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# Differential binding of IL-1α and IL-1β to receptors on B and T cells

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The interleukin 1 receptors (IL-1R) on the human B lymphoma RAJI and on the murine thymoma EL4-6.1 have been characterized. Equilibrium binding analysis using both $^{125}$I-labeled IL-1α and IL-1β showed that RAJI cells have a higher number of binding sites/cell for IL-1β (2400, $K_d$ 2.2 nM) than for IL-1α (316, $K_d$ 0.13 nM). On the other hand, EL4-6.1 cells have more receptors/cell for IL-1α (22 656, $K_d$ 1 nM) than for IL-1β (2988, $K_d$ 0.36 nM). Dexamethasone (DXM) induced on RAJI cells a time-dependent increase in binding sites for both IL-1β and IL-1α without affecting their binding affinities. However, while receptor-bound $^{125}$I-IL-1α was displaced with equal efficiency by both IL-1 forms, only unlabeled IL-1β could effectively displace $^{125}$I-IL-1β. Cross-linking experiments indicated that RAJI cells have a predominant IL-1R of about 68 kDa, while EL4-6.1 cells have an IL-1-binding polypeptide of 80 kDa. These results suggest that B and T cells possess structurally different IL-1R with distinct binding properties for IL-1α and IL-1β.

Interleukin 1 receptor; Interleukin 1α; Interleukin 1β; (RAJI B lymphoma, EL4-6.1 thymoma)

## 1. INTRODUCTION

The term interleukin 1 (IL-1) describes a family of at least two proteins, termed α and β, which share a wide array of both immunomodulatory and inflammatory activities [1]. IL-1α and IL-1β exert their biological activities through the interaction with specific receptors on the surface of target cells. Although they are only poorly homologous in their primary sequences, the two IL-1 isoforms have been reported to bind a common class of receptors on a variety of cell types including T and B cells [2] and fibroblasts [3]. The sequence of a cDNA encoding an 80 kDa polypeptide responsible for the binding of IL-1 on murine EL4-6.1 thymoma has been recently reported [4]. In the same study, COS cells expressing this recombinant IL-1R polypeptide have been shown to bind IL-1α and IL-1β with markedly different affinities [4]. It is thus conceivable that IL-1R could be formed of a complex comprising more than one polypeptide within which IL-1α and IL-1β bind in a different fashion. Moreover, it has recently been shown that differences in the molecular characteristics of IL-1R occur between B and T cells [5].

Here, we have analyzed the binding parameters and some biochemical characteristics of IL-1R on the surface of both untreated and dexamethasone (DXM)-treated human RAJI B lymphoma and on the murine EL4-6.1 thymoma using iodine-labeled IL-1α and IL-1β. Our results confirm the observation that B and T cells have structurally distinct IL-1R and suggest for the first time that the IL-1R present on the surface of these cells possess different binding properties for IL-1α and IL-1β.

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## 2. MATERIALS AND METHODS

### 2.1. *Cells*

RAJI human B cell lymphoma was obtained from Dr G.P. Pantaleo (Ludwig Institute, Lausanne, Switzerland) and cultured in RPMI 1640 medium (Gibco, Paisley, Scotland) sup-



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plemented with 10% heat-inactivated foetal bovine serum (FBS), 50 μg/ml gentamycin sulphate, 25 mM Hepes buffer and 2 mM L-glutamine. EL4-6.1 murine thymoma was obtained from Dr H.R. MacDonald (Ludwig Inst., Lausanne) and cultured as in [6]. For stimulation with glucocorticoids, RAJI cells were washed in fresh medium and then suspended at 2.5 × $10^5$ cells/ml. Dexamethasone acetate (DXM) (Sigma, St. Louis, MO) was dissolved in DMSO at 2 mg/ml and then diluted into the culture medium at the desired concentration. Control cultures received the same concentration of DMSO alone.

### 2.2. *Radiolabeling of IL-1α and IL-1β*

Human recombinant IL-1α (Biogen, Geneva) was radioiodinated using the chloramine T method exactly as described by Lowenthal and MacDonald [6]. Human recombinant IL-1β (Sclavo-De.Bi., Siena and Cassina de' Pecchi, Italy), expressed in *E. coli* and purified as in [7], was radioiodinated with [$^{125}$I]di-iodo Bolton-Hunter (4400 Ci/mmol, New England Nuclear, Boston, MA) following the manufacturer's instructions. The specific activities obtained were routinely around 1.2 × $10^6$ cpm/pmol for IL-1β and 5.4 × $10^6$ cpm/pmol for IL-1α. The biological activity of labeled IL-1 was monitored using the D10.G4.1 proliferation assay [8] and found to be the same for both $^{125}$I-IL-1α and $^{125}$I-IL-1β (not shown).

