

BINDING AND INTERNALIZATION OF INTERLEUKIN 1 BY T CELLS

Direct Evidence for High- and Low-Affinity Classes of Interleukin 1 Receptor

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IL-1, a macrophage-derived 17,500 M_r glycoprotein, has been shown to mediate a wide spectrum of biological activities (1, 2). Insofar as T cell-mediated immunity is concerned, IL-1 has been shown in several studies (3–9) to induce Th cell secretion of the growth hormone IL-2, while in other systems it has been shown to provide an obligatory signal for IL-2-R expression (10, 11). The study of the regulatory role of IL-1 in T cell proliferation has been greatly facilitated by the recent development of a direct radiolabeled IL-1 binding assay by Dower et al. (12). These authors showed that IL-1 bound to specific cell surface receptors that have a molecular weight of around 80,000. The general level of IL-1-R expression by a variety of cell types was found to be much lower than that of receptors for other hormones, such as epidermal growth factor, insulin, and IL-2. Various T cell populations, for example, were shown to express only 27–550 IL-1-R per cell (12).

We have recently characterized a mutant subline of the EL4 thymoma (EL4-6.1), which is induced to simultaneously secrete IL-2 and express IL-2-R in the presence of IL-1 (13). We show in the present report, using a direct IL-1 binding assay that these cells express extremely high numbers of IL-1-R (~20,000 per cell). The IL-1-R expressed by EL4-6.1 cells can be resolved into two classes: the vast majority of receptors bind IL-1 with a K_d of ~200–500 pM, whereas a second class of receptor, making up 1–2% of the total, binds IL-1 with a 100-fold higher affinity (K_d of 3–8 pM). This latter value is equivalent to the concentration of IL-1 that gives 50% biological activity. In addition, the two classes of IL-1-R can be distinguished on the basis of their ability to internalize IL-1; it appears that only high-affinity IL-1-R can do so. Other cell types also express both classes of IL-1-R, but the absolute number of receptors per cell is considerably less than on EL4-6.1 cells. Because of their high degree of responsiveness to IL-1 and the expression of unusually high numbers of IL-1-R, EL4-6.1 cells offer a valuable system with which regulation of IL-1-R expression and the mechanism of IL-1 action can be studied in detail.

Materials and Methods

Cell Cultures. The murine T cell lines used in this study were: EL4 thymoma sublines EL4-6.1 (14), EL4-10 (subclone of EL4-6.1), EL4-3 (an independent non-IL-2-secreting subline), EL4-RN, and EL4-RP (kindly provided by O. Kanagawa, Lilly Research Laboratories, La Jolla, CA); the IL-1-responsive T lymphoma LBRM-33-1A5B6 (LBRM, reference 15); the IL-2-dependent cytolytic T cell line CTLL (16); and the T lymphomas ST-4.2 (17), BW5147 (18), and Yac (19). Nylon wool-purified peripheral T cells from lymph nodes (LNT)¹ were prepared as described (20) and were >95% Thy-1⁺ as judged by FACS analysis. Cells were cultured in enriched DME (21) and maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. PMA was purchased from Sigma Chemical Company, St. Louis, MO. Ionomycin was obtained from Calbiochem-Behring Corp., La Jolla, CA. Human rIL-2 was provided by Biogen SA, Geneva, Switzerland, and was radioiodinated according to the method of Robb et al. (22).

Interleukin 1. Human recombinant IL-1 (α and β forms, reference 23) was kindly provided by Dr. C. Henney, Immunex Corp., Seattle, WA. A detailed description of their preparation and purity is reported elsewhere (24). The specific activity of rIL-1- α and - β was 10⁷ U/mg in both the murine thymocyte assay (1, 3) and in the EL4-6.1 IL-2 induction assay (13).

Biological Assay for IL-1. The biological activity of IL-1 was measured by its ability to stimulate IL-2 production by EL4-6.1 cells in the presence of Ionomycin (13). EL4-6.1 cells (10⁵ cells/microwell) were cultured in the presence of various concentrations of IL-1, and 0.1 μ g/ml of Ionomycin. After 24 h, the cell-free supernatant was measured for IL-2 activity using the CTLL line (16), according to the method of Landegren (25). In this assay, 1 U/ml of IL-2 supports 50% maximal proliferation during a 48-h culture period. In some experiments, IL-1 biological activity was measured by its ability to induce IL-2-R expression by EL4-6.1 cells in the presence of suboptimal concentrations of PMA. Briefly, EL4-6.1 cells (5 \times 10⁵ cells/ml) were cultured in the presence of 0.3 ng/ml of PMA plus various concentrations of IL-1. After 48 h, expression of IL-2-R was measured by using a direct IL-2 binding assay as described previously (26, 27).

IL-1 Absorption Assay. Various cell lines were extensively washed and then incubated (10⁷ cells/ml) at 4°C in the presence of a known concentration of rIL-1. After 5 h, the cell-free supernatant was measured for residual IL-1 activity in both the IL-2 production and IL-2-R expression assays using EL4-6.1 cells (13).

