Volume 260, number 2, 213-216 FEB 08047 January 1990

Identification of an interleukin-1 beta binding protein in human plasma

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Received 4 December 1989

A covalent cross-linking technique was used to bind iodinated interleukin-1 (IL1) alpha and beta to plasma proteins. One specific IL1 beta binding protein was observed, that when cross-linked to ^{125}I-IL1 beta migrated to approximately 60 kDa on SDS-PAGE. The protein did not bind IL1 alpha. The 43 -kDa protein was partially purified using a wheat germ agglutinin affinity column. The isolated factor again specifically bound IL1 beta, and appeared to consist of single chain glycoprotein. The protein was heat stable and had a rapid association time with IL1 beta. This protein may be an important carrier molecule for IL1 beta in vivo.

Interleukin 1 alpha; Interleukin 1 beta; Soluble binding protein; Interleukin 1 receptor

1. INTRODUCTION

Interleukin 1 (IL1) is a polypeptide produced by activated leukocytes and other cells. It mediates a wide range of biological activities relevant to host defence. It increases production of T and B lymphocyte growth factors and stimulates other cells involved in inflammatory and immune responses. Additionally, it plays a role in connective tissue remodelling, haematopoiesis and is an important factor in the induction of fever and acute phase protein synthesis [1].

Two distinct forms of IL1 (IL1 alpha and IL1 beta) have been described [2]. Although the two peptides have only 26% homology, they compete for the same cellular receptor with equal affinity [3,4]. The murine IL1 receptor (IL1R) has recently been sequenced and cloned [5] and is a member of the immunoglobulin gene superfamily. The 319-amino-acid extracellular domain consists of two beta-pleated sheets connected by disulphide bonds and seven potential N-linked glycosylation sites, which account for the described size heterogeneity of the IL1R.

High expression of IL1 peptides appears to be a common phenomenon in many inflammatory diseases leading to extensive tissue damage [6]. Therefore, regulatory pathways must exist to limit IL1-mediated responses. IL1-stimulated prostaglandin E_2 production post-transcriptionally blocks monocyte expression of IL1 activity [7]. In addition, a large number of IL1 inhibitory molecules have been described (reviewed by Larrick [8]), although the mechanism of inhibition in most cases is largely unknown. The most highly characterised IL1 inhibitor described is produced by macrophages in response to GM-CSF [9] and immune complexes [10] and has a molecular mass of approximately 26 kDa. This inhibitor blocks both IL1 alpha and IL1 beta but not TNF alpha binding to cell-surface receptors. Other proteins may control the distribution and bioavailability of IL1 in vivo by acting as carrier molecules. For example, treatment of plasma with trypsin or methylamine allows complex formation between alpha 2 macroglobulin and IL1 beta through disulphide bonding [11].

In this paper we describe the presence of a novel IL1 binding protein in normal human plasma. Unlike the previously known IL1 inhibitors and IL1 receptor protein, the IL1-binding protein described here appears to have affinity specifically for IL1 beta and does not bind IL1 alpha.

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2. MATERIALS AND METHODS

2.1. *Reagents*

Radioiodinated hr IL1 alpha and hr IL1 beta proteins were obtained from Du Pont (NEN Products; Hertfordshire, England). IL1 alpha was radioiodinated using the chloramine-T procedure to a specific activity of 90.4 μCi/μg (3.35 mBq/μg). IL1 beta was labelled with Bolton Hunter reagent to a specific activity of 158 μCi/μg (5.85 mBq/μg). Cold human recombinant IL1 peptides were a gift from Biogen S.A., human recombinant TNF alpha was a gift from Dr Guenther Adolf (Ernst Boehringer Institut fur Arzneimittel Forschung, A-1121 Wien, Austria). Covalent cross-linking reagents disuccinimidyl suberate (DSS), disuccinimidyl tartarate (DST) and ethylene glycolbis (succinamidyl succinate) (EGS) were obtained from Pierce and Warriner, Chester, England.

