# Purification and Characterization of a Novel Soluble Receptor for Interleukin 1

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## Summary

Affinity chromatography and reverse-phase high-performance liquid chromatography was used to purify a soluble interleukin 1 $\beta$  (IL-1 $\beta$ ) specific binding protein from the supernatant of a human B cell line, Raji. The purified protein specifically bound <sup>125</sup>I IL-1 $\beta$  forming a 60-kD complex in nonreducing conditions and a 70-kD complex in reducing conditions. Binding was found to be displaceable by mature human and murine IL-1 $\beta$  and human 31-kD IL-1 $\beta$  propeptide, but not displaceable by human and murine IL-1 $\alpha$  or human IL-1 receptor (IL-1R) antagonist. Ligand blotting revealed a 47-kD molecule that specifically bound IL-1 $\beta$ . Measurement of binding affinity of the cell surface Raji IL-1R ( $K_d = 2.2$  nm) and the Raji soluble (s)IL-1R ( $K_d = 2.7$  nm) demonstrated a similar affinity for <sup>125</sup>I IL-1 $\beta$ . Purified sIL-1R inhibited binding of IL-1 $\beta$  to cell lines with both type I (80 kD) and type II (65 kD) IL-1Rs, but did not interfere with IL-1 $\alpha$ binding. This natural sIL-1R may function as an important regulatory molecule of IL-1 $\beta$  in vivo.

<sup>1</sup>L1 $\alpha$  and IL1 $\beta$  are polypeptides with central roles in the regulation of immune and inflammatory reactions (1). IL-1 responses are mediated by specific high affinity cell surface receptors (IL-1R) of which at least two distinct forms are known to exist (2). An 80-kD (type I) receptor is found mainly on T cells and fibroblasts (3), and a lower molecular mass 65-kD, (type II) receptor is found on B cells (4) and macrophages. Many cytokine receptors are known to exist in soluble form, and release from the cell surface occurs by two distinct mechanisms. Alternative splicing of the primary RNA transcript can result in protein isoforms that lack transmembrane domains and are therefore secreted, e.g., IL-4R (5). Proteolytic cleavage of the transmembrane molecule at the cell surface, e.g., IL-2R (p55) (6), also results in receptor shedding. Other cytokine receptors that exist in soluble form include those for IL-2 (p75) IL-5, IL-6, IL-7, IFN- $\gamma$ , TNF (p75 and p55), epidermal growth factor (EGF), and M-CSF.

We have recently described a soluble binding protein specific for IL-1 $\beta$  present in normal human plasma (7), serum, synovial inflammatory exudate, activated PBMC supernatants (8), and supernatants from the human B cell line, Raji (9). Stimulation of Raji with dexamethasone increased surface expression of the IL-1R and the rate of release of soluble binding protein. Conversely, a serine protease inhibitor prevented release of the binding protein and increased IL-1R expression of the cells. Together, the data indicate that the soluble IL-1 $\beta$ binding protein is probably a proteolytically cleaved form of a surface IL-1R. Here we report the purification and characterization of this novel soluble (s)IL-1R and provide evidence that it may function as a naturally occurring regulator of IL-1 $\beta$ .

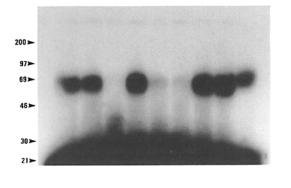
#### Materials and Methods

Purification of Raji sIL1R. The Raji cell line was obtained from the European Cell Culture Collection (Porton, Wilts, UK). Cells were maintained at 37°C in RPMI 1640 containing 5% FCS, and media was aspirated every 3–4 d, centrifuged, and stored at  $-50^{\circ}$ C. Before purification, culture supernatants were concentrated 20-fold using a Minitan ultra filtration system containing 10-kD cut-off filters (Millipore Continental Water Systems, Bedford, MA). sII-1R protein was detected by soluble covalent crosslinking as previously described (8).

Partial purification of the sIL-1R was achieved using a wheatgerm agglutinin sepharose 6MB column (Pharmacia LKB Biotechnology, UK) as previously described. sIL-1R was further purified using a IL-1 $\beta$  (mutant K138C) (10) thiol sepharose column containing 4 mg/ml IL-1 $\beta$ . Concentrated IL-1 $\beta$  binding protein preparations were applied to the column using a flow rate of 1 ml/min and continually recycled for up to 48 h at 4°C. The column was then washed in 100 mM Tris-HCl, pH 8.1 (20-column volumes), 100 mM Tris-HCl, pH 8.1, 1.0 M NaCl (20-column volumes), and subsequently eluted with 5 ml of 3 M NH<sub>4</sub>SCN in PBS. The eluate was extensively dialyzed against 100 mM Tris-HCl, pH 8.1, concentrated 10-fold with a centripep concentrator (cut-off, 10 kD; Amicon Ltd.) and stored at  $-70^{\circ}$ C.

