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Internalization of interleukin 1 (IL 1) correlates with IL 1-induced IL 2 receptor expression and IL 2 secretion of EL4 thymoma cells*

The cytokine interleukin 1 (IL 1) plays an important role in the induction of IL 2 secretion and high-affinity IL 2 receptor (IL 2R) expression by T cells. The events that follow binding of IL 1 to IL 1R, however, are still unknown. In this study we describe two variants of the murine thymoma EL4 (5D3 and D6/76) that express comparable numbers of cell surface IL 1 receptors and bind IL 1 with the same affinity, but show distinct IL 1-dependent IL 2 secretion and IL 2R expression. In the presence of the tumor promoter phorbol 12-myristate 13-acetate IL 1 augments IL 2 secretion and IL 2R expression of EL4 5D3 but not of EL4 D6/76 cells. Comparison of the internalization of IL 1 by both clones revealed that EL4 D6/76 was unable to transport cell surface-bound IL 1 to the cytoplasm. These findings suggest that internalization of receptor-bound IL 1 is required for the action of this cytokine.

1 Introduction

Secretion of interleukin 2 (IL 2) and expression of high-affinity receptors for IL 2 (IL 2R) are required for proliferation of T lymphocytes upon antigen or mitogen stimulation. The cytokine IL 1 plays a key role in the induction of these processes. It has been demonstrated that IL 1 induces IL 2 secretion by normal and neoplastic T helper cells upon mitogen stimulation [1, 2]. In addition, IL 1 induces IL 2R expression on a cloned antigen-specific helper T cell line in the presence of a monoclonal antibody (mAb) specific for the antigen receptor [3] and on murine thymocytes in the presence of IL 2 [4]. Williams et al. could demonstrate that human peripheral blood T cells secrete IL 2 and express IL 2R induced by IL 1 and mAb to the antigen receptor complex [5]. Furthermore, Lowenthal et al. [6] showed that IL 1 can augment both IL 2 secretion and IL 2R expression of the murine thymoma EL4 6.1 in the presence of the tumor promoter phorbol 12-myristate 13-acetate (PMA).

Many cells respond to growth factors by internalization of the receptor ligand complex [7]. This observation has also been made for IL 1. IL 1 is rapidly internalized, presumably as a receptor ligand complex, and transported to the nucleus in EL4 cells and Swiss 3T3 fibroblasts [8]. Whether internalization is required for a response to IL 1 leading to secretion of of IL 2 and IL 2R expression in not known. In this study we investigated this question and we describe a variant of the murine thymoma EL4 that has lost the capacity to internalize cell surface receptor bound IL 1. This variant did not react to IL 1 with enhanced IL 2 secretion and IL 2R expression in the presence of PMA. Therefore, the data presented here suggest that internalization of IL 1 is a prerequisite for the IL 1-dependent increase of PMA-induced IL 2 secretion and IL 2R expression of EL4 thymoma cells. Thus, the EL4 variants described in this study could serve as a model for investigation of the initial biochemical processes that follow IL 1 stimulation of T cells.

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Abbreviations: **BSA:** Bovine serum albumin **DSS:** Disuccinimidyl suberate **FCS:** Fetal calf serum **hu:** Human **IL:** Interleukin **IL 2R:** IL 2 receptor **rIL:** Recombinant IL **mAb:** Monoclonal antibody **PBS:** Phosphate-buffered saline **PMA:** Phorbol 12-myristate 13-acetate **SDS-PAGE:** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis **SN:** Supernatant

2 Materials and methods

2.1 Cell cultures and reagents

Cells were cultured in RPMI 1640 containing 5% fetal calf serum (FCS) and 50 μg/ml gentamycin at 37 °C in a humidified atmosphere of 5% CO_2 in air. PMA was purchased from Sigma Chemical Co. (St. Louis, MO). Disuccinimidyl suberate (DSS) was obtained from Pierce Chemical Co. (Rockville, IL).

2.2 IL 1

Human recombinant (hu r) IL 1α was kindly provided by Drs. A. Stern and P. Lomedico (Hoffmann-LaRoche, Nutley, NJ). The specific activity was 5×10^6 U/mg protein determined by the lymphocyte-activating factor assay.

