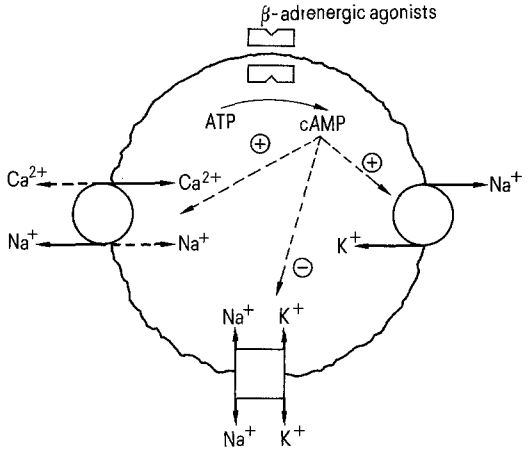
Na^+ and K^+ fluxes were measured in fresh macrophages incubated in Mg^{2+}, sucrose medium (see Garay et al.[4,9] for previously published methods for human red cells) containing (mM): 1) *K^+ medium:* 2 KCl, 2) *ouabain medium:* 1 ouabain, 3) *bumetanide medium:* 1 ouabain + 0.02 bumetanide and *Ca^{2+} medium:* 1 ouabain + 0.02 bumetanide + 1 $CaCl_2$. The difference in Na^+ efflux between K^+ medium and ouabain medium is a measure of Na^+, K^+ pump activity. The flux difference between ouabain medium and bumetanide medium is a measure of outward Na^+, K^+-cotransport. The difference in Na^+ efflux between Ca^{2+} medium and bumetanide medium is a measure of Na^+, Ca^{2+} exchange. In control experiments we observed that all these fluxes deviate from initial rate conditions after 2–10 min incubation. In all the experiments reported in this paper, we measured the initial rate of Na^+ and K^+ efflux. In 12 different experiments, the ouabain-sensitive Na^+ efflux varied between 4 and 10 mmoles $(l \cdot cells \cdot h)^{-1}$. Bumetanide-sensitive Na^+ efflux varied between 2.5 and 7.6 mmoles $(l \cdot cells \cdot h)^{-1}$ and bumetanide sensitive K^+ efflux lied between 7 and 15 mmoles $(l \cdot cells \cdot h)^{-1} \cdot Ca^{2+}$-stimulated, Na^+ efflux varied from 2 to 9 mmoles $(l \cdot cells \cdot h)^{-1}$. Most of the total Na^+ efflux was resistant to ouabain and bumetanide (20 ± 8 mmoles $(l \cdot cells \cdot h)^{-1}$, mean $\pm$ SD).

The table shows that the addition of 2 mM exogenous cAMP to the incubation medium is associated with: 1) a strong stimulation of ouabain-sensitive Na^+ efflux, 2) a significant decrease in bumetanide-sensitive Na^+ efflux and to a lesser extent in bumetanide-sensitive K^+ efflux and 3) enhanced Ca^{2+}-stimulated Na^+ efflux. The effects of exogenous cAMP were potentiated by inhibition of phosphodiesterase with 0.5 mM methylisobutylxanthine (MIX). MIX alone showed little or no effect (not presented in the table). In addition, isoproterenol, a beta-adrenergic agonist, is able 1) to completely block bumetanide-sensitive Na^+ and K^+ efflux, 2) to stimulate ouabain-sensitive Na^+ efflux and 3) to significantly increase Ca^{2+}-stimulated Na^+ efflux.

Transmembrane Na^+ and K^+ movements appear to play an important role for many vital functions of macrophages[7,8]. Inspection of data presented in the table clearly shows that ion fluxes catalyzed by different transport systems are quantitatively and in some cases qualitatively different from those classically recognized in human red cells. First, Na^+ efflux through the cotransport system is of similar magnitude to that catalyzed by the Na^+, K^+-pump. Secondly, the Na^+ to K^+ stoichiometry of outward cotransport is about 1:2 (a ratio which is close to one in human red cells, see Garay et al.[9]). Third, in contrast to the human red cell, external Ca^{2+} stimulates a ouabain- and bumetanide-resistant Na^+ efflux. The results presented here indicate that ion transport systems in macrophages may be modulated by catecholamines and other agents acting on adenylate cyclase (fig.). This may be important for the rapid recovery of a normal cell Na^+ and Ca^{2+} content after macrophage activation.



Cyclic AMP-dependent regulation of cell cation content in macrophages.

