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# Identification of a specific receptor for interleukin-1 on rat bone marrow cells

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The interleukin-1 (IL-1) receptor on rat bone marrow cells was investigated using $^{125}$I-labeled IL-1$\alpha$ and -1$\beta$. These radiolabeled ligands bound to the rat bone marrow cells in a specific and saturable manner with $K_d$ values of $0.36 \pm 0.072$ nM and $1.9 \pm 0.27$ nM, respectively. In a competitive binding experiment, IL-1$\alpha$ and -1$\beta$ inhibited the binding of $^{125}$I-IL-1$\alpha$ with $K_i$ values of $0.35 \pm 0.041$ nM and $2.9 \pm 1.0$ nM. The binding of $^{125}$I-IL-1$\beta$ was inhibited by IL-1$\alpha$ and -1$\beta$ with $K_i$ values of $0.27 \pm 0.020$ nM and $0.74 \pm 0.12$ nM. In cross-linking experiments, $^{125}$I-IL-1$\alpha$ was covalently incorporated into two proteins of 163 kDa and 63 kDa. These results suggested the presence of two binding proteins for IL-1 on the rat bone marrow cells.

Interleukin-1 receptor; Interleukin-1$\alpha$; Interleukin-1$\beta$ (Rat bone marrow cell, Mouse BALB/3T3 cell)

## 1. INTRODUCTION

IL-1$\alpha$ and IL-1$\beta$ are two distinct cytokines functioning as the regulators of inflammatory reactions or immune responses [1]. The distribution of the IL-1 receptor on fibroblasts, T cells and B cells is consistent with the known biological effects of IL-1 on these cells [2,3]. In spite of low homology (26%) of amino acid sequence between human IL-1$\alpha$ and IL-1$\beta$ [4], both bind to the same cell-surface receptor on T cells [5,6]. Molecular weight of the IL-1 receptor has been reported for various types of cell; 80 kDa for EL-4 cells [7], LBRM-33-1A5 cells [2] and BALB/3T3 cells [8], 68 kDa for Raji cells [7] and 60 kDa for human Epstein Barr virus-transformed B lymphocytes [9]. The IL-1 receptor of 80 kDa was purified to homogeneity from EL-4 cells [10], and the cDNA clone was isolated by Dower et al. [11].

In recent years, many investigators have been interested in the regulation of hematopoiesis by IL-1. The addition of IL-1 induces the in vitro production of granulocyte/macrophage colony-stimulating factor (CSF) and granulocyte CSF by mouse bone marrow stromal cells [12], fibroblasts [13], endothelial cells [14–16] and mononuclear phagocytes [17] of human origin. IL-1 enhances the ability of several kinds of CSF to stimulate colony formation by primitive hematopoietic progenitors in vitro [18–20]. However, the IL-1 receptor on primitive hematopoietic progenitor cells has not yet been identified. In this paper, we will report the first demonstration of IL-1 binding protein in rat bone marrow cells by the use of $^{125}$I-labeled human recombinant IL-1$\alpha$ and IL-1$\beta$.

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*Abbreviations:* IL-1, interleukin-1; PBS, Dulbecco's phosphate-buffered saline

## 2. MATERIALS AND METHODS

### 2.1. *Materials*

Recombinant human IL-1$\alpha$ and IL-1$\beta$ were expressed in *E. coli* and purified to homogeneity as previously described [21]. BALB/3T3 fibroblast (the American Type Culture Collection) maintained in a Dulbecco's minimum essential medium (MEM) containing 10% fetal calf serum (FCS). Bone marrow cells were obtained by flushing the femora of Sprague-Dawley rats (6–10 weeks) with an $\alpha$-MEM containing 10% FCS followed by incubation for 2 h in a $CO_2$-incubator. The non-adherent cells were collected and used for the IL-1 binding assay.

### 2.2. *IL-1 receptor assay*

Recombinant human IL-1$\alpha$ was labeled with $^{125}$I by utilizing Iodogen reagent (Pierce Chemical Co.) [22]. The radioactive IL-1$\alpha$ was purified by gel filtration. The specific activity was 130–150 $\mu$Ci/$\mu$g. Rat bone marrow cells ($5 \times 10^6$) were incubated with the $^{125}$I-IL-1 in the presence of various concentrations of unlabeled IL-1 in 200 $\mu$l of $\alpha$-MEM containing 10% FCS. The incubation was terminated by centrifugation layering on 200 $\mu$l of a phthalate oil mixture in polyethylene centrifuge tubes as described [2]. Non-specific binding was determined in the presence of 1 $\mu$g/ml unlabeled IL-1$\alpha$. The biologic activity of the radiolabeled IL-1 as assayed by its growth inhibitory activity against human melanoma A375 cell [21] was the same as that of unlabeled IL-1. For the IL-1 receptor assay in situ BALB/3T3 cells were incubated with 500 $\mu$l of Dulbecco's MEM containing 10% FCS in a 12-well plate (Corning) for 2 h at 4°C. The cell monolayers were then rinsed with 3 changes of PBS (1 ml), and

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solubilized with 1 ml of a mixture of 1% SDS and 0.2 N NaOH. The radioactivity was counted in a gamma counter.

