Cytotoxic Activities of Lymphokine-activated Killer Cells and Chemotherapy-activated Killer Cells are Inhibited by Cholera Toxin

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Abstract: Cholera toxin added to an assay mixture significantly inhibited the cytotoxic activity of lymphokine-activated killer (LAK) cells derived from normal human peripheral blood leukocytes (PBLs). LAK cells preincubated with the toxin lost 90% of their cytolytic activity. *In vitro* activation of killer cells by Interleukin−2 from PBLs was not affected by the toxin. These results indicate that LAK cells are directly impaired of the cytolytic activity by the toxin and that the generation of LAK cells is not inhibited by it. LAK cells from a HIV carrier were more sensitive to the toxin than those from normal controls. Chemotherapy-activated killer (CAK) cells (Kiyohara *et al.*, 1988) which had been activated *in vivo* were also impaired of the cytotoxic function by the toxin.
Key words: Cholera toxin, Lymphokine-activated killer cells, Chemotherapy-activated killer cells, AIDS

INTRODUCTION

Various kinds of exotoxins are known to be produced by bacteria. These toxins directly cause pathological signs of infectious diseases and supposed to modulate clinico-pathological features in patients with complicated diseases such as AIDS and cancer. Cholera toxin is composed of A subunit (which penetrates into target cells and ADP-ribosylates G proteins) and B subunits (which bind to GM1 on the intestinal epithelia). The toxin bound to epithelial cells induces efflux of water to intestinal lumen resulting in voluminous diarrhea (Gill and Meren, 1978). Few are known however about the biological effects of the toxin to the immune surveillance systems of otherwise healthy or immunocompromised hosts. We therefore analysed the effect of the toxin to cytotoxic activities of LAK cells obtained from normal

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volunteers and a HIV-carrier, and the activity of killer cells endogenously induced by chemotherapy in tumor-bearing patients (CAK cells) (Kiyohara *et al.*, 1988). Our results indicate that these killer cells are directly impaired of the cytolytic activities by cholera toxin regardless these have been activated *in vitro* or *in vivo* and are likely to become more susceptible to the toxin in a carrier status of HIV.

MATERIALS AND METHODS

Effector cells: PBLs from normal volunteers and an asymptomatic HIV carrier were isolated from the heparinized blood samples by separation on Ficoll-Hypaque gradients (Lymphoprep, Daiichi Chemical Co., Tokyo, Japan). After separation, PBLs were cultured in IL−2 containing (5UTGP3/ml, Takeda Pharmaceutical Co., Osaka) RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo) supplemented with 10% fetal calf serum (FCS) (Cell Culture Laboratories. Ohio, USA) for 48hrs. After harvesting and washing 3 times, the cells were used as LAK effector cells.

PBLs were isolated as above from patients with lung cancer who received combined chemotherapy (Carboplatin, Vindesin and Mitomycin C) a month before and used as CAK effector cells without any *in vitro* incubation with IL−2 because the cells had been shown to be activated *in vitro* (Kiyohara *et al.*, 1988).

Target cells: Daudi cells, a human Burkitt lymphoma cell line, maintained in 10% FCS containing RPMI 1640 medium were used as NK resistant and LAK sensitive target cells. Mycoplasma contamination was regularly checked and proved to be negative.

Cholera toxin: Cholera toxin and its B subunits were purified from a 569B Classic Inaba strain by gel filtration.

Cytotoxic assay: Cytotoxicity assays were performed by a standard ^{51}Cr-release technique as described (Roder *et al.*, 1978) and % specific lysis was calculated from an equation; $100 \times$ (cpm test − cpm medium)/(cpm maximum-cpm medium). Cpm medium is a count of 100 μl of supernatant obtained from a well containing target cells but not effector ones and kept in rest for 24 hours. Cpm maximum is counted as above but the sample has been taken after thourough resuspension. One lytic unit (L.U.) is defined as the ability of 10^7 effector cells to lyse 20% of 5×10^3 target cells.

RESULTS AND DISCUSSION

Cholera toxin has been used widely for activation of cAMP-dependent adenylate cyclase in various cell types (Holmgren *et al.*, 1974). However actual roles of this toxin in the establishment of bacterial infection are obscure as those of other A−B type exotoxins such as *Pseudomonas aeruginosa* toxin. Instead of a general question, "Are bacterial exotoxins essential for establishing infections?", we raised an individual question, "Does cholera exotoxin paralyze any immune surveillance system? If killer cells are affected, which step(s) is the target of the toxin?". In this paper, we answer in part to the question on the basis of *in vitro*

experiments.

Although murine T cell blastogenesis and proliferation (Woogen *et al.* 1987; Nutman *et al.*, 1987), and antibody production were reported to be inhibited by the exotoxins of periodontogenic bacteria (Ochiai *et al.*, 1989), any effect of these exotoxins to LAK cell cytotoxic activity has not been reported. LAK cells are defined as lymphocytes activated *in vitro* by an Interleukin, IL−2 (Grimm *et al.*, 1982) or IL−7 (Alderson *et al.*, 1990).

