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A soluble form of the interleukin-1 receptor produced by a human B cell line

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A soluble protein that binds specifically to interleukin-1 (IL-1)β was released from a B cell line (Raji). The covalently cross-linked binding protein/[^{125}I]IL-1β migrated at 60 kDa by SDS-PAGE. The IL-1 receptor (IL-1R) on Raji cells had the same ligand specificity. Stimulation of Raji with dexamethasone increased surface expression of the IL-1R and the rate of release of soluble binding protein. A serine protease inhibitor prevented release of the binding protein and increased IL-1R expression on the cells. These results suggest that the soluble IL-1β binding protein is a proteolytically cleaved form of the novel B cell IL-1R.

Interleukin-1; Interleukin-1 receptor; B cell; Soluble receptor

1. INTRODUCTION

Interleukin 1 (IL-1)α and β are polypeptide cytokines with proinflammatory and immuno-potentiating effects in vivo and in vitro [1]. IL-1 is produced by a variety of cells and acts via specific high affinity cell surface receptors. At least two IL-1 receptor (IL-1R) proteins exist [2,3]. An 80 kDa protein cloned from murine [4] and human [5] T cells that is also present on fibroblasts binds both IL-1α and IL-1β with equal affinity [6]. The murine and human receptors are 69% homologous at the amino acid level and are members of the immunoglobulin gene superfamily [5].

A lower molecular mass IL-1R protein (60–70 kDa) is expressed on B cells and macrophages [7]. Like the 80 kDa receptor the low molecular mass receptor is heavily glycosylated containing approximately 13 kDa of N-linked carbohydrate [8]. Interestingly, some B cells expressing the 60 kDa receptor appear to bind IL-1α and IL-1β with different affinities [9,10]. Many cytokine receptor proteins are known to exist in soluble form either by alternative splicing of mRNA to give a secreted isoform e.g. IL-4R [11] or by proteolytic cleavage of the transmembrane molecule at the cell surface e.g. IL-2R (p55) [12]. Other cytokine receptors shown to exist in soluble form include those for IL-6, γ-IFN, TNF, IL-7, EGF and M-CSF.

We have recently described the presence of a soluble binding protein specific for IL-1β in normal human plasma [13], serum, synovial inflammatory exudate and activated mononuclear cell supernatants [14]. In this paper we describe the presence of this protein in the supernatant of a human B cell Burkitt lymphoma cell line (Raji), a cell that expresses the 60 kDa receptor protein and present evidence that it is a soluble form of this receptor. There have been no previous reports of a naturally-occurring soluble IL-1 receptor.

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2. MATERIALS AND METHODS

2.1. *Reagents and cell culture*

Radioiodinated human recombinant (hr) IL-1α and hr IL-1β were obtained from DuPont (NEN Products, Herts, UK; spec. act. 85.9 μCi/μg and 158 μCi/μg respectively). Human recombinant (hr) cytokines were a gift from Glaxo Institute for Molecular Biology, Geneva, Switzerland. All hr peptides were regularly assessed for bioactivity. Disuccinimide suberate (DSS) was obtained from Pierce and Warriner Ltd (Chester, UK), and all other chemicals from Sigma (Dorset, UK). The human Burkitt lymphoma B cell line, Raji and the murine thymoma EL-4 NOB.1 T cell line were obtained from the European Cell Culture Collection (Porton, Wilts, UK). Cells were maintained at 37°C in RPMI 1640 as described previously [13,14]. Raji cells were washed twice in fresh serum-fre medium and cultured at 2.5×10^6/ml in 1 ml aliquots for up to 72 h. Cell-free supernatants obtained by centrifugation at $900 \times g$ for 5 min were stored at −20°C until used. The remaining cells were washed and resuspended in binding medium (RPMI 1640 containing 1% (w/v) bovine serum albumin, 0.1% (w/v) sodium azide and 20 mM HEPES, pH 7.2) to assess surface IL-1 receptor expression.

2.2. *Cell surface affinity crosslinking*

Raji and EL-4 NOB.1 cells (1×10^7) were covalently crosslinked to [^{125}I]IL-1 as previously described [14]. After crosslinking the cells were solubilised in PBS containing SDS (1% w/v) and protease inhibitors and analysed in 10% SDS-PAGE gels [14].