2.3. *Chemical cross-linking of cells and SDS-polyacrylamide gel electrophoresis*

Bone marrow cells ($5 \times 10^7$) were incubated for 4 h with $^{125}$I-IL-1$\alpha$ at 4°C in the presence or absence of 1 $\mu$g/ml IL-1$\alpha$. BALB/3T3 cells ($5 \times 10^7$) were incubated for 2 h under the same conditions. The incubation mixture was centrifuged, and the pellet was washed 3 times with PBS. The cells were cross-linked with $^{125}$I-IL-1$\alpha$ by disuccinimidyl suberate (Pierce Chemical Co.) (1 mg/ml) at room temperature for 1 h. The labeled and cross-linked cells were extracted with PBS containing 1% Triton X-100 and a mixture of protease inhibitors [10] on ice for 15 min. The mixture was centrifuged at 10000 $\times$ *g* for 30 min at 4°C. The supernatant was mixed with an equal volume of the 1% SDS and 5% 2-mercaptoethanol. The mixture was boiled for 3 min and then subjected to 7.5% SDS-polyacrylamide gel electrophoresis according to the method of Laemmli [23]. The gels were fixed, dried, and exposed to a Kodak XAR-5 film for 3–7 days at −70°C.

## 3. RESULTS AND DISCUSSION

Rat bone marrow cells were incubated with $^{125}$I-labeled recombinant human IL-1$\alpha$ or IL-1$\beta$ (600 Ci/mmol, Amersham). The specific binding of $^{125}$I-IL-1$\alpha$ reached an equilibrium after 4 h incubation at 4°C. As shown in fig.1a, the binding of $^{125}$I-IL-1$\alpha$ to the rat bone marrow cells at 4°C was saturable, and the non-specific binding of the radiolabeled ligand in the presence of 1 $\mu$g/ml unlabeled IL-1$\alpha$ was 15–50% of the total binding. Scatchard analysis of the data of fig.1a showed the presence of a single class of IL-1$\alpha$ binding site (241 sites/cell) with a $K_d$ of 0.31 nM (fig.1b). On the other hand, $^{125}$I-IL-1$\beta$ also bound to the rat bone marrow cells (fig.1c). Scatchard analysis showed that the rat bone marrow cells had a single class of IL-1$\beta$ binding site (577 sites/cell) with a $K_d$ of 1.4 nM (fig.1d). Results averaged from three similar experiments are listed in table 1.

Table 1

Properties of IL-1 receptor on rat bone marrow cells

| | | $^{125}$I-IL-1$\alpha$ | $^{125}$I-IL-1$\beta$ |
|---|---|---|---|
| A | $K_d$ | 0.36 ± 0.07 nM ($n$ = 3) | 1.9 ± 0.27 nM ($n$ = 3) |
| | sites/cell | 190 ± 32 | 350 ± 110 |
| B | $K_i$ IL-1$\alpha$ | 0.35 ± 0.041 nM ($n$ = 4) | 0.27 ± 0.02 nM ($n$ = 3) |
| | IL-1$\beta$ | 2.9 ± 1.0 nM ($n$ = 4) | 0.74 ± 0.12 nM ($n$ = 3) |

(A) Parameter values were obtained by analysing the equilibrium binding data in fig.1 and 2 other experiments. (B) $K_i$ values were obtained from the data of fig.2 (A and a) and 2 or 3 other experiments

As shown in fig.2A and a, the rat bone marrow cells were incubated with various concentrations of unlabeled IL-1$\alpha$ and IL-1$\beta$ for the displacement of a known amount of $^{125}$I-IL-1$\alpha$ or IL-1$\beta$ which was bound to the rat bone marrow cells. The experimental results indicated that the IL-1$\alpha$ and IL-1$\beta$ molecules bound to a common class of receptor site. However, the $K_i$ values for $^{125}$I-IL-1$\alpha$ were different by almost one order of magnitude between IL-1$\alpha$ (0.340 nM) and IL-1$\beta$ (5.7 nM). The $K_i$ values for $^{125}$I-IL-1$\beta$ were 0.29 nM with IL-1$\alpha$ and 0.69 nM with IL-1$\beta$. The average $K_i$ values are summarized in table 1. Thus, the affinity of the receptor on the rat bone marrow cells for IL-1$\alpha$ was several times higher than that for IL-1$\beta$. The difference

