*, 1989; Chizzonite *et al.*, 1989; Spriggs *et al.*, 1990). Here we report the isolation from human and murine B cells of cDNA clones encoding a novel IL-1 receptor, and some characteristics of the receptor proteins encoded by these clones. We propose to call this new receptor the type II IL-1 receptor, to distinguish it from the previously cloned p80 or type I receptor.

Results

Expression cloning method

The cloning of an IL-1 receptor from B cells was facilitated by several improvements to a method we have used previously (Sims *et al.*, 1988). In the earlier version, a cDNA library constructed in an expression vector containing the SV40 origin of replication was transfected into COS cells. The SV40 T antigen constitutively expressed in these cells allowed extensive replication of the DNA, which, in combination with elements directing efficient transcription and translation, led to robust expression of cDNAs inserted in the vector. Plasmids containing full-length IL-1 receptor cDNAs were identified by their ability to confer high-level binding of [¹²⁵I]IL-1 on the recipient COS cells, which was detected by placing the COS cell monolayer directly in contact with X-ray film.

The current method uses a plasmid–host replication system derived from Epstein–Barr virus (Yates *et al.*, 1984; Lupton and Levine, 1985) in place of the SV40-based system. A host cell line, CV1/EBNA, was derived, which constitutively expresses the EBV replication protein EBNA-1 (see Materials and methods). In addition, a segment of the EBV genome containing the origin of replication was included in an expression vector, pDC406 (Figure 1A), which also contains an SV40 origin of replication. This plasmid is capable of low copy number extrachromosomal replication in cell lines expressing EBNA-1 (Lupton and Levine, 1985), and of high copy number replication in COS cells.

Plasmid pDC406 containing a human type I IL-1R cDNA

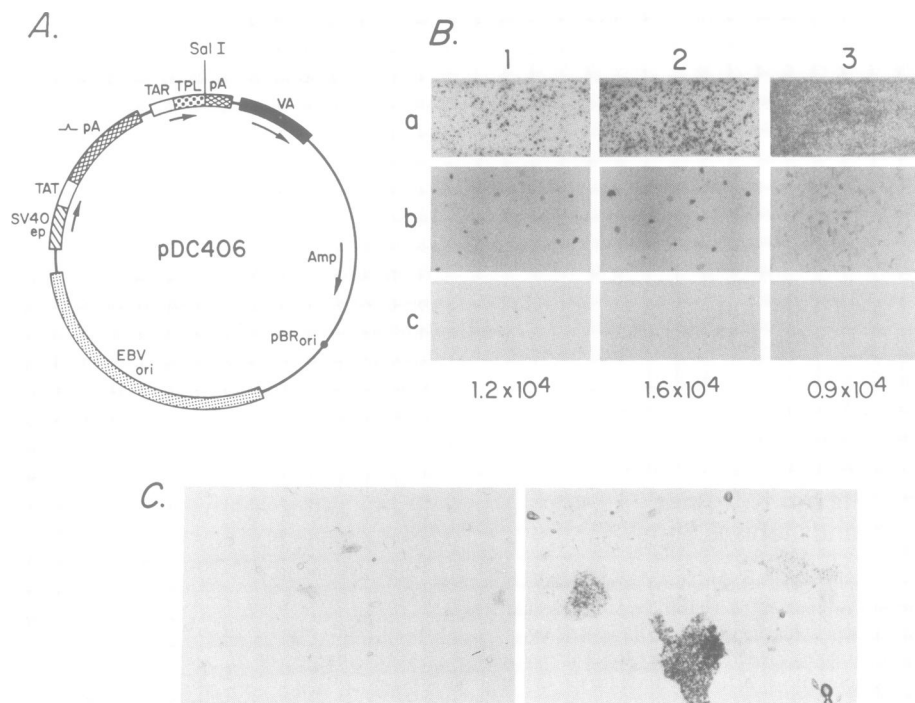


Fig. 1. (A) Schematic diagram of the expression plasmid pDC406. cDNA molecules inserted at the *Sal*I site are transcribed and translated using regulatory elements derived from HIV and adenovirus. pDC406 contains origins of replication derived from SV40, Epstein-Barr virus and pBR322. (B) Contact autoradiography (rows a and c) or slide autoradiography (row b) of cell monolayers transfected on glass slides with pDC406 containing a type I (p80) IL-1R cDNA insert and screened using [¹²⁵I]IL-1 α . Column 1 shows transfections of COS cells using the method described in Sims *et al.* (1988). Columns 2 and 3 show transfections of COS cells and CV1/EBNA cells, respectively, using the method described in Materials and methods. Transfections in row a were done with a saturating amount of DNA (~2 μ g per microscope slide). Transfections in row b were done with 0.2 ng of the IL-1 receptor-containing pDC406 and 2 μ g of pDC406 containing no insert ('empty vector'). Transfections in row c were done with 2 μ g of pDC406 empty vector. The autoradiographic signal for each spot is stronger when COS cells are transfected, but there are many more detectable spots in the CV1/EBNA transfection. The numbers below the columns show the results (c.p.m. of [¹²⁵I]IL-1 α bound) from a similar experiment, in which cells plated in 6-well dishes were transfected with saturating amounts of DNA, and, after incubation with [¹²⁵I]IL-1 α , trypsinized and counted in a gamma counter. (C) Light micrographs of CV1/EBNA cells transfected on slides with a pool of 4500 clones from a CB23 cDNA library in pDC406, one of which is the type II IL-1R clone (clone HuII75), and subjected to *in situ* autoradiography after incubation with [¹²⁵I]IL-1 β . Left, 40 \times magnification. Right, 200 \times magnification.

clone (Sims *et al.*, 1989) as a test receptor was transfected either into COS cells (Figure 1B, column 2) or into CV1/EBNA cells (Figure 1B, column 3), which after a 3 day expression period were incubated with [¹²⁵I]IL-1 α . Comparison of columns 2 and 3 in Figure 1B reveals that detectably positive cells are much more numerous in the CV1/EBNA transfection, although the intensity of the positives is somewhat less than in the COS cell transfection. Figure 1B also demonstrates the effect of modifying the transfection protocol used previously (Figure 1B, column 1; Sims *et al.*, 1988) to the improved version described in Materials and methods (Figure 1B, column 2). The new protocol results in a 2- to 3-fold increase in the number of detectably transfected cells. Finally, a method was devised to transfect cells directly on microscope slides, and then to use the technique of Gearing *et al.* (1989) to visualize the positive cells after incubation with ligand, by coating the slides with photographic emulsion and performing *in situ* autoradiography. Subsequent examination of the slides using a light microscope reveals a cluster of silver grains covering cells transfected with the IL-1 receptor clone (Figure 1B, row b; Figure 1C). The slide autoradiography technique (Gearing *et al.*, 1989) offers a more sensitive means of detection of weakly positive cells than contact autoradiography (Sims *et al.*, 1988), as well as distinguishing better between true receptor-expressing cells and false positives.

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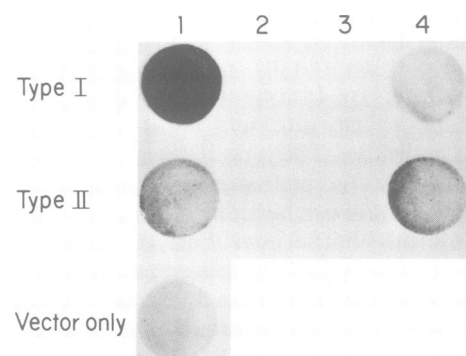


Fig. 2. Clone HuII75 encodes a non-type I IL-1 receptor. DNAs were transfected into CV1/EBNA cells and screened by plate autoradiography (Sims *et al.*, 1988) using 3×10^{-9} M [¹²⁵I]IL-1 β as the ligand. The three rows represent DNA from (top) a human type I IL-1R clone in pDC406; (middle) a pool of 75 colonies, one of which contains a human type II IL-1R clone (clone HuII75), in pDC406; (bottom) pDC406 vector with no insert. Unlabeled competitor present during the incubation with radioactive ligand was: column 1, no competitor; column 2, 1×10^{-6} M IL-1 α ; column 3, 1×10^{-6} M IL-1 β ; column 4, 1×10^{-6} M monoclonal antibody M1, which blocks binding of IL-1 to the human type I receptor.

