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

A. Yagi, K. Machii, H. Nishimura, T. Shida and I. Nishioka

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima 729-02 (Japan), Oyodo Research Laboratories, Maruho Co., Ltd, Osaka 531 (Japan), Clinical Research Center for Reumato-Allergology, National Sagamihara Hospital, Sagamihara, Kanagawa 228 (Japan), and Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812 (Japan), 18 April 1984

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),

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dissolved in 0.3 M NaCl, was applied to a Sepharose 6B (Pharmacia Fine Chemicals) column which was eluted with the same solution. Two fractions, glycoprotein fr. 1 (44 mg) and glycoprotein fr. 2 (86 mg) were recovered. The neutral polysaccharide fraction (30 mg), dissolved in 0.3 M NaCl, was applied to a Sepharose 6B column which was eluted with the same solution. Two fractions, polysaccharide fr. 1 (2.4 mg) and 2 (1.3 mg) were obtained.

Methods of analysis. Elution of the columns was monitored by measuring the absorbance of the effluents at 280 nm or at 490 nm by the phenol-sulfuric acid method[9]. Glycoprotein fr. 1 was hydrolyzed in 2 N H_2SO_4 at 100 °C for 6 h in a sealed tube. Excess sulfuric acid was removed by precipitation as barium sulfate. The filtrate was passed through Dowex 50 W, and neutral and amino sugars were obtained. Gas chromatography of the trimethyl silylated sugars from the hydrolysate was performed as previously described[10]. Acid hydrolysis of glycoprotein fr. 1 was carried out in 6N HCl at 100 °C for 6 h, and the quantitative analysis of the hexosamine was accomplished by the Elson-Morgan method[11]. The amino acid analysis was done as previously described[12].

Culture method and determination of DNA content. BHK 21 cells were obtained from Flow laboratories Inc. Cultures were started from frozen stocks. The cells were grown and maintained in Eagle's minimum essential medium (MEM, Nissui) to which 10% fetal bovine serum (FBS, Flow Lab.) and L-glutamine (2 mM) were added. The pH was adjusted to about 7.2 under 5% CO_2 with $NaHCO_3$. Cells were subcultured in 25 cm^2 tissue flasks (F 25, Corning) at a density of 6.0×10^4 cells/ml and maintained at 37 °C in a 95% air, 5% CO_2, humidified atmosphere. After 3 days of incubation, cells were trypsinized with 0.25% trypsin (Difco, dilution 1:250). Cell counting was performed in a Coulter counter model ZB. Experimental cultures were inoculated at approximately 1.5×10^3 cells per microplate

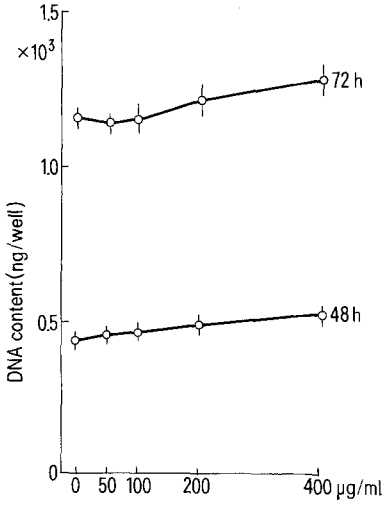

Figure 1. Effect of nondialyzable fraction on DNA synthesis in BHK-21 cells. Experimental procedures are described in 'material and methods'. Each point is the mean of the values obtained from the six microplate wells each containing 1.5×10^3 cells.

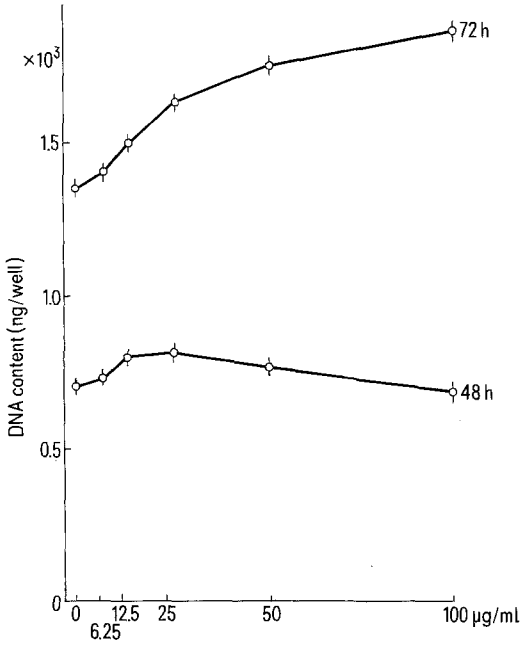

Figure 2. Effect of the glycoprotein fraction on DNA synthesis in BHK-21 cells. Experimental procedures are described in 'material and methods'. Each point is the mean of the values obtained from the six microplate wells each containing 1.5×10^3 cells.