### 2.3. *Binding assay*

Cells were suspended at 1 × $10^7$/ml in RPMI containing 10% FBS and 0.02% sodium azide (binding medium). Aliquots of 5 × $10^5$ cells were incubated for 3 h at room temperature (RT) under constant gentle agitation. At the end of the incubation unbound radioactivity was separated from cell-bound radioactivity with centrifugation on a microfuge (Beckman, Fullerton, CA) through an oil gradient (80% silicone and 20% paraffin). Non-specific binding was determined by adding a 500-fold molar excess of unlabeled IL-1α or IL-1β. All the calculations and Scatchard analysis were performed according to Munson and Rodbard [9].

### 2.4. *Covalent cross-linking and gel electrophoresis*

$^{125}$I-IL-1α and $^{125}$I-IL-1β were cross-linked to intact cells with disuccinimidyl suberate (DSS) or dithiobis(disuccinimidyl propionate) (DSP) (Pierce, Rockville, MD) as described in [3]. Briefly, cells suspensions ($10^7$ cells/ml) in binding medium were incubated with $^{125}$I-IL-1α or -β (2.3 nM) for 3 h at room temperature with or without a 500-fold molar excess of unlabeled IL-1β. After washing the unbound IL-1, DSS or DSP (50 mg/ml in DMSO) were added to a final concentration of 2 mg/ml, and the reaction was allowed to proceed for 45 min at room temperature. Cells were lysed with 50 μl of PBS containing 7.5 mM 3-(3-cholamidopropyl)dimethylammonio-1-propanesulphonate (CHAPS) (Serva, Heidelberg), 1 mM PMSF, 10 μg/ml of aprotinin, leupeptin, chymostatin and antipain (Boehringer Mannheim, Mannheim, FRG), and centrifuged in an Eppendorf apparatus for 5 min. The supernatant was denatured with Laemmli sample buffer [10], with the exception that 2-mercaptoethanol was omitted for samples cross-linked with DSP. SDS-PAGE was run on 8 or 10% gels using coloured molecular mass markers (Amersham, Little Chalfont, England). Dried gels were exposed at −40°C using Kodak XAR-5 autoradiographic films.

## 3. RESULTS

The specific binding of increasing amounts of $^{125}$I-IL-1α or $^{125}$I-IL-1β added to aliquots of 5 × $10^5$ RAJI cells is shown in fig.1. Scatchard analysis (see insets) of the typical experiment shown indicated that RAJI cells have 316 receptors/cell ($K_d$ = 0.13 nM) for IL-1α and 2400 receptors/cell ($K_d$ = 2.2 nM) for IL-1β. The same kind of experiments were then performed on the murine thymoma EL4-6.1. In a representative experiment reported in fig.2, the specific binding obtained with increasing amounts of $^{125}$I-IL-1α (A) or $^{125}$I-IL-1β (B) is plotted. Scatchard analysis (insets) showed that EL4-6.1 cells have 22656 receptors/cell ($K_d$ = 1 nM) for IL-1α and 2988 receptors/cell ($K_d$ = 0.36 nM) for IL-1β.

Glucocorticoid hormones have been recently reported to increase the number of binding sites for IL-1 in human peripheral B lymphocytes [11]. RAJI B lymphoma cells were thus incubated with 400 nM DXM and, after different time intervals, the specific binding of a fixed amount of $^{125}$I-IL-1β (70000 cpm) was determined. As shown in fig.3A the specific binding remained similar to the control level for 6 h after DXM addition and constantly increased thereafter. In all subsequent experiments RAJI cells were thus stimulated with DXM for

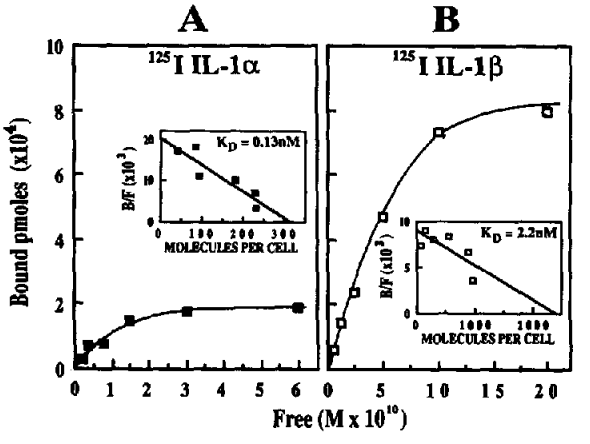


Fig.1. Equilibrium binding analysis on RAJI cells. Specific binding of increasing amounts of $^{125}$I-IL-1α (A) or $^{125}$I-IL-1β (B) to aliquots of 5 × $10^5$ cells was determined by subtracting non-specific binding obtained in the presence of a 500-fold molar excess of unlabeled IL-1α or -β. Insets: Scatchard analyses.