2.2. *Cell culture*

Murine 3T3 fibroblasts were cultured in RPMI-1640 containing

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00145793/90/$3.50

penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM) and 5% foetal calf serum (FCS; heat inactivated; 56°C, 30 min). All cell culture reagents were obtained from Northumbria Biologicals (Northumbria, England).

2.3. *Covalent cross-linking and PAGE analysis*

Plasma or semi-purified protein (50 μl) was incubated overnight at 4°C with 10 ng/ml of ^{125}I-IL1 alpha or IL1 beta, with or without 100-fold excess of cold IL1 alpha, IL1 beta or TNF alpha. Subsequently, covalent cross-linker, DSS, DST or EGS (final concentration 1 mg/ml) freshly prepared in DMSO, was added and incubated at 4°C for 30 min. Cross-linked complexes were identified in 10% polyacrylamide gels according to Laemmli [12]. Samples to be tested under reducing conditions were treated with dithiothreitol (100 mM; DTT) and boiled for 10 min.

Monolayers of 3T3 fibroblasts in 25 cm^3 flasks were incubated for 4 h at 8°C with 1 ng/ml labelled IL1 alpha or IL1 beta in RPMI 1640 containing 1% BSA, 0.1% sodium azide and 20 mM Hepes, pH 7.4 (binding buffer) in a final volume of 5 ml. Specificity of binding was controlled by the addition of excess cold cytokine as above. Unbound radioactivity was removed by washing three times in protein-free, ice-cold binding buffer. Five millilitres of this buffer was then added to each flask, cross-linker (DSS) was added to a final concentration of 1 mg/ml and incubated on ice for 45 min. The cross-linker was then removed and replaced with 5 ml of quenching solution (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). The cells were scraped using a rubber policeman into the buffer and centrifuged at 800×*g* for 10 min at 4°C. The cells were subsequently dissolved in 100 μl of 1% (w/v) SDS containing protease inhibitors PMSF (10 mM), EDTA (2 mM), pepstatin (2 mM) and 1,10 phenanthroline (2 mM). Samples were analysed by SDS-PAGE as described above. Iodinated cytokines were routinely assessed for self-association in cross-linking experiments.

2.4. *Partial purification and characterisation*

IL1 binding proteins were partially purified using a wheat-germ agglutinin sepharose 6MB column (Pharmacia LKB Biotechnology, England). Briefly, plasma was equilibrated by extensive dialysis against PBS and then loaded on the column using a flow rate of 4 ml/h. The column was subsequently washed with PBS/0.3 M NaCl, PBS/10% (v/v) ethylene glycol and finally PBS. Specifically bound glycoproteins were then eluted from the column with PBS containing 500 mM *N*-acetyl-glucosamine and 0.3 M NaCl. Two-millilitre fractions were collected and screened for IL1 bindig activity by covalent cross-linking.

Heat stability of the partially purified binding protein was tested by incubating aliquots for 30 min at temperatures ranging from 4°C to 70°C. Treated samples were then incubated with iodinated IL1 beta and cross-linked as previously described. Kinetics of binding of IL1 beta to the protein was tested by incubating labelled cytokine with binding protein for various lengths of time at 4°C, room temperature or 37°C before cross-linking.

3. RESULTS

3.1. *Covalent cross-linking*

Binding proteins were identified by cross-linking plasma previously incubated with iodinated IL1. When IL1 alpha cross-linked to human plasma was analysed by SDS-PAGE, no specific binding was observable (fig.1A). A small amount of material that just entered the gel showed binding, but this appeared to be non-specific. To demonstrate that ^{125}I-IL1 alpha retained receptor binding activity, we cross-linked it to 3T3 fibroblasts. Fig.2 shows that IL1 alpha specifically bound to its 80 KDa receptor on 3T3 cells.

Fig.1. ^{125}I-IL1 alpha (A) and ^{125}I-IL1 beta (B) cross-linked to plasma (lane 1) with excess cold IL1 alpha (lane 2), IL1 beta (lane 3) and TNF alpha (lane 4).