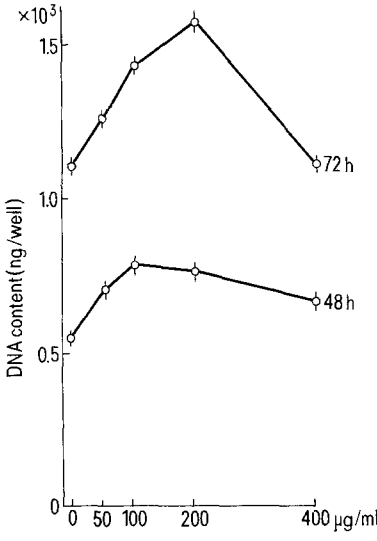

Figure 3. Effect of glycoprotein fr. 1 on DNA synthesis in BHK-21 cells. Experimental procedures are described in 'material and methods'. Each point is the mean of the values obtained from the six microplate wells each containing 1.5×10^3 cells.

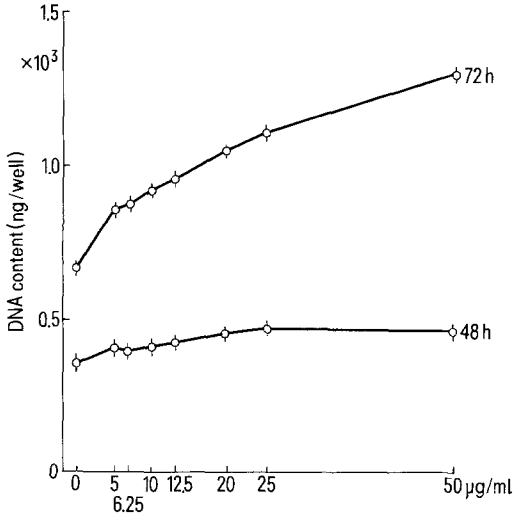

Figure 4. Effect of glycoprotein fr. 1 on DNA synthesis in BHK-21 cells. Experimental procedures are described in 'material and methods'. Each point is the mean of the values obtained from the six microplate wells each containing 1.5×10^3 cells.

well (Linbro). Cells grown for 1 day were used for the experiments. The medium was removed from the wells, and a solution (0.2 ml) of MEM and 10% FBS containing the *Aloe* sample was added. Six wells for each *Aloe* sample and blank wells containing MEM with 10% FBS were cultured for 2–3 days. At the completion of culture, cells were washed twice with Ca^{2+}, Mg^{2+} free phosphate buffered saline (PBS). Cell monolayers were then rinsed once with 0.2 ml of cold 10% trichloroacetic acid (TCA), and twice with 0.2 ml of ethanol and allowed to dry. DNA content was determined by a modified method of Kissane and Robins[13]. Briefly, to blank wells and to those containing the dried sample, 30 µl of freshly prepared 20% diaminobenzoic acid was added. The microplates were heated at 60 °C for 50 min in an incubator. To each of the wells, 0.37 ml of 0.5 N perchloric acid was added with mixing and then transferred to a microcells. Fluorescence was determined using a fluorometer (Hitachi, type MPF-4) under the following conditions: excitation wave length; 412 nm, emission wave length; 505 nm; slit (excitation), 6 nm; slit (emission), 10 nm.

Phytohemagglutination. Agglutination tests were performed in microtiter plates by mixing serial two-fold dilutions of glycoprotein fr. 1 (10 mg/ml) and 1% sheep blood cells (25 µl) in buffered saline. Agglutination was evaluated visually after 3 hr at room temperature. The ability of sugars to inhibit the interaction between glycoprotein fr. 1 and sheep blood cells was tested. Phytohemagglutinin P (Wako) was used as a positive control.