Radioiodination of rIL-1. rIL-1- α was radioiodinated using the chloramine T method (28). The method used was similar to that described for IL-2 by Robb et al. (22), and was according to the procedure of Dr. S. K. Dower, with some modifications. Small quantities of rIL-1- α (500 ng in 50 μ l of PBS pH 7.2) were added to a mixture of 15 μ l of chloramine T (30 μ g/ml in PBS) and 1 mCi (10 μ l) of Na¹²⁵I (Amersham International, Amersham, United Kingdom). The reaction was carried out in an Eppendorf tube at 4°C for 10 min. The labeled rIL-1 (¹²⁵I-IL-1) was separated from free Na¹²⁵I on a Sephadex G-25 column (PD-10; Pharmacia Fine Chemicals, Uppsala, Sweden). ~90% of the radioactivity present in the pooled fractions 5-11 (Fig. 1), was TCA-precipitable. Biological activity of ¹²⁵I-IL-1 was measured as described above. ~60-70% of the initial protein was recovered as measured from experiments using mock-labeled IL-1. The specific activity of the ¹²⁵I-IL-1 was 10⁶ cpm/ng and produced a single band of 17,000 M_r when analyzed by SDS-PAGE.

¹²⁵I-IL-1 Binding Assay. Binding of ¹²⁵I-IL-1 was measured according to the method of Dower et al. (12) and our previously published procedures for radiolabeled IL-2 (27). Briefly, aliquots of 10⁶ cells were incubated in the presence of various concentrations of ¹²⁵I-IL-1 at 4°C for various periods of time (equilibrium binding occurs within 2-4 h). Free radioactivity was separated from bound by centrifugation through an oil gradient (26). Nonspecific binding was measured in the presence of a 50- to 100-fold excess of unlabeled IL-1. Scatchard plot analysis of equilibrium binding data was performed as previously described (27).

Internalization of IL-1. Cells were washed thoroughly and incubated (5 \times 10⁶ cells/ml)

¹ Abbreviation used in this paper: LNT, nylon wool-purified lymph node T cells.

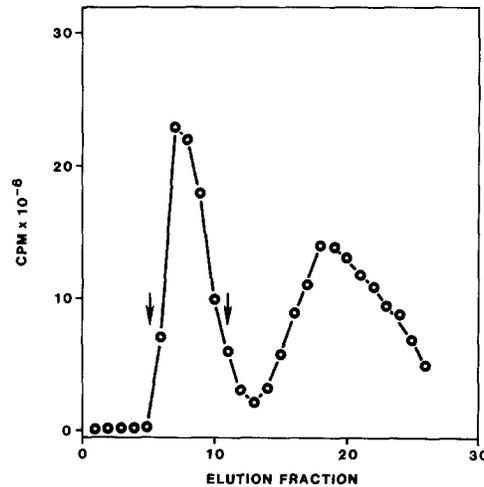


FIGURE 1. Separation of ^{125}I -IL-1 from free Na^{125}I . The ^{125}I -IL-1-reaction mixture was placed on top of a 1-ml Sephadex G-25 column that had been pretreated with BSA (10 mg/ml) and then extensively washed with PBS. ^{125}I -IL-1 was eluted with PBS (pH 7.2) and 50 μl fractions were collected and measured for radioactivity. Elution fractions 5-11 (arrows) were pooled and diluted in medium containing 10% FCS as carrier protein.

in the presence of ^{125}I -IL-1 at 4°C for 3-4 h. The cells were then either washed extensively in medium or not washed and then transferred to 37°C . The amount of internalized IL-1 and total cell-associated IL-1 was measured at various times by centrifugation of cells through an oil gradient. Internalized IL-1 was distinguished from surface-bound IL-1 by its resistance to treatment with tissue culture medium adjusted to pH 3 by adding 1N HCl. At pH 3, >95% of surface-bound IL-1 dissociated within 1 min, whereas the level of intracellular IL-1 was not affected. The rate of increase in the pH 3-resistant IL-1 binding with time at 37°C was therefore taken as a measure of the rate of IL-1 internalization. The level of radioactivity remaining after pH 3 treatment at the end of the 4°C incubation period was taken as background. No IL-1 internalization occurred at 4°C . Internalization of radiolabeled rIL-2 was measured according to the same method. In other experiments, the exclusively internal localization of pH 3-resistant radioactive ligand was directly confirmed by quantitative autoradiography on electron microscopy sections (J. Lowenthal and B. Iacopetta, unpublished observations).

Results

Identification of IL-1-R⁺ Cells by Absorption Experiments. In preliminary studies we used IL-1 absorption experiments to distinguish between IL-1-R⁺ and IL-1-R⁻ cell types. In these experiments, cells were incubated at 4°C in the presence of a known concentration of rIL-1 for 4-6 h. The cell-free supernatants were then measured for residual IL-1 activity, either by their ability to induce IL-2 secretion or IL-2-R expression by EL4-6.1 cells. Fig. 2 shows that cells from IL-1-responsive lines could absorb IL-1 activity, whereas nonresponsive cell lines such as CTLL and ST4 could not. This was not due to secretion of inhibitory products by these cells, as revealed by mixing experiments. EL4-6.1 and cells of its subclone EL4-10 were very efficient at absorbing IL-1 activity. Results from experiments in which increasing numbers of EL4-6.1 cells were incubated in the presence of a constant concentration of IL-1 revealed that these cells bound about 10,000-20,000 IL-1 molecules per cell (data not shown).

