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Modulation of intracellular Cl^- homeostasis by lectin-stimulation in Jurkat T lymphocytes

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Abstract

We investigated changes in intracellular Cl^- concentration ($[Cl^-]_i$) during lectin-induced activation and proliferation in human Jurkat T lymphocytes. $[Cl^-]_i$ was measured using Cl^- fluorescence dye (*N*-(6-methoyquinolyl) acetoxy-acetyl-ester, MQAE) methods. Lectins, phytohemagglutinin and concanavalin A, dose-dependently increased $[Cl^-]_i$ and triggered intracellular Cl^- oscillation in human Jurkat T lymphocytes. However, some mitochondria metabolism inhibitors, such as *m*-chlorocarbonylcyanide phenylhydrazone (CCP) and 2,4-dinitrophenol, increased $[Cl^-]_i$ without triggering any Cl^- oscillation. Furthermore, both lectins and metabolism inhibitors-induced elevation in $[Cl^-]_i$ were blocked by removal of extracellular Cl^- from perfusion solution or by application of anthracene-9-carboxylate, a blocker of Cl^- channels. Since an extracellular Cl^--free condition and application of 9-AC also inhibited PHA-induced proliferation, we suggested that elevation of $[Cl^-]_i$ via activation of Cl^- channels and increase in incidence of Cl^- oscillation would play an important role in modulation of Jurkat T cell activation and proliferation.

Keywords: Cl^- concentration, intracellular; Lectin; Jurkat T lymphocyte, human; Cl^--free condition; 9-AC; Stilbene derivative

1. Introduction

Intracellular Cl^- homeostasis was known to play an important role in excitation and other cellular or intracellular functions in many types of cells (Hume et al., 2000; Bretag, 1987; Lewis et al., 1993; Stakisaitis et al., 2001; Inoue et al., 1991; Nakamura et al., 1997). In cardiac muscle cells, using ion-microelectrode techniques, we observed the change in $[Cl^-]_i$ in guinea pig ventricular muscle during ischemia and reperfusion. Our studies showed that $[Cl^-]_i$ increased during ischemia and application of stilbene derivatives could suppress this elevation of Cl^- and decreased the ischemia- and reperfusion-induced injury (Lai and Nishi, 1998, 2000). In human T lymphocytes, it was known that disulfonic stilbene derivatives, such as SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid) and DIDS (4,4′-diisothiocyano stilbene-2,2′-disulfonic acid) have some inhibition effects on T lymphocyte activation and signalling (Stakisaitis et al., 2001; Phipps et al., 1996; Cardin et al., 1991; Schroeder et al., 1994) and inhibited thymocyte apoptosis (Tsao and Lei, 1996). Whereas, other reports showed that SITS and DIDS promoted T lymphocyte proliferation in T cells (Mix et al., 1992) and stimulated mitogenesis in B cells (Deane and Mannie, 1992). SITS caused a dose-dependent increase of spontaneous proliferative activity as well as of proliferation in response to the antigenic stimulus bovine peripheral myelin (Mix et al., 1992). In contrast, the drug caused a decrease of proliferation of cells stimulated with phytohemagglutinin. For explanation of mechanism of this Cl^- effect, activation of Cl^- channels and Cl^- transporters was speculated to maintain a sufficiently negative membrane potential to drive Ca^{2+} influx (Kerschbaum et al., 1997) and Cl^- blockers can affect intracellular Ca^{2+} signalling by modulation of $[Cl^-]_i$. However, the exact role of intracellular Cl^- homeostasis during T cell activation is still not investigated.

To investigate whether intracellular Cl^- plays any role in T cell activation, we observed change in $[Cl^-]_i$ after application of lectins in human Jurkat T lymphocytes using Cl^- fluorescence (*N*-(6-methoyquinolyl) acetoxy-acetyl-es-

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ter, MQAE) methods. Our results showed that $[Cl^-]_i$ and the incidence of Cl^- oscillation increased in lectin-activated human Jurkat T lymphocytes. Since this oscillation disappeared in a Cl^--free condition and lectin-induced increase in $[Cl^-]_i$ and proliferation in Jurkat T lymphocytes was blocked by Cl^- transporter blocker, we considered that intracellular Cl^- would play an important role in Jurkat T cell activation and proliferation.