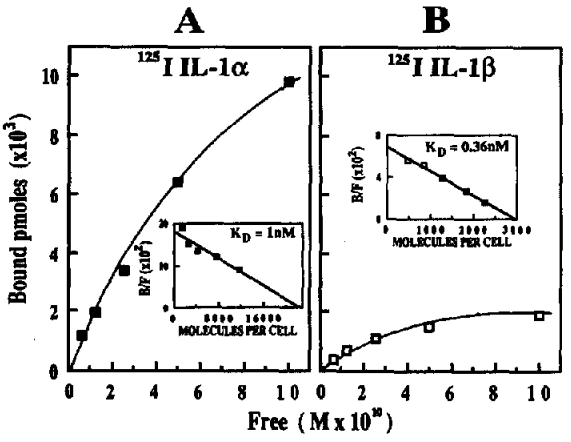


Fig.2. Equilibrium binding analysis on EL4-6.1 cells. Specific binding of increasing amounts of $^{125}$I-IL-1$\alpha$ (A) or $^{125}$I-IL-1$\beta$ (B) was determined as described in fig.1.

48 h. The specific binding of increasing amounts of $^{125}$I-IL-1$\alpha$ (fig.3B) or $^{125}$I-IL-1$\beta$ (fig.3C) was then determined on RAJI cells incubated with 400 nM DXM for 48 h. Scatchard analysis (see insets) showed that DXM-stimulated RAJI cells have 1800 receptors/cell ($K_d$ = 0.12 nM) for IL-1$\alpha$ and 9960 receptors/cell ($K_d$ = 2.1 nM) for IL-1$\beta$. Thus incubation with the glucocorticoid hormone induced an increase in specific binding for both isoforms of IL-1 without affecting their affinities for the ligand.

To characterize further the IL-1R on DXM-treated RAJI cells, competition binding experiments were performed. Fig.4 demonstrates the displacement of receptor-bound $^{125}$I-IL-1$\alpha$ (A) or $^{125}$I-IL-1$\beta$ (B) by a wide range of doses of either unlabeled IL-1$\alpha$ or IL-1$\beta$. Both isoforms were equally potent in the competition with $^{125}$I-IL-1$\alpha$, but only unlabeled IL-1$\beta$ could efficiently displace the receptor-bound $^{125}$I-IL-1$\beta$.

A partial biochemical characterization of the polypeptides involved in binding of IL-1$\alpha$ and IL-1$\beta$ on the surface of both RAJI and EL4-6.1 cells was then undertaken. The electrophoretic pattern on 8% SDS-PAGE of $^{125}$I-IL-1$\alpha$ or $^{125}$I-IL-1$\beta$ cross-linked to RAJI cells using DSS is shown in fig.5A. With both $^{125}$I-IL-1$\alpha$ and $^{125}$I-IL-1$\beta$, a major high molecular mass complex of about 85 kDa was observed which was not present when cells were incubated in the presence of a 500-fold molar excess of unlabeled IL-1. Treatment with DXM resulted in an increase in intensity of the specific bands obtained with both $^{125}$I-IL-1$\alpha$ and $^{125}$I-IL-1$\beta$, in agreement with the increase in receptor number observed in the equilibrium binding experiments. The electrophoretic pattern on 8% SDS-PAGE of the cross-linked products obtained with DSP on RAJI cells is reported in fig.5B. With $^{125}$I-IL-1$\beta$ a predominant specific band at about 85 kDa was obtained, in agreement with the results