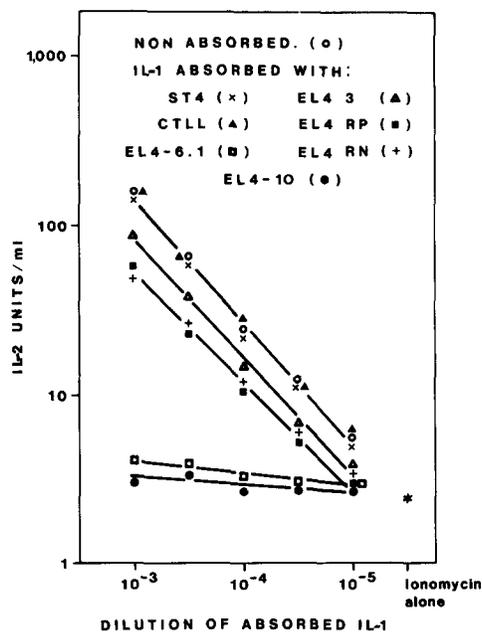


FIGURE 2. Absorption of rIL-1 by various cell lines. 10^7 cells/ml were incubated in the presence of rIL-1- α (5 ng/ml) for 4 h at 4°C. The IL-1 content of the cell-free supernatant was then measured at the indicated dilutions by its ability to stimulate IL-2 production by EL4-6.1 cells in the presence of 0.1 μ g/ml Ionomycin. Similar results were obtained using rIL-1- β or a mixture of α and β .

Radioiodinated rIL-1 Retains Biological Activity. IL-1- α retained full biological activity after radioiodination, as measured by its ability to induce IL-2 secretion by EL4-6.1 cells in the presence of Ionomycin (Fig. 3A). We saw half-maximum biological activity at an IL-1 concentration of 6 pM. Furthermore, Fig. 3B shows that both α and β forms of unlabeled IL-1 competed with 125 I-IL-1- α for binding, although IL-1- β had about threefold lower affinity. Competition for 125 I-IL-1- α binding by IL-1 was specific since <5% inhibition of binding was observed when unlabeled rIL-2 was added at concentrations of up to 100 ng/ml (i.e., a 1,000-fold molar excess; Fig. 3B).

Association Kinetics of 125 I-IL-1 with EL4-6.1 Cells. Fig. 4A shows the time course of association of 125 I-IL-1 with EL4-6.1 cells at 4°C over a 100-fold range in IL-1 concentrations. Binding reached equilibrium within 2–4 h over the range of concentrations tested. Logarithmic conversion of these data revealed a first order association rate constant, which was similar at all three concentrations, with a $t_{1/2}$ value of 30–40 min. Fig. 4B shows that binding of 125 I-IL-1 to EL4-6.1 cells occurred more rapidly at 37°C than at 4°C. The difference in association rate constant was about fourfold. Dissociation of IL-1 occurred slowly at 4°C ($t_{1/2} > 4$ h), which is in agreement with the results of Dower et al. (12).

Equilibrium Binding Analysis Reveals Two Classes of IL-1-R. EL4-6.1 cells were incubated at 4°C in the presence of various concentrations of 125 I-IL-1. After equilibrium binding was established (4 h), the cell-bound radioactivity was separated from the free radioactivity by centrifugation through an oil gradient, and

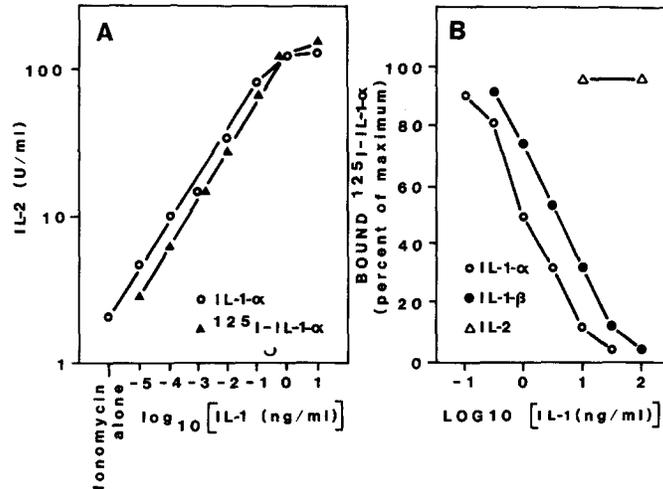


FIGURE 3. ¹²⁵I-IL-1 retains biological activity. (A) Comparison of the capacity of labeled and unlabeled rIL-1-α to induce IL-2 secretion by EL4-6.1 cells in the presence of Ionomycin. (B) Ability of ¹²⁵I-IL-1-α to compete for binding with unlabeled IL-1-α or IL-1-β. EL4-6.1 cells (10⁶ cells/well) were incubated in the presence of ¹²⁵I-IL-1-α (0.1 ng/ml) and various concentrations of unlabeled IL-1-α or IL-1-β for 4 h at 4°C. Bound radioactivity was measured as described in Materials and Methods. Maximum radioactivity was 3,660 cpm. Unlabeled rIL-2 was also added as a negative control.

