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THE ROLE OF PROTEIN KINASE C ACTIVATION IN SIGNAL TRANSMISSION BY INTERLEUKIN 2

Naofumi Mukaida, Hitoshi Yagisawa*, Tadashi Kawai and Tadashi Kasahara*[1]

Departments of Clinical Pathology and *Medical Biology & Parasitology, Jichi Medical School, Minamikawachi-machi, Tochigi-ken, Japan

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SUMMARY: Role of protein kinase C (PKC) in interleukin (IL) 2-induced proliferation was investigated by utilizing two murine IL 2-dependent cell lines, CT6 and CTLL-2 cell lines. CT6 cells showed a marked proliferative response to phorbol 12-myristate 13-acetate (PMA), while CTLL-2 did not. PMA induced PKC translocation from cytosol to membrane only in a PMA-responsive cell line. IL 2 failed to stimulate PKC translocation in both cell lines. H-7, a potent and specific PKC inhibitor, however, inhibited the proliferation of both cell lines induced by IL 2. Taken collectively, IL 2 may induce PKC activation without its translocation.

Interleukin 2 (IL 2), previously known as T cell growth factor, is realized to have a wide variety of actions on various types of cells including natural killer cells, lymphokine-activated killer cells, B cells and macrophages in addition to T cells (1). Biochemical studies have revealed the presence of two species of IL 2-binding molecules, i.e., Tac antigen and p75 on the surface of these cells (2). IL 2 receptors are converted into a high-affinity state when these two are combined. IL 2 at first binds with high-affinity IL 2 receptor or in some instance with p75 molecule. IL 2 is then internalized into cytosol fraction, which leads to its actions. The molecular events after the internalization, however, remains an open question.

Evidence has been accumulated that PKC plays an important role in the early step of signal transmission by neurotransmitters and hormones (3). PKC is present in solubilized cytosol fraction as an inactive form. When cells are stimulated with phorbol esters or physiological ligands, PKC is known to be translocated into membrane fraction and activated in the presence of phospholipid and diacylglycerol. The formation of diacylglycerol

[1]To whom all correspondence should be addressed.

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usually accompanies the breakdown of phosphoinositides (3,4). While Farrar et al. observed that PKC translocation occurs in a murine IL 2-dependent cell line in response to either PMA or IL 2 (5), this observation does not seem to be applicable to other IL 2-dependent cell lines. Only a small number of IL 2-dependent cell lines seem to proliferate in response to PMA (6, 7) and PKC translocation occurs coincidentally in PMA-responsive cell lines (8). Therefore, PKC translocation by IL 2 may be seen only in PMA-responsive IL 2-dependent cell lines and may not be generalized. To address this question, we utilized two murine IL 2-dependent cell lines which were of the same origin and differed in the responsiveness to PMA. Our results demonstrated that PMA induces PKC translocation only in a PMA-responsive cell line. In addition, we could not find a direct evidence that PKC translocation occurs in the presence of IL 2.

MATERIALS AND METHODS

Reagents: Recombinant IL 2 was a kind gift from Shionogi Pharmaceutical Company (Osaka, Japan). The complete medium consisted of RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 10 % fetal calf serum (FCS; Hyclone Laboratories, Logan, UT), 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Protein kinase inhibitors, H-7 and HA1004 were purchased from Seikagaku Kogyo (Tokyo, Japan). PMA (Sigma Chemical Co., St.Louis, MO) was dissolved in ethanol at the concentration of 1 mM and was stored at -20°C until use. All the other reagents were purchased from Sigma Chemical Co. unless otherwise indicated.

IL 2-dependent cell lines Both CT6 cell line (9) and CTLL-2 cell line (10) were established as IL 2-dependent cytotoxic T cell clones from C57BL/6 spleen cells. These cell lines were provided kindly to us through Dr. E. Shevach (NIADH, NIH, MD) and have been maintained on the complete medium containing 20 % rat factor (equivalent to 10 to 20 u/ml of IL 2) since 1983 in our laboratory as previously described (6).