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Natural killer-like activity in human cultured lymphoid cells propagated in the presence of interleukin-2: acquired resistance to prostaglandin E_2- or dexamethasone-mediated suppression

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Summary. The cytotoxic activity of human peripheral blood lymphocytes against the natural killer-sensitive target K562 was suppressed both by prostaglandin E_2 and dexamethasone. On the other hand, cultured lymphoid cells propagated in the presence of interleukin-2 showed strong cytotoxic reactivity against K562 targets, and were resistant to prostaglandin E_2- or dexamethasone-mediated suppression.

Key words. Lymphoid cells, human; cytotoxic activity; Killer-sensitive target; interleukin-2; prostaglandin E_2; dexamathasone.

Natural killer (NK) cells, present in the peripheral blood lymphocytes (PBL) of normal donors, lyse a variety of tumor and virus-infected cells without prior antigenic stimulation[1,2]. PBL cultured in medium alone rapidly lose NK activity[3]. However, recent studies have shown that in vitro incubation of lymphocytes with interleukin-2 (IL-2), a soluble mediator which is produced by activated T cells, could lead to the propagation of cultured lymphoid cells (CLC-IL-2) with substantial cytotoxic

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reactivity against various tumor cells that are resistant to lysis by NK cells as well as NK-sensitive tumor cells[4,5].

Immunosuppressive agents, such as prostaglandins (PGs) and glucocorticosteroids, have been demonstrated to suppress endogenous NK activity[6,7]. On the other hand, Leung et al.[8] have reported that interferon-activated NK cells are partially resistant to the PGE_2-mediated suppression. It therefore seems of interest to investigate the effects of immunosuppressive agents on NK-like cytotoxic activity of CLC-IL-2. In this study we report that CLC-IL-2 exhibit strong cytotoxic reactivity against the NK-sensitive K562 target, and are partially resistant to PGE_2- or dexamethasone (DEX)-mediated suppression.

Materials and methods. Media. RPMI-1640 medium (Nissui Seiyaku Co., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (Grand Island Biological Co., Grand Island, N.Y., USA), 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml) (RPMI/HI-FBS) was used unless otherwise noted. Click's medium was prepared as described[9].

Preparation of PBL. Peripheral blood was obtained from healthy human volunteers. PBL were separated by means of Ficoll-Isopaque density-gradient centrifugation[10]. Adherent mononuclear cells were removed by incubation for 1 h in 55-cm^2 tissue culture dishes (Falcon 3003, Div. Becton, Dickinson and Co., Cocekysville, MD, USA) at 37°C in a 5% CO_2 atmosphere.

CLC-IL-2. PBL were precultured at 5×10^5 cells/ml in Click's medium containing 10% FBS, 10 µg/ml phytohemagglutinin (PHA) (Difco Labs., Detroit, MI, USA), and 10% lectin-free IL-2 (Associated Biomedic Systems Inc., Buffalo, N.Y., USA) for 2 days. The cells were washed, then subcultured every 3 days at 1×10^5 cells/ml in fresh Click's medium containing 10% FBS and 10% IL-2. On day 12 of culture, the cells were washed, resuspended in RPMI/HI-FBS, and cytotoxic activity was assessed against K562 targets.

Assay for cytotoxic activity. K562 cells, a myeloid cell line[11], were labeled with 100 µCi of $Na_2{}^{51}CrO_4$ (Daiichi Radioisotope Labs, Ltd, Tokyo) for 45 min in 1 ml of RPMI/HI-FBS and used as targets. In all assays, 2.5×10^5 effector cells and 1×10^4 ^{51}Cr-labeled target cells were combined in a total volume of 1 ml of RPMI/HI-FBS in plastic tubes (Falcon 2058). The tubes were centrifuged for 5 min at 80 × g and then incubated for 4 h. After incubation, the tubes were recentrifuged for 5 min at 800 × g, and 0.5-ml aliquots of the supernatant were removed for assay of the percentage of radioactivity released. The percent specific lysis was calculated as follows:

$$\% \text{ specific lysis} = \frac{\text{experimental cpm} - \text{control cpm}}{\text{total cpm}} \times 100,$$

where experimental cpm is the mean cpm released in the presence of effector cells, control cpm is the mean cpm released from target cells incubated with medium alone, and total cpm is the total amount of ^{51}Cr incorporated into target cells.

Treatment of cells with monoclonal antibodies and complement (C). Cells (2.5×10^5 cells/100 µl) were incubated with 10 µl of monoclonal antibodies (OKT3 from Ortho Pharmaceutical Corp., Raritan, NJ, and Leu-7 from Becton Dickinson and Co., Mountain View, CA, USA) for 30 min at 37°C. Afterwards, the cells were incubated with 50 µl of rabbit C (Cappel Labs., Cochranville, PA, USA) for 1 h at 37°C, after which the cells were washed twice, resuspended in RPMI/HI-FBS, and used for cytotoxic tests. We did not correct cell concentration for the remaining live cells to allow a quantitative evaluation of the decrease in cytotoxicity due to the eliminated subpopulation.