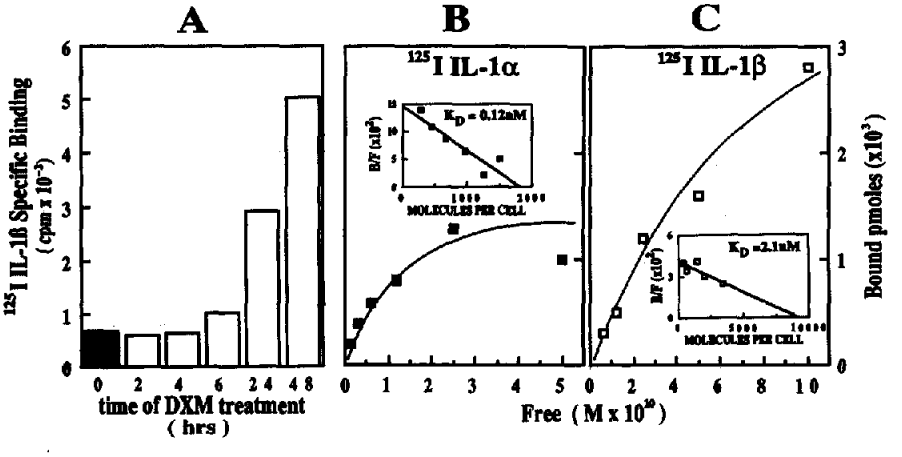


Fig.3. Characterization of IL-1R on 48 h DXM-stimulated RAJI cells. (A) Specific binding of $^{125}$I-IL-1$\beta$ (70000 cpm) to RAJI cells at different time intervals after stimulation with 400 nM DXM. Each bar represents the mean of duplicate determinations. The specific binding of increasing amounts of $^{125}$I-IL-1$\alpha$ (B) or $^{125}$I-IL-1$\beta$ (C) to RAJI cells stimulated for 48 h with DXM was determined as described in fig.1.

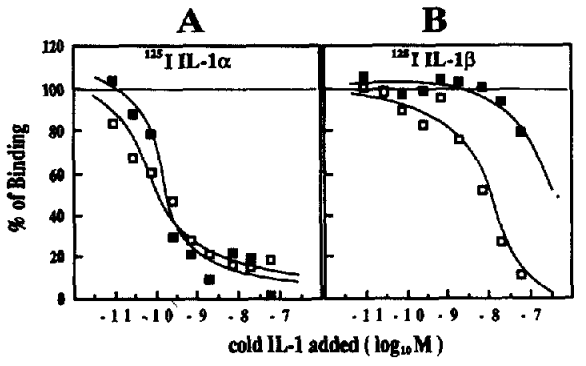


Fig.4. Competition binding analysis on RAJI cells stimulated with DXM for 48 h. Cells were incubated with 30000 cpm $^{125}$I-IL-1$\alpha$ (A) or 41000 cpm $^{125}$I-IL-1$\beta$ (B) with or without increasing concentrations of unlabeled IL-1$\alpha$ (■) or IL-1$\beta$ (□). After subtraction of non-specific binding the amount of bound radioligand at each dose was compared to that obtained in the absence of the unlabeled competitor. Each point is the mean of duplicate determinations.

obtained using DSS, while cross-linking of $^{125}$I-IL-1$\alpha$ gave a predominant high molecular mass complex of about 145 kDa and a much less intense band at 85 kDa. Treatment with DXM resulted in an increase in intensity of the bands at 145 and 85 kDa for $^{125}$I-IL-1$\alpha$, and of the 85 kDa product obtained with $^{125}$I-IL-1$\beta$.

In order to compare the structural properties of the IL-1R on RAJI B lymphoma and EL4-6.1 thymoma, the same amounts of $^{125}$I-IL-1$\alpha$ or -$\beta$ were incubated with EL4-6.1 in the presence of DSS. The electrophoretic pattern of the cross-linked products on 10% SDS-PAGE is reported in fig.5C. A major product at about 98 kDa is present with EL4-6.1 for $^{125}$I-IL-1$\alpha$ and $^{125}$I-IL-1$\beta$. The intensity of the bands obtained with IL-1$\alpha$ was much higher than those obtained with IL-1$\beta$, again in agreement with the equilibrium binding analysis that showed a greater number of binding sites for IL-1$\alpha$ on the surface of EL4-6.1 thymoma. The specificities of the bands were assessed in the presence of a 500-fold excess of unlabeled IL-1 (not shown).

## 4. DISCUSSION

In this study, evidence is provided that the IL-1R have distinct binding properties for IL-1$\alpha$ and IL-1$\beta$ on the surface of both B and T cells. In fact, the B lymphoma RAJI has a low number of receptors for IL-1$\alpha$ ($\simeq$300 sites/cell) and a higher number of receptors for IL-1$\beta$ ($\simeq$2500 sites/cell) (fig.1), while EL4-6.1 thymoma (fig.2) possesses a greater number of binding sites for IL-1$\alpha$ ($\simeq$20000 sites/cell) than for IL-1$\beta$ ($\simeq$3000 sites/cell). It was also observed that RAJI B lymphoma binds IL-1$\alpha$ with much higher affinity ($\simeq$0.1 nM) than IL-1$\beta$ ($\simeq$2 nM) while EL4-6.1 thymoma binds IL-1$\alpha$ with lower affinity ($\simeq$1 nM) than IL-1$\beta$ ($\simeq$0.4 nM).

