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Immunology

Cloning the interleukin 1 receptor from human T cells

(cytokine/lymphokine/fibroblast)

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ABSTRACT **cDNA clones of the interleukin 1 (IL-1) receptor expressed in a human T-cell clone have been isolated by using a murine IL-1 receptor cDNA as a probe. The human and mouse receptors show a high degree of sequence conservation. Both are integral membrane proteins possessing a single membrane-spanning segment. Similar to the mouse receptor, the human IL-1 receptor contains a large cytoplasmic region and an extracellular, IL-1 binding portion composed of three immunoglobulin-like domains. When transfected into COS cells, the human IL-1 receptor cDNA clone leads to expression of two different affinity classes of receptors, with K_a values indistinguishable from those determined for IL-1 receptors in the original T-cell clone. An IL-1 receptor expressed in human dermal fibroblasts has also been cloned and sequenced and found to be identical to the IL-1 receptor expressed in T cells.**

The cytokines interleukins 1α and 1β (collectively IL-1) play a central role in mediating both immune responses and inflammatory reactions (for review, see refs. 1 and 2). IL-1 elicits its activities by binding to a specific receptor molecule on the surface of responsive cells (3). cDNA clones of the IL-1 receptor expressed in murine T cells have shown it to be a 557-amino acid transmembrane protein possessing a single membrane-spanning segment (4). The extracellular, ligand-binding portion of the molecule consists entirely of three immunoglobulin-like domains. The 217-amino acid intracellular portion, while large enough in principle to possess some enzymatic function, bears no compelling resemblance to any sequence currently in standard data bases. The cloned murine IL-1 receptor is capable, in transfection assays, of fully reconstituting both the ligand binding and the signal transduction properties of the native receptor. For example, when expressed transiently at very high levels in COS cells, the recombinant receptor possesses IL-1 binding characteristics that are indistinguishable from those of the natural receptor expressed in EL4 cells (4). Indeed, a secreted, soluble form of the IL-1 receptor, containing only the extracellular part of the molecule, binds IL-1 with an affinity identical to that of the receptor in EL4 cells, demonstrating that this is the only molecule involved in IL-1 binding in these cells (5). In addition, high-level stable expression of the recombinant receptor in Chinese hamster ovary (CHO) cells results in significantly enhanced sensitivity of these cells to IL-1 (6). The increase in sensitivity does not occur if the cytoplasmic domain of the receptor has been deleted and is most simply accounted for if all of the transfected receptors are functional in signaling. Thus, the recombinant murine IL-1 receptor is fully functional in signal transduction as well as in IL-1 binding.

We have used the murine IL-1 receptor cDNA clone as a probe to isolate cDNA clones of the human IL-1 receptor from a human T-cell line and find the mouse and human receptors to be very similar molecules.† In addition, we have isolated a cDNA clone encoding the IL-1 receptor expressed in human dermal fibroblasts and have determined that its nucleotide sequence is identical to that of the IL-1 receptor expressed in human T cells.

RESULTS

We have analyzed the IL-1 receptor expressed in a $CD4^+$ $CD8^-$ human T-cell line (clone 22) (7). Resting clone 22 cells constitutively displayed two affinity classes of IL-1 receptor. The predominant class, present at 510 ± 80 receptors per cell, had a K_a of $1.2 \pm 0.5 \times 10^9\ M^{-1}$, similar to that of the IL-1 receptors found on murine T-cell lines such as EL4 (8–10). A second class of receptors, present at 50 ± 20 receptors per cell, had a K_a of $2 \pm 3 \times 10^{11}\ M^{-1}$ [this second class has also been reported for some subclones of EL4 (9)]. Upon stimulation of the clone 22 cells with IL-2 and anti-CD3 antibody, both types of receptor increased in number to 2800 ± 100 and 200 ± 100 receptors per cell, respectively. The difference in the nature of these two affinity classes of receptor, and their relevance, is at present not known. It should be noted that in our laboratory the murine T-lymphoma line EL4 possesses only the lower-affinity class of IL-1 receptors and yet is fully capable of IL-2 production in response to subpicomolar concentrations of IL-1.