Overall, model experiments using the CV1/EBNA host cell line, the improved transfection technique, and slide autoradiography, showed that the type I IL-1R cDNA clone

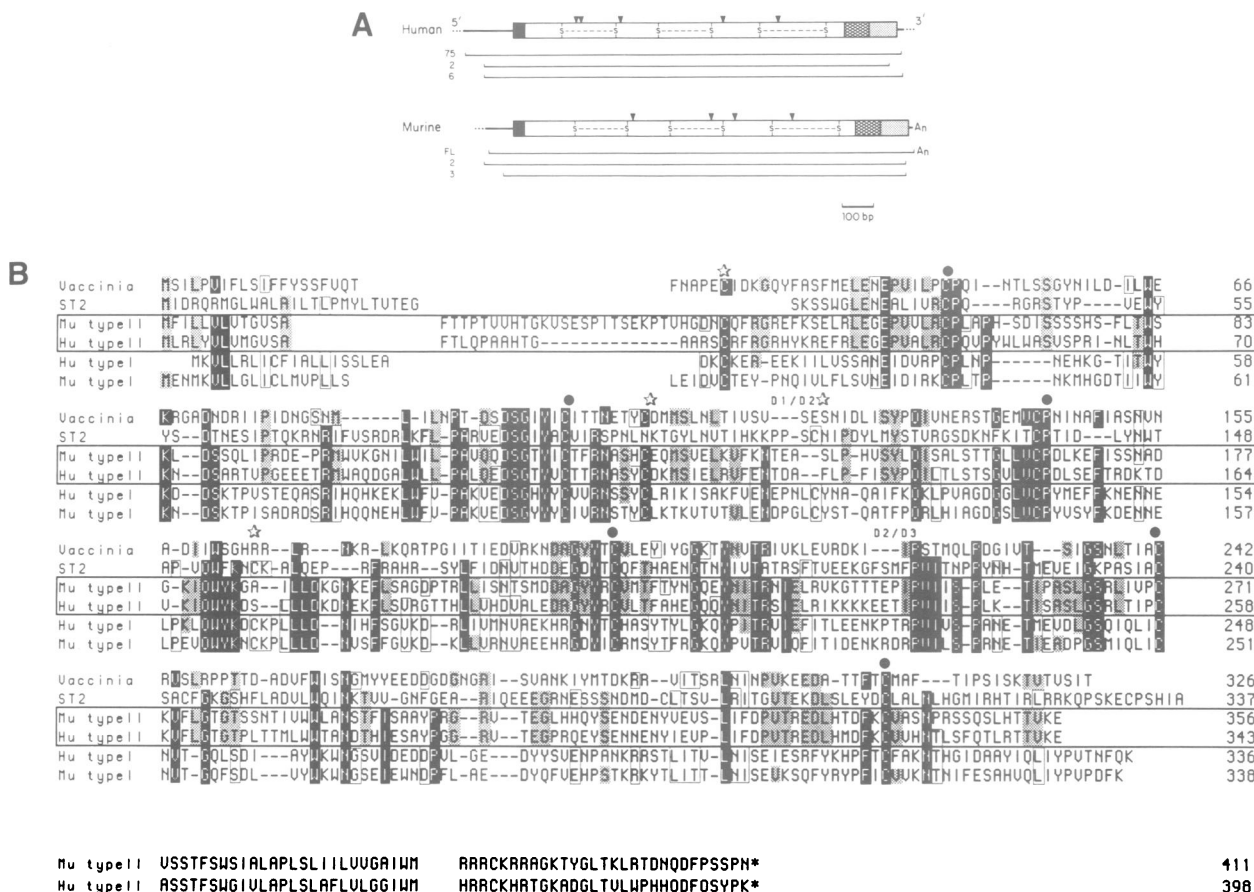


Fig. 3. (A) Schematic diagram of the human and murine type II IL-1 receptors and the cDNA clones used to determine the sequences. Thin lines represent untranslated regions, while the coding region is depicted by a box. The section representing the signal peptide is filled in; the transmembrane region is cross-hatched; and the cytoplasmic portion is stippled. Potential N-linked glycosylation sites are marked by inverted triangles. The postulated immunoglobulin-like disulfide bonds are indicated. (B) Amino acid sequences of the human and murine type I IL-1 receptors, as deduced from the cDNA clones, compared with the amino acid sequences of the human and murine type II IL-1 receptors (Sims *et al.*, 1988, 1989) and to two similar proteins found in searches of the Genbank database. Numbering begins with the initiating methionine. The postulated position of signal peptide cleavage in each sequence is that deduced as described (von Heijne, 1986), and is indicated by a gap between the putative signal peptide and the main body of the protein. The postulated transmembrane and cytoplasmic regions for the type II receptors are shown on the bottom line, and are separated from one another by a gap. Residues conserved in all four IL-1 receptor sequences are presented in white on a black background. Residues conserved in type II receptors that are also found in one of the other sequences are shaded; residues conserved in type I receptors that are found in one of the other sequences are boxed. Cysteine residues involved in forming the disulfide bonds characteristic of the immunoglobulin fold are marked with solid dots, while the extra two pairs of cysteines found in the type I receptor and in some of the other sequences are indicated by stars. The approximate boundaries of domains 1, 2 and 3 are indicated above the lines. The ST2 sequence is from Tominaga (1989) and differs by one amino acid from that called T1 in Klemenz *et al.* (1989). The vaccinia virus sequence is the B15R sequence from the WR strain (Smith and Chan, 1991), and corresponds to coordinates 174164–175141 of the complete viral sequence of the Copenhagen strain (Goebel *et al.*, 1990a,b; essentially, the B16R open reading frame). The WR strain B15R sequence differs by nine amino acids from the Copenhagen strain B16R sequence. The similarities between ST2, B15R and the type I IL-1R have previously been noted (Tominaga, 1989; Smith and Chan, 1991). The method of von Heijne (1986) predicts signal peptide cleavage in the type II receptors following Ala13, resulting in an unusually short signal peptide and an N-terminal extension of 12 (human) or 23 (mouse) amino acids beyond the point corresponding to the mature N-terminus of the human or mouse type I IL-1R, identified by protein sequencing (Sims *et al.*, 1988; K. van Ness and C. March, personal communication). The method accurately predicts the cleavage site for the murine and human type I receptors. There are several less favored but still acceptable sites of cleavage predicted by von Heijne's rules in the murine type II receptor, after Thr15 or Pro17. It is possible that cleavage occurs at one of these sites, although this would still leave a long N-terminal extension. There are no good secondary sites in the human type II receptor sequence. The alignment was done by hand and does not represent an objectively optimized alignment of the sequences. The nucleotide sequences of the murine and human type II receptor cDNAs have been deposited with EMBL (accession numbers X59769 and X59770).

in pDC406 could be diluted 50 000-fold into empty pDC406 vector and still allow detection of 5–10 positive cells per slide. In order to get 5–10 positive cells after transfection into COS cells, the same plasmid could be diluted no more than 5000-fold. These model experiments suggested that a cDNA library could be screened in relatively large pools using the current approach, saving considerable time and effort.

Isolation of cDNA clones

The human B lymphoblastoid cell line CB23 was derived by infection of cord blood with Epstein–Barr virus

(Benjamin *et al.*, 1990). A cDNA library generated from this line in the vector pDC406 was screened in pools of 4000 as described above using [¹²⁵I]IL-1 β as the ligand, and one positive pool was found after screening 350 000 clones. Characterization of the pool by Southern blot analysis using a human type I IL-1R probe (data not shown), and by antibody inhibition using a blocking antibody specific for the type I receptor (Figure 2), indicated that the positive phenotype was not due to the presence of a type I IL-1R clone. Figure 2 also indicates that the binding of IL-1 to cells transfected with this pool was specific. The original pool was then broken down into smaller subpools and re-screened.