Affinity-purified, sIL-1R was injected onto a reverse-phase, RP300 Aquapore  $30 \times 2.1$ -mm C8 column (Applied Biosystems Inc., Foster City, CA). Sample was eluted in a 10–70% (vol/vol) acetonitrile gradient with 0.3% (vol/vol) trifluoroacetic acid over a 45-min period at a flow rate of 0.2 ml/min. After neutralization with Tris, fractions were screened for IL-1 binding activity by soluble covalent crosslinking.

Ligand Blotting of sIL-1R. Purified sIL-1R (5  $\mu$ l) was subjected to electrophoresis on 10% SDS-polyacrylamide gels and trans-



1 2 3 4 5 6 7 8 9

Figure 1. Specificity of ligand binding of the sIL-1R. Purified sIL-1R was incubated with <sup>125</sup>I IL-1 $\beta$  in the absence or presence of a 500-fold excess cold cytokine. After crosslinking and SDS-PAGE, complexes were identified by autoradiography. Lane 1, no competing agent (nonreducing conditions); lane 2, excess murine IL-1 $\alpha$ ; lane 3, excess murine IL-1 $\beta$ ; lane 4, excess human IL-1 $\alpha$ ; lane 5, excess human IL-1 $\beta$ ; lane 6, excess human IL-1 $\beta$ ; lane 7, excess human IL-1 $\beta$ ; lane 8, excess human IL-1 $\beta$ ; lane 9, no competing agent (reducing conditions). Protein markers are in kilodaltons.

ferred electrophoretically to 0.45- $\mu$ m nitrocellulose filters (Biorad, Herts, UK). Ligand blotting was then performed as previously described (11).

Soluble and Cell Surface <sup>125</sup>I IL-1 Binding Assay. Soluble binding assays with purified sIL-1R and cell surface binding assays using  $5 \times 10^6$  cells were performed as previously described (8).

Inhibition of Cell Surface IL-1 Binding by sIL-1R. Raji and EL-4 NOB.1 cells (10<sup>7</sup>) were incubated with 5 ng/ml<sup>125</sup>I IL-1 $\alpha$  or <sup>125</sup>I IL-1 $\beta$  with or without decreasing concentrations of purified sIL-1R at 4°C for 3 h with constant agitation. Cell surface <sup>125</sup>I IL-1 binding was assessed as before.

#### Results

Purification of Raji sIL-1R. Wheat germ agglutinin and IL-1 $\beta$  affinity-purified, soluble IL-1R was injected onto a reverse-phase HPLC column. sIL-1R eluted with  $\sim$ 43%

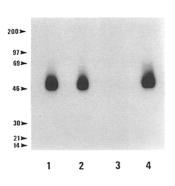


Figure 2. Ligand binding of Raji sIL-1R with <sup>125</sup>I IL-1 $\beta$ . Purified sIL-1R was subjected to electrophoresis on a 10% SDS polyacrylamide gel and transferred onto 0.45- $\mu$ M nitrocellulose filters. After blocking, filters were probed with <sup>125</sup>I IL-1 $\beta$  (5 ng/ml) alone (lane 1), with excess IL-1 $\beta$  (lane 3), and with excess TNF $\alpha$  (lane 4). Protein markers are in kilodaltons.

(vol/vol) acetonitrile as determined by soluble crosslinking and was associated with three overlapping protein peaks (data not shown).

Specificity of sIL-1R Ligand Binding. The specificity of the Raji sIL-1R was investigated by adding a 500-fold excess of cold cytokine to purified sIL-1R incubated with <sup>125</sup>I IL-1 $\beta$ . The results shown in Fig. 1 show a 60-kD complex that was formed between <sup>125</sup>I IL-1 $\beta$  and the purified sIL-1R in the absence of a competing agent (lane 1). Addition of excess human IL-1 $\alpha$  (lane 2) or murine IL-1 $\alpha$  (lane 4) did not inhibit binding, however, addition of excess human IL-1 $\beta$  (lane 3) or murine IL-1 $\beta$  (lane 5) displaced <sup>125</sup>I IL-1 $\beta$  binding, as did the addition of 500-fold excess human 31-kD IL-1 $\beta$  (lane 6). Excess TNF- $\alpha$  (lane 8) or human rIL-1R antagonist (lane 7) did not inhibit <sup>125</sup>I IL-1 $\beta$  binding. Lane 9 shows the effect of reducing conditions on the apparent molecular mass of the <sup>125</sup>I IL-1 $\beta$ /sIL-1R complex, reduction of disulphide bonds causing the complex to migrate at ~69 kD.

