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Two-chain structure of the interleukin 1 receptor

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By crosslinking radioiodinated recombinant human IL1 α to mouse EL4 thymoma cells we have identified in addition to the known IL1-binding proteins of 80 kDa, a second IL1-binding protein of about 40 kDa. This second binding protein could be demonstrated most easily when crosslinking to higher protein complexes was inhibited. This finding suggests that the IL1 receptor, similar to the receptor for other cytokines such as interleukin 2, is composed of a heterodimer, of which both polypeptides contribute to ligand binding.

Interleukin 1 receptor; Recombinant interleukin 1; Cross-linking

1. INTRODUCTION

Interleukin 1 (IL1) is a cytokine secreted by several cell types, mainly by macrophages, that plays a crucial role in the activation of T-lymphocytes. The IL1 activity resides in two different proteins, IL1 α and IL1 β, which have both been cloned molecularly with a molecular mass of 17 kDa [1,2]. Like many other polypeptide hormones IL1 activity is mediated via high-affinity binding to plasma membrane receptors. Because the IL1 receptor is not very abundant on cells its structure has not yet been elucidated completely. IL1-binding proteins have been characterized by crosslinking of radioiodinated IL1 to different cells. These binding proteins have been shown to have molecular masses in the range of about 60–97 kDa [3–6]. Part of the heterogeneity appears to be due to a variable degree of glycosylation [7]. Here, we have identified an additional binding protein of about 43 kDa by crosslinking radioiodinated IL1 α to E14 mouse thymoma cells.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

EL4 cells (a mouse T-lymphoma cell line) were cultured in RPMI 1640 (Gibco, Karlsruhe) supplemented with 10% FCS (Gibco), 2 g/l $NaHCO_3$ (Merck, Darmstadt), 2 mM L-glutamine (Gibco), 10 U/ml penicillin (Gibco), 0.1 mg/ml streptomycin (Gibco) and 4.5×10^{-5} M 2-mercaptoethanol (Serva, Heidelberg).

2.2. *Radiolabeling of recombinant human IL1 α*

9 μg recombinant human IL1 α (rh IL1 α, Biogen, Geneva) dissolved in 20 μl of 0.1 M borate buffer, pH 8.5, was added to 1 mCi ^{125}I-labeled Bolton-Hunter reagent (1 mCi, diiodo derivative, 4000 Ci/mmol, Amersham Buchler, Braunschweig) which had been dried under nitrogen. The mixture was incubated on ice for 1 h and then the reaction was stopped with 0.5 ml of 0.5 M glycine in 0.1 M borate buffer, pH 8.5. After an additional incubation on ice for 5 min the sample was fractionated on a small Sephadex G-25 column, which was equilibrated with PBS, pH 7.4, 0.1% gelatin. The radioactivity of each fraction was counted using a gamma-counter. The specific activity of ^{125}I-IL1 α was about 10 μCi/μg protein.

2.3. *Crosslinking of ^{125}I-IL1 α to EL4 cells*

Crosslinking was performed as follows: 10^7 EL4 cells were incubated with 0.1 μCi (~10 ng, 200 U) human recombinant ^{125}I-IL1 α in Hepes-RPMI 1640 with 1% BSA (Sigma, Munich), 0.7 mM bacitracin (Serva), 1 mM leupeptin, 1 mM iodoacetamide, 1 mM benzamidine, 1 mM PMSF (Sigma) at 4°C for 1 h. The cells were then washed twice with PBS at 4°C and crosslinked with 0.5 mM disuccinimidylsuberate (DSS,

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Pierce, Geisenheim-Johannisberg) in PBS with the above-mentioned protease inhibitors at 25°C for 60 min. The reaction was stopped with excess glycine. The cells were washed twice with PBS at 4°C and lysed with 1% Triton in PBS, pH 7.4, at 4°C for 30 min. The solubilised cells were then centrifuged at 12000 × g at 4°C for 15 min in a microfuge. The supernatant was denatured with a buffer containing 0.06 M Tris, pH 8.0, 3% SDS, 30% glycerol, 3 mM EDTA, 1 mM PMSF, 15% 2-mercaptoethanol at 25°C overnight. The membrane proteins were separated by SDS-PAGE (7.5%) at 40 mA constant current at 25°C. Gels were fixed, stained and then dried under heat and vacuum. Autoradiography was performed at −80°C for 7–10 days using X-ray films in a cassette equipped with an intensifying screen.

