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Murine interleukin-1 receptor: differences in binding properties between fibroblastic and thymoma cells and evidence for a two-chain receptor model

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The concentration of porcine interleukin-1β (pIL1β) required to elicit half-maximal IL2 production from NOB-1, a subline of murine thymoma EL4, was 100-fold greater than for p1Lα. In contrast, similar doses of each type of IL1 stimulated increased lactate production by Balb/C 3T3 fibroblasts. Receptor-bound ^{125}I-IL1α was displaced with equal efficiency by both unlabelled forms from 3T3 cells, but a 20-fold lower affinity for p1L1β was observed using NOB-1. Crosslinking experiments suggested that the IL1 receptors on each line consisted of two polypeptides of 80 and 100 kDa. The results provide the first evidence for a multiple-component IL1 receptor within which IL1α and IL1β may bind at different loci, and suggest the receptors may have evolved differently in the two lines.

Interleukin-1 receptor; Interleukin-1; (Mouse thymoma cell; 3T3 fibroblast)

1. INTRODUCTION

Interleukin-1 (IL1) is the name given to a family of polypeptides produced by activated monocytes and macrophages which share multiple biological activities [1]. Originally defined as a lymphocyte-activating factor [2], IL1 also acts as a local hormone, mediating many of the effects of inflammation and regulating local tissue remodelling [3]. There are at least two forms of IL1, called α and β, which although sharing only limited homology, interact with a common receptor on all cell types studied to date [4–7].

A crucial question concerns the similarity between IL1 receptors on cells of different lineages. In a recent study [5] of the binding of recombinant human IL1β to murine 3T3 fibroblasts and LBRM-33-1A5 T lymphoma cells it was concluded that the molecular sizes of the receptors and their binding affinities were broadly similar.

Here, we have exploited the differential responses of a murine fibroblast line and a T-cell line to natural porcine IL1α and IL1β in order to demonstrate differences in the IL1-binding characteristics of the two cell types. For experiments designed to reveal possible structural differences in IL1 receptors of the two cell types we used a novel crosslinking procedure which revealed a previously unreported IL1-receptor polypeptide.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

Balb/C 3T3 fibroblasts (clone A31) were cultured in Dulbecco's modified medium containing 10% foetal calf serum (FCS). The NOB-1 subline of EL4-6.1 thymoma [8] was cultured in

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RPMI 1640 containing 5% FCS. All cultures were maintained at 37°C in a humidified atmosphere of air enriched with 5% CO_2.

2.2. *Reagents*

IL1α and β were purified from culture supernatants of concanavalin A-stimulated porcine buffy coat leucocytes [9]. pIL1β was radioiodinated with Bolton-Hunter reagent to around 500 Ci/mmol exactly as in [4]; pIL1α was labelled using a modified chloramine-T procedure [10] to 1800 Ci/mmol. Both preparations migrated as single bands of 21 kDa upon SDS-PAGE and were fully active in a cartilage resorption assay [9]. Recombinant murine IL1 was a gift from Dr P. Lomedico (Hoffmann-La Roche, Nutley, NJ). The covalent crosslinking reagents ethylene glycol bis(succinimidyl succinate) (EGS) and sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate (SS) were from Pierce (Pierce & Warriner, Chester, England).

2.3. *Biological assays*

The two-stage assay for IL1 using NOB-1 has been fully described elsewhere [8]. Very briefly, washed NOB-1 cells (2×10^5) were incubated with IL1 samples in 200 μl RPMI 1640 containing 5% FCS. After 24 h, aliquots of the medium were transferred to microtitration wells containing the IL2-responsive cytotoxic mouse T-cell line CTLL (4×10^3 cells/well). 18 h later, proliferation was measured by pulsing the cells for 3 h with 0.5 μCi [^{3}H]thymidine per well. For measurement of glycolysis, newly confluent monolayers of 3T3 fibroblasts (2×10^5 cells/well) were washed in two changes of maintenance medium and incubated for 48 h in 0.5 ml of fresh medium containing IL1 samples. Following deproteinization of the supernatants using perchloric acid, their lactate content was measured by the lactate dehydrogenase method using a kit supplied by Sigma (St. Louis, MO).