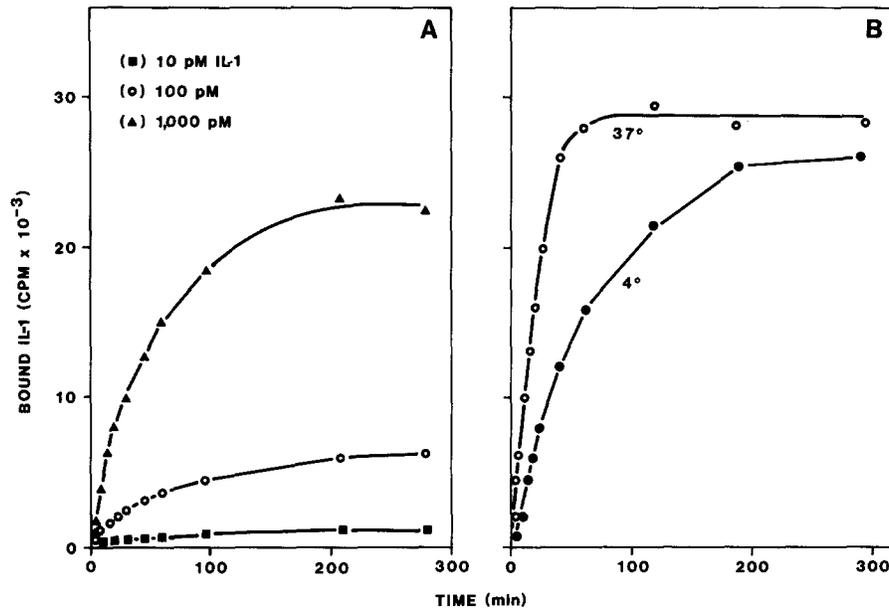


FIGURE 4. Association kinetics of ¹²⁵I-IL-1 with EL4-6.1. (A) Cells were incubated at 4°C in the presence of the indicated concentrations of ¹²⁵I-IL-1 for various periods of time. (B) Cells were incubated at 4°C or 37°C in the presence of ¹²⁵I-IL-1 (1 nM) and sodium azide (0.02%) for various periods of time. Nonspecific binding has been subtracted in A and B.

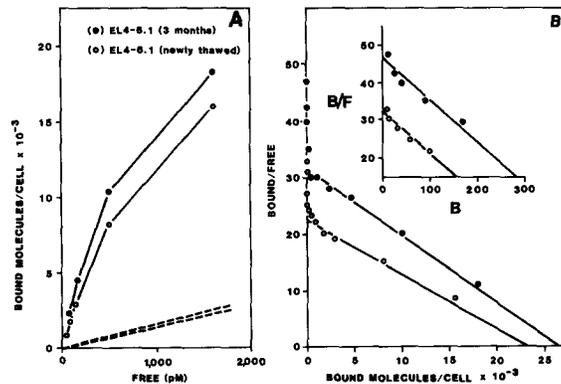


FIGURE 5. Equilibrium binding analysis of ^{125}I -IL-1 to EL4-6.1. Cells were incubated at 4°C in the presence of various concentrations of ^{125}I -IL-1 (0.2–2,000 pM) for 4 h. Freshly thawed EL4-6.1 cells (O) were compared to EL4-6.1 cells that had been cultured for 3 months (●) in their ability to bind ^{125}I -IL-1. (A) Specific equilibrium binding of ^{125}I -IL-1 (solid lines) after subtraction of nonspecific binding (dashed lines). (B) Scatchard plot analysis of the binding data shown in A. Inset shows the binding of ^{125}I -IL-1 to the high-affinity class of IL-1-R in more detail. Similar results were obtained in six independent experiments.

TABLE I
Expression of High- and Low-Affinity IL-1-R by Different Types of T Cells

Cells	Number of experiments	IL-1-R per cell (mean \pm SEM)		Dissociation constant (mean \pm SEM; pM)	
		High affinity	Low affinity	High affinity	Low affinity
EL4-6.1	5	340 \pm 120	17,700 \pm 4,890	4.6 \pm 2.5	380 \pm 140
EL4-16	2	280 \pm 80	11,100 \pm 3,330	3.9 \pm 1.8	440 \pm 170
EL4-RP	2	195 \pm 105	5,700 \pm 1,060	4.7 \pm 1.2	335 \pm 110
EL4-3	5	<2	<2		
LBRM	4	46 \pm 18	2,550 \pm 660	5.3 \pm 2.7	360 \pm 160
Yac	2	35 \pm 16	1,650 \pm 300	3.3 \pm 1.2	290 \pm 60
CTLL	5	<2	<2		
BW5147	3	<2	<2		
ST4	3	<2	<2		
LNT*	2	12 \pm 4	180 \pm 40	5.0 \pm 1.5	405 \pm 145
LNT day 2 [†]	2	23 \pm 6	580 \pm 180	5.5 \pm 1.8	340 \pm 90

* Freshly isolated LNT.

[†] LNT cultured 2 d in the presence of PMA (1 ng/ml) and Ionomycin (0.25 $\mu\text{g}/\text{ml}$).

then quantitated. Fig. 5A shows the specific equilibrium binding after subtraction of the nonspecific binding. Scatchard plot analysis of these data (Fig. 5B) revealed that EL4-6.1 cells express two classes of IL-1-R. ~98–99% of the total IL-1-R (~18,000/cell) bound IL-1 with a K_d of 380 \pm 140 pM (Table I). A second class of IL-1-R that is expressed at a low level (340 \pm 120/cell) bound IL-1 with a much higher affinity (K_d = 4.6 \pm 2.5 pM; Table I and Fig. 5B, inset). IL-1-R expression by EL4-6.1 cells was stable over a 3-mo culture period (Fig. 5).