2. Materials and methods

2.1. Preparation of Jurkat T lymphocytes

Jurkat T lymphocytes, a human T cell leukemia cell line which can be stimulated to produce interleukin-2 under a variety of conditions, were used in the present study, and cultured with RMPI-1640 medium with 10% fetal calf serum. The measurement of $[Cl^-]_i$ was made after 3-day culture.

2.2. Measurements of intracellular chloride concentrations

2.2.1. Loading Jurkat T lymphocytes with MQAE

The Jurkat T lymphocyte was resuspended in 5 mM MQAE (Dojindo Laboratories, Kumamoto, Japan) in Tyrode solution and loaded for 60–90 min at 37 °C. The Tyrode solution containing (in mM) 127 NaCl, 4.7 KCl, 0.25 $MgCl_2$, 1.2 KH_2PO_4, 5.5 glucose, 2.5 $CaCl_2$ was equilibrated with 5% CO_2/95% O_2 at 37 °C, and pH was adjusted with $NaHCO_3$ (20 to 25 mM) to 7.4. After loading, the suspension was centrifuged at 1000 × *g* for 15 min at 4 °C, and then, was resuspended in Tyrode solution with glucose and 0.1% bovine serum albumin, and kept on ice. An appropriate amount was subsequently centrifuged for each experiment. To see lectin-induced changes in $[Cl^-]_i$ in HCO_3^--free condition, a HEPES-buffered Tyrode solution was used in some experiments and contained (in mM) 140 NaCl, 5.0 KCl, 0.25 $MgCl_2$, 5.5 glucose, 1.5 $CaCl_2$, 10 HEPES and was equilibrated with 100% O_2 at 37 °C, and pH was adjusted to 7.4 with NaOH.

