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Identification of the plasma membrane receptor for interleukin-1 on mouse thymoma cells

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The plasma membrane receptor for interleukin-1 (IL-1) has been characterized from mouse EL4-6.1 thymoma cells. Following binding of IL-1 to surface labeled EL4-6.1 cells, the IL-1 binding molecule was immunoprecipitated using a rabbit antiserum against the hormone. The putative IL-1 receptor is a membrane-associated glycopeptide of $M_r = 82000$ containing probably two or three N-linked glycan units as indicated by its conversion into a $M_r = 60000$ polypeptide upon deglycosylation with endo-β-*N*-glycosidase F.

Interleukin-1 receptor; Recombinant interleukin-1; (Mouse EL4-6.1 cell)

1. INTRODUCTION

Interleukin 1 (IL-1) is a family of at least 2 polypeptide hormones released by macrophages which act as major mediators of inflammatory responses [1–4]. IL-1 biological activity is mediated via binding to specific cell surface receptor(s), the functional properties of which have recently been reviewed [5]. Although IL-1 binding sites have been identified on a variety of cell types [4,5], the low number of receptors observed even on highly IL-1-responsive cell lines and the lack of specific anti-receptor antibodies so far has hampered detailed structural studies. However, an IL-1-binding membrane polypeptide was identified on both murine and human cells by treatment of surface-bound radioiodinated ^{125}I-IL-1 with bivalent water-soluble cross-linkers [6–9].

Recently, we have described a mutant subclone of the EL-4 mouse thymoma (EL4-6.1) [10] that expresses an unusually high number of IL-1 binding sites (~20.000 per cell) [11], thus representing a suitable model for further biochemical studies. In this report, we have used this cell line in conjunction with recombinant IL-1 (rIL-1) [12] and a rabbit antiserum to the hormone in order to identify and partially characterize the plasma membrane-associated rIL-1-binding protein.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

The murine T cell lines used in this study were the EL4 thymoma sublines EL4-6.1 [10] and EL4-3 (an independent non-IL-2 secreting subline). Cells were cultured in enriched DME and maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

2.2. *Reagents*

The α form of purified human recombinant interleukin-1 (rIL-1α) [13] and hyperimmune rabbit antiserum anti-rIL-1α were provided by Biogen SA, Geneva, Switzerland. One microgram of the IgG fraction of this antiserum completely inhibited the binding of ^{125}I-rIL-1α to EL4-6.1 cells whereas 50% inhibition was obtained with 0.1 μg/ml (not shown).

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2.3. *Cell labeling and rIL-1α binding*

Cells were labeled by the enzyme-catalysed surface iodination [14] with previously described modification [15]. After labeling, 10×10^6 cells were incubated for 4 h at 4°C with or without 1 μg/ml of rIL-1α in 1 ml of DME with 20% fetal calf serum (FCS). This represented approximately ten times the saturating concentration for low affinity IL-1 receptor [11]. Cells were then washed five times with cold PBS and lysed in 25 mM Tris-HCl, pH 8.4, buffer supplemented with 0.5% Nonidet-P40 (NP-40), 0.5% sodium deoxycholate (DOC), 50 mM NaCl, 0.01% NaN_3, 2 mM PMSF and 10 μg/ml of leupeptin, pepstatin and antipain (Sigma, St. Louis, MO). Alternatively, the incubation with 1 μg/ml of rIL-1α was performed after lysis of labeled cells for 4 h at 4°C followed by overnight dialysis in the cold.

2.4. *Immunoprecipitation*

The lysates of $15–20 \times 10^6$ cells were precleared with 10 μl of normal rabbit serum followed by 50 μl of protein A-coupled to Sepharose 4B (Pharmacia, Uppsala). The cleared lysates were filtered through 0.2 μm Millipore filters before addition of 20 μl (~ 100 μg of protein A binding IgG) of rabbit anti-rIL-1α antiserum. After 45 min of incubation at room temperature, 50 μl of protein A-Sepharose were added and incubation prolonged for 30 min. Immunoprecipitates were washed 3 times in 10 mM phosphate buffer at pH 8.2 supplemented with 0.05% SDS, 0.05% DOC, 0.5% NP-40, 10 mM EDTA and alternatively 3 times with 120 mM Tris-HCl buffer at pH 8.2 containing 100 mM NaCl, 0.5% NP-40 and 10 mM EDTA.