Cholera whole toxin (5 μg/ml) present in the incubation mixture for the significantly decreased LAK cytotoxicity by 59% (Fig. 1a), but B subunits of the toxin did not (data not shown). Following two experiments indicate LAK cells but not target cells to have been affected by cholera whole toxin. In the first experiment, normal LAK cells were preincubated with the toxin for 1 hr, washed 3 times and used for cytolytic assay in the absence of the toxin (Fig. 2). The preincubated LAK cells significantly lost cytolytic activity to the extent enough for causing the inhibition shown in Fig 1a. In the second experiment, target cells were incubated with 100 times concentrated (0.5 mg/ml) toxin and examined for viability by Trypan blue dye exclusion. No increase in the number of dead cells was observed even after an incubation for 60 min (data not shown). These results indicate that cholera toxin directly binds to LAK cells and inhibits their cytolytic activity. As the preincubation of the killer cells with the toxin can be shortened to 1 min without any decrease in the effect (Fig. 3), cholera toxin bound to the cells can effectively keep its inhibitory activity throughout the incubation for effector-target interaction. These results suggest that LAK cells have certain pathway for negative regulation of signal transduction as shown in NK cells (Watanabe *et al.*, 1992)

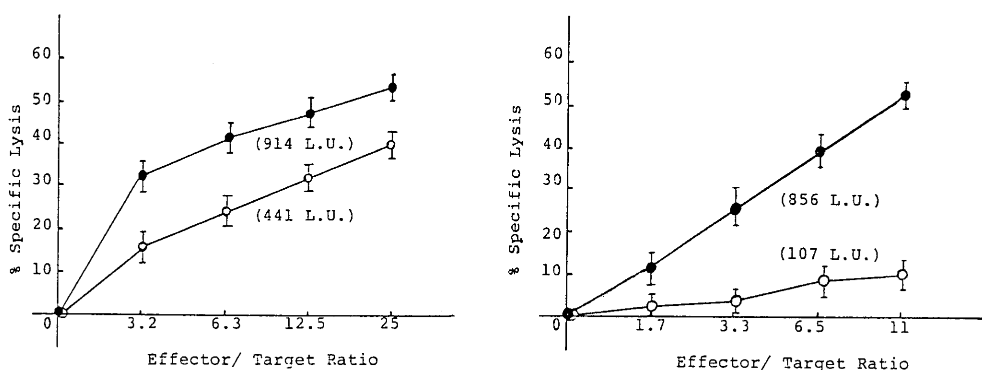

Fig. 1a. Inhibition of IL−2 activated human PBL LAK activity by cholera whole toxin during 18 hrs assay.
●—● Not treated, ○—○ 5 μg/ml of cholera whole toxin was mixed to the assay system. ● or ○ shows standard deviation in all the figures.

Fig. 1b. Inhibition of IL−2 activated HIV asymptomatic carrier PBL LAK activity by cholera whole toxin during 18 hrs assay.
●—● Not treated, ○—○ 5 μg/ml of cholera whole toxin was mixed to the assay system.

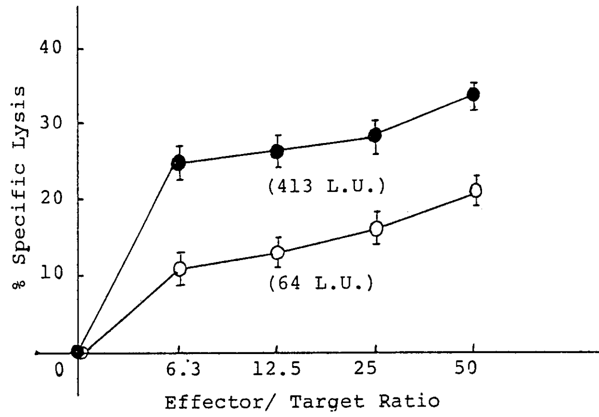

Fig. 2. Inhibition of IL−2 activated human PBL LAK activity by cholera whole toxin after 1 hr preincubation.
●—● Not treated, ○—○ LAK cells were pretreated with 5 μg/ml of cholera whole toxin and after washing 3 times, 18 hrs ^{51}Cr release assay against Daudi cells was performed.

