Activation of the Na^+/K^+-ATPase by interleukin-2

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Activated B61.SF.1 and CTLL-2 T lymphocyte clones which are strictly dependent on interleukin-2 (IL-2) for growth were used to study the activation of Na^+/K^+-ATPase. 50% of [^{3}H]thymidine maximal incorporation was obtained when the extracellular concentration of Na^+ or K^+ was reduced to 50 or 2 mM, respectively. 'Quiescent' CTL clones stimulated with IL-2 showed an increase of 48–380% in ouabain-sensitive ^{86}Rb uptake. Furthermore, this stimulation was completely inhibited by a monoclonal antibody PC.61 directed at the IL-2 receptor. The activation of the pump was dependent on the dose of IL-2, took place at the same doses of IL-2 that were required to stimulate cell proliferation and was linear for at least 30 min.

Interleukin *$(Na^+ + K^+)$-ATPase* *T lymphocyte activation*

1. INTRODUCTION

Activation of lymphocytes by mitogens or antigens results in the secretion of interleukin-2 (IL-2) [1]. Antigen binding also results in an increase in the level of expression of high-affinity receptors for IL-2 [2]. Once the receptor is expressed on the surface of T cells, the binding of IL-2 to its receptors is sufficient to promote cell growth [1,2]. In the plasma membrane of mammalian cells, there is an Na^+/K^+-ATPase which pumps Na^+ out of the cells against a chemical gradient [3] and one of the earliest mitogenic signals in many systems is an increase in the activity of this pump [4,6]. The activity of the Na^+/K^+-ATPase in intact fibroblast is thought to be regulated by the supply of Na^+ [7,8]. It has been postulated that growth factors stimulate the activity of this pump by increasing Na^+ entry [7–11].

Several results indicate that IL-2-mediated T cell growth resembles that induced by other growth factors [12–14]. Thus, it has been shown recently that IL-2 induces Na^+ entry by activating an Na^+/H^+ antiporter [15]. Furthermore, ouabain, an inhibitor of Na^+/K^+-ATPase, specifically inhibits lymphocyte transformation [16].

Here, we have investigated directly the activation of the Na^+/K^+-ATPase using cytotoxic T cell clones, whose growth is dependent on IL-2, and pure IL-2.

2. MATERIALS AND METHODS

2.1. *Reagents*

Human recombinant IL-2 (IL-2r) was purified from extracts of *E. coli* containing a plasmid with the cDNA coding for human IL-2 and was a generous gift from Dr J.L. Barbero (Antibiotics). Mouse IL-2 (IL-2pp) was obtained from concanavalin A-stimulated mouse spleen cell supernatant and partially purified as described [16]. Ouabain was purchased from Sigma. The monoclonal antibody PC.61, specific for the IL-2 receptor, was a generous gift from Dr M. Nabholz [17].

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Abbreviations: CTL, cytotoxic T lymphocyte; FBS, fetal bovine serum; IL-2, interleukin 2; 2-ME, 2-mercaptoethanol

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2.2. *Cell culture*

Murine IL-2-dependent T cell lines, CTLL-2 and B6.1.SF.1 were maintained in continuous culture with IL-2 containing media as in [18].

2.3. *Assay of cell proliferation*

For the determination of DNA synthesis cloned CTL (10000 cells in 100 μl) were cultured in 96-well plates in DME containing 2% FBS, 10 μM 2-ME and the indicated amounts of IL-2. After 20 h the cells were pulsed with 1 μCi [^{3}H]thymidine for 4 h and then harvested on glass-fiber filters [18].

2.4. *Measurement of Na^+/K^+-ATPase pump*

Quiescent CTL clones (2×10^6 in 1 ml) were incubated in duplicate for 45 min except in the kinetic experiments with IL-2 in DME, 10% FBS at 37°C, then ouabain (1 mM) was added to half of the samples. 15 min later ^{86}Rb (1 μCi/ml) was added. After various times of incubation, the cells were washed 3 times either by centrifugation (CTLL-2) or by washing directly the plates (adherent B61.SF.1) with 0.1 M $MgCl_2$ (pH 7.0) and the radioactivity was estimated.

3. RESULTS AND DISCUSSION

The CTLL-2 and B61.SF.1 mouse cytotoxic cell lines are strictly dependent on IL-2 for their growth which can be blocked by ouabain (fig.1). They can be grown continuously in the presence of a source of IL-2 and serum [18]. In this sense, they resemble activated T cells since they do not need antigen or mitogens to grow [1,2]. Under these culture conditions both cell lines were highly dependent on the Na^+ or K^+ concentration in the growth medium. When the Na^+ concentration was reduced and replaced by choline or sucrose to maintain the isotonicity of the medium a severe decrease in DNA synthesis, measured using [^{3}H]thymidine, was observed (fig.2A). For both cell lines only 50% of the maximal growth was obtained with 45–50 mM Na^+. For K^+, 50% of maximal stimulation was observed at 2 mM in the medium (fig.2B). These values are remarkably similar to those obtained on the growth of fibroblasts induced by serum or growth factors [6,7].

