

Activation of T Cells by Interleukin 1 Involves Internalization of Interleukin 1

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ABSTRACT

The cytokine interleukin-1 plays an important role in the induction of IL-2 secretion and IL-2 receptor expression. The events that follow binding of IL-1 to its receptor are not known. We found that in a purified T cell population (comprising the Lyt_2^- and L_3T_4^- T cells) IL-1 in the absence of antigen or mitogen induced strong proliferation and *de novo* expression of IL-2R light chain mRNA. This was accompanied by high affinity IL-2 binding. Production of IL-2 or IL-2 mRNA was not detected under these conditions. As a model system for IL-1 action two EL4 subclones were isolated. EL4 5D3 responded to IL-1 by augmentation of PMA induced IL-2 secretion and IL-2R expression. EL4D6/76 bound IL-1 with the same affinity but did not respond. We found that this line was unable to internalize surface bound IL-1. The finding suggests that in T cells internalization of IL-1 is required for its activity.

INTRODUCTION

Interleukin-1 (IL-1) exerts two main effects on T cells. Together with mitogen or antigen it induces the secretion of interleukin-2 (IL-2) (1) and the expression of IL-2 receptors (IL-2R)(2). When mouse thymocytes were stimulated with IL-1 in the absence of added mitogen or antigen expression of IL-2R was observed(3). This effect was only seen in the presence of IL-2 and was determined by binding of a monoclonal antibody against the IL-2R and by proliferation. We concluded from these experiments that in the absence of mitogen or antigen IL-1 induces only expression of IL-2R and no IL-2 secretion. To obtain insight into the mechanism of these phenomena we attempted 1. to identify a purified target cell population for this effect, and 2. to establish a model system to be able to study the mechanism on a biochemical and molecular level.

A subpopulation of Lyt_2^- and L_3T_4^- thymocytes (DNT) strongly responded to the combination of both cytokines in a synergistic fashion. We found that the main effect of IL-1 was to stimulate *de novo* expression of high affinity IL-2R. Since the murine thymoma EL4 can respond to IL-1 by augmentation of IL-2 secretion and IL-2R expression (4) we subcloned EL4 and selected two subclones that differed in responsiveness to IL-1 with respect to both properties. Using clones we show that internalization of IL-1 correlates with IL-1 activity.

MATERIALS AND METHODS.

Cell Cultures: Cells were cultured in RPMI 1640 containing 5% FCS and 50 μ l/ml gentamycin at 37°C in a humidified atmosphere of 5% CO₂ in air. Thymocytes had 10% FCS and 3x10⁻⁵M 2-mercaptoethanol in addition.

Preparation of DNT: DNT were prepared from thymuses of 4-week old C3H mice by antibody and complement treatment (5). The mixture included antibodies against the antigens L3T₄, Lyt₂, Ia^k, and MAC-1. The yield was 1% of total thymic cells. They were 80-90% Thy-1 positive and 45-50% IL-2R positive.

Proliferation Assays: Proliferation was assessed by using standard methods as described (5).

Recombinant Cytokines: Human (hu) rIL-1 α was kindly provided by Drs. A. Stern and P. Lomedico, Hoffmann-LaRoche, Nutley, NJ, USA. The specific activity was 5x10⁶U/mg protein determined by the lymphocyte activating factor assay. Purified human recombinant IL-2 from E. coli was kindly supplied by Cetus Corporation, Emeryville, USA.

Assay for IL-2 Receptor Expression: Affinity purified mab 7D4 (6) was radioiodinated using the chloramin T method. Cells were washed and resuspended in 90 μ l binding medium (RPMI 1640, 0.006M Hepes, 1% BSA, 0.1% Na-Azide). mab 7D4 was added in 10 μ l binding medium to a final concentration of 4 μ g/ml. Bound radioactivity was determined as described (7).