Results and discussion. Phenolic constituents of the pale yellow crude extract, such as barbaloin, were selectively removed by absorption with nonionic porous resin (Amberlite XAD-2). The water eluate of the column of Amberlite XAD-2 was dialyzed in a Visking tube; and neutral and polar biopolymers were obtained from the nondialyzable fraction. The polar biopolymer (glycoprotein fr.) was subjected to gel-filtration to give glycoprotein fractions 1 and 2. Both fractions were examined by disk electrophoresis on 7% polyacrylamide gels, pH 9.5, and 10% sodium dodesylsulfate polyacrylamide gels, pH 7.2. When the gels were stained with coomassie brilliant blue and periodate-Schiff reagents, glycoprotein fr. 1 appeared as a single band while glycoprotein fr. 2 appeared as several bands. On analytical ultracentrifugation using a schlieren optical system in a single sector at 20 °C and 56,000 rpm, glycoprotein fr. 1 appeared as a single symmetrical peak with a sedimentation coefficient ($C_{20,w} = 3.34$ S at a concentration of 1.2% in 0.02 M NH_4HCO_3). Therefore, it appears that glycoprotein fr. 1 is homogeneous. The mol. wt, estimated from column chromatography on Sepharose 6B with a series of dextrans as standards, was determined to be 40,000[10].

The carbohydrate, hexosamine, and protein contents of glycoprotein fr. 1 containing 5% ash, were determined to be 34%[9], 4%[11] and 57%[14], respectively. On acid hydrolysis and gas chromatographical analysis, glycoprotein fr. 1 showed mannose, glucose, galactose, glucosamine, galactosamin, and N-acetylglucosamine in ratio of 2:2:1:1:4:1. Amino acid analysis showed the following composition: Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Phe, Orn, Lys, Arg, and Pro. A study of the chemical and physical properties of glycoprotein fr. 1 indicated that it is a homogeneous glyoprotein. Maximal agglutination by glycoprotein fr. 1 was observed at a concentration of 1.25 mg/ml, whereas phytohemagglutinin P showed maximal agglutination at a concentration of 0.04 mg/ml. The solution of glycoprotein fr. 1 was adjusted to 5 mg/ml, and inhibition by sugars was tested. The maximum inhibition by glucose, mannose, and galactose occurred at a concentration of 3.4, 1.7, and 1.7 mM, respectively. Figure 1 shows the effect of the nondialyzable fraction on DNA synthesis. The dialysate demonstrated no effect. Among the nondialyzable fractions, only the glycoprotein fraction showed an effect on DNA synthesis (fig. 2). The maximum activity of glycoprotein fr. 1 occurred at a concentration of 100 µg/ml in a 48-h culture and 200 µg/ml in a 72-h culture (fig. 3). Glycoprotein fr. 2 showed no effect. Glycoprotein fr. 1 (aloe lectin) stimulated DNA synthesis in BHK 21 cells at a concentration of 5 µg/ml (fig. 4), resulting in active growth and division of blast-like cells without any morphological change in the cells even at a high concentration. Heat-treated aloe lectin (100 °C for 30 min), however, did not stimulate DNA synthesis, which suggests that the active component is the native protein moiety of aloe lectin. The present experiments show the characterization of the chemical and physical properties of a glycoprotein which has a lectin property and which stimulates DNA synthesis in BHK 21 cells. Aloe lectin, which induces blastmitogenesis, may be responsible for the therapeutic effect of aloe on burns.

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The potency of heparin-like activity of glycosaminoglycans released by human endothelial cells in culture

K. Shimada, I. Kusumoto and T. Ozawa

Department of Medicine and Geriatrics, Kochi Medical School, Kochi 781-51 (Japan), 28 May 1984

Summary. Glycosaminoglycans isolated from culture medium conditioned by human endothelial cells showed heparin-like antithrombin III cofactor activity measured by Xa inhibition. Their activity was relatively weak, 0.1% of the potency of heparin, but was approximately 5-fold more potent than that of glycosaminoglycans derived from vascular smooth muscle cells.
Key words. Endothelial cells; glycosaminoglycan; heparin, smooth muscle cells.

It has been postulated that the endogenous heparin-like inhibitor could be derived from the vascular endothelium and take part in the nonthrombogenic properties of blood vessels[1]. To prove this hypothesis, recent studies have tested the effects of