Isolation and Analysis of cDNA Clones. To isolate clones encoding the human IL-1 receptor, we screened an oligo(dT)-primed cDNA library made from clone 22 RNA with a murine IL-1 receptor cDNA probe (see Fig. 1 legend). Several different cDNA clones were isolated. The cDNA from the clone with the longest insert, λ4, was sequenced in its entirety. Comparison with the mouse IL-1 receptor sequence revealed that this clone contained with entire 3′ untranslated region and ≈90% of the coding region. A 450-base-pair restriction fragment from the 5′ end of this clone was used to screen a second, randomly primed cDNA library made from clone 22 RNA, and a number of new clones were isolated. These were characterized by restriction mapping and by hybridization to probes from different parts of the coding region of λ4, and portions of several of them were eventually sequenced. Two of these clones contained the entire coding region (based on sequence homology to the murine IL-1 receptor). Fig. 1 diagrams the cDNA clones analyzed in detail, and Fig. 2 shows the composite sequence of the human IL-1 receptor mRNA deduced from the cDNA clones isolated.

The human IL-1 receptor protein is very closely related to the mouse IL-1 receptor, both in sequence and in overall organization. Table 1 shows that the extracellular portions are identical in size, and the signal peptides, transmembrane regions, and cytoplasmic portions are similar in length. The overall sequence identity is 69% at the amino acid level and 75% at the nucleotide level.

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Abbreviation: IL-1, interleukin 1.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M20658 and M27492).

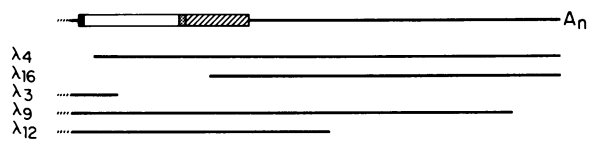

FIG. 1. Diagram of the extent of various cDNA clones used to determine the sequence of the human T-cell IL-1 receptor. Clones λ4 and λ16 were obtained from an oligo(dT)-primed cDNA library, and clones λ3, λ9, and λ12 were from a randomly primed cDNA library. Both libraries were made in λgt10 from clone 22 cell RNA taken 48 hr after stimulation. Dotted lines at the left indicate further sequence to the 5′ side not dealt with in this report. Plaque-lift filters from the oligo(dT)-primed library were hybridized and washed at 50°C in 0.9 M NaCl, using the entire insert of the murine IL-1 receptor clone 78 (4) as a probe.

As for the murine IL-1 receptor, the extracellular, IL-1 binding part of the human IL-1 receptor is a member of the immunoglobulin gene superfamily. It is organized into three domains, each of which would be predicted to adopt an immunoglobulin-like fold (11) based on the conservation of critical amino acid residues. Both the signal peptide and the transmembrane region are only modestly conserved between human and mouse. The cytoplasmic segments of the molecules are the most highly conserved, sharing 78% of their amino acids.

The 3′ untranslated regions for both murine and human IL-1 receptor mRNAs are unremarkable. There is very little nucleotide similarity between them. The 3′ untranslated region is 3118 nucleotides in the human and ≈2800 nucleotides in the mouse. The human 3′ untranslated region contains an *Alu* sequence at nucleotides ≈1900 to ≈2170. Based on analysis of the 3′ ends of a number of cDNA clones, the human seems to have a single poly(A) site, whereas the murine gene has two closely spaced poly(A) sites. Each of the three sites of polyadenylylation is preceded by the canonical sequence (12) AATAAA.