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2.3. *Soluble covalent cross-linking and SDS-PAGE analysis*

Analysis of soluble IL-1 binding proteins in Raji cell culture supernatants (50 μ)l was performed as previously described in detail [13,14].

2.4. *Soluble and cell surface [^{125}I]IL-1 bindings assay*

Soluble binding assays on Raji supernatants were performed as previously described [14]. Supernatants (100 μl) were incubated with 5 ng/ml [^{125}I]IL-1β in a final volume of 0.2 ml for 2 h at 20°C. All experiments were performed in duplicate and results were expressed as specific binding activity after subtraction of non-specific binding.

Cell surface binding assays were performed according to Dower et al. [15]. Cells (1×10^6) were incubated with 5 ng/ml [^{125}I]IL-1β in 150 μl binding medium or binding medium containing 100-fold excess unlabelled IL-1β for 3h at 4°C with constant agitation. The cells were then washed twice in ice-cold protein-free buffer and resuspended in 100 μl of the same buffer. Bound and free [^{125}I]IL-1β were separated by layering duplicate 50 μl aliquots on to 300 μl of a phthalate oil mixture (1.5 parts dibutyl phthalate (Sigma) and 1 part dioctyl phthalate (Aldrich Chemical Co.)). Tubes were centrifuged for 90 s at 10 000 × g the tips excised and counted in a gamma counter.

3. RESULTS

3.1. *Covalent crosslinking to cell surface binding proteins*

Iodinated IL-1β was covalently crosslinked to Raji cells and the detergent extracts analysed by SDS-PAGE (Fig. 1A). One species of binding protein was observed that, when cross-linked to IL-1, migrated at approximately 82 kDa, representing a cell surface protein of 65 kDa. This band was detectable in the presence of excess cold hr IL-1α but not in the presence of excess hr IL-1β. In contrast, IL-1α crosslinked to EL-4 NOB.1 T cells revealed a 97 kDa band representing an 80 kDa receptor protein. Binding of [^{125}I]IL-1α to this protein was inhibited both by cold IL-1α and IL-1β (Fig. 1B).

3.2. *Covalent crosslinking to soluble binding proteins*

Soluble IL-1 binding proteins in Raji cell supernatants were identified by crosslinking to iodinated IL-1. When IL-1α cross-linked to Raji supernatant was analysed by SDS-PAGE, no specific binding was observed (Fig. 2A). In contrast, when [^{125}I]IL-1β was covalently crosslinked by Raji supernatant a major [^{125}I]IL-1β-protein complex migrated as a broad band at approximately 60 kDa (Fig. 2B). This binding was displaceable by 100-fold excess cold IL-1β but not by other cold cytokines. EL-4 NOB.1 cells did not release any detectable specific IL-1 binding proteins (data not shown).

3.3. *Time course of IL-1 receptor expression and release*

Raji cells were incubated with either media or dexamethasone-acetate (500 nM) for up to 72 h. At each time point supernatants and cells were assessed for specific IL-1β binding. Raji cell surface IL-1R expression did not change during the 72 h culture period when the cells were incubated in medium alone. However, there was an accumulation of soluble binding protein over the same culture period (Fig. 3). In contrast, when Raji cells were incubated with 500 nM dexamethasone, cell surface receptor expression was significantly increased over cells incubated in media alone at 48 and 72 h of culture. Concurrently soluble binding levels were also elevated at these time periods compared to the control culture supernatants (Fig. 3).

3.4. *Effect of metabolic and protease inhibitors on Raji IL-1R*

To elucidate the mechanism of Raji IL-1R release, Raji cells were incubated for 48 h with or without a range of metabolic or protease inhibitors. Subsequently both cell surface and supernatant receptor levels were assessed by IL-1β binding (Fig. 4). Cyclohexamide (1