2.4. *Binding assays*

3T3 cells were seeded into 25-well plates at 2×10^4 cells/well and used 4–5 days later when confluent. The monolayers were washed twice with binding buffer [Hank's balanced salts supplemented with 20 mM Hepes and 0.1% (w/v) BSA] and incubated, with gentle shaking, for 4 h at 12°C in 0.4 ml of fresh binding buffer containing labelled and unlabelled ligands as required. The plates were then rinsed rapidly with three changes of ice-cold binding buffer, and bound radioactivity was counted following solubilization of the cell layers in 1% SDS/0.1 N NaOH. NOB-1

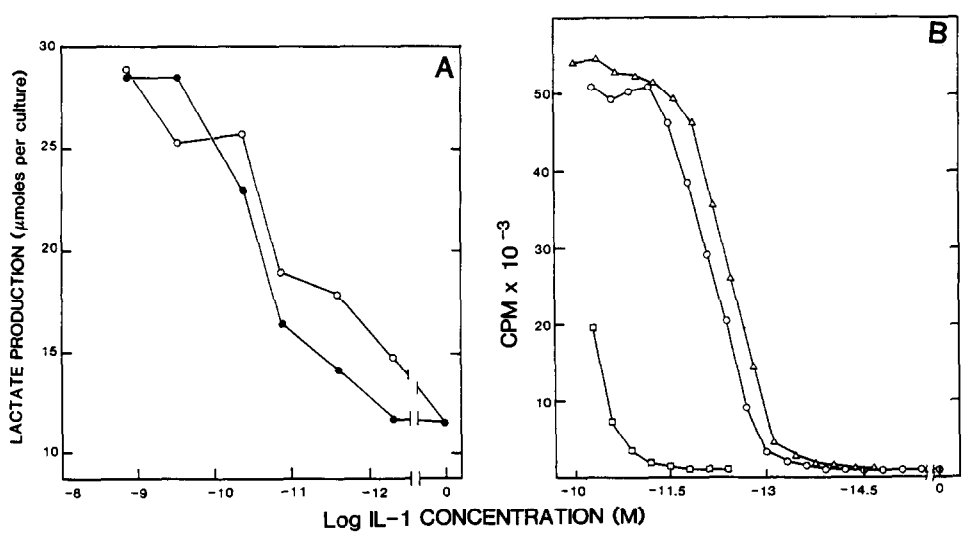


Fig.1. Comparison of the concentration dependence of pig IL1α and IL1β activity in 3T3 cells and NOB-1 cells. (A) Lactate production was measured in Balb/C 3T3 fibroblasts as described in the text; triplicate wells were incubated for 48 h with pIL1α (○) or β (●). (B) Two-stage assay of IL2 production by NOB-1 in response to pIL1α (○) or β (□); the effect of recombinant murine IL1α is shown for comparison (△). All points are means of triplicate determinations.

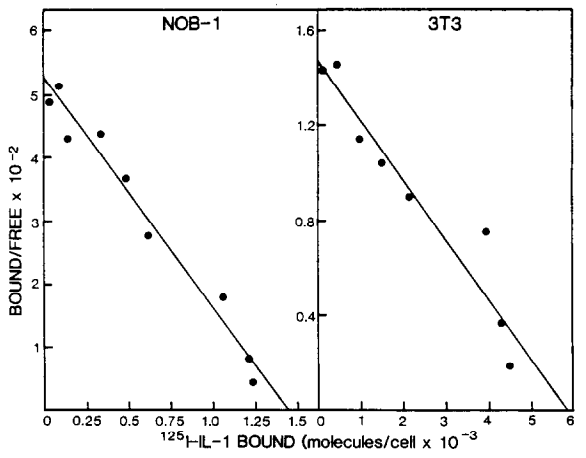


Fig.2. Scatchard analysis of equilibrium binding of ^{125}I-pIL1α to NOB-1 and 3T3 cells. Binding assays were carried out as described in the text, each point being derived from the mean of triplicate determinations from which non-specific binding (measured in the presence of 1 μg/ml unlabelled IL1) has been subtracted. Straight lines were fitted to the points by a least-squares method (r = 0.984 for NOB-1 and 0.959 for 3T3).