2.5. *Enzyme digestions*

Digestions of immunoprecipitates with endo-β-*N*-acetylglucosaminidase H (endo-H) were performed as previously described [15]. In brief, immunoprecipitates were heated with 0.1 M Tris-HCl buffer, pH 7.5, 1% SDS, and 1% 2-β-mercaptoethanol for 5 min. This solution was diluted with 9 vols of 0.15 M sodium citrate buffer, pH 5.5, containing 4 mM PMSF, 10 μg/ml of pepstatin, leupeptin and antipain, and 3 mIU of endo-H (New England Nuclear, Boston, MA) and was incubated at 37°C for 16 h. Proteins were recovered by precipitation with an equal volume of 30% (w/v) trichloroacetic acid. The sediment was washed twice with ice-cold acetone. Digestions with endo-β-*N*-acetylglucosaminidase F (endo-F) were carried as follows: the immunoprecipitates were solubilized as for endo-H digestion and then diluted nine times with 0.1 M sodium phosphate buffer, pH 6.1, containing 50 mM EDTA. 1% NP-40, 1% 2-β-mercaptoethanol, 4 mM PMSF, and 10 μg/ml of pepstatin, leupeptin and antipain, 0.4 U of endo-F preparation (New England Nuclear, Boston, MA) were then added. The mixture was incubated at 37°C overnight. Proteins were recovered as described for endo-H digestion.

2.6. *Polyacrylamide gel electrophoresis*

One-dimensional polyacrylamide gel electrophoresis in SDS (SDS-PAGE) was carried out on 10% or 10–13% gradient gels according to Laemmli [16]. Immunoprecipitates were dissolved in 80 mM Tris-HCl buffer, pH 6.8, 0.1 M dithiothreitol, 4% SDS, 10% glycerol, and 0.01% bromphenol blue (sample buffer) at 100°C for 5 min before layering onto the gel. The following molecular mass standards (in Da) were used: β-galactosidase (116 000), phosphorylase *b* (94 000), transferrin (78 000), bovine serum albumin (69 000), ovalbumin (46 000), glyceraldehyde-3-P-dehydrogenase (34 000), α-chymotrypsinogen (25 000) and cytochrome *c* (12 500).

3. RESULTS AND DISCUSSION

In view of the slow dissociation rate of IL-1 from its receptor on intact cells ($t_{1/2} \gg 4$ h) [6,11], we decided to immunoprecipitate the putative plasma membrane receptor for IL-1 from surface iodinated EL4-6.1 cell lysates previously incubated with rIL-1α (see section 2, using a rabbit antiserum against human rIL-1α. Thus a single labeled polypeptide of an apparent molecular mass of 80–85 kDa under both reducing and non reducing conditions could be specifically immunopurified after preincubation of the labeled cells with rIL-1α (fig.1A, lane 1). This component was not detected when the incubation of the cells with rIL-1α was omitted prior to immunoprecipitation (fig.1A, lane 2). In addition, it was not found when rIL-1α was added to EL4-3 cells, a thymoma line recently shown to be negative for the expression of the IL-1 receptor [11] (fig.1B, lanes 3–4). Moreover, the polypeptide partitioned into the detergent phase of