3. RESULTS AND DISCUSSION

The results of crosslinking ^{125}I-IL1 α to EL4 cells are shown in fig.1. When the cells were incubated with ^{125}I-IL1 α at 4°C for 1 h, washed twice and then crosslinked with 0.5 mM DSS at 25°C for an additional 5 min (fig.1A) three bands with apparent molecular masses of 60, 102–126 and 130–151 kDa were specifically labeled under reducing conditions. The appearance of these bands was inhibited in samples incubated with a 10-fold excess of unlabeled rhIL1 α (Biogen) and rhIL1 β (Biogen). Complete inhibition could not be achieved, because of the lack of sufficient unlabeled IL1 (fig.2G,H). Taking into account that the molecular mass of rhIL1 α is 17 kDa, the molecular masses of the binding proteins (the three labeled proteins minus the molecular mass of rhIL1 α) were 43, 85–109 and 113–134 kDa. The same results were obtained under non-reducing conditions (not shown). In the further discussion the molecular masses of the specifically labeled bands will be referred to those of the IL1-binding proteins minus 17 kDa.

Recently Dower et al. [3] have also described the two high-molecular-mass proteins under reducing and non-reducing conditions, however, they failed to detect the 43 kDa binding site. They postulated the 85–109 kDa protein to be the IL1-binding protein and the 113–134 kDa form to be an association between the 85 kDa IL1-binding protein and an adjacent membrane protein of about 40 kDa which in their study did not bind IL1. However, our data indicated clearly that this 40 kDa protein is also an IL1-binding protein. This apparent discrepancy may be due to the different experimental conditions used. Dower et al. [3] always

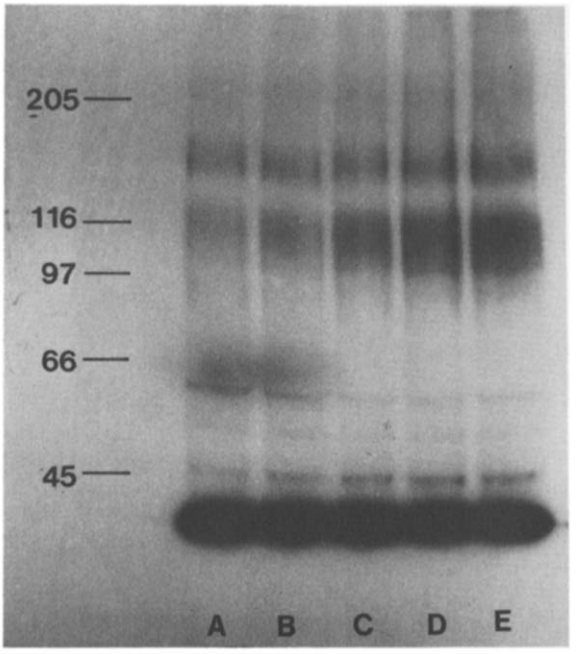

Fig.1. 10^7 EL4 cells were incubated with 0.1 μCi human recombinant ^{125}I-IL1 α at 4°C for 1 h, washed twice and crosslinked with 0.5 mM DSS at 25°C for 5, 15, 30, 60 and 120 min (lanes A–E). The cells were washed twice and lysed with 1% Triton in PBS, pH 7.4. The membrane proteins were denatured and separated by SDS-PAGE as described in section 2.

crosslinked IL1 at substantially higher concentrations of DSS (2.7 mM). Extended crosslinking may completely crosslink the 40 kDa protein to higher complexes (e.g. 80 or 120 kDa). Thus in the experiment of fig.1 a very short crosslinking time led to the most prominent labeling of the 43 kDa protein. To clarify this issue the following experiments were designed. First the cells were incubated with ^{125}I-IL1 α at 4°C for 1 h, washed twice and then crosslinked with 0.5 mM DSS at 25°C for different time periods (5–120 min). Fig.1 shows that the intensity of the 113–134 and 85–109 kDa bands increased with time of crosslinking, whereas that of the 43 kDa band decreased. These results suggested that the 43 kDa proteins indeed crosslinked to higher protein aggregates with increasing crosslinking time.

Fig.2 shows the results of another type of experiment where the EL4 cells were preincubated with DSS at 25°C for different time periods and then ^{125}I-IL1 α was added for 30 min. The aim was to