The number of specific binding sites for both IL-1 isoforms on RAJI cells can be increased by in-

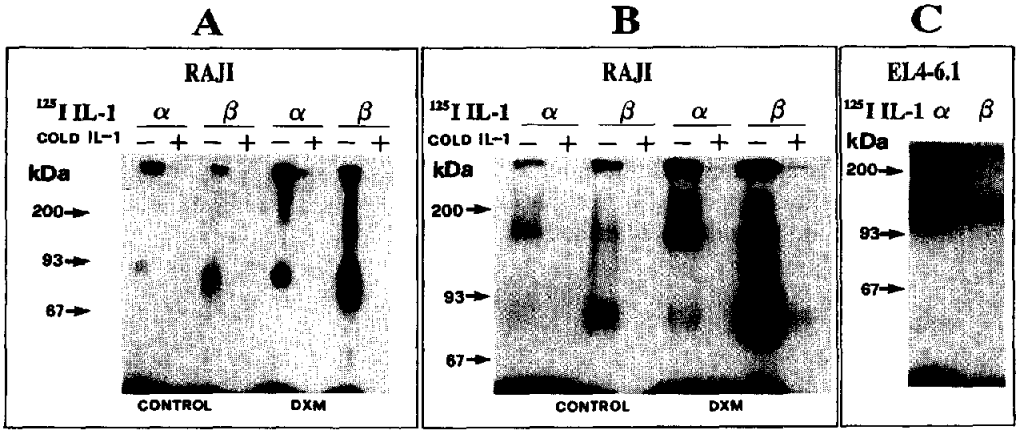


Fig.5. Autoradiography of the 8% SDS-PAGE of cross-linked products obtained using DSS (A) or DSP (B) on control and 48 h DXM-stimulated RAJI cells incubated with $^{125}$I-IL-1$\alpha$ or $^{125}$I-IL-1$\beta$ in the absence (−) or presence (+) of a 500-fold molar excess of IL-1$\beta$. (C) Autoradiography of the 10% SDS-PAGE of the cross-linked products obtained using DSS on EL4-6.1 incubated with $^{125}$I-IL-1$\alpha$ or $^{125}$I-IL-1$\beta$.

cubation with glucocorticoids, but the binding parameters of IL-1$\alpha$ and IL-1$\beta$ are still distinct. In fact, after 48 h treatment with DXM, the number of receptors for IL-1$\alpha$ ($\simeq$2000 sites/cell; $K_d \simeq$ 0.1 nM) is still lower than that of receptors for IL-1$\beta$ ($\simeq$10000 sites/cell; $K_d$ = 2 nM).

This apparent dissociation was further confirmed by the results of competition binding experiments. In fact, while the receptor-bound $^{125}$I-IL-1$\alpha$ could be displaced by both forms of unlabeled IL-1, $^{125}$I-IL-1$\beta$ was effectively displaced only by unlabeled IL-1$\beta$.

Taken together the results of binding experiments showed that human B cells and murine T cells have contrasting properties in the binding of the two IL-1 isoforms, and suggested that RAJI cells have an IL-1R to which IL-1$\alpha$ and IL-1$\beta$ bind at distinct although partially overlapping sites.

Some preliminary information on the structural characteristics of the specific binding sites for IL-1 on both RAJI and EL4-6.1 cells was provided by affinity cross-linking experiments. On RAJI cells, the IL-1R, after subtraction of the molecular mass of IL-1, appeared to consist of a major component of about 68 kDa binding both IL-1$\beta$ and IL-1$\alpha$. Our results are thus in agreement with the recent report of a 68 kDa IL-1R on RAJI cells observed by using $^{125}$I-IL-1$\beta$ cross-linked with DSS [5]. An IL-1-binding polypeptide of about 130 kDa was also observed mainly with $^{125}$I-IL-1$\alpha$ cross-linked using DSP. The identity of this high molecular mass component is presently unknown, however we cannot exclude the possibility that it could be a dimer of the IL-1/IL-1R generated during the cross-linking procedure.

The cross-linking experiments confirmed the reported difference in molecular mass of IL-1R between human RAJI B lymphoma and murine T cells [5]. In fact the predominant band observed with EL4-6.1 using both IL-1$\alpha$ and -$\beta$, after subtraction of the molecular mass of IL-1, accounted for the presence of an 80 kDa IL-1R, as described previously in this cell line [12].

In conclusion, the present data suggest that the IL-1R present on both B and T cells possess distinct binding parameters for IL-1$\alpha$ and IL-1$\beta$ and also confirm and extend the notion of structural heterogeneity between the IL-1R of different cell lineages.

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