Table I. ALIGN scores

	Human type II	Murine type II	Human type I	Murine type I	Vaccinia B15R	Murine ST2	Vaccinia B18R	Human <i>flg</i>	Human <i>bek</i>
Human type II	–	41.06	16.15	16.62	21.36	11.77	9.52	3.12	4.08
Murine type II	59	–	14.42	14.01	19.66	9.28	10.92	3.64	3.90
Human type I	28	26	–	56.71	9.04	15.09	4.63	5.70	5.23
Murine type I	30	27	63	–	10.46	11.32	6.28	4.57	6.29
Vaccinia B15R	31	32	24	24	–	8.63	3.89	6.55	6.37
Murine ST2	22	23	25	24	24	–	3.61	7.40	5.18
Vaccinia B18R	18	18	15	16	19	18	–	1.10	2.34
Human <i>flg</i>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	42.30
Human <i>bek</i>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–

The numbers above the diagonal represent the ALIGN (Dayhoff *et al.*, 1983) scores for the indicated pairs of protein sequences. Scores >3.00 are generally taken to indicate significant similarity ($P < 0.001$). The numbers below the diagonal represent the percent identity between each pair of proteins within the three Ig-like domains, which was calculated by summing the number of matches found in the alignment of Figure 3B and dividing by the average of the number of residues in this portion of the two proteins. n.d. not determined.

This process continued until a single clone capable of conferring high-level IL-1 binding ability on CV1/EBNA cells was isolated.

Sequence analysis of the resulting clone, clone HuII75, revealed a 1.4 kb cDNA insert with a single long open reading frame and short 5' and 3' untranslated regions. To confirm the representative nature of this clone, we used the insert as a probe to isolate another two cDNA clones from the human B lymphoblastoid line Raji. In addition, the human cDNA was used to isolate two cDNA clones from the murine pre-B lymphoma 70Z/3, and a third mouse clone was generated (also from 70Z/3 RNA) by anchored PCR using an oligo(dT) adaptor primer to fix the 3' end of the clone. A schematic diagram of the various clones used to determine the characteristics of the human and murine type II IL-1 receptors is given in Figure 3A. The composite amino acid sequences are presented in Figure 3B.

The type II IL-1 receptor is similar to the type I receptor in its overall structure. There is a signal peptide, an extracellular portion comprised of three immunoglobulin-like domains, a single transmembrane region and a cytoplasmic domain. The extracellular regions of the two receptors are clearly but distantly related in amino acid sequence (28% identity). The major difference between the type I and type II receptors lies in the cytoplasmic portion. The type I receptors have ~215 amino acid residues intracellularly, while the mouse and the human type II receptors have only 29. The M_r predicted from the deduced amino acid sequence of the human type II receptor, minus the signal peptide, is 43 987. Presumably, glycosylation at some or all of the five potential N-linked carbohydrate addition sites accounts for the difference between this figure and the size of 60–68 kDa typically seen on cross-linking to [¹²⁵I]IL-1.

Related genes

Searches of the Genbank and EMBL nucleic acid sequence databases revealed substantial similarity of the human and

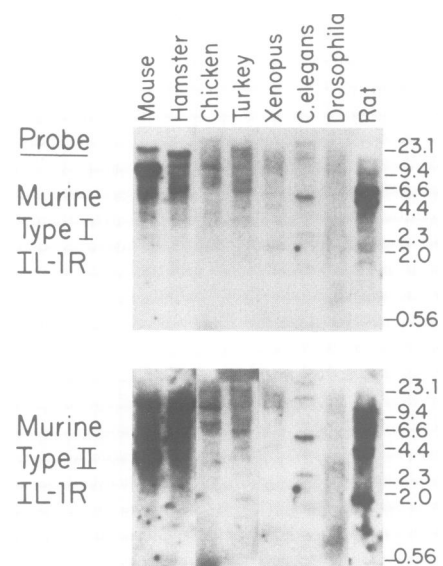


Fig. 4. Genomic Southern blots of DNA from various organisms, digested with *Hind*III and hybridized to a probe from the extracellular region of (top) the murine type I IL-1 receptor or (bottom) the murine type II IL-1 receptor. The numbers on the right indicate the sizes, in kilobase pairs, of marker DNA fragments run in parallel.

murine type II IL-1 receptor coding regions to an open reading frame present in a murine cDNA called ST2 (Tominaga, 1989), and to an open reading frame in vaccinia virus termed B15R (Smith and Chan, 1991). These similarities are depicted in Figure 3B and quantitated in Table I. Both genes consist of an apparent signal peptide and three immunoglobulin-like domains, but no transmembrane or cytoplasmic segment; i.e. they appear to code for secreted receptor analogues. It is not known whether either molecule binds IL-1.

More distant matches of the type II receptor ligand binding

portion were seen to several other molecules in the database. There was similarity to a second vaccinia virus gene (Table I), apparently encoding another triple Ig-domain secreted molecule [called S antigen in Ueda *et al.* (1990), B19R in Goebel *et al.* (1990a,b) and B18R in Smith and Chan (1991), who also noted its similarity to the type I IL-1R]. Antibodies to this protein reduce the severity of viral infection (Ueda and Tagaya, 1973). Another match was to Ig domains 2, 3 and 4 of the human DCC molecule (Fearon *et al.*, 1990), a low abundance surface protein found in many tissues but absent in a large proportion of colon carcinoma cells. Similarity was also observed to a single Ig domain flanked by potential splice signals encoded on the antisense strand of the *Drosophila melanogaster zeste* gene (Mansukhani *et al.*, 1988).

Finally, use of the transmembrane region in database searches revealed a noteworthy similarity to the membrane-spanning segment of the Epstein-Barr virus protein BHRF1 (Pfitzner *et al.*, 1987), which resembles the cellular oncogene *bcl-2*. The two transmembrane regions (VSST-FSWSIAPLSLILLVVGAIWM in the murine type II IL-1R, FSWTLFLAGLTLVICSYLFIS in BHRF1) show 10 identities and four conservative substitutions in a 17 amino acid stretch. This suggests interaction of both of these transmembrane regions with a common second subunit. The cytoplasmic portion of the type II IL-1R possesses no significant similarity with any sequence in the GenBank and EMBL databases.

Evolutionary conservation and chromosomal mapping

IL-1-like activities have been reported in a number of lower eukaryotes, including fish, starfish, tunicates and molluscs (Beck *et al.*, 1989, and references therein; Hughes *et al.*, 1990). Given these reports, it was of interest to ask whether an IL-1 receptor gene might also be conserved in lower eukaryotes. Genomic Southern blots performed at reduced stringency with DNAs from a variety of organisms show strong conservation of sequences which hybridize to type I and type II IL-1R probes (Figure 4). In mammals (human, mouse and rat), *Hind*III digests of genomic DNA show different band patterns when probed separately with the extracellular portions of the two receptor types. Avian (chicken, turkey), amphibian (*Xenopus laevis*) and invertebrate (*Caenorhabditis elegans*, *D.melanogaster*) DNAs each show the same pattern of hybridizing bands with either type I or type II probes. The hybridizing bands do not correspond to any major bands visible on the ethidium bromide stained gel. These results suggest that a molecule or molecules similar to the type I and type II IL-1 receptors of mouse may be encoded in the genomes of most eukaryotes, and that the duplication of an ancestral IL-1R gene occurred at the time mammals emerged. While invertebrates are thought not to possess immune systems, the pro-inflammatory action of IL-1 may be evolutionarily ancient.