2.3 Cloning of EL4 cells

Cells were cloned under limiting dilution conditions. EL4 5D3 was selected for high PMA-induced IL 2 secretion. EL4 D6/76 was selected for low incorporation of ^{125}I-labeled hu rIL 1α at 37 °C.

2.4 Assay for IL 2

IL 2 activity in supernatants (SN) was quantitated by the ability of the SN to support growth of the IL 2-dependent murine

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T cell clone W2 as described elsewhere [9]. Units of IL 2 are based on a standard purchased from Biogen (Geneva, Switzerland; Batch No. RNB85738/09Y). IL 2 secretion was induced with 5×10^5 cells/ml in culture medium (2-ml cultures) with the addition of hu rIL 1α (10 U/ml) and PMA (see Table 1) for 32 h. SN were removed for IL 2 determination.

2.5 Assay for IL 2R expression

Affinity-purified mAb 7D4 [10] was radioiodinated using the chloramine-T method [11]. Cells induced either with IL 1 and PMA or PMA alone for 30 h were washed and resuspended in 90 µl binding medium (RPMI 1640, 0.006 M Hepes, 1% bovine serum albumin, 0.1% sodium azide). mAb 7D4 was added in 10 µl binding medium to a final concentration of 4 µg/ml. After incubation of ice for 1 h unbound radioactivity was removed by extensive washing with phosphate-buffered saline (PBS). The cells were lysed in 200 µl PBS containing 0.1% sodium dodecyl sulfate (SDS). Aliquots of 180 µl were removed and radioactivity was determined in a gamma counter.

2.6 RNA extraction and hybridization procedures

Cytoplasmic RNA was prepared by the method of Cheley and Anderson [12]. Cells ($1 \times 10^6 - 2 \times 10^6$) were lysed with 1 ml 7.6 M guanidinium hydrochloride (Fluka, Neu-Ulm, FRG) in 0.1 M potassium acetate, pH 5. DNA was sheared by aspiration (6 ×) through a 21 gauge needle. RNA was precipitated over night at − 20 °C after addition of 0.6 ml of ethanol. After centrifugation the pellet was dissolved in 200 µl of 15% formaldehyde in water. To this solution 200 µl of 20 × SSC was added and RNA was denatured by heating to 50 °C for 15 min. Aliquots were applicated onto nylon membranes in serial 3-fold dilutions (Compas, Genofit, Heidelberg, FRG). RNA was fixed on the membranes by UV irradiation for 2 min. The first well of the RNA dot blots represents cytoplasmic RNA equivalent to 5×10^5 cells. RNA for Northern analysis was prepared by the guanidine isothiocyanate/cesium chloride method. RNA was denatured with formaldehyde and electrophoresed in a 1% agarose gel. The blotting was done on nylon membranes (Compas). RNA was fixed by UV irradiation. Hybridizations were done according to the manufacturer's protocol in the presence of dextran sulfate and formamide at 42 °C. The filters were washed twice under high stringency conditions (65 °C, 30 min, 2 × SSC containing 1% SDS). The probe for the mouse IL 2 was generously provided by Dr. A. Schimpl (Würzburg, FRG) and contains the complete exon 4 of the IL 2 gene [13]. The probe for the mouse IL 2R was generously provided by Dr. T. Malek (Miami, FL). It represents the Eco RI-Pst I fragment of the IL 2R cDNA (clone pmIL 2pr1) [14]. Probes were labeled overnight with ^{32}P-GTP and ^{32}P-CTP (spec. act. 3000 Ci/mmol = 111 TBq/mmol; Amersham, Frankfurt, FRG) by the random primer method using a hexamer (Pharmacia Fine Chemicals, Uppsala, Sweden) as primer.

2.7 Iodination of hu rIL 1α

Purified hu rIL 1α was labeled with ^{125}I by utilizing the Enzymobead reagent according to the manufacturer's instructions (Bio-Rad, Richmond, CA). The ^{125}I-labeled rIL 1α was separated from unbound Na^{125}I by chromatography on a Sephadex G-25 column (PD10; Pharmacia) which was equilibrated with RPMI 1640 containing 5% FCS prior to use. Biological activity of the iodinated material was 80% to 90% of the original material (data not shown). IL 1 activity of this material was determined using the standard phytohemagglutinin (PHA)-costimulator assay with MTT as described [15].