Results. The effect of PGE_2 on the cytotoxic activity of fresh PBL or CLC-IL-2 against K562 targets is shown in table 1. When PGE_2 was added to cytotoxic assays directly, NK activity of fresh PBL was suppressed in a dose-dependent manner (70% inhibition at 10^{-6} M). In contrast, CLC-IL-2 showed strong cytotoxic reactivity and resistance to the PGE_2-mediated suppression (15% inhibition at 10^{-6} M).

The kinetics of the suppression of NK activity by DEX are shown in the figure. The in vitro suppression of NK activity of PBL was time-dependent, requiring at east 5 h of incubation with DEX to achieve maximal levels of suppression. In preliminary experiments, no differences were observed in NK activity of freshly obtained PBL and PBL cultured for 5 h in medium alone. In addition, it was also observed that CLC-IL-2 maintained its strong NK-like activity for 5 h of incubation in medium without IL-2 supplementation. Therefore, we examined the suppressive effects of DEX on the cytotoxic activity of fresh PBL or CLC-IL-2 after preincubation with medium containing various concentrations of DEX for 5 h. As shown in table 1, NK activity of fresh PBL was suppressed by DEX in a dose-dependent manner, while the suppressive effects of DEX on NK-like activity of CLC-IL-2 was not significant (5.4% inhibition at 10^{-6} M).

Table 1. Effect of PGE_2 or DEX on cytotoxic activity of fresh PBL or CLC-IL-2 against K562 targets

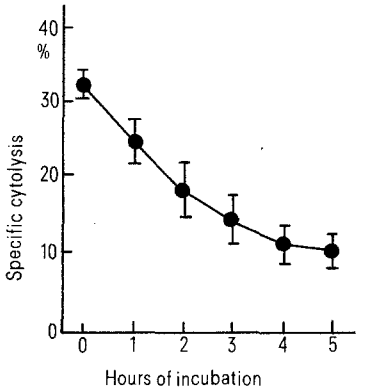
Concentration	Treatment			
	PGE_2[a]		DEX[b]	
	PBL	CLC-Il-2	PBL	CLC-IL-2
Control	38.8 ± 2.5[c]	79.5 ± 1.7	34.6 ± 7.1	74.5 ± 1.5
10^{-10} M	–	–	30.9 ± 7.7	74.6 ± 2.9
10^{-9} M	35.8 ± 3.8	77.5 ± 2.1	24.5 ± 6.5	75.5 ± 2.2
10^{-8} M	31.8 ± 6.8	77.2 ± 3.2	16.5 ± 6.2	71.5 ± 3.4
10^{-7} M	23.0 ± 6.0	73.7 ± 2.0	15.3 ± 3.5	69.3 ± 3.0
10^{-6} M	11.3 ± 4.2	67.2 ± 3.5	13.9 ± 3.8	70.8 ± 4.3

[a] Cytotoxic activity of fresh PBL or CLC-IL-2 was assessed in the absence or presence of various concentrations of PGE_2 at an effector cell:target cell ratio of 25:1. [b] Fresh PBL or CLC-IL-2 were incubated with medium containing various concentrations of DEX for 5 h. After incubation, the cells were washed and cytotoxic activity was assessed at an effector cell:target cell ratio of 25:1. [c] Percent cytolysis. Mean ± SD of six healthy human volunteers.

Table 2. Cytotoxic activity of fresh PBL or CLC-IL-2 after treatment with monoclonal antibody and C[a]

Treatment	PBL	CLC-IL-2
Untreated	35.1 ± 6.3	66.5 ± 10.1
C-treated	30.0 ± 5.4	63.7 ± 8.9
OKT3 + C	31.3 ± 5.5	60.0 ± 8.4
Leu-7 + C	10.9 ± 2.6	38.7 ± 5.4

[a] PBL or CLC-IL-2 were treated with monoclonal antibody plus C as described in materials and methods. [b] Percent cytolysis. Mean ± SD for seven healthy human volunteers.



Kinetics of suppression of NK activity by DEX. Fresh PBL were incubated with medium containing 10^{-7} M DEX for various periods. After incubation, the cells were washed and NK activity was assessed at an effector cell:target cell ratio of 25:1. Each point represents mean ± SD for five healthy human volunteers.

Two monoclonal antibodies were used for detection serologically defined markers on NK-like activity of CLC-IL-2 using C-mediated lysis (table 2). NK activity of fresh PBL was significantly diminished by treatment with Leu-7 (human NK cell marker) plus C but not OKT3 (human T cell marker). Similarly, NK-like activity of CLC-IL-2 was significantly diminished by Leu-7 but not OKT3.