RNA Extraction and Hybridization Procedures: Cytoplasmic RNA was prepared by the method of Cheley and Anderson (8). RNA for Northern analysis was prepared by the guanidine-isothiocyanate/cesiumchloride method. Hybridizations were done in the presence of dextran sulphate and formamide at 42°C. 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 (9). The probe for the mouse IL-2R was generously provided by Dr. T. Malek (Miami, FL, USA). It represents the EcoRI-PstI fragment of the IL-2R cDNA (clone pmIL-2pr1) (10).

Iodination of hu rIL-1 α : Purified hu rIL-1 α was labeled with ¹²⁵I by utilizing the Enzymobead reagent according to the manufacturer's instructions (BioRad Richmond, CA). Biological activity of the iodinated material was 80 to 90% of the original material (data not shown).

Binding of Labeled Cytokines to Cells: Cells were washed and incubated with labeled cytokines as described (5). T cells were incubated for 2h at 37°C in fresh medium prior to binding. ¹²⁵I-IL-2 was purchased from NEN (NEX 229, Dreieich, FRG) or Amersham (IM 197, Frankfurt, FRG). The binding reaction was terminated by centrifuging the cells through an oil mixture. Specific binding is represented as the difference in cpm bound by cells with and without nonlabeled cytokine.

Internalization of ¹²⁵I-rIL-1 α : 4x10⁶ cells and 8 U of ¹²⁵I-rIL-1 α were preincubated in 500 μ l 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 μ l pH 3 buffered medium. After incubation on ice for 5 min to remove surface bound radioactivity cells were centrifuged again and lysed in 200 μ l lysis buffer (10 mM Tris HCl, pH 7.4; 150 mM NaCl; 1% Triton X-114; 2mM PMSF; 2% Isopropanol). After 10 min incubation on ice the nuclear and cytoplasmic fractions were separated by centrifugation at 10000xg at 8°C for 10 min. Radioactivity was determined in a gamma counter.

RESULTS AND DISCUSSION

We were prompted to investigate DNT for a IL-1 response in the absence of mitogen or antigen because it was published that DNT carry receptors for IL-1 and respond to IL-1 in the presence of mitogens (11). We knew from our previous experiments that there was a low percentage of IL-1-responder cells among thymocytes. Therefore, DNT were prepared from thymuses of adult mice and tested for a synergistic response to the combination of IL-1 and IL-2. It was shown that the synergistic effect in the presence of saturating concentrations of IL-2 was IL-1-dose dependent (5). Neither IL-1 nor IL-2 alone had any effect on growth.

In other experiments (not shown) we showed that the proliferative response was dependent on IL-2 (the response was inhibited to 90% by mab against the IL-2R)

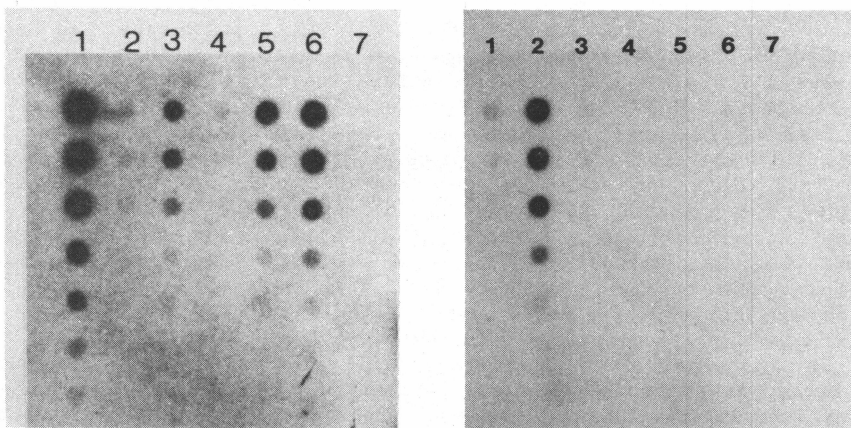


Figure 1: Kinetics of IL-2 and IL-2R mRNA. DNT were cultured with IL-1 (10 U/ml) and IL-2 (50 U/ml) for 1 to 3 days. Cytoplasmic RNA from such cells was blotted in serial twofold dilutions and probed with probes for the light chain of the IL-2R and IL-2. The left panel of Fig. 2 shows that there was relatively little IL-2R mRNA on day 0 (lane 3), no detectable mRNA on day 1 (lane 4) and dramatically increasing amounts on days 2 and 3 (lanes 5 and 6).