cells were washed twice with binding buffer, resuspended at 5 × 10^6/ml, and aliquots (0.3 ml) incubated with IL1 samples for 2.5 h at 12°C. Bound radioligand was separated from free using the phthalate oil method described by Dower et al. [10]. All binding data were analysed by the LIGAND program [11].

2.5. *Covalent cross-linking and gel electrophoresis*

^{125}I-pIL1s were crosslinked to intact cells using the homobifunctional reagent EGS exactly as in [4]. For photoaffinity labelling, cells were incubated with ^{125}I-IL1 (9.5 × 10^{-10} M) as described above, washed free of unbound ligand and suspended in PBS (4 × 10^5/ml). SS was added to a final concentration of 2.5 mM and the reaction was allowed to proceed in total darkness for 20 min at 0°C before photolysis for 5 min using the 366 nm output of a hand-held ultraviolet lamp at 10 cm from the samples. After the reaction had been quenched by the addition of 2 vols of 10 mM Tris-HCl, pH 7.5/1 mM EDTA, cells were pelleted and prepared for electrophoresis as in [4]. Crosslinked complexes were identified in 7.5% polyacrylamide gels according to Laemmli [12].

3. RESULTS AND DISCUSSION

Fig.1A shows that the half-maximal doses of each form of pIL1 required to stimulate increased glycolysis in confluent 3T3 cells were very similar; we chose to assay lactate production since, in our hands, 3T3 fibroblasts did not proliferate in response to pIL1 as has been reported using recom-

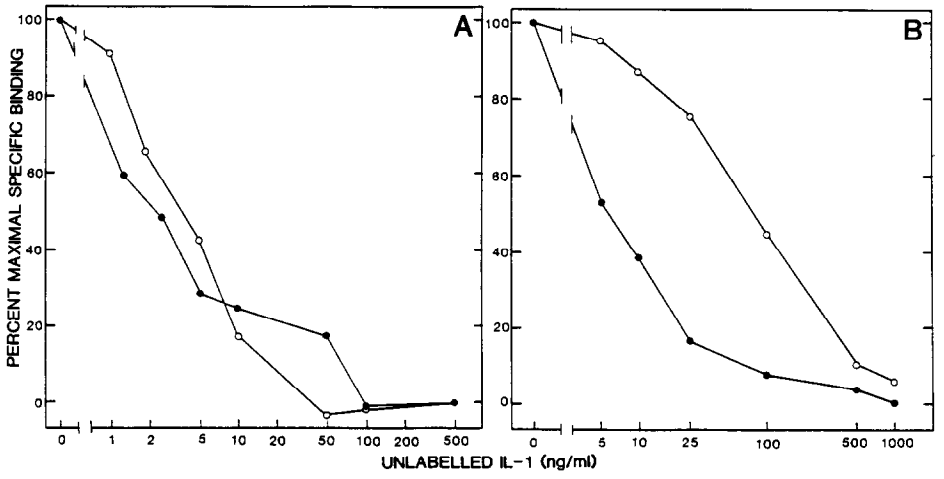
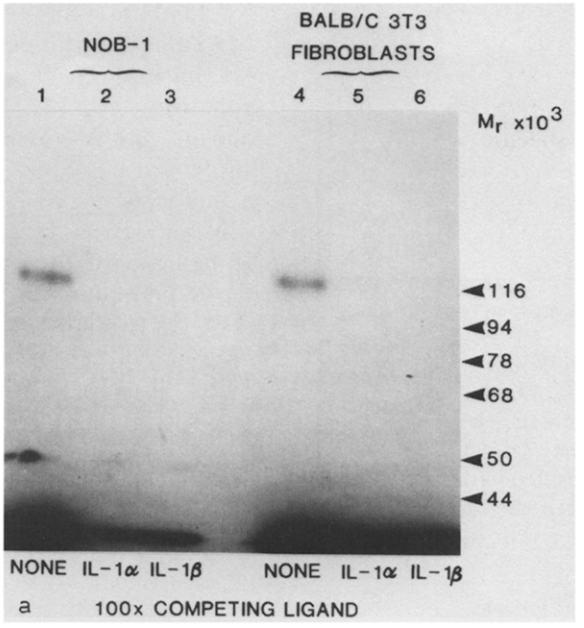