The level of high-affinity IL-1-R expressed by EL4-6.1 cells was compared with that of other T cell lines, as well as with normal peripheral T cells (Fig. 6 and Table I). Other, independently derived EL4 cell lines varied in their expression of IL-1-R, which correlated well with their ability to respond to IL-1

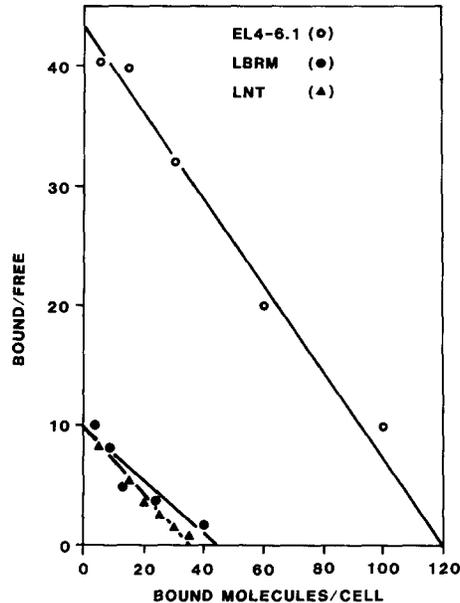


FIGURE 6. Quantitation of high-affinity IL-1-R expressed by different cell types. EL4-6.1 (○), LBRM (●) and LNT cultured for 2 d in the presence of PMA and Ionophore, (▲) were incubated in the presence of various concentrations of ^{125}I -IL-1 (0.2–10 pM) for 4 h at 4°C. After subtraction of nonspecific binding, the number of binding sites per cell and the dissociation constants were quantitated by Scatchard plot analysis.

and absorb IL-1 activity. Cells of the EL4-3 line did not express IL-1-R (level of detectability is two receptors per cell), whereas EL4-RP.1 (subclone of EL4-RP) and EL4-16 (parental line of EL4-6.1) expressed intermediate levels of IL-1-R. Two other thymomas, LBRM and Yac, expressed lower numbers of IL-1-R (30–50 high-affinity IL-1-R per cell). Cells of the IL-2-dependent cytolytic T cell line CTLL, and two other thymomas, ST4 and BW5147, were negative for IL-1-R expression as was already suggested by their inability to absorb IL-1 activity (Fig. 2). In addition, activated normal T cells expressed a total of ~600 IL-1-R per cell, of which about 20 were of the high-affinity type. Interestingly, normal resting T cells also expressed detectable numbers of IL-1-R (~10 high-affinity and 180 low-affinity receptors per cell).

Internalization of ^{125}I -IL-1. We used the ability to internalize IL-1 as an independent criterion for the expression of functional IL-1-R. EL4-6.1 cells were incubated at 4°C in the presence of ^{125}I -IL-1 for 4 h to achieve equilibrium binding of IL-1 to both high- and low-affinity IL-1-R. Treatment with pH 3-buffered medium resulted in the rapid dissociation (95–98% within 1 min) of surface-bound IL-1. The remaining radioactivity was taken as background. When the cells were transferred to 37°C there was a time- and temperature-dependent increase in the proportion of cell-bound radioactivity that was resistant to acid treatment. This represents IL-1 that had been internalized. Internalization is a specific process, which requires the expression of IL-1-R. Fig. 7 shows that EL4-6.1 cells (IL-2-R⁻) had the capacity to internalize ^{125}I -IL-1, but failed to inter-

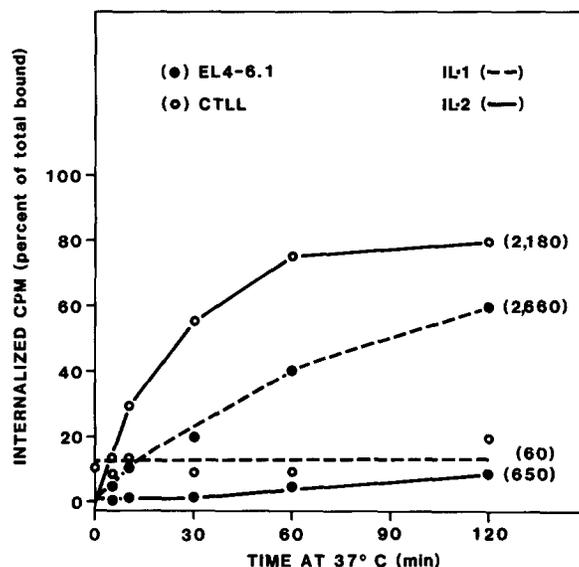


FIGURE 7. Capacity to internalize IL-1 correlates with the expression of specific IL-1-R. EL4-6.1 (●) and CTLL (○) cells were incubated at 4°C in the presence of 30 pM of ^{125}I -IL-1 (dashed lines) or ^{125}I -IL-2 (solid lines). After 4 h the cells were transferred to 37°C and internalized radioactivity was measured at different times, as described in Materials and Methods. Internalized IL-1 and IL-2 is expressed as a percentage of the total cell-associated ligand. The numbers in brackets refer to the total cell-bound cpm at time 0.

nalize ^{125}I -IL-2. In contrast, CTLL cells (IL-2-R⁺/IL-1-R⁻) rapidly internalized ^{125}I -IL-2, but could not internalize ^{125}I -IL-1.