2.8 Binding of ^{125}I-rIL 1α

Cells (2×10^6) were incubated with various concentrations of ^{125}I-rIL 1α in 200 µl binding medium (RPMI 1640, 0.006 M Hepes, 1% BSA, 0.1 Sodium azide) at 8 °C for 4 h. Two 95 µl aliquots were removed and centrifuged through a mixture of dibutylphthalate: phthalic acid bis(2-ethylhexylester) (1.5 : 1). The assay was done in duplicate. Radioactivity of the cell pellet and the SN was determined in a gamma counter. Non-specific binding was measured in the presence of a 100-fold excess of unlabeled rIL 1α. Scatchard plots [16] of equilibrium data were analyzed by using a computer program (Dr. L. Erkell, German Cancer Research Center, Heidelberg, personal communication).

2.9 Affinity cross-linking and SDS-polyacrylamide gel electrophoresis (PAGE)

Cells were preincubated at 37 °C for 30 min in the presence of 0.1% sodium-azide to inhibit internalization of receptor ligand complexes. Then 1×10^7 cells were incubated for 2 h at room temperature with ^{125}I-rIL 1α in 500 µl binding medium in the presence or absence of unlabeled rIL 1α. Subsequently, the cells were washed twice by centrifugation with PBS, pH 8.4, and resuspended in 100 µl PBS, pH 8.4. 4 µl DSS (50 mg/ml in dimethyl sulfoxide) was added to give a final concentration of 2 mg/ml, and the mixture was incubated at room temperature for 1 h. The cells were then washed twice with PBS and lysed in 40 µl buffer (PBS 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride) on ice for 5 min. Samples were centrifuged at 10000 × g at 8 °C for 15 min to remove cell debris. The SN was mixed with 40 µl 2 × dissociation buffer (0.14 M Tris-HCl, pH 6.8; 4% SDS; 20% glycerol; 0.008% bromophenol blue). Samples were boiled for 3 min and subjected to 10% SDS-PAGE using the procedure of Laemmli [17].

2.10 Internalization of ^{125}I-rIL 1α

To determine the kinetics of internalization of ^{125}I-rIL 1α 4×10^6 cells and 8 U of ^{125}I-rIL 1α were preincubated in 500 µl RPMI 1640 containing 5% FCS at 8 °C for 1 h and then transferred to 37 °C. Samples were removed at the indicated times. Cells were pelleted by centrifugation and resuspended in 500 µl, pH 3, buffered medium. After incubation on ice for 5 min to remove surface-bound radioactivity [18] cells were centrifuged again and lysed in 200 µl lysis buffer (10 mM Tris HCl, pH 7.4; 150 mM NaCl; 1% Triton X-114; 2 mM phenylmethylsulfonyl fluoride; 2% isopropanol). After 10 min incubation on ice the nuclear and cytoplasmic fractions were separated by centrifugation at 10000 × g at 8 °C for 10 min. Radioactivity in both fractions was determined in a gamma counter. Purity of the nuclear preparation was controlled microscopically after staining with M + D Diff Quick according to the manufacturer's instructions (Merz-Dade, AG, Düdingen, Switzerland).

3 Results

3.1 IL 1-induced IL 2 secretion and IL 2R expression

The tumor promoter PMA induces the murine thymoma EL4 to secrete IL 2 and to express IL 2R. Both activities can be augmented by IL 1 in a dose-dependent manner [6]. To investigate the mechanisms that lead to IL 1-induced IL 2 secretion and IL 2R expression, two EL4 subclones (5D3 and D6/76) were selected by cloning under limiting dilution conditions. These subclones differ in their capacity to respond to IL 1 in the presence of PMA. Table 1 shows that IL 1 augments the PMA-induced IL 2 secretion of EL4 5D3, and that the effect of IL 1 is more pronounced under suboptimal PMA concentrations (2 ng/ml PMA). This is consistent with the data published by Lowenthal et al. [6]. In contrast, IL 2 secretion of EL4 D6/76 was not enhanced by addition of IL 1 to the cultures. IL 1 alone did not have any effect on either cell line (data not shown).