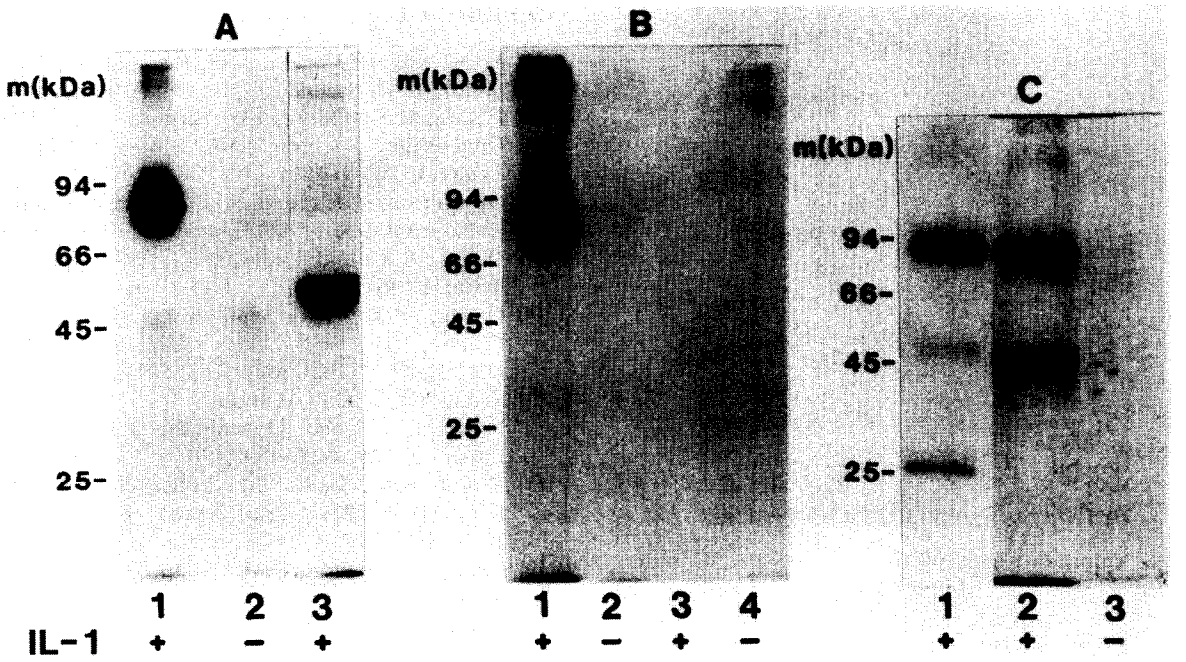

Fig.1. (A) Autoradiograph of 10% SDS-PAGE under reductive conditions. Immunoprecipitates with rabbit anti-rIL-1α of surface-labeled EL4-6.1 cells incubated with (+) or without (−) rIL-1α (lanes 1,2). Lane 3: preclearing with normal rabbit serum. (B) Autoradiographs of 10% SDS-PAGE under non-reductive conditions. Immunoprecipitates with rabbit anti-rIL-1α of surface-labeled cells incubated with (+) or without (−) rIL-1α. Lanes 1,2: EL4-6.1 cells; lanes 3,4: EL4-3 cells. (C) Autoradiographs of 10% SDS-PAGE under reductive conditions. Immunoprecipitates with rabbit anti-rIL-1α. Lane 1: surface labeled cells incubated with rIL-1α and extracted with TX-114 prior to immunoprecipitation; lanes 2,3: surface labeled cells extracted with NP 40/DOC. Lysates were incubated with (+) or without (−) rIL-1α prior to immunoprecipitation.

Triton X-114 which is suggestive for the existence of a hydrophobic detergent-binding domain of the protein [17] (fig.1C, lane 1).

The same 80–85 kDa polypeptide could also be immunopurified when rIL-1α was incubated with cell lysates, thus indicating that the interaction of IL-1α with its putative surface-expressed receptor also occurs after detergent extraction of membrane proteins (fig.1C, lanes 2,3).

In some experiments two surface-labeled polypeptides of apparent molecular mass of 45–50 and 29–30 kDa, respectively, co-precipitated with the 80–85 kDa component. Their possible relationship to the IL-1 receptor cannot be excluded. However, they represented $<5\%$ of the total immunoprecipitated radioactivity. Moreover, the 45–50 kDa polypeptide also found in the absence of IL-1α was a major component of the preclearing step (fig.1A, lane 3). It is therefore likely that it represented a contaminant of the specific immunoprecipitates.

Digestion of the immunopurified putative IL-1 receptor with endo-β-*N*-glycosidase F generated a band of apparent molecular mass of 60 kDa (fig.2A). In contrast, treatment with endo-β-*N*-glycosidase H had no effect (fig.2B). In addition, a minor polypeptide of $\sim$30 kDa was generated upon endo-F treatment (fig.2A). This probably represented the deglycosylated form of the 45–50 kDa contaminant described above.

These preliminary structural studies indicate that the 80–85 kDa polypeptide is glycosylated and the shift in apparent molecular mass observed is consistent with the presence of two or three N-linked glycan units of the endo-H-resistant (i.e. complex) type.

In conclusion, these data represent the first direct identification of the plasma membrane component reacting specifically with IL-1α. In addition, they confirm and extend the preliminary biochemical characterization of the putative IL-1

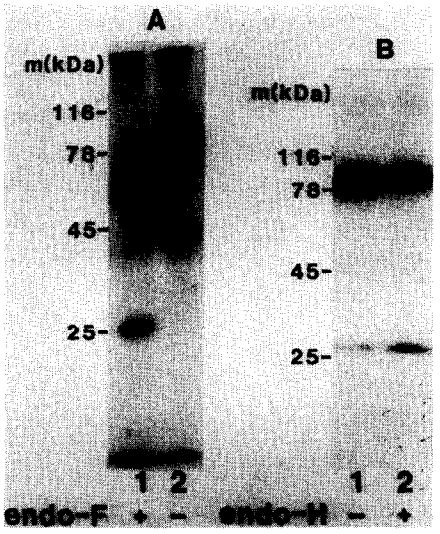

Fig.2. Autoradiographs of 10–13% SDS-PAGE under reductive conditions. Immunoprecipitates with rabbit anti-rIL-1α of surface labeled EL4-6.1 cells incubated with rIL-1α. (A) Digestion with endo-F (lane 1) and control (lane 2). (B) Digestion with endo-H (lane 1) and control (lane 2).

receptor detected by chemically cross-linking radioactive IL-1 to the cell surface [6–9].

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