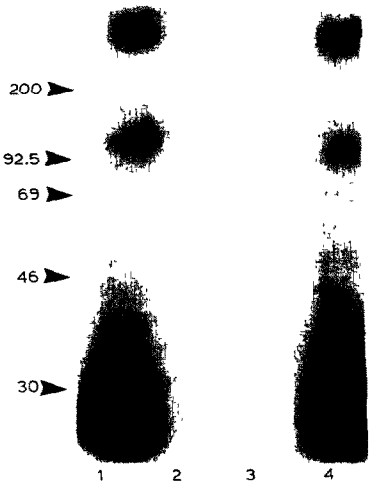

Fig.2. Cross-linking of iodinated IL1 alpha to 3T3 cells (lane 1) with excess cold IL1 alpha (lane 2), IL1 beta (lane 3) and TNF alpha (lane 4).

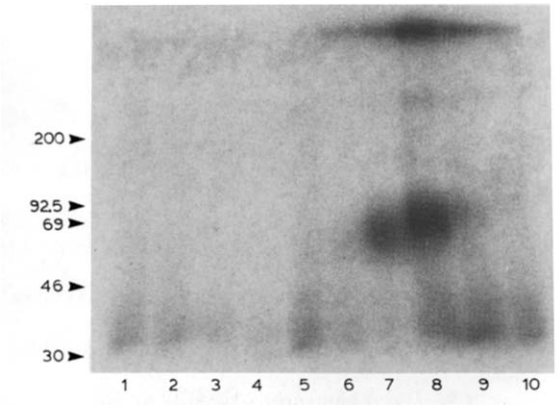

Fig.3. Cross-linking of ^{125}I-IL1 beta to fractions eluted from a WGA column.

In contrast when ^{125}I-IL1 beta was cross-linked to plasma, an IL1 beta/protein complex was observed to migrate at approximately 60 kDa (fig.1B). This binding was displacable by 100-fold excess of cold IL1 beta but not IL1 alpha or TNF alpha, and was seen with all three cross-linkers used. Some non-specific binding was also seen to high-molecular mass material.

3.2. *Purification and biochemical characterisation*

The 60 kDa IL1 beta/binding protein complex migrated as a broad band from 50–70 kDa suggesting the possibility of variable glycosylation. We therefore used lectin affinity chromatography for partial purification of the binding protein. Experiments revealed that the binding protein could be specifically eluted from a wheat germ agglutinin (WGA) affinity column. The result of this purification is shown in fig.3. The binding protein eluted from the column with *N*-acetyl glucosamine and when cross-linked to ^{125}I-IL1 beta, migrated at the same molecular mass as the unpurified plasma protein. The specificity of this protein for IL1 beta was retained (fig.4) as it failed to bind ^{125}I-IL1 alpha. Further ^{125}I-IL1 beta binding was only competed by 100-fold excess cold IL1 beta. Separation of plasma on the WGA column resulted in a 25-fold purification.

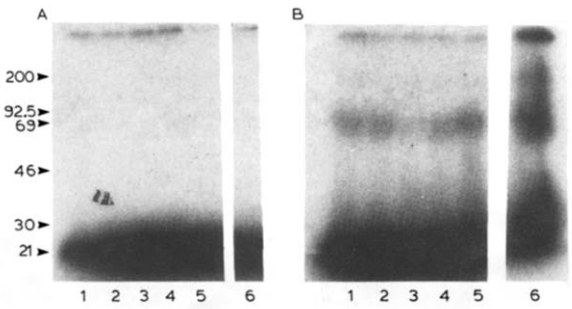

Fig.4. ^{125}I-IL1 alpha (A) and ^{125}I-IL1 beta (B) cross-linked to fraction 8 of the affinity column (lane 1), with excess cold IL1 alpha (lane 2), IL1 beta (lane 3) and TNF alpha (lane 4) and also under reducing conditions (lane 5) iodinated proteins are also cross-linked to plasma (lane 6).