The 5′ untranslated regions are also not conserved between mouse and human. Comparison of several human cDNA clones shows that there is a common 5′ untranslated region shared between all human mRNAs that extends ≈85 nucleotides upstream of the initiating ATG. Further to the 5′ side, different mRNAs seem to have different sequences (J.E.S., unpublished data). Whether this is also true in the mouse is not yet known. A detailed study of this phenomenon will be published elsewhere. On Northern blots, both the murine (4) and human (not shown) IL-1 receptor coding region probes hybridize to an RNA that migrates just slower than 28S rRNA, consistent with a messenger RNA of ≈5 kilobases, as would be estimated from the sizes of the coding and 3′ untranslated regions and a few hundred bases of 5′ untranslated region.

IL-1 Binding Properties. To establish unequivocally that the cDNA clones we had isolated did indeed encode the human IL-1 receptor, we subcloned a restriction fragment containing the presumptive coding region into a mammalian expression vector (see Fig. 3 legend). This plasmid was introduced into COS cells and the IL-1 binding characteristics of the transfected cells were examined.

The transfected COS cell population expressed an average of 33,000 IL-1 binding sites per cell, with a K_a of $7 \pm 1 \times 10^8$ M^{-1} (Fig. 3*B*). This is similar to the K_a of $1.2 \pm 0.5 \times 10^9$ M^{-1} found for the major class of IL-1 receptors on clone 22 cells. Binding of ^{125}I-labeled IL-1α to the transfected COS cells was completely inhibitable by a 100-fold excess of unlabeled IL-1α or IL-1β. Since in a typical experiment ≈5% of the COS cells become transfected (as measured by flow cytometry using fluorescein isothiocyanate-conjugated IL-1α as a detection reagent) (4), each cell that has actually taken up the IL-1 receptor plasmid would be expressing ≈600,000 receptors per cell. The background level of IL-1 receptor expression on untransfected COS cells, or on COS cells transfected with the pDC201 vector plasmid alone, is ≈500 receptors per cell. Thus, the cDNA clone we have isolated does indeed encode an IL-1 receptor.

Interestingly, the transfected COS cells also expressed a small number of higher-affinity receptors with a K_a of $\approx 5 \times 10^{11}$ M^{-1} (Fig. 3*B*). This is in contrast to COS cells transfected with the murine IL-1 receptor cDNA, which only express a single affinity class of receptor. The high-affinity receptors were present in the population at an average of 60 receptors per cell, or, correcting for the frequency of cells that have actually taken up DNA, at a level of ≈1200 receptors per transfected cell. This is a substantially greater number of K_a $\approx 10^{11}$ M^{-1} receptors than are found on the clone 22 T cells, although as a fraction of the total IL-1 receptors present it is lower than the percentage of high-affinity sites on clone 22 cells. The untransfected COS cells possess only the lower-affinity sites. Thus, transfection of a single type of cDNA molecule into the COS cells can lead to expression of both high- and low-affinity IL-1 receptors.

Fibroblast IL-1 Receptor. The IL-1 receptor expressed by B lymphocytes appears to be smaller than the T-lymphocyte IL-1 receptor and to have a different relative affinity for IL-1α and IL-1β (13–15). This raises the question of whether there are different types of IL-1 receptor expressed in different tissues. We have started to address this question by examining the IL-1 receptor expressed in fibroblasts. The size of human fibroblast receptors, estimated from cross-linking experiments, is ≈79 kDa, very similar, but not identical, to the size of the T-cell IL-1 receptor (16). Some measurements have estimated the K_a of the fibroblast receptor to be 3×10^9 M^{-1} (16, 17), identical to the K_a of the T-cell IL-1 receptor, whereas others have suggested that the receptor on fibroblasts has a K_a of 1×10^{11} M^{-1} (18). In addition, there is one report of a considerable difference in the relative affinity for IL-1α and IL-1β of the receptors on fibroblasts and on T cells (19). We have isolated cDNA clones of the IL-1 receptor expressed in human dermal fibroblasts by hybridization at moderate stringency with a probe derived from the human T-cell IL-1 receptor cDNA clone. The frequency of the clones in the cDNA library [made from total poly(A)$^+$ RNA] was ≈1 in 5000. Fourteen of these were picked at random and tested by Southern blotting for similarity to probes made from both the extracellular and cytoplasmic regions of the T-cell IL-1 receptor. All 14 clones hybridized to the probes as well as did the T-cell type receptor clone, even when washed to very high stringencies (68°C; 0.015 M NaCl). Next, the restriction maps of three of the clones were characterized in some detail and were found to be identical to each other and to the restriction map of the T-cell IL-1 receptor. Finally, the entire coding region was sequenced from one of the fibroblast clones and was identical to that of the IL-1 receptor clones isolated from human T cells, with the exception of an A to C change at nucleotide 1681, which does not alter the amino acid sequence. This difference seems more likely to be a polymorphism between individuals or an error introduced during cDNA synthesis than an indication of different genes being expressed in the two cell types. Thus, we conclude that the IL-1 receptor expressed in human dermal fibroblasts is the same as that made by the clone 22 human T-cell line and that such differences as have been observed in size and affinity for ligand are probably due to posttranslational modifications and/or methodological differences.