The data shown in Fig. 8 support the hypothesis that IL-1 internalization occurs via high-affinity but not low-affinity IL-1-R. EL4-6.1 cells were incubated at 4°C in the presence of 100 pM ^{125}I -IL-1 for 4 h. According to the Scatchard data shown in Fig. 5, this allowed occupancy of virtually all high-affinity receptors (~250 per cell) and ~2,000 low-affinity receptors. The cells were then washed to remove all of the unbound ^{125}I -IL-1. There was no change in the level of cell-bound ^{125}I -IL-1 when the cells were subsequently transferred to 37°C and incubated for an additional 4 h (the level of cell-bound ^{125}I -IL-1 stayed constant because there was no rebinding of IL-1, nor was there any appreciable dissociation from either class of IL-1-R). Under these conditions, there was a rapid internalization of ^{125}I -IL-1, which reached a maximum level within 20 min (Fig. 8). This represented the internalization of ~200 IL-1 molecules per cell, close to the value of initially occupied high-affinity IL-1-R. There was no additional internalization of IL-1 after this time, despite the fact that ~2,000 IL-1-R per cell were still occupied. If, on the other hand, the cells were not washed before their transfer to 37°C, the level of internalized IL-1 continued to increase with time (as does the total cell-associated IL-1). This may reflect the rebinding of free ^{125}I -IL-1 to recycled or newly expressed high-affinity IL-1-R. After 60 min at 37°C, the level of internal IL-1 in the unwashed cells was three times that of the washed cells. Assuming that only the 200–300 high-affinity IL-1-R expressed by these cells can be internalized, and that these receptors return to the cell

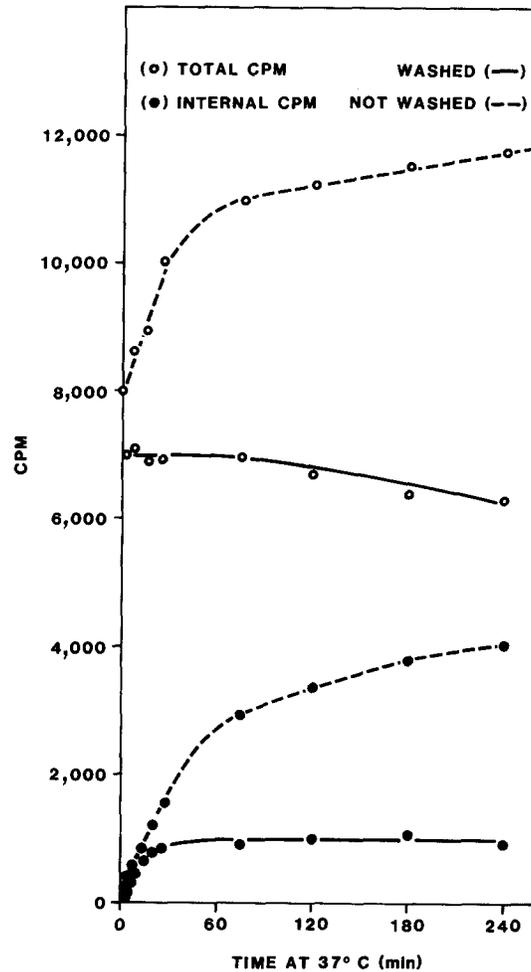


FIGURE 8. Evidence that internalization of ^{125}I -IL-1 occurs via high-affinity receptors. EL4-6.1 cells were incubated at 4°C in the presence of 100 pM ^{125}I -IL-1. After 4 h, half of the cells were washed three times to remove unbound ^{125}I -IL-1 (solid lines), whereas the remainder were not washed (dashed lines). The cells were then incubated at 37°C . Total cell-associated (○) and internalized ^{125}I -IL-1 (●) was measured at different times using duplicate aliquots of 10^6 cells, as described in Materials and Methods.

surface, the data would be consistent with a receptor recycling time of 10–20 min.

Finally, Fig. 9 shows that the capacity of cells to internalize IL-1 correlates with the number of high affinity IL-1-R expressed per cell. IL-1-R⁻ cells such as EL4-3, CTLL, and BW5147 could not rapidly internalize ^{125}I -IL-1. LBRM cells, which express ~50 high-affinity IL-1-R per cell internalized fewer molecules than did EL4-6.1 cells, which express ~200–400 such receptors per cell.