Ligand Blotting of sIL-1 $\beta$  Binding Protein. Purified sIL-1R was separated on a 10% SDS-PAGE gel under nonreducing conditions and blotted onto nitrocellulose. Probing with <sup>125</sup>I IL-1 $\beta$  revealed a band migrating at 47 kD (Fig. 2). This band was also seen when the blots were incubated with 100-fold excess cold IL-1 $\alpha$  or TNF- $\alpha$  but not when incubated with excess cold IL-1 $\beta$ . No binding was seen when the sIL-1R was separated under reducing conditions (data not shown).

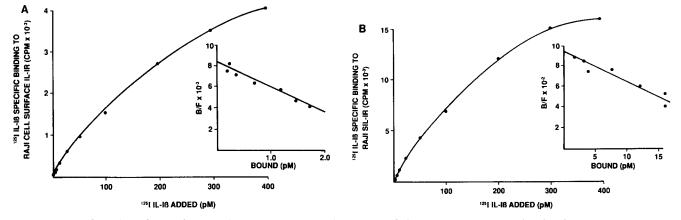


Figure 3. Specific binding of cell surface and soluble Raji IL-1R. Raji cells (A) or purified Raji sIL-1R (B) were incubated with varying concentrations of  $^{125}$ I IL-1 $\beta$  for 4 h at 8°C and ligand-receptor complexes separated from free ligand by centrifugation through phthalate oil mixture (A) or by precipitation with polyethylene glycol (B). Binding shown represents specific binding Scatchard analysis (*inset*) gives  $K_d$  of (A) ~2.2 nM and (B) ~2.7 nM. Results are representative of two experiments.

# 1252 Characterization of a Natural Soluble Interleukin 1 Receptor

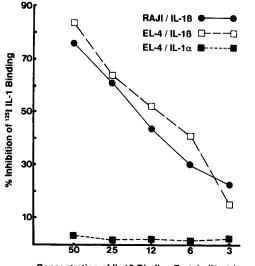
Cell Surface and Soluble Raji IL-1R Binding. Analysis of <sup>125</sup>I IL-1 $\beta$  binding to the Raji cell surface IL-1R and sIL-1R showed that both exhibited specific and saturable binding (Fig. 3). Scatchard analysis revealed that the Raji cell surface IL-1R bound <sup>125</sup>I IL-1 $\beta$  with an apparent  $K_d$  of 2.2 nM (Fig. 3 A), while the soluble receptor protein bound <sup>125</sup>I IL-1 $\beta$  with a  $K_d$  of 2.7 nM (Fig. 3 B).

Inhibition of IL-1R Binding by sIL-1R. Raji (type II IL-1R bearing) and EL-4 NOB.1 (type I IL-1R bearing) cells were incubated with <sup>125</sup>I IL-1 $\alpha$  and <sup>125</sup>I IL-1 $\beta$  in the presence or absence of decreasing concentrations of purified sIL-1R (Fig. 4). Raji did not bind <sup>125</sup>I IL-1 $\alpha$  (data not shown), however, <sup>125</sup>I IL-1 $\beta$  binding was inhibited in a dose-related fashion by the sIL-1R preparation. The sIL-1R also inhibited <sup>125</sup>I IL-1 $\beta$  binding to the EL-4 NOB.1 cell line, however, <sup>125</sup>I IL-1 $\alpha$  binding was not affected by incubation with sIL-1R.

### Discussion

The present study described the purification and characterization of a sIL-1R derived from the supernatant of the human B cell Burkitt lymphoma cell line Raji. The protein binds IL-1 $\beta$  but not IL-1 $\alpha$ . We have previously described a protein with the same properties in normal human plasma, serum, synovial exudate, and supernatants from activated PBMC (7, 8).