We also tested whether EL4 5D3 and EL4 D6/76 showed a different response to IL 1 with respect to IL 2R expression. Cells were activated with IL 1 and PMA or PMA alone, and after 30 h of activation binding of ^{125}I-labeled mAb 7D4 was measured. mAb 7D4 specifically recognizes the p55 protein of the IL 2R complex [10]. The results of these experiments are summarized in Table 2. The amount of radioactivity bound to PMA-stimulated EL4 5D3 cells was significantly enhanced by IL 1 in a dose-dependent manner. On the contrary, in the presence of PMA IL 1 did not augment the binding of radioactive mAb 7D4 to EL4 D6/76 cells. The data presented in Tables 1 and 2 clearly demonstrate that both EL4 clones were capable of IL 2 secretion and IL 2R expression in the presence of PMA. Only clone EL4 5D3, however, and not clone EL4 D6/76, was further stimulated by IL 1.

Table 1. Induction of IL 2 secretion by IL 1 and PMA

Additions to culture[b]	IL 2 secretion (U/ml)[a] by EL4 subclones	
	EL4 5D3	EL4 D6/76
PMA (2 ng/ml)	5	6
PMA (20 ng/ml)	75	30
PMA (2 ng/ml) IL 1α (10 U/ml)	30	6
PMA (20 ng/ml) IL 1α (10 U/ml)	100	30

a) Units of IL 2 in the SN of the clones were based on the standard purchased from Biogen, Geneva, Switzerland (Batch No. RNB85738/09Y).
b) Cell concentration 5×10^5/ml. Cells were incubated with IL 1 and PMA for 32 h.

Table 2. Induction of IL 2R expression by IL 1 and PMA

Additions to culture		Binding of ^{125}I-labeled mAb 7D4 to cells (cpm)[a]	
IL 1	PMA[b]	EL4 5D3[c]	EL4 D6/76[c]
–	–	232	282
–	+	1296	1922
0.625 U/ml	+	4885	2121
10	+	7565	1819

a) ^{125}I-labeled mAb 7D4 was added at a final concentration of 4 μg/ml to 1×10^5 cells.
b) PMA was added to a final concentration of 10 ng/ml.
c) IL 2R expression was measured after a 30-h activation of cells.

3.2 Quantity of induced IL 2 and IL 2R mRNA in EL4 clones

To investigate whether the IL 1-induced increase in IL 2 and IL 2R expression on EL4 5D3 was due to increased amounts of mRNA rather than to increased translation, we compared the induction of specific mRNA in EL4 5D3 and EL4 D6/76. Cells were induced with IL 1 and PMA or PMA alone for 24 and 48 h, and cytoplasmic RNA extracts were probed for IL 2 and IL 2R mRNA, respectively. The results of these experiments are shown in Fig. 1. After 24 h of induction EL4 5D3 showed a significant amount of IL 2-specific mRNA induced by PMA (Fig. 1A, lane 1). The amount of mRNA was further augmented by addition of IL 1 (Fig. 1A, lane 2). In contrast, the amount of IL 2 mRNA synthesized by EL4 D6/76 could not be further augmented by IL 1 (Fig. 1A, lanes 3 and 4, respectively). After 48 h of induction EL4 5D3 also expressed a significant amount of IL 2R mRNA (Fig. 1B, lane 1). Again, in the presence of IL 1 a substantial increase of IL 2R mRNA was observed for EL4 5D3 (Fig. 1B, lane 2) but not for EL4 D6/76 (Fig. 1B, lanes 3 and 4, respectively). The IL 1-induced increase of IL 2 mRNA of clone EL4 5D3 as shown by dot blot analysis in Fig. 1A was also evident from the Northern blot in Fig. 1C. From these data it can be concluded, that the effect of IL 1 was most likely due to increased transcription of IL 2 and IL 2R genes.

3.3 Number and affinity of IL 1R on EL4 5D3 and EL4 D6/76 cells

It has been shown previously that IL 1 binds to specific cell surface receptors [18, 19]. The lack of response of EL4 D6/76 to IL 1 could therefore be explained by the absence of cell surface IL 1R. To test this hypothesis, we determined receptor numbers and dissociation constants for IL 1 for both clones by Scatchard plot analysis [16]. We found that EL4 D6/76 and EL4 5D3 expressed 2300 to 2400 cells surface IL 1R per cell (r^2 = 0.98 and 0.99, respectively) with an apparent dissociation constant in the range of $1.2 \times 10^{-10} - 2.5 \times 10^{-10}$ M. This dissociation constant is in good agreement with the data published for EL4 by Kilian et al. [19]. Thus, our data indicate that the two clones do not differ in IL 1R density of affinity.