The chromosomal locations of the murine and human type II IL-1 receptors have been determined by techniques similar to those used to map the type I IL-1 receptors (Copeland *et al.*, 1991) (data not shown). The human type II receptor gene (*IL-1R2*) is located on the long arm of chromosome 2, at position 2q12 – 2q22. The human type I receptor maps to 2q12 (Copeland *et al.*, 1991) and the human *IL1 α* and *IL1 β* genes have been mapped to 2q13 – 2q21 (Webb

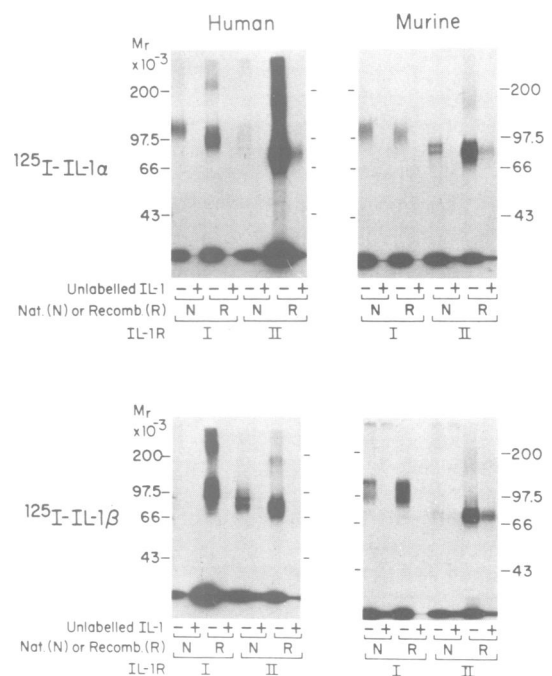


Fig. 5. Characterization of IL-1 receptors by affinity cross-linking. Cells expressing IL-1 receptors were cross-linked to [¹²⁵I]IL-1 in the absence or presence of the cognate unlabeled IL-1 competitor, extracted, electrophoresed and autoradiographed as described in Materials and methods. Recombinant receptors were expressed transiently in CV1/EBNA cells. The cell lines used for cross-linking to natural receptors were KB (human type I), CB23 (human type II), EL4 (murine type I) and 70Z/3 (murine type II).

et al., 1986; Modi *et al.*, 1988; Lafage *et al.*, 1989). This linkage is intriguing, but its significance is unclear.

In the mouse, the type II IL-1 receptor gene (*Il-1r2*) also maps to the same position as the type I IL-1 receptor gene (*Il-1r1*), at the centromere-proximal region of chromosome 1 (Copeland *et al.*, 1991). Interestingly, the *St2* gene (*St2*) also maps in same region (S.Tominaga, N.A.Jenkins, D.J.Gilbert, N.G.Copeland and T.Tetsuka, in preparation). No recombinants were found between *Il-1r1* and *Il-1r2* in 86 mice typed in common, while one recombinant was found between *St2* and the IL-1 receptor genes in 168 mice typed in common. These results are suggestive of extensive duplication in this region. In contrast to the human, the mouse IL-1 genes map to a separate chromosome, chromosome 2 (D'Eustachio *et al.*, 1987; Boulton *et al.*, 1989).

Structural analysis by affinity crosslinking

Figure 5 shows the results of an affinity crosslinking experiment, using radiolabeled IL-1 α and IL-1 β , which compares the sizes of the recombinant murine and human type II IL-1 receptor proteins with their natural counterparts, and with natural and recombinant murine and human type I receptors. In general, the sizes of the transiently expressed recombinant receptors are similar to the natural receptors, although the recombinant proteins tend to migrate slightly faster and as slightly broader bands, presumably owing to differences in glycosylation pattern when overexpressed in CV1/EBNA cells. In all cases the type II receptors are distinctly smaller than the type I receptors. One combination (natural human type I receptor with IL-1 β) failed to give

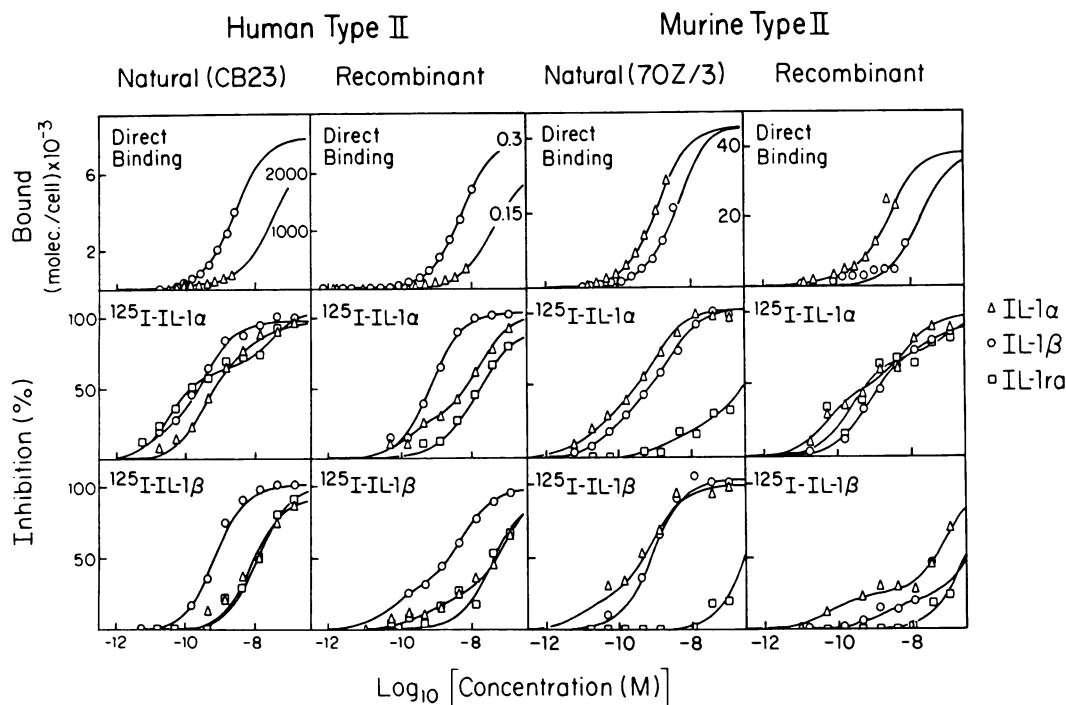


Fig. 6. Binding properties of type II IL-1 receptors. The top row shows direct binding experiments in which increasing concentrations of [125 I]IL-1 α or [125 I]IL-1 β were bound to cells expressing natural or recombinant type II IL-1 receptors. The lower two rows show competition experiments in which increasing amounts of unlabeled IL-1 α , IL-1 β or IL-1ra were used to inhibit the binding of [125 I]IL-1 α or [125 I]IL-1 β to cells.

specific crosslinking products. Since approximately equal amounts of label were loaded into each experimental lane, as evinced by the intensity of the free ligand bands at the bottom of the gels, this combination must crosslink relatively poorly.

The lane showing natural human type II receptor-bearing cells crosslinked with [125 I]IL-1 α reveals a component in the size range ($M_r \sim 100\,000$) of complexes with natural and recombinant type I receptors. No such complex can be detected in the lane containing recombinant type II receptor [Figure 5 and a lighter exposure (not shown)]. It is possible that this represents a low level of expression of type I receptors on the CB23 cells, since these cells contain trace amounts of type I receptor mRNA (see Figure 7C). This interpretation is uncertain, however, because several other ligand-receptor combinations in this experiment also gave multiple crosslinked products which, because of their size or because they occur with recombinant receptors, are unlikely to represent crosslinking to both receptor types.

Binding properties of IL-1 receptors

Figure 6 shows a survey of the binding of human IL-1 α , IL-1 β and IL-1ra to human and murine type II IL-1 receptors, in both their natural and recombinant forms. IL-1 α and IL-1 β are the agonist forms of the cytokine, whereas IL-1ra, the IL-1 receptor antagonist, competes with IL-1 α and IL-1 β for receptor binding but elicits no biological response (Hannum *et al.*, 1990; Eisenberg *et al.*, 1990; Carter *et al.*, 1990). Tables II and III summarize these results, and the results of analogous experiments with the type I receptor, in quantitative fashion. Direct binding was tested for [125 I]IL-1 α and for [125 I]IL-1 β . Also, inhibition assays were used to test all three unlabeled ligands for their ability to compete for binding with either [125 I]IL-1 α or [125 I]IL-1 β .

One general theme that emerges from the direct binding

data is that cells expressing type I IL-1 receptors show a single class of binding sites for IL-1 α and two classes of binding sites for IL-1 β . Conversely, cells expressing type II IL-1 receptors show a single class of binding sites for IL-1 β and two classes of binding sites for IL-1 α . These different patterns of binding for the two agonist forms of IL-1 arise from shared rather than separate populations of binding sites, however, since each ligand can compete completely for the other's binding to either type of receptor. Furthermore, the binding patterns seem to be an intrinsic property of the type I and type II receptor chains, since the recombinant receptors expressed at high levels in CV1/EBNA cells, in general, behave similarly to the corresponding natural receptors.