DISCUSSION

We have isolated cDNA clones of the IL-1 receptor from a human T-cell line by using the murine IL-1 receptor cDNA as

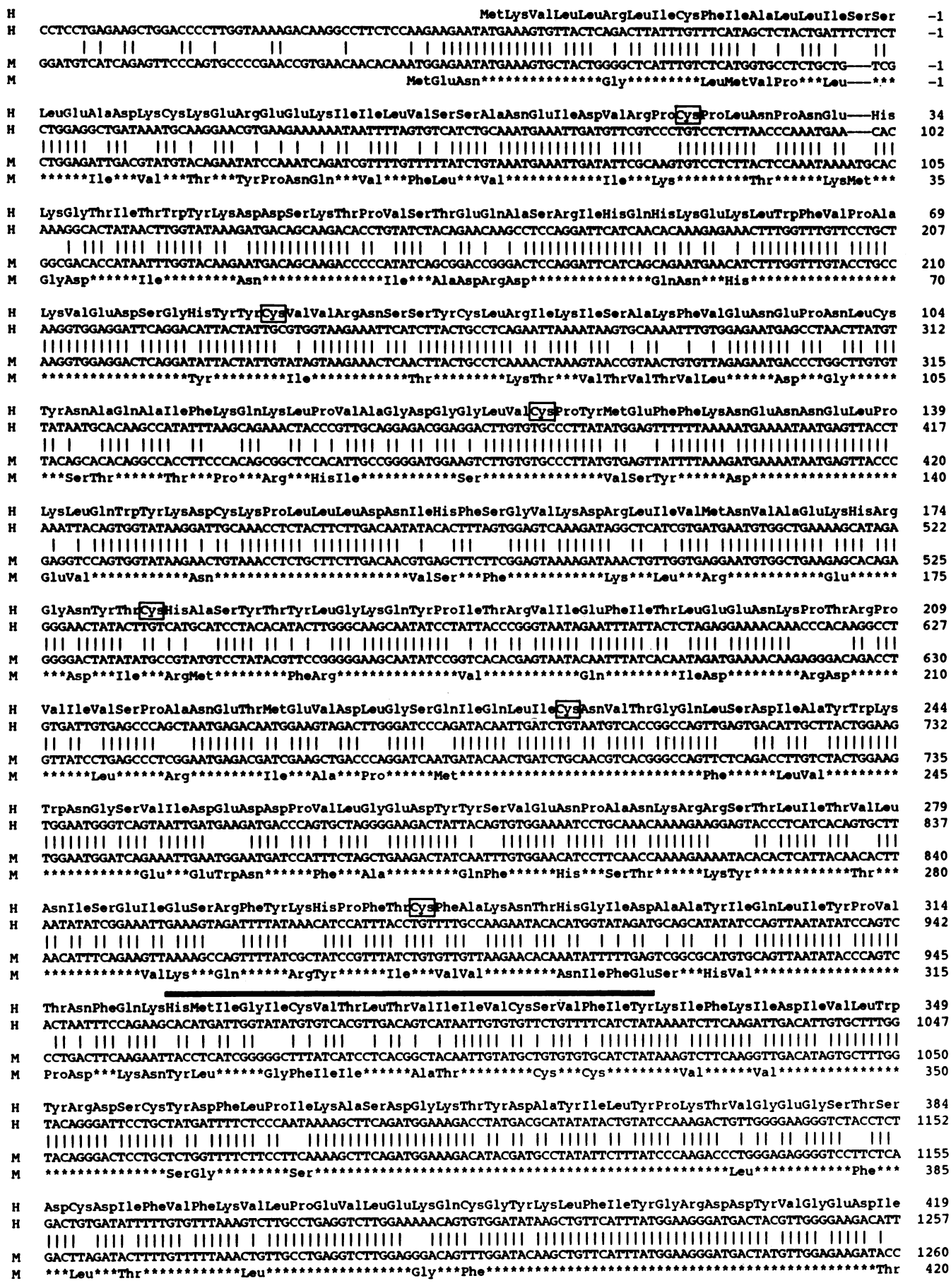

FIG. 2. (*Figure continues on the opposite page.*)