3.4 Affinity cross-lining of ^{125}I-IL 1 to the cell receptors

The Scatchard plot analysis ruled out that EL4 D6/76 was unreactive to IL 1 as a consequence of missing IL 1R. A deletion in the cytoplasmic tail of the receptor molecule, however, could account for the lack of IL 1-dependent signal transduction in EL4 D6/76. Therefore, to detect any major differences of the receptor molecules on both clones, the size of the receptor ligand complex was determined by SDS-PAGE after affinity cross-linking of cell surface receptors to ^{125}I-rIL 1α. EL4 5D3 and EL4 D6/76 cells were incubated with ^{125}I-rIL 1α in the presence or absence of unlabeled IL 1 followed by addition of

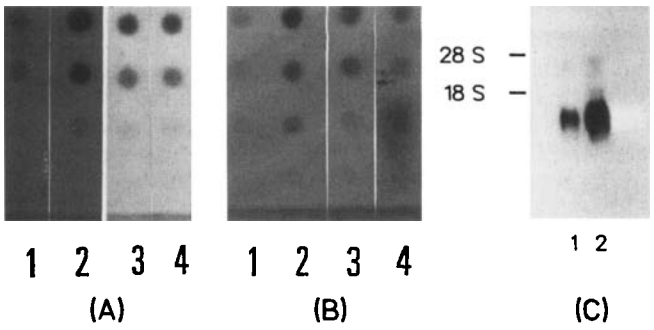

Figure 1. Quantity of IL 1-induced IL 2 and IL 2R mRNA in EL4 clones. (A) Activation of EL4 5D3 (lanes 1 and 2) and EL4 D6/76 (lanes 3 and 4) with 5 ng/ml PMA (lanes 1 and 3) or 5 ng/ml PMA and 5 U/ml rIL 1α (lanes 2 and 4) for 24 h. Dot blot with the IL 2 probe. (B) Activation of EL4 5D3 (lanes 1 and 2) and EL4 D6/76 (lanes 3 and 4) with 20 ng/ml PMA (lanes 1 and 3) or 20 ng/ml PMA and 5 U/ml rIL 1α (lanes 2 and 4) for 48 h. Dot blot with the IL 2R probe. Cytoplasmic RNA *from* the macrophage cell line PU5-1.8 did not hybridize with the IL 2 and IL 2R probes. (C) Northern analysis of EL4 5D3 RNA after stimulation with PMA (5 ng/ml, lane 1) or PMA and hu rIL 1α (10 U/ml, lane 2) for 8 h. The IL 2 probe was used. No hybridization using the IL 2 or IL 2R probe was obtained with RNA from unstimulated EL4 cells.

the bivalent lysine directed crosslinker DSS. Detergent extracts were analyzed under nonreducing conditions on SDS-PAGE as shown in Fig. 2. Cross-linking of ^{125}I-rIL 1α to EL4 D6/76 (Fig. 2, lane 1) or EL4 5D3 (Fig. 2, lane 4) cells resulted in a radioactive band with a molecular size of approximately 100 kDa revealing a mol. mass of 80–85 kDa for the receptor molecule. This mol. mass is consistent with the data published by Bron and MacDonald [20] for EL4 6.1 and by Dower et al. for LBRM-33-1A5 [21]. In the presence of cold rIL 1α the radioactive band was either drastically reduced for EL4 D6/76 (Fig. 2, lane 2) or completely absent for EL4 5D3 (Fig. 2, lane 5). In the absence of the crosslinker no bands could be observed (Fig. 2, lanes 3 and 6). These data show that the mol. wt. of the IL 1R of the two clones are identical. However, small deletions or amino acid substitutions in the receptor molecule expressed by EL4 D6/76 would not be detected by this method.