Proliferation assay Proliferation was assayed by a rapid colorimetric assay described by Mosmann (11) with some modifications. Ten thousand cells were incubated in 96-well flat bottom microplate in 100 µl of the complete medium in the presence of the indicated concentrations of IL 2 or PMA. After 40 hr at 37°C, 100 µg of 3-(4,5-dimethylthiazol-2yl)2,5-diphenyl tetrazolium bromide (MTT) in 20 µl of phosphate buffered saline (PBS) was added into each well. After 4 hours, 200 µl of 0.04 N HCl in isopropranol was added and the absorbance at 570 nm was read.

Subcellular fractionation 5×10^6 cells in 1 ml of serum-free RPMI 1640 medium were stimulated with either 100 u/ml IL 2 or 20 nM PMA for the indicated time intervals at 37°C. Subcellular fractionation was then performed according to the method described by Abraham (12). All the samples were stored at -80°C until the measurement and their protein concentrations were determined according to the method of Bradford (13) using bovine serum albumin as the standard.

Determination of PKC activities PKC activities were determined in the same manner as previously described (14) except that the reaction was carried out for 5 min. PKC activity was expressed as the amount of the incorporation of phosphate from ATP into histone per milligram of protein per min.

RESULTS

Proliferative responses of IL 2-dependent cell lines to IL 2 and PMA We have chosen two murine IL 2 dependent cell lines, CT6 and CTLL-2 , both of which were derived from the same strain, C57BL/6 mice, to look at the response to IL 2 and PMA. Both CT6 and CTLL-2 cell lines showed a significant proliferative response to IL 2 with a similar manner (Table 1). However, only CT6 cell line showed a marked proliferative response to PMA, while CTLL-2 cell line failed to proliferate in response to PMA. An another murine IL 2-dependent cell line, NK clone 7 (provided by Professor Kumagai, Tohoku University, Japan) did not proliferate in the presence of PMA as well (data not shown). These results were consistent with our previous results (6), indicating that the PMA-responsiveness of CT6 was kept for at least 5 years and not a transient phenomenon.

Subcellular distribution of PKC in IL 2-dependent cell lines by stimulation with PMA or IL 2 We next examined the effects of IL 2 and PMA on the distribution of PKC in CTLL-2 and CT6. PMA failed to change PKC activity in both cytosol and membrane fraction of PMA-unresponsive CTLL-2 (Fig. 1A). Similarly, no apparent change in PKC activity of both fractions was observed in response to IL 2 (Fig. 1B). In contrast, on PMA-responsive CT6, PMA triggered the translocation of PKC activity from cytosol to membrane fraction rapidly during 5 to 10 min incubation, resulting in a complete transposition of the distribution of their activities (Fig. 2A). Under these conditions, IL 2 never induced such translocation (Fig. 2B). These results indicated that IL 2 did not trigger PKC translocation at least in these murine IL 2-dependent cell lines.

Effects of protein kinase inhibitors on IL 2-induced proliferation We looked at the effects of protein kinase inhibitors on IL 2-induced proliferation of these IL 2-dependent cell lines. Figure 3 showed that H-7, a

Table 1. Proliferative response of CT6 and CTLL-2 to IL 2 and PMA

Stimulants	Proliferation (net absorbance at 570 nm)[a]	
	CT6	CTLL-2
none	0.196 ± 0.026	0.009 ± 0.003
IL 2 100 u/ml	0.722 ± 0.180	1.023 ± 0.023
IL 2 10 u/ml	0.422 ± 0.018	0.623 ± 0.045
IL 2 1 u/ml	0.210 ± 0.024	0.112 ± 0.005
PMA 100 nM	0.738 ± 0.116	0.009 ± 0.005
PMA 20 nM	0.858 ± 0.102	0.007 ± 0.003
PMA 4 nM	0.506 ± 0.096	0.010 ± 0.004

a) Proliferation of CT6 and CTLL-2 was determined by a colorimetric assay using MTT and a net absorbance at 570 nm was expressed. Mean ± 1 S.D. in a quadriplicate experiment are shown.