We purified the Raji sIL-1R by sequential wheat germ agglutinin affinity chromatography, IL-1 $\beta$  affinity chromatography, and reverse-phase HPLC. The specificity of this material was characterized by using soluble covalent crosslinking and confirmed our previous findings that the sIL-1R specifically



Concentration of IL-18 Binding Protein (% v/v)

Figure 4. Inhibition of cell surface IL-1R binding by sIL-1R. Raji and EL-4 NOB.1 cells (10<sup>7</sup>) were incubated with 5 ng/ml <sup>125</sup>I IL-1 in the presence of various concentrations of purified sIL-1R. After incubation at 8°C for 4 h, bound and free ligand were separated by centrifugation through a phtalate oil mixture. Binding in the absence of sIL-1R was 6,852 cpm for IL-1 $\alpha$  and EL-4, 4,339 cpm for IL-1 $\beta$ , and EL-4 and 3,052 cpm for IL-1 $\beta$  and Raji. Results are representative of three individual experiments.

bound to IL-1 $\beta$ . Murine IL-1 molecules showed the same binding specificity to the purified sIL-1R. The human IL-1R antagonist (12) failed to inhibit binding of <sup>125</sup>I IL-1 $\beta$  to the sIL-1R, and it has been reported that this molecule fails to bind the type II cell surface IL-1R. Interestingly, we found that excess human 31-kD IL-1 $\beta$  propertide could displace the mature 17-kD molecule from the sIL-1R. Previously published studies have shown that the IL-1 $\beta$  propeptide fails to bind the type I IL-1R and has no biological activity on cells with this receptor (13). The finding that the sIL-1R binds to the propeptide may have important implications for the in vivo handling of IL-1 $\beta$ . Treatment of the sIL-1R/<sup>125</sup>I IL-1 $\beta$  complex with reducing agents revealed an apparent change in the molecular mass of the complex from  $\sim 60$  to  $\sim 70$  kD. As II-1 $\beta$  contains no disulphide linkages, it is likely that the sII-1R is held in its conformational shape by disulphide bonds.

Further characterization of the sII-1R was achieved by ligand blotting, previously used to study a number of cell surface receptors, including the type I IL-1R (11). Ligand blotting demonstrated a 47-kD molecule in nonreducing conditions that, again, specifically bound IL-1 $\beta$ . Use of reducing agents led to loss of binding activity (data not shown), indicating that the disulphide-bonded cysteine residues probably hold the receptor in a functional conformation.

Scatchard analysis of cell surface and soluble <sup>125</sup>I II-1 $\beta$ binding showed the  $K_d$  of the cell surface II-1R to be 2.2 nM. This is in good agreement with previous studies (14). We have previously demonstrated that a sII-1 $\beta$  binding protein semi-purified from synovial fluid (SF) had a  $K_d$  of ~0.4 nM (8). Analysis of the binding of II-1 $\beta$  to the Raji sII-1R revealed a  $K_d$  of 2.7 nM very similar to the Raji cell surface II-1R, although others have found Raji sII-1R to have a lower affinity (15). The sixfold difference in the affinity of the SF sII-1R and the Raji-derived sII-1R might be explained by other II-1 binding factors in the SF preparations. However, pancreatic islet  $\beta$  cells also possess II-1R specific for II-1 $\beta$ and appear to express both high ( $K_d = 0.2$  nM) and low ( $K_d = 1.4$  nM) sites (16), therefore synovial cells may shed a higher affinity sII-1R than the Raji clone.

Given the high affinity for IL-1 $\beta$ , the molecule may function as a specific inhibitor of IL-1 $\beta$  in vivo. To test this, we performed binding studies using EL-4 NOB.1, a T cell line with a type I IL-1R, and Raji cells that only possess a type II IL-1R. The results showed that the sIL-1R inhibited IL-1 $\beta$ binding to both cell lines in a dose-dependent fashion, however, IL-1 $\alpha$  binding to EL-4 NOB.1 was not inhibited. Soluble cytokine receptors may have considerable therapeutic potential. Recent studies have used a recombinant truncated type I IL-1R to inhibit rejection of heart allografts (17) and IL-1 induced B cell function (18). The natural soluble IL-1R may play an important role in modulating IL-1 $\beta$  activities in vivo.

The finding of certain cell types able to discriminate between IL-1 $\alpha$  and IL-1 $\beta$  has important biological implications. It has been noted that IL-1 $\beta$  is more potent than IL-1 $\alpha$  in the brain, pancreas, ovarian granulosa cells, leydig cells, and immunostimulatory activity in vivo. Differential expression of IL-1R types may explain these observations. Additionally release of the sIL-1R could be induced from normal human PBMC after stimulation with mitogen (8), indicating that this IL-1 $\beta$ -specific IL-1R probably plays a role in normal immune responses. The natural sIL-1R may be useful in

modulating the actions of IL-1 $\beta$  in vivo, especially where immunopathogenesis is associated specifically with IL-1 $\beta$ .