Discussion. The ability of human CLC-IL-2 to lyse various tumor cells in vitro has been reported by several investigators[4,5,12]. Kedar et al.[4] have shown that without prior specific antigenic stimulation, CLC-IL-2 are highly cytotoxic to K562 targets and various tumor cells, both with low and with high susceptibility to lysis by fresh NK cells. In the present study we have also demonstrated that CLC-IL-2 exhibit strong cytotoxic reactivity against K562 targets. In addition, it was shown that CLC-IL-2 were partially resistant to the PGE_2- or DEX-mediated suppression. The mechanism(s) of reduced sensitivity to PGE_2 or DEX by CLC-IL-2 are at present unclear. Further studies are necessary to understand these phenomena.

The nature of NK-like cytotoxic cells grown in the present culture conditions has not been fully defined. Kedar et al.[4] have reported that much of the cytotoxicity seen in CLC-IL-2 is attributable to propagation of NK cells. Sugamura et al.[13] have shown that human NK cells can proliferate in the presence of IL-2, and the cloned T cell lines have NK activity. In this study, although we did not use purified large granular lymphocytes (which have been shown to be closely associated with human NK activity), NK-like cytotoxic activity of present CLC-IL-2 was significantly diminished by treatment with Leu-7 and C. Therefore, we reasoned that human endogenous NK cells or their precursor cells could proliferate in vitro in the presence of IL-2. However, it remains unanswered whether NK-like cells grown in IL-2 containing medium are identical with NK cells occurring naturally in PBL, or not.

Our results indicate a protection of natural host resistance by IL-2 in view of the presented finding that NK-like cytotoxic activity of CLC-IL-2 is hardly suppressed by PGE_2 or DEX.

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Effect of aloe lectin on deoxyribonucleic acid synthesis in baby hamster kidney cells

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Summary. A homogeneous glycoprotein (mol.wt 40,000) containing 34% carbohydrate was isolated from *Aloe arborescens* var. *natalensis*. At a concentration of 5 μg/ml, this glycoprotein was shown to stimulate deoxyribonucleic acid (DNA) synthesis in baby hamster kidney (BHK) cells and to have the properties of a lectin which reacts with sheep blood cells. The chemical and physical properties of the glycoprotein (aloe lectin) are also discussed.

Key words. Aloe arborescens var *natalensis:* glycoprotein, aloe lectin; deoxyribonucleic acid synthesis; baby hamster kidney cells.

As a traditional medicine in many countries, *Aloe* species have been commonly used for the treatment of radiation injury[1] skin diseases[2] eye afflictions[3,4] and intestinal disorders[5]. Recently, Lushbaugh and Hale reported that the use of fresh *Aloe vera (A. barbadensis)* gel in the treatment of experimental radiodermatitis in rabbits hastened both the degradative and regenerative phases of the lesions so that complete healing occurred in half the time required for untreated lesions[6]. Brasher, Zimmermann and Collings reported the effects of *Aloe vera* gel on the Gey strain of Hela cells and rabbit kidney fibroblasts in actively growing monolayer cultures[7]. The effect of *A. arborescens* var. *natalensis* gel on wound healing is closely associated with stimulation of the rapid formation of granulation tissue, fibrosis, followed by proliferation of the fibroblasts, and regeneration of the epidermis[8]. The present experiment was undertaken to isolate the active substance in *Aloe* by screening for DNA synthesis for DNA in BHK 21 (clone 13) cells and to investigate its chemical and physical properties.

Material and methods. Preparation of *Aloe* samples. Fresh leaves (1 kg) were homogenized and centrifuged at 10,000 rpm 30 min. The supernatant was lyophilized to a pale yellow curde extract (8 g), an aliquot of which (3 g) was applied to a column of Amberlite XAD-2 and eluted with distilled water. The eluate was concentrated under reduced pressure and lyophilized to a colorless powder (2 g). The powder was dialyzed against distilled water. The dialysate was evaporated to dryness, and the nondialyzable material was concentrated on a Sartorius hollow fiber dialyzer concentrator. The nondialyzable fraction with a mol.wt higher than 10,000 was lyophilized to a colorless fibrous material (0.3 g). Nondialyzable material (1 g), dissolved in 0.02 M NH_4HCO_3, was gel-filtrated on a column of DEAE-cellulofine (Chisso Corp.). The same buffer was used to elute the neutral polysaccharide fraction (30 mg). The column was then eluted with 0.3 M NaCl; the eluate was concentrated on a Sartorius hollow fiber dialyzer concentrator and lyophilyzed to a pale brown powder (400 mg). An aliquot of this glycoprotein fraction (300 mg),