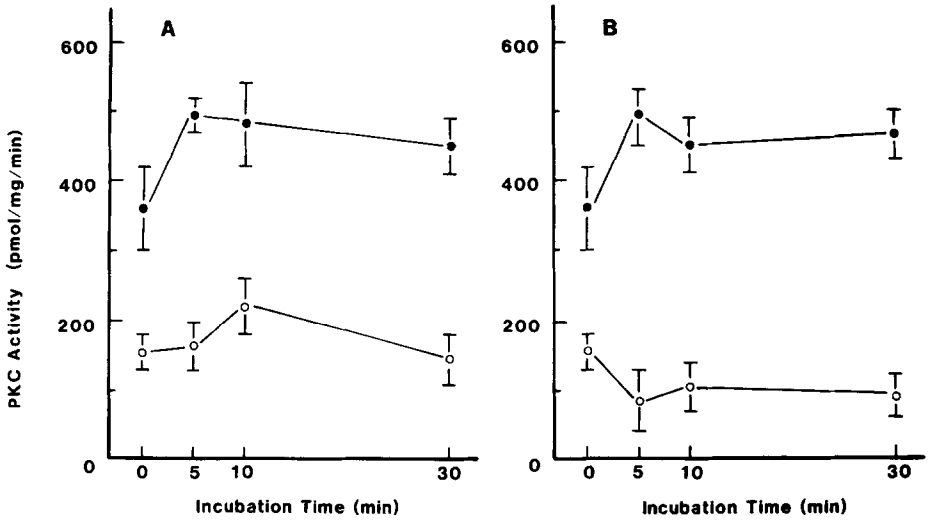

Fig.1. PKC activities of cytosols (closed circles) and membranes (open circles) of CTLL-2 was determined after the stimulation with PMA (A) and IL 2 (B) at the indicated time interval. Mean ± 1 S.D. in a triplicate experiment are shown.

potent PKC inhibitor with a Ki value of 6.0 μM (15), inhibited IL 2-induced proliferation of both cell lines at 25 μM, while a control drug, HA1004 (a Ki value of 40 μM), failed to inhibit the proliferation even at 50 μM. PMA-induced proliferation of CT6 cell line was also inhibited by H7 in a

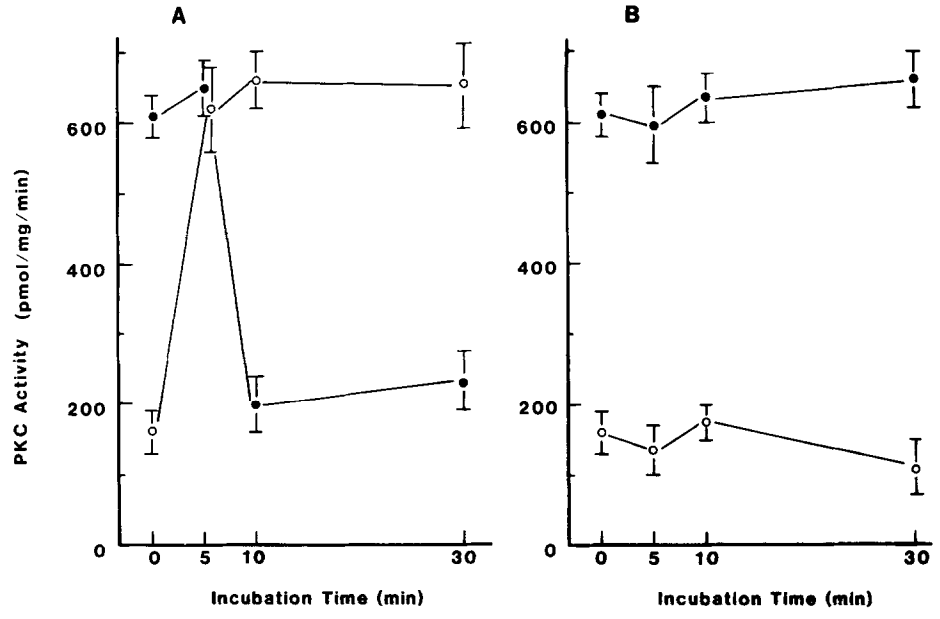

Fig.2. PKC activities of cytosols (closed circles) and membranes (open circles) of CT6 were determined after the stimulation with PMA (A) and IL 2 (B) at the indicated time interval. Mean ± 1 S.D. in a triplicate experiment are shown.