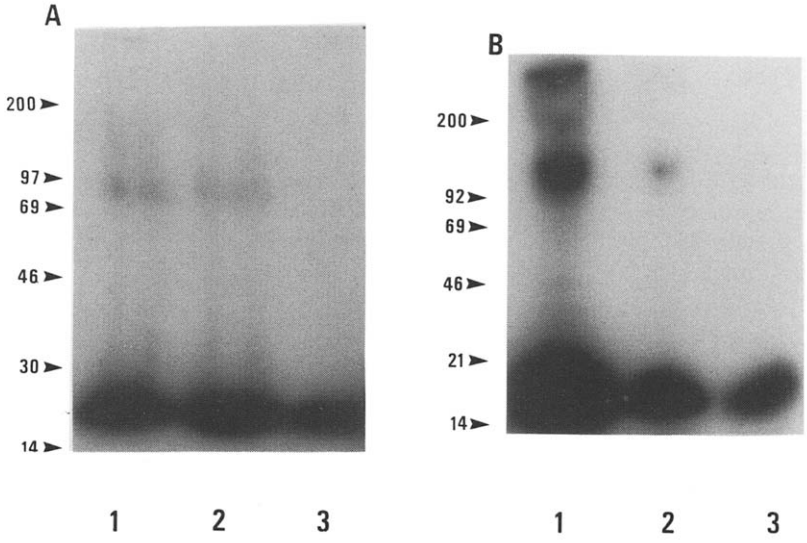

Fig. 1. Affinity cross-linking of [^{125}I]IL-1β to Raji cells (A) and [^{125}I]IL-1α to EL-4 NOB.1 cells (B). In each case, Lane 1 with no competing agent. Lane 2 with excess IL-1α. Lane 3 with excess IL-1β. Protein markers are in kDa.

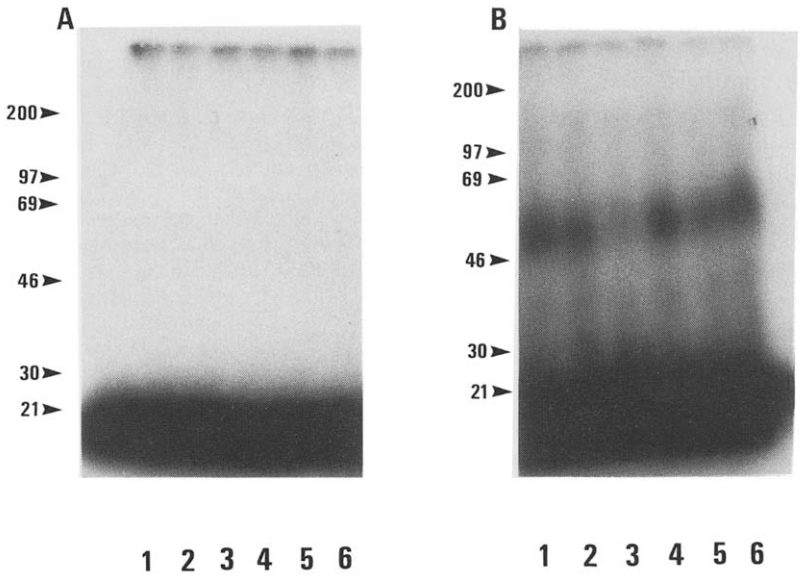

Fig. 2. Affinity cross-linking of [^{125}I]IL-1 to Raji supernatant proteins. (A) With [^{125}I]IL-1α, (B) with [^{125}I]IL-1β. In each case lane 1 with no competing agent; lane 2 with excess IL-1α. Lane 3 with excess IL-1β, lane 4 with excess TNF-α, lane 5 with excess TNF-β, lane 6 with excess IL-6.

μg/ml) and actinomycin D (5 μg/ml) reduced cell surface IL-1R expression and reduced soluble binding to undetectable levels. Aprotinin (50 μg/ml), a serine protease inhibitor, doubled the level of cell surface IL-1R expression and at the same time reduced soluble binding to undetectable levels. In contrast chymostatin (50 μg/ml) had no significant effects on cell surface or soluble binding levels. Chloroquine (200 μM), an agent that blocks receptor internalisation and lysosomal degradation increased Raji cell surface IL-1R expression 8-fold and soluble binding 2-fold. Monensin (2 μM) had no effect on cell surface IL-1R expression but decreased soluble binding protein levels by 60%. None of the treatments significantly reduced cell viability.