Fig.3. Displacement of ^{125}I-pIL1α by unlabelled IL1α and β. 3T3 cells (A) and NOB-1 cells (B) were incubated with 120 pM ^{125}I-IL1α and increasing concentrations of unlabelled IL1α (●) or IL1β (○). After subtraction of non-specific binding the amount of radioactivity bound at each dose was compared to that bound in the presence of tracer alone (3306 cpm in A, 3059 cpm in B). Each point is the mean of triplicate determinations.

binant human IL1 [5]. In contrast, pIL1β was less potent, by two orders of magnitude, in stimulating IL2 production by NOB-1 (fig.1B). The ED_{50} for pIL1β was 0.7 pM, comparable to recombinant murine IL1α; the ED_{50} for pIL1β was 71 pM. These findings prompted us to examine the binding



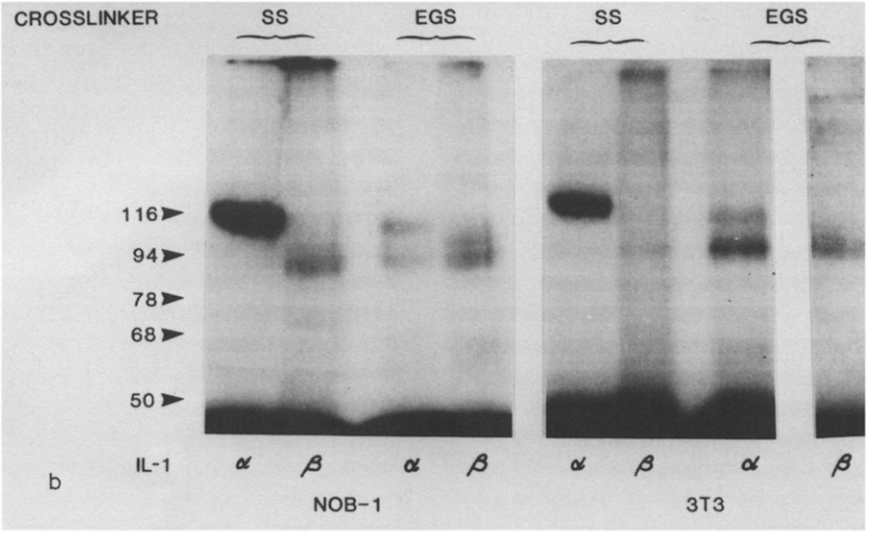


Fig.4. (A) Photoaffinity crosslinking of ^{125}I-pIL1α to intact cells. 10^7 NOB-1 cells (lanes 1–3) and 6×10^5 3T3 cells (lanes 4–6) were incubated at 12°C with 950 pM ^{125}I-IL1α either alone (lanes 1,4) or in the presence of 95 nM unlabelled pIL1α (lanes 2,5) or β (lanes 3,6). After crosslinking with 2.5 mM SS as described, the cells were solubilized and run under reducing conditions on 7.5% SDS-polyacrylamide gels. Autoradiographs of the dried gels are shown together with the migration positions of protein markers (β-galactosidase, phosphorylase *a*, transferrin, bovine serum albumin, IgG, ovalbumin). (B) Comparison of crosslinking patterns obtained using ^{125}I-labelled pIL1α and β. Experiments using SS were carried out as described above; crosslinking using EGS (0.5 mM) was as described in section 2. Gels were run under reducing conditions.