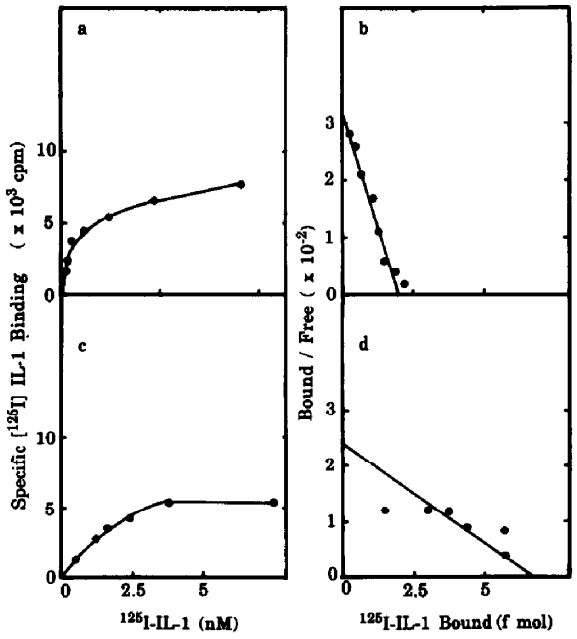


Fig.1. Binding of $^{125}$I-IL-1$\alpha$ (a) and IL-1$\beta$ (c) to rat bone marrow cells. Data from (a) and (c) were replotted in the Scatchard coordinate system (b and d).

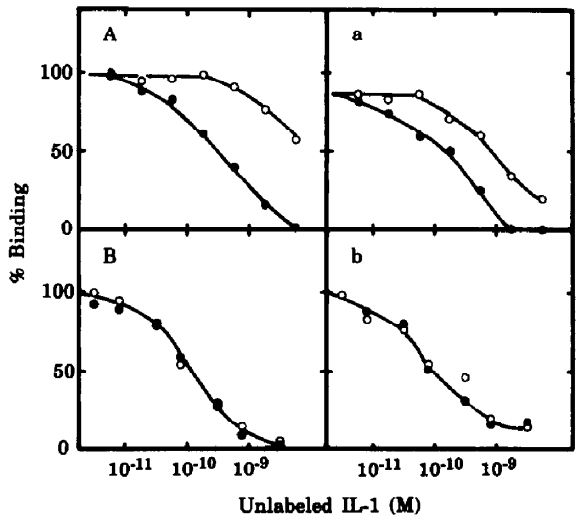


Fig.2. Cross-competition between IL-1$\alpha$ (•) and IL-1$\beta$ (○) on rat bone marrow cells and BALB/3T3 cells. Rat bone marrow cells (A, $5 \times 10^6$ cells; a, $7.6 \times 10^6$ cells) were incubated with 140 pM $^{125}$I-IL-1$\alpha$ (A) and 714 pM $^{125}$I-IL-1$\beta$ (a). BALB/3T3 fibroblasts ($1 \times 10^6$ cells) were incubated with 70 pM $^{125}$I-IL-1$\alpha$ (B) and 397 pM $^{125}$I-IL-1$\beta$ (b). Maximal binding was measured in the presence of the $^{125}$I-ligands alone. (A) $^{125}$I-IL-1$\alpha$ 1354 cpm; (a) $^{125}$I-IL-1$\beta$ 1817 cpm; (B) $^{125}$I-IL-1$\alpha$ 3412 cpm; (b) $^{125}$I-IL-1$\beta$ 3770 cpm.

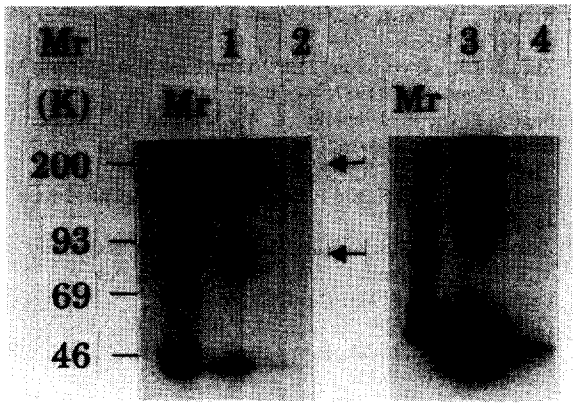


Fig.3. SDS-polyacrylamide gel electrophoresis of IL-1 receptors of rat bone marrow cells (lanes 1 and 2) and BALB/3T3 fibroblasts (lanes 3 and 4) covalently cross-linked with $^{125}$I-IL-1$\alpha$. Lanes 2 and 4 contained 1 $\mu$g/ml unlabeled IL-1$\alpha$. Lanes Mr are for the molecular mass markers. Positions of 180 kDa and 80 kDa are indicated with arrows.