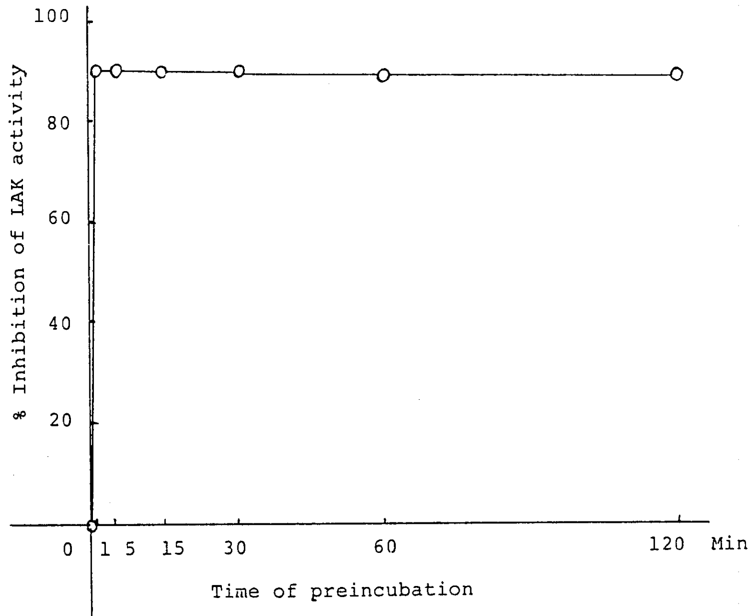

Fig. 3. Kinetics of inhibition of IL−2 activated human PBL LAK activity by cholera whole toxin.
○—○ LAK cells were pretreated with 5 μg/ml of cholera whole toxin and after washing 3 times, 18 hrs ^{51}Cr release assay against Daudi cells was performed.

LAK cells from an asymptomatic HIV carrier were more sensitive to the toxin than those from normal volunteers (Fig. 1b). His LAK activity showed 87% inhibition in the presence of 5 μg/ml whole toxin. An immune surveillance function is therefore suggested to be worsened in HIV carriers by the superinfection of cholera, which is expected in tropical countries.

We have shown that CAK cells are *in vivo* activated LAK-like cells found in PBLs of tumor bearers who received combined chemotherapy a month before examination (Kiyohara *et al.*, 1988). CAK cells were therefore used to determine whether cytotoxic activity of *in vivo* activated killer cells are inhibited by cholera toxin as *in vivo* activated ones or not. CAK cells were shown to be susceptible to the toxin (Fig. 4) as LAK cells (Fig. 1a).

We observed NK and CAK activities become undetectable at the terminal stages of AIDS and malignancies, respectively. The activation of these killer cells *in vivo* should be a factor for longer life span. We therefore examined whether cholare whole toxin modulates or not an *in vivo* activation process of normal PBLs to LAK cells. The toxin added to IL−2 containing activation mixture of normal PBLs to LAK cells. The toxin added to IL−2 containing activation mixture of normal PBL suspensions neither increased nor decreased the activation of killer cells (Fig. 5). In conclusion, cholera toxin inhibits a cytotoxicity stage but not an activation stage of LAK and CAK cells. It implies that killer cells activated *in vivo* into effector cells without inhibition are paralyzed by exotoxins in bacterial infections.

How and what kinds of molecular steps are blocked by cholera toxin should be elucidated in future. It must be noteworthy to give a comment that the toxin blocks the signal transduction for killing at a level lying after phosphoinositide turnover in NK cells (Watanabe *et al.*, 1992).

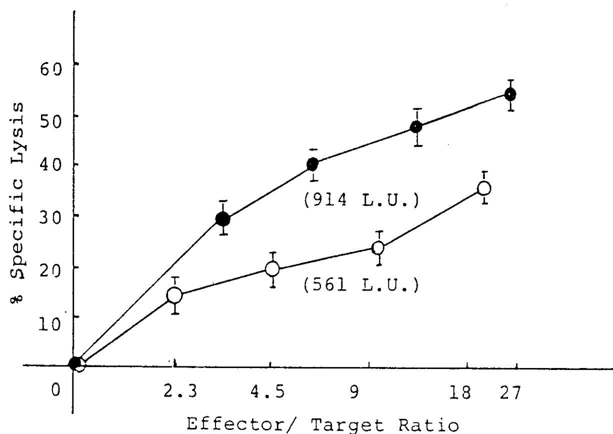

Fig. 4. Inhibition of tumor bearer CAK activity by cholera whole toxin during 18 hrs assay.
●—● Not treated, ○—○ 5 μg/ml of cholera whole toxin was mixed to the assay system.

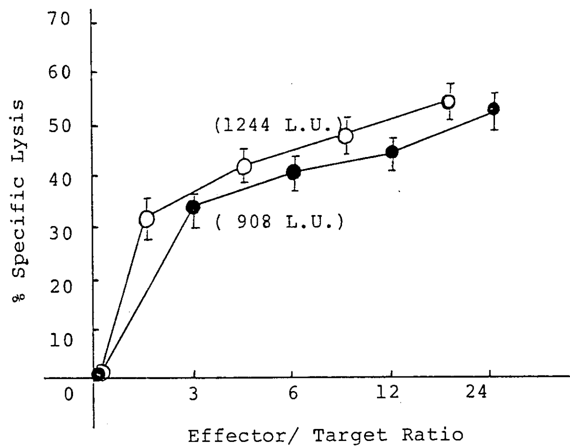

Fig. 5. No inhibition of activation phase of PBL LAK activities by cholera whole toxin. ●—● Not treated, ○—○ 5 μg/ml of cholera whole toxin was mixed before 48 hrs LAK activation system, washed 3 times and assayed against ^{51}Cr labelled target Daudi cells.

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