The human IL-1 receptor antagonist, IL-1ra, binds poorly if at all to the natural murine type II IL-1 receptor (Figure 6, Table III), as reported previously (Hannum *et al.*, 1990). Curiously, IL-1ra binds to the recombinant murine type II receptor with an affinity comparable with that of IL-1 α (Figure 6, Table III). The reason for the inability of the natural murine type II receptor to bind IL-1ra is unclear. IL-1ra binds to human type II IL-1 receptors, both natural and recombinant, with an affinity similar to that of IL-1 α (Figure 6, Table II), and is capable of essentially complete inhibition of the binding of both [125 I]IL-1 α and [125 I]IL-1 β to the human type II receptor. In preliminary studies, IL-1ra lacked IL-1 agonist activity on type II receptor-bearing human cells which did respond to IL-1 α and IL-1 β (M. Alderson, personal communication). These data suggest that in the human system, IL-1ra is an antagonist for both IL-1 receptors.

Expression of type I and type II IL-1R mRNA

The human type II IL-1 receptor cDNA hybridizes on Northern blots to a single species of RNA which migrates slightly faster than 18S rRNA (Figure 7). This RNA is

Table II. Binding properties of human IL-1 receptors

		Type I receptor							
		Natural				Recombinant			
		$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$		$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$		$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$		$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$	
	Site 1	1.24 \pm 0.01	3.21 \pm 0.06	0.27 \pm 0.06	3.2 \pm 0.7	600 \pm 9	5.3 \pm 0.3	200 \pm 30	0.5 \pm 0.1
	Site 2			0.98 \pm 0.06	0.24 \pm 0.03			400 \pm 30	<0.001
Unlabeled competitor	Inhibition								
		$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$
IL-1 α	Site 1	56 \pm 11	30 \pm 8	100 \pm 1	24 \pm 2	98 \pm 2	2.7 \pm 0.3	97 \pm 2	1.2 \pm 0.1
	Site 2	44 \pm 11	2.6 \pm 0.9						
IL-1 β	Site 1	54 \pm 9	24 \pm 6	43 \pm 20	70 \pm 60	75 \pm 16	6 \pm 2	98 \pm 3	0.7 \pm 0.1
	Site 2	48 \pm 8	1.2 \pm 0.4	60 \pm 20	4 \pm 2	22 \pm 16	0.2 \pm 0.2		
IL-1ra	Site 1	99 \pm 2	17 \pm 2	17 \pm 3	100 \pm 100	100 \pm 2	8 \pm 1	98 \pm 3	2.3 \pm 0.4
	Site 2			84 \pm 3	13 \pm 2				
		Type II receptor							
		Natural				Recombinant			
		$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$		$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$		$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$		$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$ $K_A(\text{M}^{-1}) \times 10^{-9}$	
	Site 1	0.22 \pm 0.04	3.0 \pm 0.7	7.9 \pm 0.3	0.24 \pm 0.04	12 \pm 1	90 \pm 30	2590 \pm 50	0.19 \pm 0.01
	Site 2	6.8 \pm 0.1	0.03 \pm 0.002			2000 \pm 700	0.03 <0.01		
Unlabeled competitor	Inhibition								
		$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$
IL-1 α	Site 1	75 \pm 8	11 \pm 3	93 \pm 4	0.13 \pm 0.02	28 \pm 4	16 \pm 7	22 \pm 5	2.0 \pm 1.0
	Site 2	20 \pm 8	0.09 \pm 0.08			72 \pm 4	0.06 \pm 0.01	74 \pm 5	0.013 \pm 0.0003
IL-1 β	Site 1	72 \pm 10	10 \pm 3	102 \pm 2	1.4 \pm 0.2	101 \pm 2	4.0 \pm 0.4	30 \pm 3	19 \pm 8
	Site 2	24 \pm 10	0.9 \pm 0.09					68 \pm 3	0.2 \pm 0.2
IL-1ra	Site 1	62 \pm 3	110 \pm 10	101 \pm 3	0.09 \pm 0.01	24 \pm 20	1 \pm 1	90 \pm 5	0.03 \pm 0.01
	Site 2	42 \pm 4	0.04 \pm 0.02			65 \pm 20	0.04 \pm 0.01		

Affinity and inhibition constants (K_A , K_I) determined for human IL-1 receptors (type I and type II, natural and recombinant) with human IL-1 α , IL-1 β and IL-1ra.

present at varying levels in a number of human B lineage cell lines, including CB23, Raji, ARH77 and RPMI1788 (Figure 7), but is not present in three B cell lines (EW36, CA46 and HRI) that are negative for surface IL-1 receptors by radioreceptor assay (Benjamin *et al.*, 1990). It is present in greater abundance in a subline of Raji that expresses 2500 IL-1 receptors/cell than in another subline expressing only 35 receptors/cell (Figure 7). Interestingly, all of these B cell lines except Raji also express type I IL-1R mRNA, although at a much lower level than type II receptor mRNA (Figure 7C and data not shown). The murine type II IL-1R cDNA hybridizes to an RNA of similar size in the mouse pre-B cell line 70Z/3 (not shown). This cell line has previously been shown to have no type I IL-1R mRNA (Bomszyk *et al.*, 1989).

The human type II IL-1R cDNA probe also hybridized to RNA extracted from monocytes (M.K.Spriggs, S.K.Dower and J.E.Sims, in preparation), total bone marrow, keratinocytes, placenta, peripheral blood T cells and the hepatoma cell line HepG2 (Figure 7). All of these RNAs also contained type I IL-1R mRNA, but at different relative abundance. HepG2 cells have roughly equal amounts of type I and type II mRNA, whereas placenta is enriched in message for the type I receptor. Bone marrow, keratinocytes and T cells have substantially more type II than type I mRNA (Figure 7C). In both keratinocytes and peripheral blood T cells, the two IL-1R mRNAs are strongly induced

when the cells are treated with phorbol ester. Endothelial cells possess a small amount of type II RNA; if type I mRNA is present, it is below our level of detection (not shown). Fibroblasts (both gingival and foreskin) and cultured aortic smooth muscle cells express only the type I IL-1R mRNA.

Discussion

The type II IL-1 receptor, whose cloning is described in this report, resembles the type I IL-1 receptor in having a ligand binding portion comprised of three immunoglobulin-like domains and a single membrane-spanning segment. The sequence identity between the ligand binding portions of the two human receptors is only 28%, and that between IL-1 α and IL-1 β , IL-1 α and IL-1ra and IL-1 β and IL-1ra is 26%, 19% and 26%, respectively (March *et al.*, 1985; Eisenberg *et al.*, 1990; Carter *et al.*, 1990). Nevertheless, both receptors can bind all three ligands. It might be speculated that in the absence of strong sequence conservation, structural elements would play a large role in the receptor-ligand interaction. Presumably some sequence-specific contacts are made, however, and the areas of modest conservation between the two receptors near the C-terminus of domain 2 and the N-terminus of domain 3 would be candidate regions to be involved in these contacts.

In addition to the conservation of amino acid residues found among most immunoglobulin family members

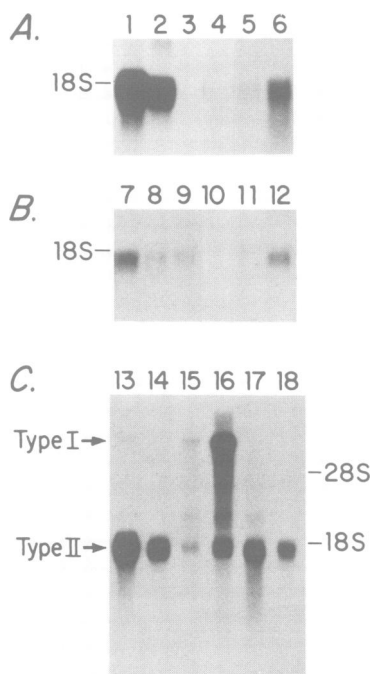


Fig. 7. Northern blots hybridized with a probe for the human type II IL-1R (A and B) or with a mixture of probes for the human type I and type II IL-1R (C). The filters shown in panels A and C contain poly(A)⁺ RNA, while that in panel B used total RNA. The lanes contain human RNA from: lane 1, CB23; lane 2, Raji; lane 3, smooth muscle; lane 4, foreskin fibroblasts; lane 5, resting peripheral blood T cells; lane 6, activated peripheral blood T cells; lane 7, CB23; lane 8, Raji; lane 9, total bone marrow; lane 10, HRI, lane 11, resting keratinocytes; lane 12, activated keratinocytes; lane 13, CB23; lane 14, Raji; lane 15, HepG2; lane 16, placenta; lane 17, activated peripheral blood T cells; lane 18, ARH77.

