

INTERNALIZATION OF IL-1 IS REQUIRED FOR IL-2 GENE ACTIVATION IN EL4 THYMOMA CELLS.

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INTRODUCTION

Secretion of IL-2 and expression of high affinity IL-2R are required for proliferation of T cells upon mitogen or antigen stimulation. The cytokine IL-1 plays a key role in induction of these processes. It has been demonstrated that IL-1 induces IL-2 expression by normal and neoplastic T helper cells after stimulation. (Farrar et al., 1980; Kasahara et al., 1985) Also, IL-1 induces IL-2R expression on a helper T cell after stimulation of the antigen receptor (Kaye et al., 1984) and on murine thymocytes in the presence of IL-2 (Männel et al., 1985). Similar effects can be observed with selected subclones of EL4 thymoma cells in the presence of PMA (Lowenthal et al., 1986). The mode of action of IL-1 is not known. IL-1 stimulates cAMP accumulation pointing to an involvement of a G protein (Chedid et al., 1989). There is conflicting evidence with respect to the PKC pathway. Also, it was reported recently that IL-1 activates the nuclear factor κ B (Osborn et al., 1989).

Like many other growth factors IL-1 is rapidly internalized and its transport to the nucleus was reported by Mizel et al. (1987). Whether internalization of IL-1 is required for a response to IL-1 was not known. By selection of two variants of EL4 that differ in their IL-1 responsiveness we show in this report that IL-1 internalization correlates with IL-1 responsiveness and that as a consequence the IL-2 promoter is activated.

METHODS

The selection of EL4 subclones and the measurement of IL-2 secretion and IL-2R expression were described by von Hoegen et al. (1989)

Cross linking of the IL-1R with labeled IL-1 and PAGE was done as described (von Hoegen et al.,1989). Internalization and separation of membrane and cytoplasm associated IL-1 was described (Von Hoegen et al.,1989).

Northern analysis and the probes used were described by Falk et al. (1989). Transfection of EL4 was done by the DEAE dextran method. Cat activity was measured 48h after transfection by conventional methods. The construction of the plasmids used for transfection was described by Serfling et al. (1989).

RESULTS AND DISCUSSION.

In an attempt to determine whether IL-1 internalization was required for activity of IL-1 on T cells subclones of EL4 that differed in their response to IL-1 were selected by limiting dilution. The parameters used for distinction of the clones were the production of IL-2 protein in the cell supernatant and the expression of the IL-2R on the cell surface. One selected subclone, EL4 5D3, responded to IL-1 whereas the other one, EL4D6/76, showed no IL-1 response. The cells were stimulated with either PMA or with PMA + IL-1. The ratios of PMA + IL-1/PMA (SI) stimulation with respect to both parameters were determined. The SI for IL-2R was 5.8 for EL4 5D3 and 1.0 for EL4D6/76. Correspondingly, the SI for IL-2 was 3.2 for EL4 5D3 and 1.0 for EL4D6/76. To ascertain that this difference in responsiveness was not simply due to a lack of IL-1R in the nonresponsive clone EL4D6/76 IL-1R biochemical characteristics were analyzed by Scatchard analysis. No difference was found between the two clones ($K_d = 1.2-2.5 \times 10^{-10}$, 2300-2400 receptors/cell). Cross linking of the receptor with its labelled ligand and PAGE also revealed no difference in molecular mass (85 kD). Both clones responded to PMA with production of IL-2 and expression of IL-2R suggesting no major difference in the PKC pathway. It was shown that in IL-1 responsive cells IL-1 is internalized and transported to the nuclear membrane (Mizel et al.,1987). To investigate whether the defect of EL4D6/76 would be based on its inca-

It was clear from this and similar experiments that the addition of IL-1 to the reaction did not result in a shifted production of IL-2mRNA. The effect of IL-1 was to increase the amount of IL-2mRNA at a given time after stimulation. In experiments aimed at determining the half life of this mRNA we could also find no difference due to the effect of IL-1. The most reasonable explanation of these data was that IL-1 did not affect posttranscriptional events in EL4 5D3. The increase in IL-2mRNA could therefore be explained best by the assumption that IL-1 exerted its effect on the level of transcription of the IL-2 gene. We therefore decided to investigate the effect of IL-1 on the mouse IL-2 promoter. Various constructs were prepared that all contained the CAT reporter gene including the Tk promoter. Different segments of the mouse IL-2 promoter were cloned 5' of the Tk promoter in these constructs. pILCAT 0 contained the segment from -7 to -2000, pILCAT 1 contained the segment from -7 to -483, and pILCAT 2/1+ contained the segment from -7 to -293. The positive control for transfection was pTkCAT 21a containing the MSV enhancer, the negative control was pBLCAT 2 containing no enhancer (Serfling et al., 1989). These constructs were used to transfect the IL-1 responsive cell EL4 5D3 by the DEAE dextran method. 48h after transfection the cells were stimulated with IL-1 or PMA alone or with the combination of the 2 reagents. After overnight stimulation the supernatants were removed for the measurement of the IL-2 produced. The cell pellet was lysed to determine the CAT activity.