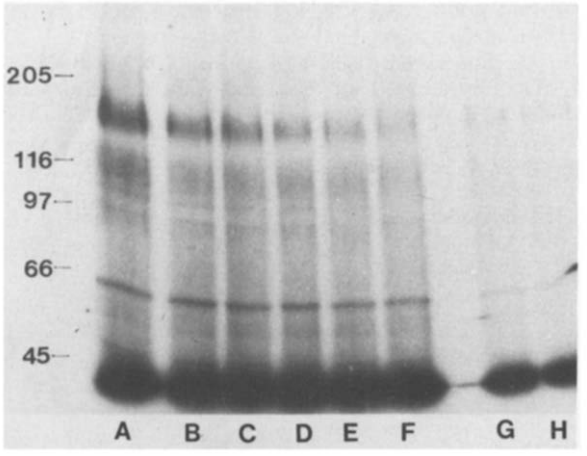

Fig.2. 10^7 EL4 cells were preincubated with 0.5 mM DSS at 25°C for 5, 15, 30, 60, 120 and 180 min (lanes A–F), then 0.1 μCi ^{125}I-rh IL1 α was added, and the cells were crosslinked at 25°C for an additional 30 min. Cells were washed twice and processed as described in the legend to fig.1. Lanes G,H show the results of an experiment in which the cells were treated as described for lane A with the difference that crosslinking was performed with a 10-fold excess of unlabeled IL1 α (lane G) or IL1 β (lane H).

'freeze' the membrane proteins by crosslinking before labeled IL1 was crosslinked and thus prevent the formation of IL1-labeled protein aggregation of higher molecular mass. With increasing preincubation times with DSS the intensity of the 113–134 and 85–109 kDa bands indeed decreased whereas that of the 43 kDa band remained unchanged. The most likely explanation is that DSS reacted first with intramolecular amino groups of a protein. After a preincubation period of 2 h, probably most of the amino groups of one protein have reacted with DSS so that intermolecular crosslinking of two proteins is no longer possible.

In the third type of experiments the cells were incubated with ^{125}I-IL1 α at 4°C for 1 h, washed twice and crosslinked with DSS at 4°C for 15 min. Under these conditions fig.3 shows a major band of 43 kDa. As the plasma membrane of the cells is rigid at 4°C the lateral movement of proteins as well as collision between proteins is very low. Thus binding of labeled IL1 to the 43 kDa protein can be detected, which at higher temperature may be overlooked as crosslinking complexes of higher molecular mass are formed.

These three sets of independent experiments

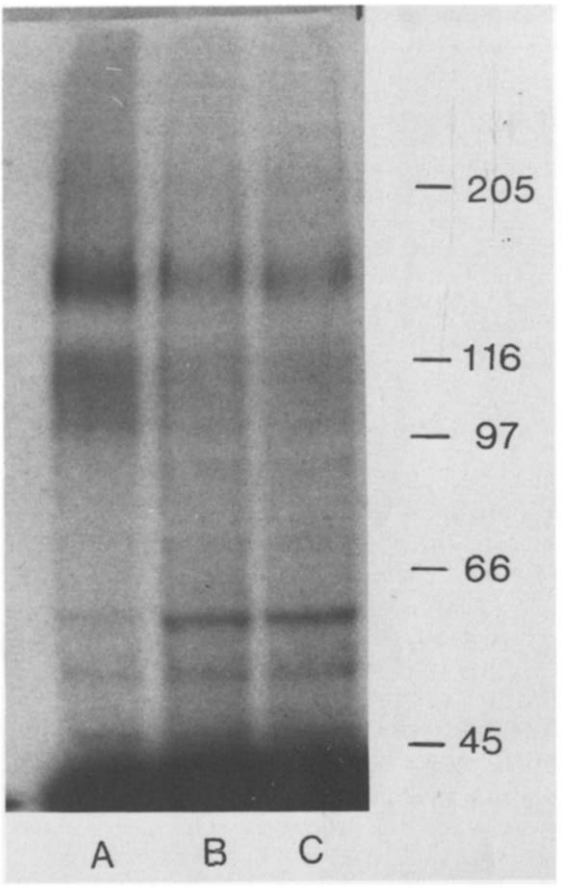

Fig.3. 10^7 EL4 cells were incubated with 0.1 μCi rh IL1 α at 4°C for 60 min, washed twice and crosslinked at 25°C for 30 min (lane A), 4°C for 15 min (lane B), and 4°C for 30 min (lane C). The cells were washed twice and processed as described in the legend to fig.1.

clearly demonstrated that IL1 binds to the 43 kDa protein. Our data can be reconciled with those of Dower et al. [3] and Bron and MacDonald [6], who both in addition to a 80 kDa binding protein suggested a second protein of 40–50 kDa to be associated with the receptor. The important difference is that we can demonstrate that this second protein also contributes to IL1 binding. It should be mentioned that our experiments do not exclude the possibility that this 43 kDa protein is the only IL1-binding protein, and that the apparent 80 kDa binding protein may represent dimers preexisting in the cell. In conclusion, we propose that the IL1 receptor consists of two chains of about 80 and 43 kDa which both contribute to IL1 binding. This

structure is very similar to the recently elucidated two-chain structure of the IL2 receptor [7].

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