(Williams and Barclay, 1988), there are other hallmark residues more specifically present in IL-1 receptors (Figure 3B). Among the more notable of these are an extra pair of cysteine residues in the first domain, as well as another extra pair in the second domain of the type I receptors; prolines following the first cysteine of the Ig disulfide bond in both the first and second domains; a ProAla sequence preceding by 11 amino acids the second cysteine of the Ig disulfide bond in the first domain; two prolines near the N-terminus of the third domain; and the Gly-X-X-Tyr-X-X-ThrArg-X-Ile sequence near the C-terminus of the second domain. The extra pairs of cysteines in the first and second domains almost certainly form intra-domain disulfide bonds (J.McGourty and K.Stremler, personal communication).

Three-dimensional structures have been determined for two members of the fibroblast growth factor family (Zhu *et al.*, 1991), and have been found to be very similar to the three-dimensional structures of IL-1 α and IL-1 β . Interestingly, however, the two known FGF receptors (Dionne *et al.*, 1990; Houssaint *et al.*, 1990; Reid *et al.*, 1990; Miki *et al.*, 1991), while immunoglobulin superfamily members, are not closely related to the IL-1 receptors (Table I). The FGF receptors lack virtually all of the hallmark residues noted above. They also require only two Ig domains in order to bind FGF (Johnson *et al.*, 1990; Mansukhani *et al.*, 1990; Reid *et al.*, 1990; Hou *et al.*, 1991; Miki *et al.*, 1991), whereas all three Ig domains are necessary for the type I IL-1 receptor to bind either IL-1 α or IL-1 β (Dower and Sims, 1990).

The major difference between the type I and type II IL-1 receptors lies in their cytoplasmic portions. This segment of the type I receptor is required for induction of biological responses to IL-1 (Curtis *et al.*, 1989), and by analogy, it might be expected that signal transduction by the type II receptor will also be mediated by the cytoplasmic portion. Whereas the ~215 amino acid cytoplasmic domain of the type I receptor is large enough potentially to serve an enzymatic function, the 29 amino acid cytoplasmic domain of the type II receptor presumably must function via association with another molecule or molecules. IL-1-mediated signal transduction is poorly understood, and it is not clear whether the signaling pathways used by the type I and type II receptors are entirely separate or overlap, and if so to what extent. It is clear that the type II IL-1 receptor is capable of signaling, since biological responses to IL-1 can be observed in 70Z/3 (Giri *et al.*, 1984) and Raji (Horuk and McCubrey, 1989) B cell lines, which express only the type II receptor.

Related genes

The closest relatives of the two IL-1 receptors found in the GenBank and EMBL databases are a cellular gene termed ST2, and a vaccinia virus open reading frame called B15R. These genes encode apparently secreted immunoglobulin family members that also contain many of the hallmark residues of the IL-1 receptor family described above. It is not known whether either gene product binds IL-1.

The ST2 gene is expressed in fibroblasts stimulated to grow by serum or oncogenic transformation, but not in quiescent cells. Possibly it neutralizes a growth inhibitory molecule whose structure is similar to that of IL-1. IL-1 itself has a modest growth-promoting effect in fibroblasts, by virtue of its induction of PDGF-A chain synthesis (Raines *et al.*, 1989).

There have been several recent reports of virally encoded (hijacked cellular?) genes potentially capable of modulating host responses to infection. For example, the Shope fibroma virus, another pox virus, encodes a molecule whose sequence resembles a soluble TNF receptor and which has been demonstrated to bind TNF (Smith *et al.*, 1991). In addition, the major secretory protein of vaccinia virus inhibits the complement cascade (Kotwal *et al.*, 1990), and Epstein-Barr virus produces an interleukin-10-like molecule that inhibits production of γ -interferon by T cells and possibly by NK cells (Hsu *et al.*, 1990). Given that we have shown recently that a soluble type I IL-1 receptor can suppress responses to allogeneic cells *in vivo* (Fanslow *et al.*, 1990), it will be of interest to determine whether the B15R gene, and/or the more distantly related B18R gene, are capable of binding any of the IL-1s, and whether they can thereby promote viral infection by acting as an immunosuppressive agent.

RNA analyses

The type II IL-1 receptor has previously been thought to be primarily a B cell receptor, while the type I receptor has frequently been termed the 'T cell/fibroblast' receptor. Given this history, the presence of type II IL-1R mRNA in T cells (Figure 7) is surprising. Several cross-linking (Savage *et al.*, 1989; Solari, 1990) or antibody blocking (Munoz *et al.*, 1991) studies have suggested that more than one type of IL-1R might be present in the murine T cell line D10, albeit without positive identification or characterization of the non-

Table III. Binding properties of murine IL-1 receptors

		Type I receptor							
		Natural				Recombinant			
		$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$	$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$	$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$	$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$
	Site 1	10.8 \pm 0.01	15.9 \pm 0.5	1.4 \pm 0.1	8.4 \pm 1.2	878 \pm 25	5.4 \pm 0.6	89 \pm 33	1.3 \pm 0.6
	Site 2			9.6 \pm 0.1	0.08			762	0.03
Unlabeled competitor	Inhibition								
		$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$
IL-1 α	Site 1	101 \pm 2	27 \pm 2	30 \pm 8	800	101 \pm 2	6.3 \pm 0.8	102 \pm 2	7.9 \pm 0.8
	Site 2			72 \pm 8	5 \pm 2				
IL-1 β	Site 1	97 \pm 2	8.2 \pm 0.1	98 \pm 4	10 \pm 1	99 \pm 1	2.0 \pm 0.1	101 \pm 1	2.7 \pm 0.2
	Site 2								
IL-1ra	Site 1	97 \pm 3	17 \pm 2	80 \pm 20	6 \pm 2	100 \pm 2	15 \pm 2	103 \pm 1	15 \pm 1
	Site 2			20 \pm 19	0.1 \pm 0.03				
		Type II receptor							
		Natural				Recombinant			
		$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$	$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$	$[^{125}\text{I}]\text{IL-1}\alpha$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$	$[^{125}\text{I}]\text{IL-1}\beta$ sites/cell $\times 10^{-3}$	$K_A(\text{M}^{-1}) \times 10^{-9}$
	Site 1	0.32 \pm 0.01	0.74 \pm 0.04	0.33 \pm 0.02	0.21 \pm 0.07	1.45 \pm 1.7	70 \pm 110	38	0.05 \pm 0.01
	Site 2					37 \pm 11	0.37 \pm 0.25		
Unlabeled competitor	Inhibition								
		$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$	$I_{\text{max}}(\%)$	$K_I(\text{M}^{-1}) \times 10^{-9}$
IL-1 α	Site 1	32 \pm 4	78 \pm 25	24 \pm 4	100	48 \pm 6	170 \pm 70	24 \pm 4	20 \pm 20
	Site 2	68 \pm 4	1.1 \pm 0.2	74 \pm 4	1.2 \pm 0.3	48 \pm 6	0.13 \pm 0.06	76 \pm 4	0.01 \pm 0.03
IL-1 β	Site 1	38 \pm 5	17 \pm 5	102 \pm 2	1.3 \pm 0.1	67 \pm 5	16 \pm 3	20 \pm 7	0.8 \pm 0.5
	Site 2	62 \pm 5	0.4 \pm 0.1			22 \pm 5	0.04 \pm 0.01	78 \pm 7	<0.01
IL-1ra	Site 1	22 \pm 4	0.3 \pm 0.2	100 \pm 20	0.006	67 \pm 11	40 \pm 20	not detectable	<0.01
	Site 2	78 \pm 4	0.002			30 \pm 30	0.01 \pm 0.01		

Affinity and inhibition constants (K_A , K_I) determined for murine IL-1 receptors (type I and type II, natural and recombinant) with human IL-1 α , IL-1 β and IL-1ra. In the experiments reported here, 70Z/3 shows only one class of binding sites for [^{125}I]IL-1 α ; on other occasions (for example, see Bomsztyk *et al.* 1989), the 70Z/3 cell line shows a biphasic binding curve for [^{125}I]IL-1 α .

type I IL-1 receptor. In addition, the extent to which the phenotype of the D10 cell line reflects that of normal T cells has been questioned. Perhaps for these reasons, the possible presence of the type II receptor in T cells has not been fully appreciated. We have not demonstrated translation and surface expression of the type II IL-1R mRNA found in human peripheral blood T cells in this study, but we have shown that the type II receptor protein is present on the surface of antigen-responsive murine T_H2 T cell clones subsequent to crosslinking of CD3 (D.McKean, J.L.Slack, S.K.Dower and J.E.Sims, unpublished data).