and that this inhibition was counteracted by IL-1 (5). It was also shown that an 18h pulse with IL-1 was sufficient to induce maximal proliferation suggesting a differentiation signal given by IL-1. (5). These data led to the conclusion that IL-1 only stimulated IL-2R expression but not IL-2 secretion in DNT. This hypothesis was confirmed on the mRNA level and by IL-2 binding.

The controls included in the dot blot were RNA from an IL-2-dependent cell line (lane 1), RNA from PMA stimulated EL4 (lane 2), and RNA from the stimulated macrophage cell line P388 D1 (lane 7). The right panel of Fig.2 shows that IL-2 mRNA under these conditions was undetectable in DNT. This finding was corroborated by the binding of ^{125}I -IL-2 under high affinity conditions. DNT were cultured in IL-1 plus IL-2 for 4 days and each an aliquot was removed for binding. 6×10^5 cells per assay well were incubated in 20nM ^{125}I -IL-2 and the amount of radioactivity bound was determined. IL-2 binding exactly followed the pattern of IL-2R mRNA expression. Specific cpm bound on days 0, 1, 2, and 3 were 1501, 132, 2712, and 3482, respectively. Scatchard analysis performed on day 3 of culture showed that the IL-1 induced *de novo* expressed receptor was of high affinity (K_D 23 to 38 pM) (5). We concluded from these experiments that IL-1 in the absence of mitogens only stimulates high affinity IL-2R expression. For the secretion of IL-2 by T cells a second signal like a mitogen is required. In our experimental system it might be possible that a second signal for IL-2R expression is provided by IL-2. It is known that IL-2 upregulates its own receptor (12). It was impossible in our system to study the effect of either cytokine alone since the cells did not survive without IL-2. Whether the p75 chain of the IL-2R was also regulated cannot be answered by our experiments. There is good evidence, however, for the its constitutive expression thymocytes (13).

The mechanism of action of IL-1 in T cells is not known. Like other growth factors IL-1 is rapidly internalized (14). To study early events in IL-1 triggered T cell responses we cloned by limiting dilution two EL4 cell lines that differed in their IL-1 response. EL4 5D3 responded to IL-1 with augmentation of IL-2 secretion and IL-2R expression whereas EL4D6/76 did not respond to IL-1. Cells of both lines were stimulated at $10^6/\text{ml}$ with PMA (10ng/ml) and PMA plus IL-1 (10U/ml). After 24h of culture IL-2 titers were measured in the supernatant and IL-2R expression on the cells was determined. In EL4 5D3 IL-1 augmented IL-2R expression (measured by binding of labeled 7D4 anti-IL-2R antibody) by a factor of 5.8 and IL-2 secretion by 3.2. These numbers varied between experiments (range 2-10). No IL-1-induced increase in both parameters was observed in EL4D6/76. Both

lines produced comparable amounts of IL-2 (220 U/ml versus 130 U/ml when stimulated with PMA) and bound a comparable amount of antibody (1.300 cpm/ 10^5 cells versus 1.929 cpm). It was shown before that IL-1 binds to specific cell surface receptors (15). The most obvious explanation, therefore, for this observed lack of IL-1 responsiveness of clone EL4D6/76 was a missing IL-1R on this cell. This question was investigated by determining number and affinity of IL-1R on both clones by evaluation of Scatchard plots of ^{125}I -IL-1 binding to cells. It was found that EL4D6/76 and EL4 5D3 expressed 2300 to 2400 cell surface IL-1 receptors per cell, with an apparent dissociation constant in the range of $1.2 - 2.5 \times 10^{-10}\text{M}$. This dissociation constant is in good agreement with the data published for EL4 by Kilian et al. (16). Also, no differences were found in affinity cross-linking experiments with ^{125}I -IL-1 (7). Both clones showed a band at 80 - 85 kD which is consistent with published data (17). Mizel et al. (14) have shown that labeled IL-1 is transported to the nucleus in EL4 and Swiss 3T3 cells. To test the possibility that EL4D6/76 might have lost the capacity to internalize IL-1, we monitored the uptake of ^{125}I -rIL-1 α by these cells over a time period of 6 h.