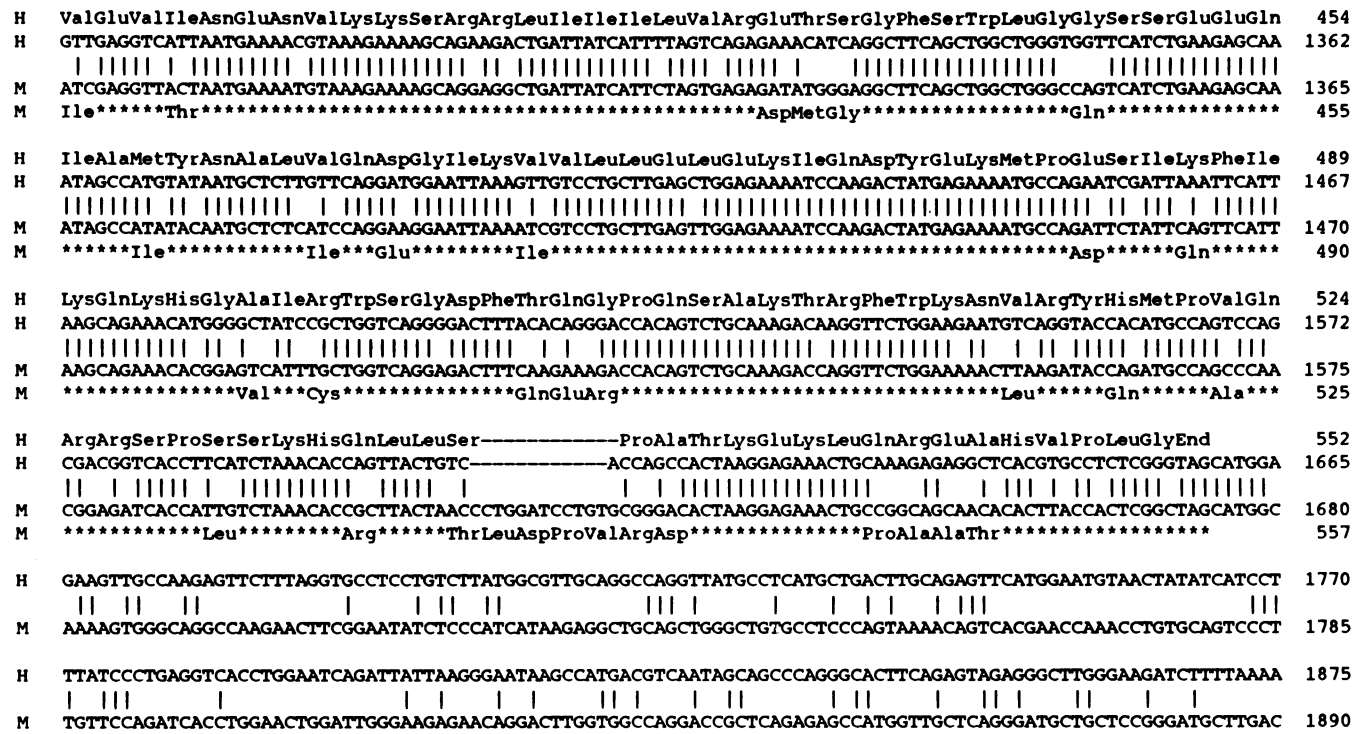

FIG. 2. Sequence of the IL-1 receptor expressed in human T cells. The deduced amino acid sequence is shown above the cDNA sequence. The third and fourth lines show the nucleotides and amino acids in the murine IL-1 receptor sequence (4) that differ from the human sequence. Both the nucleic acid and amino acid sequences are numbered from the first residue of the mature proteins, which for the human was determined by analogy with the sequenced N terminus of the natural murine IL-1 receptor protein. Amino acid residues −1 through −17 form a presumptive signal peptide. The putative transmembrane region is overlined. The cysteines postulated to form the immunoglobulin domain disulfide bonds are boxed. Only the sequence of the coding region and areas immediately surrounding it are shown. The sequences of the entire murine and human IL-1 receptor coding and 3′ untranslated regions have been deposited with GenBank.† All sequencing was performed on both strands and across all internal restriction sites used in subcloning.

a probe. The human and murine receptors are very similar molecules. Both contain an extracellular, ligand-binding segment composed of three immunoglobulin-like domains, a single transmembrane region, and a cytoplasmic portion of ≈215 amino acids that does not obviously resemble other sequenced molecules of known function. There is also a high degree of sequence conservation between the murine and human receptor, particularly in the cytoplasmic portion of the molecule. Possibly the cytoplasmic portion interacts with some other molecule inside the cell and as a consequence is significantly constrained in its ability to mutate.