The RNA analyses suggest that both types of IL-1 receptor may be expressed in several different cell lines, including B lymphoblastoid lines and HepG2 cells, and also in certain tissues or bulk cultures such as peripheral blood T cells, keratinocytes and placenta. The reason for this may be to increase the range of responses that can be elicited by IL-1 from a given cell type, since the very different cytoplasmic portions suggest that the two receptors utilize different signal transduction pathways. The existence of two affinity classes of IL-1 binding sites is not likely to be a consequence of co-expression, since these can be exhibited by individual recombinant receptors in transient transfection experiments (Figure 6, Tables II and III). Furthermore, the similarity of the ligand binding portions of the two receptors suggests

that they bind IL-1 in the same way, unlike the subunits of the IL-2 receptor, which interact with different segments of the IL-2 molecule. Preliminary experiments support the idea that the two receptors cannot bind IL-1 simultaneously (J.L.Slack, C.J.McMahan, J.E.Sims and S.K.Dower, unpublished data).

Materials and methods

Cells

The B lymphoblastoid line CB23 was derived by Epstein-Barr virus infection of cord blood as described (Benjamin *et al.*, 1990) and at the time of harvest for RNA expressed 17 000 IL-1 receptors/cell using [^{125}I]IL-1 β . One subline of Raji cells ('Raji-high') was obtained from the ATCC (CCL 86); these cells had ~2500 IL-1 receptors/cell using [^{125}I]IL-1 α . Another subline ('Raji-low') was from the Immunex Corporation collection; these cells had 35 IL-1 receptors/cell. 70Z/3 cells (~1000 IL-1R/cell using [^{125}I]IL-1 α) were obtained from Dr K.Bomsztyk. HepG2 (~2100 IL-1R/cell using [^{125}I]IL-1 α) cells were from Dr J.Bauer. Normal human placenta was obtained at delivery. Human bone marrow was obtained from healthy volunteer donors. Aortic smooth muscle cells were a gift from Dr E.Raines. Human foreskin keratinocytes were a gift from Dr R.Blanton and harvested for RNA after 6 h of culture either in medium or in medium containing 10 ng/ml PMA. Human foreskin fibroblasts (~5000 receptors/cell) were also from Dr R.Blanton; human gingival fibroblasts were a gift from Dr. E.Qwarnstrom. Normal human peripheral blood T cells, purified by E-rosetting, were precultured for 6 days in 10 ng/ml IL2 and 10 ng/ml OKT3 antibody, and then stimulated for 6 h in 1% PHA and 10 ng/ml PMA.

Other cells and cell lines used (and their IL-1 receptor levels at time of harvest for RNA, if known) include the human B lineage lines RPMI1788 (240 receptors/cell), CB33 (30 receptors/cell) and ARH77; EW36, CA46 and HRI (Benjamin *et al.*, 1990), three human B cell lines with no detectable IL-1 receptors: the human epidermoid carcinoma cell line KB (ATCC CCL 1717) (~1200 receptors/cell); and the murine T cell lymphoma EL-4 6.1 C10 (~10 000 receptors/cell). All cells were grown in RPMI1640 medium containing 10% fetal bovine serum.

CV1/EBNA cell line

A *HindIII*–*AhaII* restriction fragment spanning EBV coordinates 107 932 to 109 894 (Baer *et al.*, 1984) and encoding Epstein–Barr virus nuclear antigen I (EBNA-1), was cloned into the expression vector pDC302 (Mosley *et al.*, 1989) and co-transfected with pSV2gpt (Mulligan and Berg, 1981) into the African green monkey kidney cell line CV1 (ATCC CCL 70), using calcium phosphate precipitation. Cell lines capable of growing in medium containing hypoxanthine, aminopterin, thymidine, xanthine and mycophenolic acid were screened for expression of functional EBNA-1 by their ability to support the replication of plasmids containing the EBV origin of replication and a drug (G418) resistance marker.

Antibody

The anti-human type I IL-1 receptor monoclonal antibody M1, used in Figure 2, is a mouse IgG1 which blocks binding of IL-1 to the receptor. The immunogen was a line of C127 cells stably expressing the recombinant human type I receptor at ~3 × 10⁵ receptors/cell.

Expression vector pDC406

pDC406 is identical to pDC402, which is the plasmid called HAV-EO in Dower *et al.* (1989), except for the elimination of an intron within the adenovirus tripartite leader. This intron contained elements which unexpectedly promoted expression of the cDNA inserts in *Escherichia coli*.

cDNA library generation

Double-stranded, blunt-ended, random-primed cDNA was prepared from CB23 poly(A)⁺ RNA essentially as described (Gubler and Hoffman, 1983) using a Pharmacia cDNA kit. Adapters were added to the cDNA as described (Haymerle *et al.*, 1986) using the oligonucleotide pair:

```
5' TCGACTGGAACGAGACGACCTGCT 3'
3' GACCTTGCTCTGCTGGACGA 5'
```

Low molecular weight material was removed by passage over Sephacryl S-1000 at 65°C, and the cDNA was ligated into the mammalian expression vector pDC406 (see above) which had previously been cut with *SalI* and ligated to the same oligonucleotide pair. DNA was electroporated (Dower *et al.*, 1988) into *E. coli* DH5 α , and after 1 h growth at 37°C, the transformed cells were frozen in 1 ml aliquots in SOC medium (Hanahan, 1983) containing 20% glycerol. One aliquot was titered to determine the number of ampicillin-resistant colonies. The resulting CB23 library had 3.9 × 10⁶ individual clones. cDNA libraries were constructed in λ gt10 using RNA from the human B lymphoblastoid cell line Raji or from the murine pre-B lymphoma line 70Z/3, using standard techniques (Mosley *et al.*, 1989).

Transfection procedure

One-chambered glass slides (Nunc # 177372) were treated with 1 ml of human fibronectin (Boehringer Mannheim; 10 ng/ml in PBS) for 30 min at room temperature and rinsed with PBS. CV1/EBNA cells were seeded at 2 × 10⁵ cells per slide in 3 ml of medium (DMEM containing 10% fetal bovine serum) and allowed to adhere overnight at 37°C. The medium was replaced with 1.5 ml medium containing 66.7 μ M chloroquine, and a DNA mixture consisting of 2 μ g DNA, medium containing chloroquine to 175 μ M, and 25 μ l of DEAE dextran (4 mg/ml in PBS) was added to the cells. Cells and DNA were left at 37°C for 5 h. The DNA mix was removed and the cells were shocked with 1 ml medium containing 10% DMSO for 2.5 min. The medium was then replaced with 3 ml fresh medium, and the cells placed at 37°C for 3 days.

Library transfections and screening

Bacteria transformed with the CB23 library in pDC406 were plated in pools of ~4000 colonies onto plates containing ampicillin, and after overnight growth, scraped into LB (10 g tryptone, 5 g yeast extract and 10 g NaCl per liter). Miniprep (Birnboim and Doly, 1979) DNA was made from approximately one-quarter of the cells, and the remaining cells were frozen in LB containing 20% glycerol. Approximately 2 μ g of miniprep DNA was transfected into CV1/EBNA cells as described above. After 3 days at 37°C, the slides were screened by incubating them with 3 × 10⁻⁹ M [¹²⁵I]IL-1 β as described (Sims *et al.*, 1988), rinsed with PBS, and then soaked in 2.5%

glutaraldehyde for 30 min to fix the cells to the slide. After rinsing with PBS, they were dipped in liquid photographic emulsion (Kodak GTNB-2) at room temperature, dried and allowed to expose for 2–10 days in a light-proof box at 4°C before developing. Slides were examined at 25× magnification under bright-field illumination to detect positive cells.