4. DISCUSSION

Analysis of Raji supernatants cross-linked with iodinated IL-1 revealed a protein that bound to IL-1β but not IL-1α. We have previously described a protein with the same properties in normal human plasma, serum, synovial exudate and supernatants from activated blood mononuclear cells [13,14]. The Raji cell surface IL-1R had an identical specificity as has also been reported by others [23]. Stimulation of B cells with glucocorticoids increases cell surface expression of Il-1R [10,16]. We confirmed this and also found that stimulation of Raji cells with dexamethasone increased the levels of soluble IL-1β binding proteins.

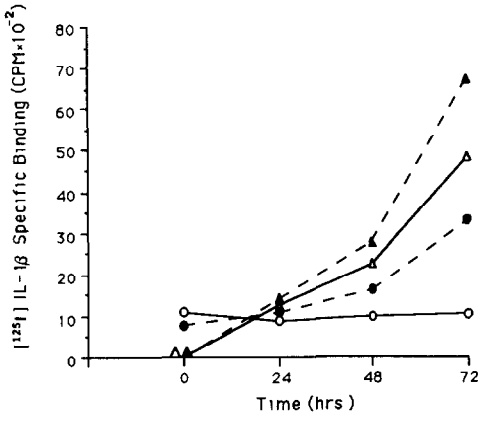

Fig. 3. Time course of Raji cell and supernatant IL-1R accumulation in response to dexamethasone (Dex). Raji cell surface IL-1R (○—○), Raji cell surface IL-1R plus Dex (●---●), Raji supernatant IL-1R (△—△), Raji supernatant plus Dex (▲---▲). Data shown is representative of 3 experiments.

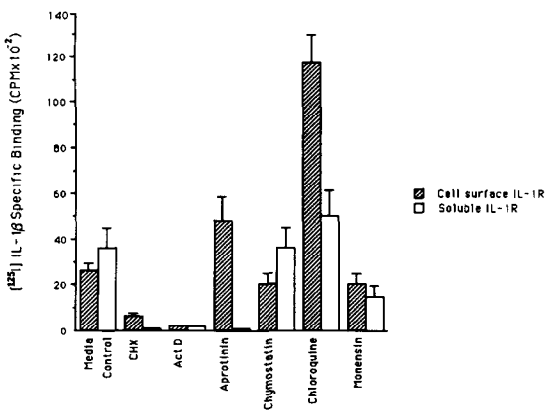

Fig. 4. Effect of metabolic and protease inhibitors on Raji IL-1R. Shaded bars represent cell surface levels, open bars supernatant levels. Binding shown represents specific binding. Bars represent mean ± SEM ($n = 3$).

One mechanism of cell surface receptor release is by proteolytic cleavage so we tested protease inhibitors on the release of the IL-1β binding protein. Aprotinin, a serine protease inhibitor increased cell surface IL-1R expression 2-fold while soluble IL-1β binding became undetectable. The lysomotropic agent chloroquine caused an 8-fold increase in cell surface IL-1R expression and a significant increase in soluble binding levels. The data suggest the cell surface IL-1 receptor is recycled and a soluble binding protein is created by the action of a serine protease at the cell surface. Further, the rate of release is related to the level of cell surface IL-1R expression.

It is now recognised that the IL-1R on T cells and fibroblasts is a separate gene product to that found on B cells [2,3], although cells expressing both have been described [9,17,23]. Recent studies have indicated that the two receptors may bind IL-1α and -β with different affinities [9,10,23]. Interestingly, there are many reports of biological systems where IL-1β is more potent than Il-1α. In the brain Il-1β is more effective in inducing fever [18] and ovarian granulosa cell differentiation is inhibited to a greater extent by IL-1β than IL-1α [19]. In vivo IL-1α and IL-1β produce similar pyrogenicity and acute phase response, but only IL-1β shows immunostimulatory activity [20]. Pancreatic β-islet cells express receptors specific for IL-1β [21] and islet cell cytotoxicity is only mediated by IL-1β [22].

Given that the soluble IL-1R appears to have a ligand affinity similar to the cell surface receptor [14] it may function as a specific inhibitor of IL-1β. There are no previous reports of a naturally-occurring soluble IL-1 receptor and the molecular structure of the 60 kDa B cell IL-1 receptor from which we believe the soluble binding molecule is derived has not yet been reported. The production of soluble binding protein by a B cell line should provide adequate material for purification and characterisation of this apparently novel IL-1 receptor.

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