Discussion

We have previously described the EL4-6.1 thymoma line as a high IL-1-responder and have shown that these cells are induced in the presence of rIL-1

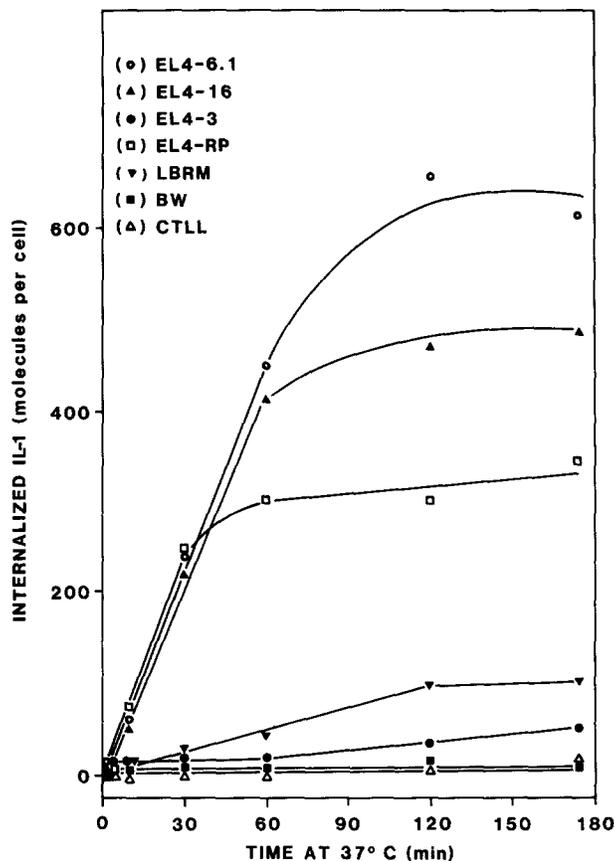


FIGURE 9. Internalization of IL-1 by various T cell lines. Cells were incubated at 37°C in the presence of 20 pM ^{125}I -IL-1 and internalized ^{125}I -IL-1 was measured at various times. See Table I for the number of high-affinity IL-1-R per cell.

(α or β form) to both secrete IL-2 and express IL-2-R, provided that a second signal is given in the form of Ionomycin or suboptimal concentration of PMA (13). The recent availability of human rIL-1 (23) and a procedure for its radiolabeling to high specific activity has allowed us to directly quantitate the number and affinity of IL-1-R expressed by various T cells. We show here that the IL-1-responsiveness of EL4-6.1 cells correlates with the expression of an unusually high number of IL-1 binding sites ($\sim 20,000$ per cell), which could be resolved into high- and low-affinity classes. Despite the large variation in the overall level of IL-1-R expression between different T cell types, the ratio of the number of high- to low-affinity IL-1-R was consistent. The results presented here further suggest that the biological activity of IL-1 is mediated exclusively via interaction with the minor class of high-affinity IL-1-R.

Radioiodination of rIL-1- α had no detectable effect on biological activity, as measured by its ability to induce IL-2 secretion and IL-2-R expression by EL4-6.1 cells. Dower et al. (12) radiolabeled biochemically purified human IL-1- β using the Bolton-Hunter reagent. The IL-1- β , incubated for 1 h with the reagent, was labeled to a high specific activity ($2\text{--}5 \times 10^{15}$ cpm/mmol) but retained only

5% of its biological activity. We radioiodinated human rIL-1- α by incubation in the presence of Na¹²⁵I and chloramine T for 10 min, thereby achieving a high level of specific activity ($1-2 \times 10^{16}$ cpm/mmol) while retaining full biological activity. ¹²⁵I-IL-1- α competed in an equimolar fashion with unlabeled IL-1- α for binding to EL4-6.1 cells. Taken together with the functional studies, these data argue that ¹²⁵I-IL-1 can be used as a valid estimate of the behavior of the natural form of the molecule. Furthermore, in agreement with the recent findings of Dower et al. (24), unlabeled rIL-1- α and rIL-1- β both competed (albeit with slightly different affinity) for the binding of labeled rIL-1- α suggesting that the two forms of rIL-1 bind to the same receptor.

Scatchard plot analysis of ¹²⁵I-IL-1 equilibrium binding revealed the presence of two classes of IL-1-R on a number of different T cell populations. The major class, making up 95–99% of the total IL-1-R, has a K_d of 300–500 pM. The minor class of receptors has an ~100-fold higher affinity for IL-1 (K_d of 2–8 pM). The level of expression of IL-1-R among different T cell lines was variable. Certain cell lines such as ST4, BW1547, CTLL, and EL4-3 were negative, whereas a variety of independently derived EL4 lines were highly positive. Other thymoma cell lines such as Yac and LBRM expressed low, but detectable numbers of IL-1-R, which is consistent with an earlier report (12) in which the number of IL-1-R expressed by a variety of T cell types ranged from 27 to 550 per cell. In the latter study, the binding affinity and receptor number they reported for the LBRM cell line were similar to what we find here for the low-affinity class of IL-1-R. The reason why these authors failed to detect the high-affinity class of IL-1-R on LBRM cells is probably technical, i.e., they may not have been able to detect binding at sufficiently low IL-1 concentration (<10 pM) because of the lower specific activity and/or biological activity of their ¹²⁵I-IL-1- β preparations as compared with the ¹²⁵I-IL-1- α preparations used in the present study.

All IL-1-R⁺ T cell populations tested expressed both high- and low-affinity classes of IL-1-R, and the affinity of IL-1 binding to either class was comparable. The existence of high- and low-affinity receptors has been reported in a number of peptide hormone systems, including nerve growth factor (29), epidermal growth factor (30), insulin (31), platelet-derived growth factor (32), and IL-2 (27, 33). In all cases, the proportion of high-affinity receptors is low (5–15% of the total) and the difference in affinity between the two classes for ligand binding is ~50–100-fold. The biological significance of this receptor dichotomy is not known.