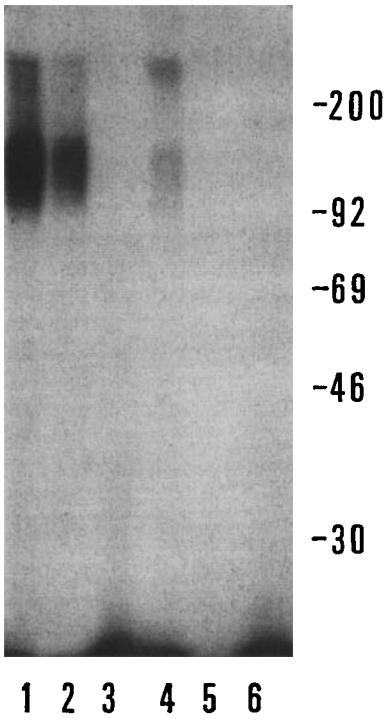

Figure 2. Characterization of IL 1R on EL4 5D3 and EL4 D6/76 by affinity cross-linking with ^{125}I-rIL 1α. ^{125}I-rIL 1α was cross-linked to cells by the lysine-directed reagent DSS. Detergent lysates of cells were analyzed by 10% SDS-PAGE under nonreducing conditions. Lanes 1, 2, 3 EL4 D6/76; lanes 4, 5, 6 EL4 5D3; Negative controls contain an excess of unlabeled rIL 1α (lanes 2 and 5) or the cross-linker was omitted (lanes 3 and 6). The mol. wt ($\times 10^{-3}$) is indicated in the right margin.

3.5 Internalization of ^{125}I-rIL 1α

Mizel et al. [8] have shown that labeled IL 1 is transported to the nucleus in EL4 and Swiss 3T3 cells. To test the possibility that EL4 D6/76 might have lost the capacity to internalize the IL 1R complex, we monitored the uptake of ^{125}I rIL 1α by these cells over a time period of 6 h. Fig. 3 shows a representative experiment out of five. Cells were incubated at 37 °C with ^{125}I rIL 1α. At the indicated times cell surface-bound radioactivity was removed by pH 3 treatment, cells were lysed and the lysate was separated into cytoplasmic and nuclear fractions as described in Sect. 2. Radioactivity accumulated in the cytoplasmic fraction of EL4 5D3 cells and reached a plateau after 3 to 4 h. In contrast, there was no significant increase of radioactivity in the corresponding cytoplasmic fraction of EL4 D6/76. The same picture arose when the amount of radioactivity in the nuclear fractions of the two clones was compared (Fig. 3). The same experiment was carried out in the presence of PMA and showed no qualitative difference to the experiment described in Fig. 3. EL4 D6/76 did not internalize IL 1

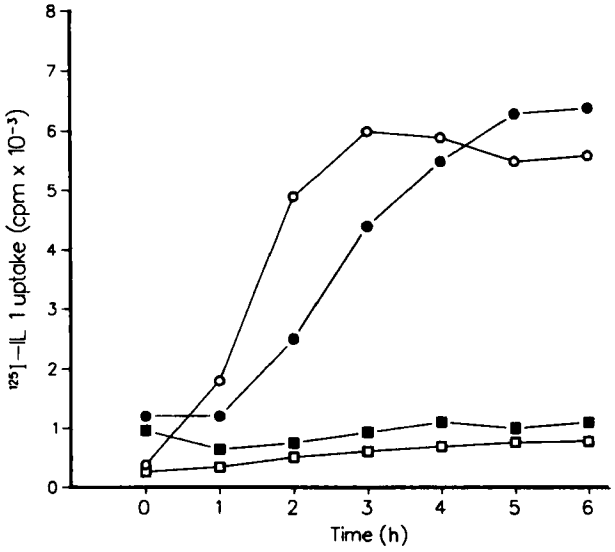

Figure 3. Internalization of ^{125}IrIL 1α. After preincubation of EL4 5D3 or EL4 D6/76 cells with ^{125}I-rIL 1α at 8 °C for 1 h samples were transferred to 37 °C. Aliquots were taken at the indicated times and the amount of radioactivity in the nuclear and cytoplasmic fractions was determined. EL4 5D3 nuclear (○—○) and cytoplasmic fraction (●—●); EL4 D6/76 nuclear (□—□) and cytoplasmic fraction (■—■).

whereas EL4 5D3 only showed an insignificant increase of IL 1 internalization (about 20%).