TABLE 1. IL-1 activates the mouse IL-2 promoter in transfected EL4 5D3

Plasmid	-	IL-1	PMA	PMA+IL-1	SI Cat	SI IL-2
pTkCat21A	12	13.5	18.1	18.7	1.03	4.0
pBLCat2	<1	<1	1.0	1.0	1.0	3.5
pILCat0	1.4	2.8	8.1	13.6	1.7	5.0
pILCat1	1	<1	5.3	9.7	1.8	3.0
pILCat2/1+	1	<1	5.0	12.1	2.4	5.2

The numbers depict % acetylation. They represent the mean of 4 experiments. Bold numbers represent stimulation index (SI). SI equals PMA+IL-1/ PMA stimulation. SI for IL-2 de-

termination is the ratio of the units IL-2 produced under the respective conditions. EL4 5D3 cells were transfected with the given plasmids and stimulated after 48h with the given stimuli.

The Table shows that IL-1 activates the IL-2 promoter. All transfected cells responded to IL-1 with increased IL-2 production irrespective of the plasmid used showing that no plasmid interfered with induction of the endogenous IL-2 gene and that the transfections had no toxic effect on the cells. The positive control pTkCAT 21A conferred constitutive expression whereas the negative control pBLCAT 2 conferred no significant CAT expression whether or not the cells were stimulated. All plasmids that contained segments of the IL-2 promoter showed stimulation-dependent CAT and IL-2 expression. In all cases this expression was enhanced by IL-1. The smallest fragment (in plasmid pILCAT 2/1+) still contained an element of the IL-2 promoter reactive to IL-1.

In summary, we deduce from the above data the following hypothesis. Interaction of IL-1 with its receptor is not sufficient for induction of a response. IL-1 needs to be internalized and may then interact with a nuclear receptor similar to the action of steroid hormones. The interaction of IL-1 with its nuclear receptor leads to the activation of IL-1 specific nuclear binding proteins which in turn activate the IL-2 promoter resulting in the production of IL-2mRNA.

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pability to internalize IL-1 cells of the two clones were incubated with labeled IL-1 at 37⁰C and the kinetics of cytosol-associated IL-1 was determined. We found that EL4 5D3 internalized IL-1 reaching plateau levels within 3 h whereas EL4D6/76 did not internalize any IL-1 into the cytosol. (Von Hoegen et al., 1989) We concluded from these data that internalization of IL-1 is a prerequisite for a response to IL-1. This interpretation was supported by the fact that chloroquine at a concentration of 10 µg/ml inhibited the IL-1 induced increase in IL-2 production by 68.5% at a cell concentration of 5x10⁵/well and by 42% at a cell concentration of 10⁶/well.

The next question was whether IL-1 had its effect on the level of translation or transcription. To this end the IL-2 mRNA levels were monitored at different times after stimulation with either PMA or PMA + IL-1. (Stimulation with IL-1 alone did never have any effect on IL-2 production). Total RNA was prepared and electrophoresed in a 1% agarose gel under denaturing conditions and the Northern blot was probed with a probe specific for IL-2.

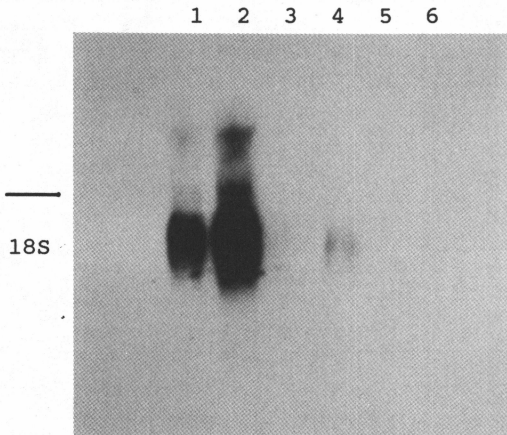


Figure 1. Kinetics of IL-2mRNA production. EL4 5D3 cells were stimulated with PMA (lanes 1, 3, 5) or with PMA + IL-1 (lanes 2, 4, 6). RNA from stimulated cells was prepared after 8h (lanes 1, 2); 24h (lanes 3, 4); and after 48h (lanes 5, 6). The Northern blot was probed with an IL-2 probe.

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