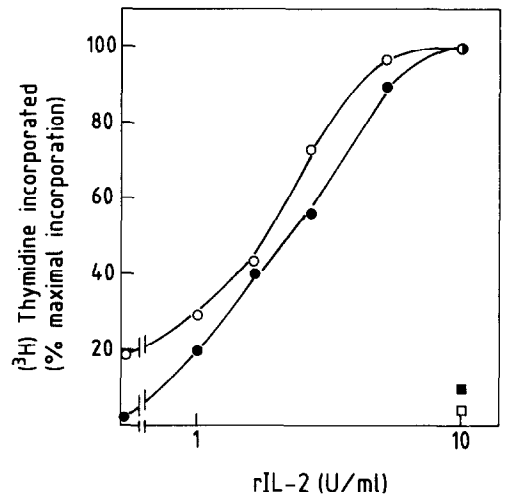

Fig.1. Effect of IL-2 on the growth of cytotoxic T cell clones. Quiescent B61.SF.1 (○, □) or CTLL-2 (●, ■) cells were stimulated with various concentrations of IL-2(r) ,in the absence (○,●) or presence of ouabain (1 mM) (□ , ■). Maximal incorporation was 54320 and 89870 cpm for B61.SF.1 and CTLL-2, respectively.

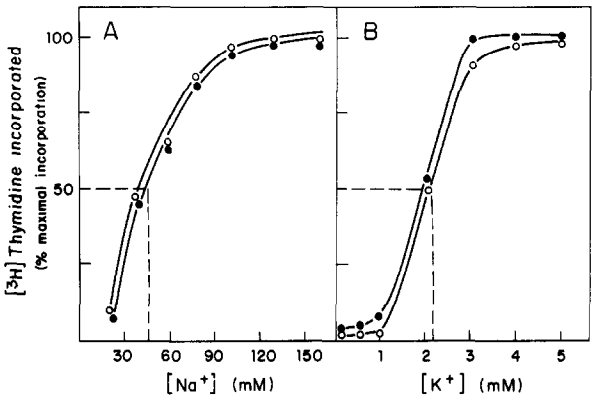

Fig.2. Effect of external Na^+ (A) and K^+ (B) concentration on the growth of cytotoxic T cell clones. Quiescent B61.SF.1 (○) or CTLL-2 (●) cells were stimulated with 10 U/ml IL-2 (pp) at different ion concentrations. Maximal incorporation was 85320 and 124810 cpm for B61.SF.1 and CTLL-2, respectively.

The requirement for high concentrations of Na^+ and K^+ in the medium suggests that activation of the Na^+/K^+-ATPase could be an early event in the stimulation of T cell growth by IL-2 as has been shown in other cell types [6,7]. To assess directly this point in our system, both cell lines were grown

in IL-2-containing media for 4 days in order to deplete IL-2 by consumption. Under these conditions most of the cells are synchronized in the G_0/G_1 phase of the cell cycle (quiescent cells, not shown) and therefore the early events of IL-2-induced T cell activation can be studied more easily.

The addition of either partially purified mouse or pure human recombinant IL-2 to cells significantly increased the activity of the Na^+/K^+-ATPase measured as ouabain-sensitive ^{86}Rb uptake by the cells. The average stimulation of B61.SF.1 cells during the first 15 min was 48% in 9 independently set experiments with mouse IL-2 and 121% with human rIL-2 (table 1). Strikingly IL-2 stimulation of quiescent CTLL-2 cells showed a much higher increase, 354 and 388%, respectively. The reason for this discrepancy is not known although it can be partially explained by the fact that CTLL-2 cells enter more easily than B61.SF.1 cells into the G_0 phase after deprivation of IL-2. Therefore more CTLL-2 cells are probably in the quiescent stage during the assay. This was also suggested by the more stringent dependency of IL-2 for growth of CTLL-2 cells than B61.SF.1 cells shown in fig.1. Noteworthily, the experimental protocols for both cell lines are different which could also contribute to explaining the observed differences. More importantly, the stimulation of Na^+/K^+-ATPase with IL-2 was completely abrogated by a monoclonal antibody PC1.61 specific for the mouse IL-2 receptor (table 1). This monoclonal antibody prevents IL-2 binding to its receptor [17] and therefore blocks IL-2-dependent proliferation of T cell lines including B61.SF.1 and CTLL-2 at the concentration listed in table 1 (not shown).

