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 \(^{125}\)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 \(^{125}\)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.

Materials and Methods

**Purification of Raji sIL-1R.** 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°C. Before purification, culture supernatants were concentrated 20-fold using a Microcon ultrafiltration system containing 10-kD cut-off filters (Millipore Continental Water Systems, Bedford, MA). sIL-1R protein was detected by soluble covalent crosslinking as previously described (8).

Partial purification of the sIL-1R was achieved using a wheat-germ 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 NH4SCN in PBS. The eluate was extensively dialyzed against 100 mM Tris-HCl, pH 8.1, concentrated 10-fold with a centrifuge concentrator (cut-off, 10 kD; Amicon Ltd.) and stored at −70°C.

Affinity-purified, sIL-1R was subjected to a reverse-phase, RP300 Aquapore 30 × 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 µl) was subjected to electrophoresis on 10% SDS-polyacrylamide gels and trans-
Figure 2. Ligand binding of Raji sIL-1R with \(^{125}\)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 \(^{125}\)I IL-1\(\beta\) (5 ng/ml) alone (lane 1), with excess IL-1\(\alpha\) (lane 2), with excess IL-1\(\beta\) (lane 3), and with excess TNF-\(\alpha\) (lane 4). Protein markers are in kilodaltons.

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\(^\circ\)C and ligand-receptor complexes separated from free ligand by centrifugation through phtalate oil mixture (A) or by precipitation with polyethylene glycol (B). Binding shown represents specific binding Scatchard analysis (inset) gives \(K_d\) of (A) \(\approx2.2\) nM and (B) \(\approx2.7\) nM. Results are representative of two experiments.

Results

Purification of Raji sIL1R. Wheat germ agglutinin and IL-1\(\beta\) affinity-purified, soluble IL-1R was injected onto a reverse-phase HPLC column. sIL-1R eluted with \(\approx43\%\) (vol/vol) acetonitrile as determined by soluble crosslinking and was associated with three overlapping protein peaks (data not shown).

Specificity of sIL1R 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 \(^{125}\)I IL-1\(\beta\). The results shown in Fig. 1 show a 60-kD complex that was formed between \(^{125}\)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 \(^{125}\)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 \(^{125}\)I IL-1\(\beta\) binding. Lane 9 shows the effect of reducing conditions on the apparent molecular mass of the \(^{125}\)I IL-1\(\beta\)/sIL-1R complex, reduction of disulphide bonds causing the complex to migrate at \(\approx69\) kD.

Ligand Blotting of sIL1\(\beta\) Binding Protein. Purified sIL-1R was separated on a 10% SDS-PAGE gel under nonreducing conditions and blotted onto nitrocellulose. Probing with \(^{125}\)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).
Cell Surface and Soluble Raji IL-1R Binding. Analysis of
$^{125}$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 $^{125}$I IL-1$\beta$ with an apparent $K_{d}$ of 2.2 nM (Fig. 3 A),
while the soluble receptor protein bound $^{125}$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 $^{125}$I IL-1$\alpha$ and $^{125}$I IL-1$\beta$ in the presence or
absence of decreasing concentrations of purified sIL-1R (Fig.
4). Raji did not bind $^{125}$I IL-1$\alpha$ (data not shown), however,
$^{125}$I IL-1$\beta$ binding was inhibited in a dose-related fashion by
the sIL-1R preparation. The sIL-1R also inhibited $^{125}$I IL-1$\beta$
binding to the EL-4 NOB.1 cell line, however, $^{125}$I IL-1$\alpha$
binding was not affected by incubation with sIL-1R.

Discussion

The present study described the purification and charac-
terization 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 ag-
glutinin affinity chromatography, IL-1$\beta$ affinity chromatog-
raphy, and reverse-phase HPLC. The specificity of this mate-
rial was characterized by using soluble covalent crosslinking
and confirmed our previous findings that the sIL-1R specifically
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 $^{125}$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$\beta$ propeptide 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 II 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/$^{125}$I IL-1$\beta$
complex with reducing agents revealed an apparent change in the
molecular mass of the complex from $\sim$60 to $\sim$70 kD. As
IL-1$\beta$ contains no disulphide linkages, it is likely that the sIL-1R
is held in its conformational shape by disulphide bonds.

Further characterization of the sIL-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 $^{125}$I IL-1$\beta$
binding showed the $K_{d}$ of the cell surface IL-1R to be 2.2
nM. This is in good agreement with previous studies (14).
We have previously demonstrated that a sIL-1$\beta$ binding pro-
tein semi-purified from synovial fluid (SF) had a $K_{d}$ of $\sim$0.4
nM (8). Analysis of the binding of IL-1$\beta$ to the Raji sIL-1R
revealed a $K_{d}$ of 2.7 nM very similar to the Raji cell surface
IL-1R, although others have found Raji sIL-1R to have a lower
affinity (15). The sixfold difference in the affinity of the SF
sIL-1R and the Raji-derived sIL-1R might be explained by
other IL-1 binding factors in the SF preparations. However,
pancreatic islet $\beta$ cells also possess IL-1R specific for IL-1$\beta$
and appear to express both high ($K_{d}$ = 0.2 nM) and low
($K_{a}$ = 1.4 nM) sites (16), therefore synovial cells may shed
a higher affinity sIL-1R than the Raji clone.

Given the high affinity for IL-1$\beta$, the molecule may func-
tion 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, how-
ever, IL-1$\alpha$ binding to EL-4 NOB.1 was not inhibited. Soluble
cytokine receptors may have considerable therapeutic poten-
tial. Recent studies have used a recombinant truncated type
II 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 be-
 tween 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

![Figure 4](image-url)

Figure 4. Inhibition of cell surface IL-1R binding by sIL-1R. Raji and
EL-4 NOB.1 cells (40) were incubated with 5 ng/ml $^{125}$I IL-1 in the pres-
ence 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 phenol 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.
PBMC after stimulation with mitogen (8), indicating that this IL-1β-specific IL-1R probably plays a role in normal immune responses. The natural sIL-1R may be useful in modulating the actions of IL-1β in vivo, especially where immunopathogenesis is associated specifically with IL-1β.

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