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#### References

- 1. Di Giovine, F.S., and G.W. Duff. 1990. Interleukin 1: the first interleukin. Immunol. Today. 11:13.
- Bomsztyk, K., J.E. Sims, T.H. Stanton, J. Slack, C.J. McMahan, M.A. Valentine, and S.K. Dower. 1989. Evidence for different interleukin 1 receptors in murine B and T cell lines. Proc. Natl. Acad. Sci. USA. 86:8034.
- 3. Bird, T.A., and J. Saklatvala. 1986. Identification of a common class of high affinity receptors for both types of procine interleukin 1 on connective tissue cells. *Nature (Lond.).* 324:263.
- Matsushima, K., T. Akahoshi, M. Yamada, Y. Furutani, and J.J. Oppenheim. 1986. Properties of a specific interleukin 1 (II-1) receptor on human Epstein Barr virus-transformed B lymphocytes: identity of the receptor for II-1 alpha and II-1 beta. J. Immunol. 136:4496.
- Mosely, B., M.P. Beckmann, C.J. March, R.L. Idzerda, S.D. Gimpel, T.V. Bos, D. Friend, A. Alpert, D. Anderson, J. Jackson, J.M. Wignal, C. Smith, G. Gallis, J.E. Sims, D. Urdal, M.B. Widmer, D. Cosman, and L.S. Park. 1989. The murine interleukin 4 receptor: molecular cloning and characterisation of secreted and membrane bound forms. *Cell.* 59:335.
- Robb, R.J., and R.M. Kutny. 1987. Structure-function relationships for the IL-2 receptor system IV analysis of the sequence and ligand binding properties of soluble Tac protein. J. Immunol. 139:855.
- 7. Eastgate, J.A., J.A. Symons, and G.W. Duff. 1990. Identification of an interleukin 1 beta binding protein in human plasma. FEBS (Fed. Eur. Biochem. Soc.) Lett. 260:213.
- 8. Symons, J.A., J.A. Eastgate, and G.W. Duff. 1990. A soluble binding protein-specific for interleukin 1 beta is produced by activated mononuclear cells. *Cytokine*. 2:190.
- Symons, J.A., and G.W. Duff. 1990. A soluble form of the interleukin 1 receptor produced by a human B cell line. FEBS (Fed. Eur. Biochem. Soc.) Lett. 272:133.
- Wingfield, P., P. Graber, A.R. Shaw, A.M. Gronenborn, G.M. Clore, and H.R. MacDonald. 1989. Preparation, characterisa-

tion and application of interleukin 1 beta mutant proteins with surface-accessible cysteine residues. Eur. J. Biochem. 179:565.

- 11. Bird, T.A., A.J.H. Gearing, and J. Saklatvala. 1988. Murine interleukin 1 receptor: Direct identification by ligand blotting and purification to homogeneity of an interleukin 1 binding glycoprotein. J. Biol. Chem. 263:12063.
- Eisenberg, S.P., R.J. Evans, W.P. Arend, E. Verderber, M.T. Brewer, C.H. Hannum, and R.C. Thompson. 1990. Primary structure and functional expression from complementary DNA of a human interleukin 1 inhibitor. *Nature (Lond.)*. 343:341.
- Mosely, B., D.L. Urdal, K.S. Prickett, A. Larsen, D. Cosman, P.J. Conlon, S. Gillis, and S.K. Dower. 1987. The interleukin 1 receptor binds the human interleukin 1 alpha precursor but not the interleukin 1 beta precursor. J. Biol. Chem. 262:2941.
- Horuk, R., J.J. Huang, M. Covington, and R.C. Newton. 1987. A biochemical and kinetic analysis of the interleukin 1 receptor: evidence for differences in molecular properties of IL-1 receptors. J. Biol. Chem. 262:16275.
- Giri, J.G., R.C. Newton, and R. Horuk. 1990. Identification of soluble interleukin 1 binding protein in cell-free supernatants: evidence for soluble interleukin 1 receptor. J. Biol. Chem. 265:17416.
- Hammonds, P., M. Beggs, G. Beresford, J. Espinal, J. Clarke, and R.J. Mertz. 1990. Insulin-secreting beta-cells possess specific receptors for interleukin 1 beta. FEBS (Fed. Eur. Biochem. Soc.) Lett. 261:97.
- Fanslow, W.C., J.E. Sims, H. Sassenfeld, P.J. Morrissey, S. Gillis, S.K. Dower, and M.B. Widmer. 1990. Regulation of alloreactivity in vivo by a soluble form of the interleukin 1 receptor. *Science (Wash. DC)*. 248:739.
- Maliszewski, C.R., T.A. Sato, T.V. Bos, S. Waugh, S.K. Dower, J. Slack, M.P. Beckmann, and K.H. Grabstein. 1990. Cytokine receptors and B cell functions I. Recombinant soluble receptors specifically inhibit IL-1 and IL-4 induced B cell activities in vitro. J. Immunol. 144:3028.