The mechanism of signal transduction by IL-1 is unknown. IL-1 does not lead to breakdown of phosphatidylinositol lipids, nor does it lead to an increase in intracellular calcium concentration (20–22). Some investigators have found an increase in phosphorylation of certain cellular proteins in response to IL-1 (23–27). The only one of these proteins whose identity is known is the epidermal growth factor receptor (25). In that instance, phosphorylation seems to be mediated by a protein kinase other than protein kinase C. It is clear from examination of the sequence of the IL-1 receptor that it does not possess many of the sequence motifs that are conserved in all other protein kinases (28). Thus, it is unlikely that the IL-1 receptor itself actually functions as a protein kinase. However, the receptor could interact with a separate protein kinase to activate it.

Table 1. Similarity between mouse and human IL-1 receptors

	Mouse	Human	Amino acid identity, %	Nucleotide identity, %
Signal peptide	19 aa	17 aa	53	63
Extracellular portion	319 aa	319 aa	64	73
Transmembrane region	21 aa	20 aa	48	59
Cytoplasmic domain	217 aa	213 aa	78	80
Mature protein	557 aa	552 aa	69	75

aa, Amino acids.

The cytoplasmic portions of the mouse and human IL-1 receptors show some resemblance to sequences that are conserved in many nucleotide binding proteins (T. P. Hopp, B. Gallis, J.E.S., and C.J.M., unpublished data). If the IL-1 receptor does bind a nucleotide, this might suggest that the receptor can function as its own G protein in stimulating protein kinase activity and perhaps other functions as well.