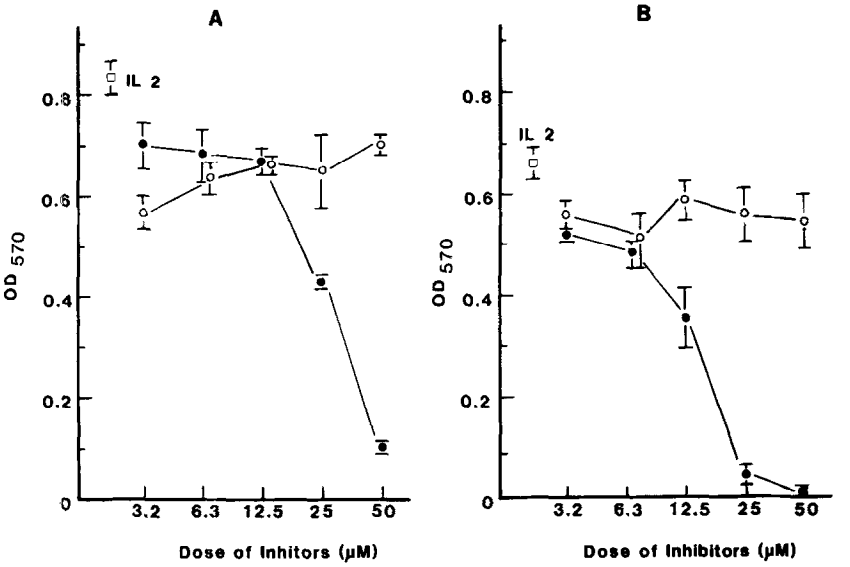

Fig.3. After treatment with H-7 (closed circles) and HA1004 (open circles) for 2 hr at 37°C, IL 2 was added to a final concentration of 10 u/ml. Then the proliferation of CT6 (A) and CTLL-2 (B) was measured. Mean ± 1 S.D. in a quadriplicate experiment are shown.

similar manner (data not shown). These results suggested that PKC activation but not PKC translocation was prerequisite for the IL 2-induced proliferation of these murine IL 2-dependent cell lines.

DISCUSSION

Receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate leads to the formation of inositol-1,4,5-trisphosphate ($InsP_3$), which serves as a mediator of Ca^{2+} mobilization from internal Ca^{2+} store (4). The other product of the hydrolysis, 1,2-diacylglycerol initiates the activation of Ca^{2+}- and phospholipid-dependent PKC without a net increase of Ca^{2+} concentration. PKC has been recognized to be identical with the receptor for tumor-promoters, phorbol esters (3). The treatment of cells with phorbol esters resulted in the translocation of PKC from cytosol to membrane fraction (16).

It was reported by Farrar et al. that IL 2 induced the hydrolysis of phosphoinositides (17) which led to the translocation of PKC (5). In contrast to their report, other investigators claimed that IL 2 failed to induce the hydrolysis of phosphoinositides (18, 19). In addition, since phorbol esters induced PKC translocation only in PMA-responsive cell lines (8) and only a minority of IL 2-dependent cell lines can respond to PMA, the cell line which translocates PKC in response to PMA may be an exceptional case. To address this point, we utilized two IL 2-dependent cell

lines which differed in the responsiveness to PMA. Our results show that both cell lines fail to translocate PKC from cytosol to membrane fraction with the stimulation by IL 2 and that PMA-induced PKC translocation occurs only in a PMA-responsive cell line. Like CT6 cell line, there are other types of cells in which only phorbol esters but not physiological ligands can translocate PKC (20-22).

That PKC inhibitor, H-7, but not HA1004, inhibited IL 2-induced proliferation of murine T cell lines is in concordance with a previous report (23). Accordingly, the level of PKC activity inhibitable by H-7 is necessary for the IL 2-induced proliferation but PKC translocation from cytosol to membrane may not be primarily necessary for its proliferation. Several proteins were reported to be phosphorylated by activation of protein kinases in response to IL 2 (24-27). The characterization of these proteins should help to elucidate the precise role of PKC and other types of protein kinases in IL 2-induced signal transmission.

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