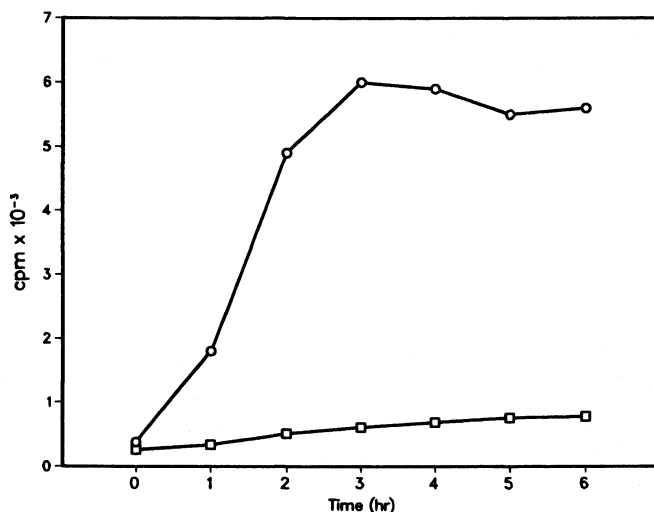


Figure 2: Kinetics of internalization of IL-1. Radioactivity accumulated in the cytoplasmic fraction of EL4 5D3 cells and reached a plateau after 3 to 4 h. In contrast, there was no significant increase of radioactivity in the corresponding cytoplasmic fraction of EL4D6/76.

When the same experiment was carried out in the presence of PMA no qualitative difference was detected. Thus, internalization of IL-1 correlates with its activity in EL4 cells. Whether internalization of IL-1 or its transport to the cytoplasm and nucleus are necessary for a response of a cell to IL-1 is not known. Nevertheless, this hypothesis is supported by our experiments showing that chloroquine (10 $\mu\text{g}/\text{ml}$) inhibited IL-1 induced IL-2 secretion of EL4 5D3 cells ($5 \times 10^5/\text{ml}$) by 40 to 70%. It might therefore be possible that IL-1 has to react with a nuclear receptor as postulated by Mizel et al. (14). If IL-1 does not influence any posttranslational parameters what is its activity? A first indication was obtained from a analysis of mRNA production for IL-2 and IL-2R. 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. Together with preliminary data showing that the half life of IL-2 mRNA was not different when EL4 5D3 cells were stimulated with PMA or PMA plus IL-1 we concluded that the effect of IL-1 was most likely due to increased transcription of IL-2 and IL-2R genes. It is totally unknown how IL-1 affects gene transcription and detailed investigation of IL-1 induced IL-2 and IL-2R gene

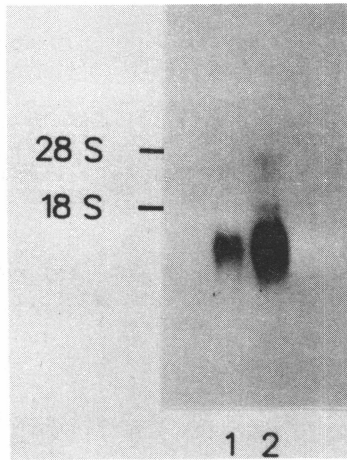


Figure 3: Northern blot of stimulated EL4 5D3 cells. After 24 h of induction EL4 5D3 showed a significant amount of IL-2 specific mRNA induced by PMA (lane 1). This amount was greatly increased when IL-1 in addition was present during stimulation (lane 2). The same was true for IL-2R mRNA (not shown).

transcription will be necessary to clarify the pathway of IL-1 dependent activation of T cells.

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