Although EL4 D6/76 expressed the same amount of cell surface receptors and bound IL 1 with the same affinity as EL4 5D3, ^{125}I-IL 1α bound to the cell surface of EL4 D6/76 was not transported into the cell. We do not know why nuclear binding of IL 1 precedes cytoplasmic accumulation. One could speculate, however, that transport of IL 1 and binding to a putative nuclear IL 1R is fast and accumulation of IL 1 in the cytoplasm is only seen when nuclear binding reaches saturation. We concluded from our data that the inability of EL4 D6/76 to respond to IL 1 with increased IL 2 secretion and IL 2R expression is a consequence of a defect of this cell line to internalize IL 1.

4 Discussion

The events that follow binding of IL 1 to the specific cell surface receptors and lead to secretion of IL 2 and expression of IL 2R during T cell activation are still largely unknown. In this study we describe two variants of the murine thymoma EL4, EL4 5D3 and EL4 D6/76, which may help us to understand the initial processes of the cellular response to IL 1. The cell surface receptors for IL 1 expressed by these two subclones show no significant differences in number, affinity for IL 1 or molecular size (Fig. 2). However, in the presence of PMA only EL4 5D3, but not EL4 D6/76, responded to IL 1 with increased IL 2 secretion (Table 1) and IL 2R expression (Table 2). This response was correlated with increased amounts of IL 1-induced IL 2 and IL 2R mRNA (Fig. 1). The most significant result of our experiments was that the response of the EL4 clones correlated with the internalization of the receptor-bound ^{125}I-rIL 1α. The EL4 clone EL4 D6/76, which did not respond to IL 1 also did not internalize ^{125}I-rIL 1α (Fig. 3). Whether internalization of IL 1 or its transport to the nucleus as demonstrated in this report and in a previous one by Mizel et al. [8] are necessary for a response of a cell to IL 1 is not known. Our data, however, support this hypothesis and show that binding of IL 1 to the IL 1R is not sufficient to trigger a biological response. Further support for the importance of IL 1 internalization for IL 1 action comes from our experiments that chloroquine (10 μg/ml) inhibited IL 1-induced IL 2 secretion of EL4 5D3 cells (5×10^5/ml) by 40 to 70%. Thus, it might be possible that a response to IL 1 can only be achieved when it reacts with a nuclear receptor as postulated by Mizel et al. [8].

We have considered the possibility that the defect in internalization of IL 1 by EL4 D6/76 might be due to a defect in the IL 1R molecule. Such defects have indeed been described for the human low density lipoprotein (LDL) receptor. LDL receptors that missed the cytoplasmic tail or differed in a single amino acid from the wild type sequence were nonfunctional (for review see [22]). Our experiments would not detect such minor differences in the IL 1R of the two clones. However, we have compared the receptor proteins expressed by EL4 5D3 and EL4 D6/76 on SDS-PAGE after cross-linking to labeled rIL 1α and found them to be identical in mol. wt. Thus, major differences of the receptor molecule could not be responsible for the internalization defect of EL4 D6/76.

On the basis of no major differences in mol. wt. of the IL 1R molecules expressed by the two subclones we considered differences in biochemical events that follow binding of IL 1 to the receptor. First, we considered that the activity of protein kinase C was different in the two clones. This explanation was made unlikely by our finding that PMA alone stimulated IL 2 secretion and IL 2R expression in both clones at about equal quantities. Our experiments do not rule out, however, that IL 1 may augment the activity of this enzyme as postulated previously [23]. The activity of protein kinase C is associated with the transport of this enzyme from the cytosol to the plasma membrane [24]. Abraham et al. showed, however, that IL 1 does not induce this process [25]. Thus, a difference in the biology of protein kinase C in these two clones is unlikely. Second, we considered a difference in a Ca^{2+}-dependent activation pathway. This was equally unlikely, since both clones showed an identical synergistic response to PMA and the Ca^{2+} ionophore ionomycin with respect to the secretion of IL 2 (I. von Hoegen unpublished).

Taken together, our data suggest that internalization of IL 1 is a prerequisite for the action of this cytokine. Since we have found no major differences in the number, affinity and mol. wt. of IL 1R and in the activity of protein kinase C or a Ca^{2+}-dependent activation pathway of the two clones, a further biochemical characterization of the processes that follow IL 1 stimulation will be needed to explain the internalization defect. The investigation of such processes may also lead to a better understanding of IL 1-dependent pathways of activation of normal T cells.

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