Using this technique, one pool (# 75) was identified as having numerous strongly positive cells. A glycerol stock of this pool was thawed and plated into 18 pools of 500, and screened on slides as described above. Two pools of 500 were positive, and one of these was used for further breakdown. Subsequent pools of 75 colonies, and then single colonies, were screened by transfecting 2 × 10⁵ CV1/EBNA cells with 2 μ g DNA in 6-well dishes essentially as described above, except that after incubation with [¹²⁵I]IL-1 β , the cells were removed from the well with trypsin and counted in a gamma counter. The positive clone thus identified was termed clone HuII75.

Additional human type II IL-1R cDNA clones were isolated from a Raji λ gt10 oligo(dT)-primed cDNA library by screening in 50% formamide/5 × SSC at 42°C with a ³²P-labeled DNA probe made by random priming from the entire insert of HuII75. The filters were washed at 63°C in 2 × SSC/1% SDS. The sizes of the cDNA inserts from the primary positive plaques were determined by Southern hybridization, using the same probe, and two phage were chosen for plaque purification, subcloning of their cDNA inserts, and sequencing to confirm the representative nature of clone 75.

Murine type II IL-1R cDNA clones were obtained by screening a 70Z/3 λ gt10 random-primed cDNA library in 35% formamide/5 × SSC at 42°C with the HuII75 probe. Two positive plaques were purified, and the cDNA inserts subcloned. When DNA sequencing showed that these clones were truncated at their 3' ends, 3'-anchored PCR (Frohman *et al.*, 1988) was used to isolate a clone extending from 5' of the initiating methionine to the poly(A) tract. This clone (MuII-FL) was sequenced, and also transferred into pDC406 for expression in CV1/EBNA cells, which confirmed that it encoded an IL-1 receptor.

Zoo blot

Southern blots containing 5 μ g/lane of *HindIII*-digested genomic DNA from various organisms were hybridized at 65°C and washed in 0.12 M NaCl at either 55°C (for the blot probed with the type I receptor probe) or 60°C (for the blot probed with the type II receptor probe). The murine type I IL-1 receptor probe was a 694 bp cDNA fragment extending from the *DraI* site (nucleotides 394–399) within the second Ig-like domain of the extracellular portion, through the *HindIII* site (nucleotides 1085–1090) within the cytoplasmic portion (Sims *et al.*, 1988). The murine type II receptor probe was a 595 bp cDNA fragment extending from the 5' end of cDNA clone MuII2 (Figure 3A) through an *EcoRI* site at the position of amino acids Glu170/Phe171 (Figure 3B), within the second Ig-like domain of the extracellular portion.

Chromosome mapping

The human type II IL-1 receptor gene (*IL-1R2*) was mapped to chromosome 2q12 → 2q22 by a combination of segregation analysis in rodent–human hybrid cells and chromosomal *in situ* hybridization. Methods and cell lines were as described (Copeland *et al.*, 1991), with additional cell lines either described in Cannizzaro *et al.* (1987), Durst *et al.* (1987) and Finger *et al.* (1988), or obtained from the NIGMS Human Genetic Mutant Cell Repository (Coriel Institute, Camden, NJ) (Hybrids 8854, 9142, 7300, 7298 and 7297). The probe was a ~750 bp restriction fragment of clone HuII75 extending from the *EcoRI* site at the position of amino acids Glu157/Phe158 (Figure 3B), within the second Ig-like domain of the extracellular portion, through the 3' end of the cDNA insert. The murine type II IL-1 receptor gene (*Il-1r2*) was mapped to the centromeric region of chromosome 1 by analysis of restriction fragment length polymorphisms in interspecific backcrosses as described (Copeland *et al.*, 1991). The probe was a ~700 bp restriction fragment of clone MuII2 extending from the *EcoRI* site at the position of amino acids Glu170/Phe171 (Figure 3B), within the second Ig-like domain of the extracellular portion, through the 3' end of the clone (Figure 3A), four amino acids prior to the translational stop codon.

RNA hybridization

RNA samples were electrophoresed and blotted as described (Sims *et al.*, 1988), using 5 μ g (Figure 7A) or 2 μ g (Figure 7C) of polyadenylated RNA, or 5 μ g of total RNA (Figure 7B), per lane. The filters were subsequently stained with methylene blue to monitor the evenness of loading and transfer. Blots were hybridized overnight in 50% formamide/5 × SSC at 63°C with antisense RNA probes, and washed at 63°C in 0.2 × SSC/1% SDS. The human type II IL-1 receptor probe was made from the entire insert of clone HuII75 (1350 nucleotides). The human type I IL-1 receptor cDNA probe was a mixture of two subclones, from the extracellular and cytoplasmic

regions, covering a total of 1030 nucleotides. Control lanes, containing Sp6 RNA polymerase sense transcripts of the two receptor cDNAs, were run in the experiment of Figure 7C to ensure that comparable hybridization signals were obtained from the type I and type II IL-1 receptor probes (not shown).

Affinity crosslinking and radioreceptor assays

Recombinant human IL-1 α and IL-1 β were expressed, purified and labeled as described previously (Dower *et al.*, 1985, 1986). Recombinant IL-1 receptor antagonist (IL-1ra) was cloned (D. Anderson, unpublished data) using the published cDNA sequence (Eisenberg *et al.*, 1990), expressed by transient transfection in COS cells, and purified by affinity chromatography on a column of soluble human type I IL-1 receptor coupled to affigel (Dower *et al.*, 1989; C.J. McMahan, J.L. Slack, S.K. Dower and J.E. Sims, unpublished results) with elution at low pH.

Affinity crosslinking was performed as described (Park *et al.*, 1987) using 1 nM [¹²⁵I]IL-1 α or [¹²⁵I]IL-1 β without or with 1 μ M competitor unlabeled IL-1 as a specificity control. KB cells and all recombinant receptors expressed in transfected CV1/EBNA cells were analyzed as adherent monolayer cultures; all other cells were analyzed in suspension at 1.6×10^7 cells/ml. Aliquots containing equal amounts (c.p.m.) of [¹²⁵I]IL-1 for all experimental samples, and equal volumes of the corresponding specificity controls, were electrophoresed on 10% polyacrylamide slab gels.

Binding assays were performed as described previously (Sims *et al.*, 1988). EL-4 6.1 C10, 70Z/3, CB23 and KB cells were analyzed in suspension at 1.6×10^7 cells/ml. CV1/EBNA cells expressing recombinant receptors were analyzed in suspension at 1.6×10^5 cells/ml with 1.6×10^7 EL4 3⁺ (IL-1 receptor-negative) cells/ml added as carriers. For competition assays [¹²⁵I]IL-1 was present at 0.3 nM. All data were corrected for non-specific binding, measured in the presence of 1 μ M IL-1.

Analysis of binding data

We and others have often observed that when [¹²⁵I]IL-1 α and [¹²⁵I]IL-1 β are individually bound to the same cells in the same experiment, the apparent site numbers for the two ligands do not correlate well. Nevertheless, when inhibition studies are performed, the two unlabeled IL-1s completely cross-compete, indicating that there are no sites present which bind one or the other form exclusively. In addition it is often observed that the equilibrium binding of one or both ligands shows complexity, consistent either with receptor heterogeneity or with some form of cooperativity. Dissociation kinetics experiments have ruled out the latter possibility, however (Dower *et al.*, 1985; Benjamin *et al.*, 1990). The apparent discrepancy between the direct binding and the inhibition experiments can be accounted for in one of two ways: (i) there are sites which bind one form of [¹²⁵I]IL-1 with such low affinity that they cannot be detected above the non-specific background in direct binding experiments; or, (ii) one ligand is damaged by the radiolabeling procedure, and as a consequence, cell surface receptors selectively bind the less heavily labeled or unlabeled molecules in the preparation. Therefore, the bound counts cannot be converted into bound molecules based on the average specific activity of the labeled preparation, and the apparent site number is underestimated. Since in many cases the inhibition data we generated could not be fit by a simple single site model, indicating that complex binding isotherms were observed even where ligand damage by radiolabeling was not an issue, we chose to treat all instances of site number discrepancy according to possibility (i) and fit the data with a non-cooperative two-site model constraining the total site number to be equal to that observed for the ligand that gave the higher site number.

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