Several investigators have observed that IL-1–IL-1 receptor complexes can be found in the nucleus several hours after incubation of cells with IL-1 (29, 30). It is believed that proteins that accumulate in the nucleus do so by virtue of a particular amino acid sequence that mediates their nuclear uptake (31). While this sequence is not well defined, comparison of several examples of sequences that serve this function in different proteins suggests that a stretch rich in basic amino acids and prolines may be essential. Such a sequence can be found near the C terminus of the mouse and human IL-1 receptors in the vicinity of amino acids 522–531. Another sequence rich in basic amino acids but lacking in prolines can be found at amino acids 428–432. At the moment, it is not known whether either of these sequences mediates transport of the IL-1–IL-1 receptor complex to the nucleus, nor is it known whether such nuclear accumulation is important in IL-1 receptor-mediated signal transduction.

IL-1 exerts a broad range of effects on a wide array of cell types. In some instances, the receptors have been shown to

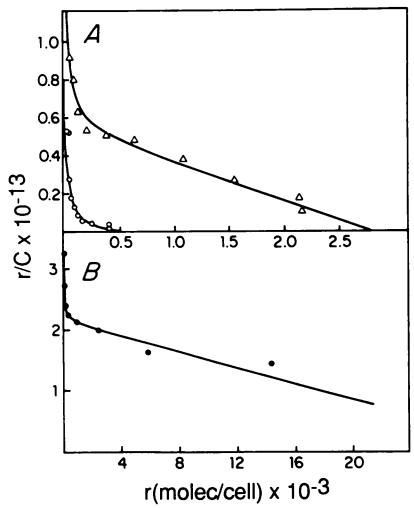

FIG. 3. (*A*) Scatchard analysis of the IL-1 receptors on clone 22 cells (7) before and after stimulation with IL-2 and anti-CD3 antibody. (*B*) Scatchard analysis of IL-1 receptors on a COS cell population 3 days after transfection with the human recombinant IL-1 receptor. ^{125}I-labeled IL-1α was incubated with cells, cell-bound IL-1 was separated from free, and the data were analyzed as described (4). In *A*, clone 22 cells were grown as described (7) and analyzed 7 days (○) or 48 hr (△) after their last stimulation. When present, IL-2 and OKT3 antibody were used at 10 ng/ml each. For *B*, an expression plasmid was generated by inserting a *Sty* I to *Bgl* II restriction fragment (nucleotides −85 to 1867 of Fig. 2) of the human IL-1 receptor cDNA into pDC205, a variant of pDC201 (4) from which the adenovirus tripartite leader has been deleted. We were unable to clone the human receptor in the sense orientation in the parental pDC201 vector. We believe that this is because low-level expression of the human IL-1 receptor in *Escherichia coli*, driven by a cryptic promoter within the tripartite leader segment, is toxic to the bacteria. Transfections were performed as described (4).

be 80-kDa proteins (4, 8, 16, 18, 32–34), whereas on B cells the IL-1 receptor appears to have a smaller size and different IL-1 binding properties than does the IL-1 receptor on T cells (13–15). This suggests that the IL-1 receptor on B cells may be a different molecule from that expressed in T cells. Indeed, given the wide variety of activities mediated by IL-1, it would not be surprising for there to be several different kinds of IL-1 receptors possessing different signaling mechanisms and expressed in different cell types. As a start on analyzing the IL-1 receptors expressed in different types of cells, we have isolated cDNA clones of the IL-1 receptor expressed in human dermal fibroblasts. Fibroblasts are known to respond to IL-1 by proliferating (16, 35) and by synthesizing a wide variety of inflammatory mediators such as IL-6, granulocyte–macrophage colony-stimulating factor, and prostaglandins (36–38). The proliferation is mediated by induction of platelet-derived growth factor (39). The cDNA clone that we have sequenced from human fibroblasts is identical in its coding region to the IL-1 receptor expressed in human T cells. While we cannot say that this is the only type of IL-1 receptor expressed in human fibroblasts, the frequency of clones isolated by hybridization at relatively high stringency was high, and restriction mapping of three of the clones indicated them all to be identical within the coding region. It seems likely, therefore, that despite the different responses of T cells and fibroblasts to IL-1, their IL-1 receptors are the same.

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