in the $K_i$ values between IL-1$\alpha$ and IL-1$\beta$ is consistent with the difference in the hemopoietin 1 activity between IL-1$\alpha$ and IL-1$\beta$ [19]. The same experiments with IL-1$\alpha$ and IL-1$\beta$ were carried out using mouse BALB/3T3 fibroblasts as presented for fig.2B and b. In the case of BALB/3T3 cells, both IL-1$\alpha$ and IL-1$\beta$ molecules bound to a common class of receptor site with an approximately equal affinity. The $K_i$ value for $^{125}$I-IL-1$\alpha$ was 109 pM (IL-1$\alpha$ and IL-1$\beta$), and those for $^{125}$I-IL-1$\beta$ were 103 pM (IL-1$\alpha$) and 143 pM (IL-1$\beta$). Similar results have been reported in several recent works with pig synovial fibroblasts and articular chondrocytes [5], and human dermal fibroblasts [6].

When $^{125}$I-IL-1$\alpha$ was incubated with the rat bone marrow cells in the presence of a homobifunctional cross-linker, two major proteins of molecular weights 180 kDa and 80 kDa were radiolabeled upon SDS-polyacrylamide gel electrophoresis (fig.3). Subtracting 17 kDa (molecular mass of IL-1), the molecular mass values of the cross-linked proteins were estimated to be 163 kDa and 63 kDa. The binding of these two proteins with $^{125}$I-IL-1$\alpha$ was reduced in parallel depending on the concentration of unlabeled IL-1$\alpha$ (fig.4a). The same results were obtained with unlabeled IL-1$\beta$ (fig.4b). Thus, the two proteins could bind to IL-1$\alpha$ or IL-1$\beta$ with an approximately equal affinity. In the same experiment, the IL-1 receptor of BALB/3T3 cells showed a molecular weight of approximately 80 kDa (97 kDa as a conjugate with IL-1$\alpha$).

The molecular weight of the IL-1 receptor was also reported to be 80 kDa for EL-4 cells [7], LBRM-33-1A5 cells [2], human normal and embryonic lung fibroblasts [24]. In contrast, the IL-1 receptors of Raji cells [7] and Epstein Barr virus-transformed B lymphocytes [9] showed a molecular mass of 68 kDa and 60 kDa, respectively. Horuk et al. [7] suggested that such a heterogeneity in molecular weight reflects a structural difference of the receptor proteins. The two main cross-linked proteins with molecular masses of 180 kDa and 80 kDa on rat bone marrow cells may be subunits composing a receptor complex. But, it should be noted that the rat bone marrow cells used in this work are not a homogeneous cell population. It is possible that the two radiolabeled proteins are attributable to two different types of cell.

Almost the same results were obtained with mouse bone marrow cells (data not shown). The IL-1 receptor reported in this paper may be a common IL-1 receptor for bone marrow cells of various animals. Namely, two binding proteins of different sizes were found. The affinity for the receptor was different between IL-1$\alpha$ and IL-1$\beta$. Further characterization of the structure and function of IL-1 receptor on bone marrow cells requires the cloning of the receptor cDNA as recently reported for the IL-1 receptor cDNA from EL-4 cells [11].

Very recently, Parker et al. suggested the presence of IL-1 receptor on granulocytes and granulocytic progenitors from bone marrow cells of untreated mice [25]. Identity of their IL-1 receptor with our binding proteins of rat bone marrow cells awaits further investigations.

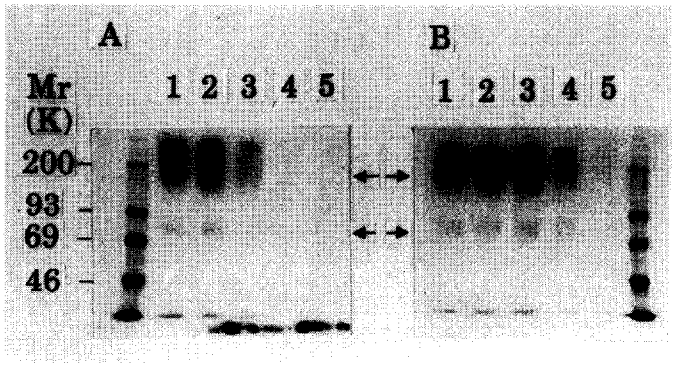


Fig.4. Inhibition of $^{125}$I-IL-1$\alpha$ binding to two binding proteins by unlabeled IL-1$\alpha$ or IL-1$\beta$. (A) Rat bone marrow cells were covalently radiolabeled with $^{125}$I-IL-1$\alpha$ in the presence of unlabeled IL-1$\alpha$ (lanes 1–5: 0, 1, 10, 100 and 1000 ng/ml). (B) Experimental conditions were the same as shown in (A) except that IL-1$\beta$ was used instead of IL-1$\alpha$. Positions of 180 kDa and 80 kDa are indicated with arrows.

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