of porcine IL1 to murine IL1 receptors. Scatchard analysis (fig.2) revealed that, in each cell type, ^{125}I-pIL1α bound to a single class of receptors. In the typical experiments shown we detected 1500 binding sites on NOB-1 (K_d = 420 pM) and 5900 sites on 3T3 (K_d 550 pM). Our finding of a single class of receptors confirms previous reports using 3T3 [5] and EL-4 cells [6]. Despite using an IL1 preparation of comparable specific activity we found no evidence to support the existence of high- and low-affinity receptor populations as was described for binding of human rIL1 to EL-4-6.1 [13], the parent line of NOB-1.

The relative potency of pIL1β in the two assays was reflected in its ability to displace ^{125}I-pIL1α (fig.3), thus in the 3T3 binding assay unlabelled IL1β was equipotent with unlabelled IL1α; in four experiments the inhibition constant for displacement ranged between 104 and 740 pM for IL1α and 156–175 pM for IL1β. The respective K_i values for NOB-1 were 110–340 pM and 1.1–2.3 nM. These data suggest that murine cells of independent lineage are able to discriminate at the level of receptor binding, between forms of porcine IL1. One implication is that the structure of the IL1 receptor must have evolved along separate lines in the two cell types resulting in an altered capacity to recognise pIL1β without alteration in their ability to bind pIL1α.

In order to explore the possibility that there might be structural differences between the receptors on the two cell types we investigated the cell surface components that could be covalently crosslinked to IL1. Other investigators [4–7] have used homobifunctional crosslinkers; the success of this approach depends upon the close proximity of amino side groups in ligand and receptor. We have also used a heterobifunctional reagent, SS, in which a highly reactive nitrene group is photochemically generated, capable of insertion into C–C and C–H bonds, resulting in less restricted crosslinking. Crosslinking of ^{125}I-pIL1α to NOB-1 and 3T3 with SS revealed a single high molecular mass complex of 120 kDa (fig.4A), which was not formed if the cells were incubated in the presence of a 100-fold excess of unlabelled IL1α or β, indicating that both ligands became associated with a polypeptide of 100 kDa. Similar results were obtained when electrophoresis was carried out without prior reduction of the samples (not shown). Crosslinking of IL1β resulted in the formation of a major band at around 97 kDa with both SS and EGS (fig.4B); in some cases, both bands were visible using the latter reagent, this was also the case with IL1α. The possibility of proteolytic artefacts was excluded by performing these experiments with or without proteinase inhibitors (pepstatin A, EDTA, phenylmethylsulfonyl fluoride, and *N*-ethylmaleimide); in each case the patterns were the same (not shown). The same patterns were also observed using SS concentrations between 0.025 and 5 mM, therefore it seems unlikely that the 120 kDa complex arose through crosslinking of two IL1 molecules to a single 80 kDa component. As far as we can ascertain, the crosslinking profiles of NOB-1 and 3T3 are identical, so whatever the structural difference between the two cell types' receptors, it is not revealed at this level. Our finding of a 97 kDa band is in agreement with previous reports [4,5] of an 80 kDa murine IL1-receptor protein, but this study is the first to identify a second, larger receptor component. Although crosslinking studies cannot unambiguously define ligand-binding polypeptides, three alternative interpretations might be considered: firstly, a precursor is differentially processed to two forms of receptor (i.e. 80 and 100 kDa); secondly, there are in fact two genetically distinct types of receptor; and thirdly, the IL1 receptor comprises two different polypeptide chains. Purification of the two components is underway in order to distinguish between these possibilities.

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