Reducing SDS-PAGE resulted in the ^{125}I-IL1 beta/binding protein complex migrating at the same molecular mass as in non-reducing conditions (fig.4). Heat treatment of semi-purified binding protein before cross-linking showed that the ^{125}I-IL1 beta binding ability was retained even at 70°C (data not shown). Incubation of labelled IL1 beta with binding protein for various lenghts of time at 4°C, room temperature and 37°C indicated that rapid association occurred, with maximal binding after 30 min at 37°C (data not shown).

4. DISCUSSION

SDS-PAGE analysis of plasma cross-linked with iodinated IL1s shows an IL1 beta/binding protein complex of molecular mass 60 kDa suggesting the presence of a 43 kDa factor in human plasma capable of specific association with IL1 beta and not IL1 alpha. Partial purification using a WGA column isolated a protein that bound IL1 beta and was not affected by reducing conditions, suggesting the presence of a single polypeptide chain. The binding factor is thought to be a heat-stable glycoprotein with rapid association kinetics with IL1 beta. A high-molecular-mass factor is also seen to bind both labelled IL1 alpha and IL1 beta; this appears to be non-specific.

Vairous factors have been described as IL1 inhibitors [8], most of which are assayed by their ability to interfere with the co-mitogenic activity of IL1 on thymocytes. A well-characterised 23 kDa inhibitor affects both IL1 alpha and IL1 beta stimulation of fibroblast prostaglandin production and blocks iodinated IL1 alpha binding to its receptor [9]. Uromodulin, an immunosuppressive glycoprotein, inhibits IL1-induced T cell proliferation. This activity depends on intact glycosylation and it appears that IL1 itself binds to uromodulin through lectin-like binding [13]. It is unlikely that either of these inhibitors are comparable to the binding protein described here, due to molecular mass and specificity differences. A granulocyte-derived 45–70 kDa molecule [14] and a 40 kDa immunosuppressive protein from epidermal cells have been described to inhibit IL1-induced thymocyte co-mitogenesis and fibroblast proliferation [15]. However, none of these factors have been shown to be specific for either IL1 alpha or IL1 beta alone.

Some groups have suggested that the proteinase inhibitor alpha 2 macroglobulin (alpha 2M) may, in an activated form, bind IL1, accounting for its various immunosuppressive actions in vitro [11]. Activated alpha 2M has been found at high levels in synovial fluid of rheumatoid arthritis patients and also acts as a binding protein for IL6 [16]. Alpha 2M may account for the high-molecular-mass factor seen to bind non-specifically both IL1 alpha and IL1 beta in the present study.

The 43 kDa specific IL1 beta binding protein described here may represent a solubilised form of the IL1 receptor, but a recombinant form of the murine IL1R comprising only the extracellular domain retains equal affinity for IL1 alpha and beta [17]. Radiolabelled IL1 beta has a half-life in vivo of approximately 5–10 min following i.v. injection [18]. Despite the presence of IL1R on many cells circulating in the blood, IL1 beta is almost entirely associated with the plasma. Distribution studies indicate that apart from the liver and kidneys, IL1 beta distributes into all tissues to approximately the same extent. The specific binding protein described here may therefore act as a carrier molecule for IL1 in body fluids and may be an important determinant of tissue distribution and bioavailability in vivo. It may also explain in part the difficulty of measuring IL1 in body fluids. With the increasing interest in the potential of recombinant IL1 beta as a therapeutic molecule or as a target for pharmacological intervention, it becomes important to understand the factors that control its handling in vivo.

Acknowledgements: We wish to thank Miss Anne S. Mitchell for the preparation of the manuscript. This work was supported by a grant from the Nuffield Foundation (Oliver Bird Fund for Research into Rheumatism) and by the Mary E. McKinnell Trust (Fellowship to J.A.S.).

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