Table 1

Activation of the Na^+/K^+-ATPase by IL-2

Cell line	Stimulus	PC.61 added	Na^+/K^+-ATPase [%stimulation ± SD] (number of expts)
B61SF1	mouse IL-2(pp)	–	48 ± 15 (9)
	mouse IL-2(pp)	+	4 ± 2 (2)
	human IL-2(r)	–	121 ± 47 (6)
	human IL-2(r)	+	13 ± 2 (2)
CTLL-2	mouse IL-2(pp)	–	354 ± 54 (2)
	human IL-2(r)	–	398 (1)

Quiescent cells were stimulated with IL-2 and 15 min later ouabain-sensitive $^{86}Rb^+$ uptake was measured. Results are presented as the mean % stimulation ± SD over the basal level of ouabain-sensitive, $^{86}Rb^+$ uptake in the absence of IL-2

The stimulation of Na^+/K^+-ATPase had a dose dependency on IL-2 added (fig.3) very similar to that observed in cell growth (fig.1). This activation of the pump occurred very rapidly after the addition of the IL-2 to quiescent cells and remained linear at least for 30 min (fig.4).

In the pathway leading to cell proliferation, the early stimulation of the Na^+/K^+-ATPase has been observed to precede and to be required for such events as DNA synthesis in many systems including the mitogenic stimulation of T lymphocytes [4–8]. The aim of our studies was to assess the relationship between IL-2 and the activation of the pump. For this purpose we have employed an in vitro system which consists only of cloned T cells and pure IL-2.

Taken together, our results show that IL-2 activates the Na^+/K^+-ATPase of cytotoxic T lym-

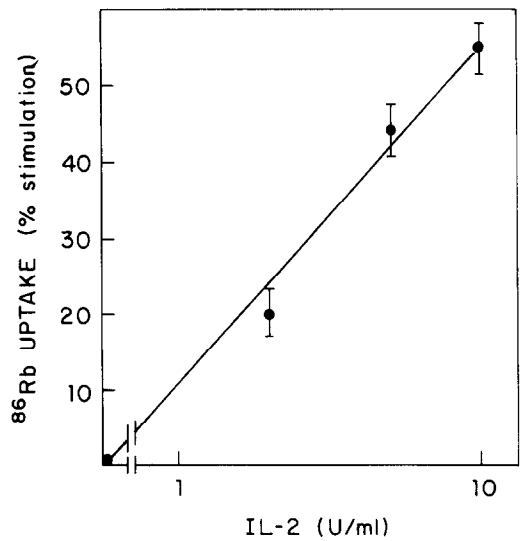

Fig.3. Dose response of IL-2 in the activation of Na^+/K^+-ATPase. Quiescent B61.SF.1 cells were stimulated with various amounts of IL-2 and the uptake of $^{86}Rb^+$ sensitive to ouabain determined 15 min later. Results are presented as % increase over the basal level (8324 cpm) of ouabain-sensitive uptake in the absence of IL-2.

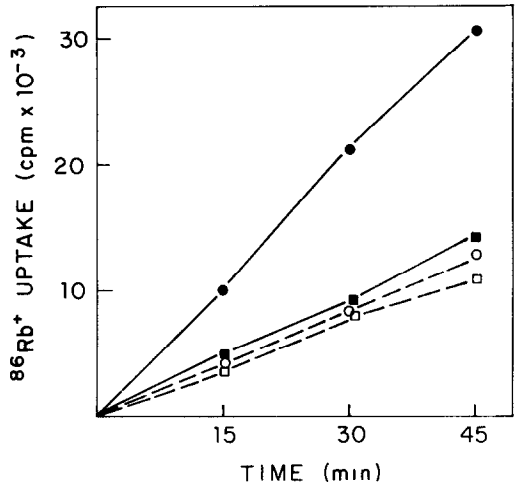

Fig.4. Kinetics of Na^+/K^+-ATPase activation by IL-2. Quiescent B61.SF.1 cells were stimulated with IL-2 (10 μg/ml) (●), anti-IL-2 receptor monoclonal antibodies (2 μg/ml) (○), IL-2 plus anti IL-2 receptor antibody (■), or medium alone (□) and the ouabain-sensitive $^{86}Rb^+$ uptake measured at various times, after the addition of IL-2.

phocyte lines and that this activation is probably related to cell growth. This is supported by several facts: (i) IL-2 from different sources increases Na^+/K^+-ATPase in two cell lines whose growth is IL-2 dependent; (ii) activation of the Na^+/K^+-ATPase occurs at a physiological dose of IL-2 similar to that required for cell growth; (iii) activation is prevented by a monoclonal antibody against the receptor at a dose similar to that blocking cell proliferation; (iv) stimulation is strictly dependent on the quiescence state of the cells; (v) inhibition by ouabain or K^+ deprivation of the Na^+/K^+-ATPase blocks IL-2-dependent growth.

Recently, it has been shown that IL-2 stimulates an Na^+/H^+ antiporter in a similar system with cloned T cells [15]. Although the authors claimed that this activation may not be necessary for cell proliferation, the Na^+ and K^+ requirements of IL-2-induced T cell growth observed in figs 2 and 3 suggest that activation by IL-2 of the Na^+/K^+-ATPase of T lymphocytes could be an early event in the stimulation pathway leading to DNA synthesis and cell division by analogy with the early monovalent cation fluxes that takes place in the stimulation of other cells by growth factors [7–11].

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