Interestingly, normal resting T cells expressed very low numbers of high-affinity IL-1-R (~10 per cell). This value is only an estimate of the average number of receptors per cell based on the population as a whole, and it is conceivable that only a subpopulation of peripheral T cells expresses IL-1-R. In this context, recent results from experiments using highly purified populations of lymph node T cells indicate that the L3T4⁺ subset is positive for IL-1-R expression, whereas the Lyt-2⁺ subset is not.² Expression of IL-1-R by resting T cells is probably constitutive, since activation by PMA and Ionomycin (which induces growth of both L3T4⁺ and Lyt-2⁺ subsets [34]) did not significantly

² Lowenthal, J. W., and H. R. MacDonald. Expression of interleukin-1 receptors is restricted to the L3T4⁺ subset of mature T lymphocytes. Manuscript submitted for publication.

enhance IL-1-R expression after 2 d of culture, suggesting that there was no positive or negative selection for IL-1-R-expressing cells.

In a number of other hormone systems, the ligand-receptor complex has been shown to be internalized upon ligand binding (35, 36), and this internalization is thought to be a necessary signal for the generation of biological responsiveness (37, reviewed in 38). For IL-2, internalization has been shown to occur via the high-affinity class of IL-2-R, whereas low-affinity IL-2-R are nonfunctional in this respect (39; 40; M. Nabholz, personal communication). We now provide evidence that a similar functional dissociation exists between high- and low-affinity IL-1-R. Internalization of IL-1 is a specific process, requiring the presence of cell surface IL-1-R. The proportion of total cell-bound IL-1 that can be endocytosed by EL4-6.1 cells corresponds to the proportion of IL-1 that is bound to the high-affinity class of IL-1-R. Furthermore, when EL4-6.1 cells were incubated at 4°C in the presence of either 10 pM ^{125}I -IL-1, a concentration that permits occupancy of high- but not low-affinity IL-1-R, or 1 nM, a concentration that allows occupancy of all IL-1-R, and then washed and transferred to 37°C, the rate and extent of IL-1 internalization was the same (data not shown). Finally, the capacity of different cell lines to internalize IL-1 correlated closely with the level of high-affinity IL-1-R expression. As in other hormone systems, the functional significance of ligand internalization and degradation is not known. One possibility is that this process allows dissociation of the ligand from its receptor and permits the receptor to recycle back to the cell surface and to bind more ligand. So far, no function has been attributed to low-affinity hormone receptors, but it is conceivable that they represent an external pool of nonfunctional receptors that can be rapidly recruited into the functional high-affinity pool. The transition between the two classes of receptors may involve a conformational change, for example by association with a second accessory molecule. Irrespective of its functional significance, it would appear that the ability to internalize IL-1 can be used as a sensitive, independent measure for the expression of high-affinity IL-1-R.

In contrast to EL4-6.1 cells, other T cell populations (including normal activated T cells) express low levels of IL-1-R. Even though they express only 10–60 high-affinity IL-1-R per cell they can still internalize and respond to IL-1. Similar findings of low numbers (15–300 per cell) of high affinity hormone receptors have been reported for granulocyte and macrophage growth factors (41 and F. Walker and A. W. Burgess, personal communication). It is significant that the concentration of IL-1 corresponding to half-maximal occupancy of the high-affinity class of IL-1-R (i.e., the K_d value) is identical to the concentration of IL-1 that gives half-maximal biological activity (2–8 pM). This is similar to the situation found for IL-2, where the K_d value of the high-affinity class of IL-2-R corresponds to the IL-2 concentration giving half-maximal biological activity (10–30 pM) (26, 27, 33, 37). Taken together, the data presented here suggest that the biological activity of IL-1 can be attributed exclusively to its interaction with high-affinity IL-1-R.

Because of the extremely high number of IL-1-R expressed by EL4-6.1, they constitute a valuable model system with which the structure, function, and regulation of IL-1-R can be studied. Furthermore, this cell line should facilitate

the isolation of the IL-1-R in purified form and cloning of the gene(s) that encode it.

Summary

In this report we describe, on the basis of direct IL-1 binding assays and IL-1 internalization studies, the existence of two classes of IL-1-R on a variety of T cell types. Cells of the EL4-6.1 thymoma express large numbers (~20,000 per cell) of IL-1-R that have a K_d of ~300 pM for IL-1. Even though these receptors make up 98–99% of the total IL-1-R per cell, they appear to be nonfunctional, based on their inability to endocytose IL-1. A minor class of IL-1-R (200–400/cell) has an ~100-fold higher affinity for IL-1 (K_d , ~5 pM) and can rapidly internalize the ligand upon binding. All of the biological activity of IL-1 can be shown to occur via binding to high-affinity IL-1-R since the IL-1 concentration giving half-maximum biological activity in EL4-6.1 cells corresponds precisely to the K_d of this class of receptor. Other cell types, including normal T cells, also express both high- and low-affinity IL-1-R, but the absolute number of receptors per cell is considerably less.

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