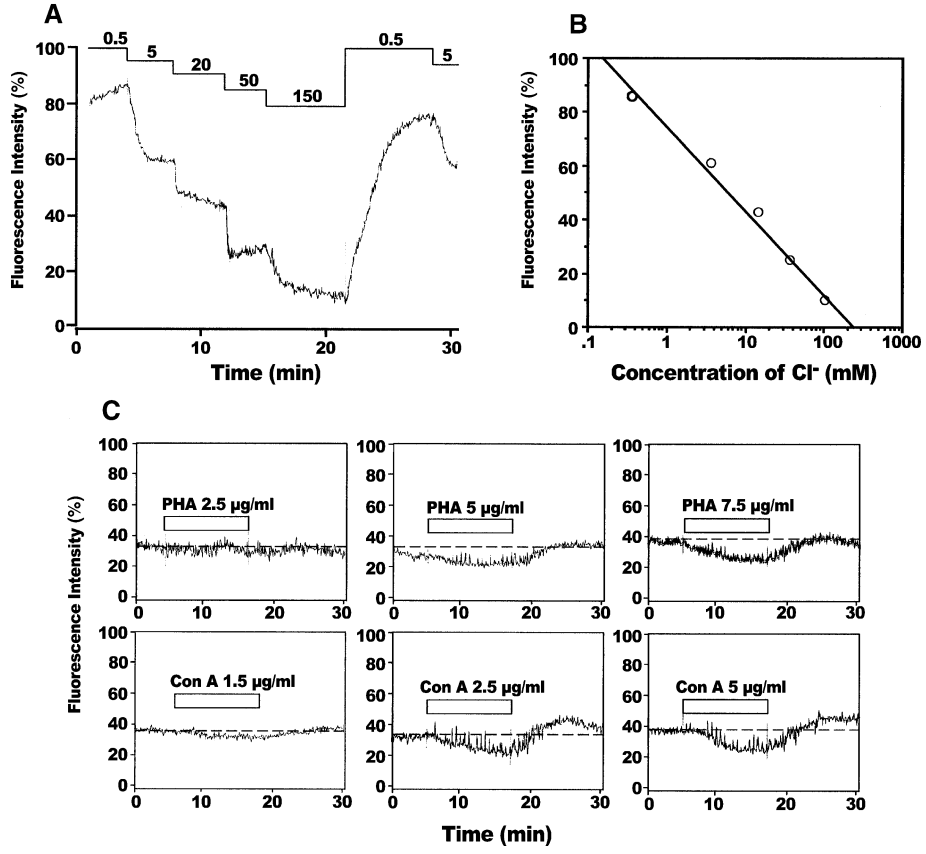

Fig. 1. Effects of lectins on intracellular Cl^- concentration ($[Cl^-]_i$) in human Jurkat T lymphocytes. (A) Change in MQAE fluorescence intensity after expose to different concentration of Cl^- in perfused solution in human Jurkat T lymphocytes. (B) Calibration of MQAE fluorescence for change of Cl^- concentration obtained from (A). (C) Effects of PHA (upper) or Con A (lower) on $[Cl^-]_i$; PHA and Con A, known as potential T cell activators which stimulated T cell signalling and proliferation, induced an increase in $[Cl^-]_i$. Dose-dependent increases in $[Cl^-]_i$ indicated by PHA from 2.5 to 7.5 μg/ml (upper), and by Con A from 1.5 to 5 μg/ml (lower) were shown in this figure. For chloride measurements, cultured Jurkat T lymphocytes were incubated with 5 mM MQAE (Dojindo Laboratories) for 60–90 min at 37 °C, then, the cells were washed with the Tyrone solution. And the cells were placed on a poly-L-lysine-coated mounted coverslip dish (35 mm diameter, MatTek) and incubated for 30 min. Then, microfluorometric measurements were made with an image processor system (ACOUSCosmos, Hamamatsu Photonics). The fluorescence intensity of MQAE was collected and stored on a MO disk every 4 s until analysis. PHA: phytohemagglutinin; Con A: concanavalin A.

2.2.2. Calibration of intracellular MQAE fluorescence

The T cells were resuspended in an experimental Cl^--free solution (NaCl was substituted by equimolar amounts of sodium gluconate, $CaCl_2$ by calcium gluconatemonohydrate, $MgCl_2$ by $MgSO_4$, KCl by potassium gluconate), centrifuged once and resuspended in a cuvette with 350 μl of Cl^--free solution plus 10 mM glucose, 0.1% bovine serum albumin. To prevent movement of cells during assay, the MQAE loaded Jurkat T cells were placed on a poly-L-lysine-coated mounted coverslip dish (35 mm diameter, MatTek Ashland, MA) and incubated for 30 min. Then, microfluorometric measurements were monitored using an image processor system (excitation, 355 nm; emission, 460 nm) (ACOUSCosmos, Hamamatsu Photonics, Hamamatsu, Japan) at room temperature (24 °C). The MQAE fluorescence image was collected and stored on a magneto-optical disk every 4 s until analysis. A fast solution change system was used to ascertain the rapid and even distribution of the added components.

Calibration of intracellular MQAE was performed by determining the correlation between fluorescence intensity and Cl^- concentration by a procedure modified from those described by Krapf et al. (1988). The maximal fluorescence in the absence of Cl^- was obtained by depleting intracellular Cl^- by adding the ionophores nigericin (7 μM) and tributylin acetate (10 μM) to the Jurkat T lymphocytes in the Cl^--free experimental medium. To see change of MQAE fluorescence intensity with different Cl^- concentrations in perfused solution, five different concentrations of Cl^- were made (0.5, 5, 20, 50, 150 mM) and added to the culture dish, respectively. Then the MQAE fluorescence intensities were recorded (Fig. 1A). At the end of experiment, 150 mM KSCN, in the presence of 5 μM of the K^+ ionophore valinomycin, was added to quench the fluorescence of MQAE. The SCN^- ion has a much high affinity for the indicator than Cl^- ion and therefore quenches most of the MQAE fluorescence at the given Cl^- concentration minus F_{KSCN}. The calibration cure of intracellular MQAE was obtained from data in Fig. 1B. The Cl^--free solution was made by replacement of Cl^- with equimolar gluconate as described previously (Lai et al., 1996; Lai and Nishi, 1998).

2.3. Lectin-induced proliferative response of the human Jurkat T lymphocytes

Proliferation response of the Jurkat T lymphocytes after application of lectins was investigated, as described (Kamikawaji et al., 1991). In brief, the cells (3×10^4) was cultured in the absence or presence of lectins, and then pulsed with 1.0 μCi/well of [^{3}H] thymidine for last 16 h. The cells were then harvested and the incorporated radioactivity was measured in a microbeta scintillation counter (Chen et al., 1996, 1997). To investigate the effect of extracellular Cl^- on proliferation response in the Jurkat T lymphocytes, Jurkat T cells was washed and cultured with normal Tyrode solution for 30 min or cultured with a Cl^--free solution for 30 min. Then, the cells were harvested and the incorporated radioactivity was measured as described above.

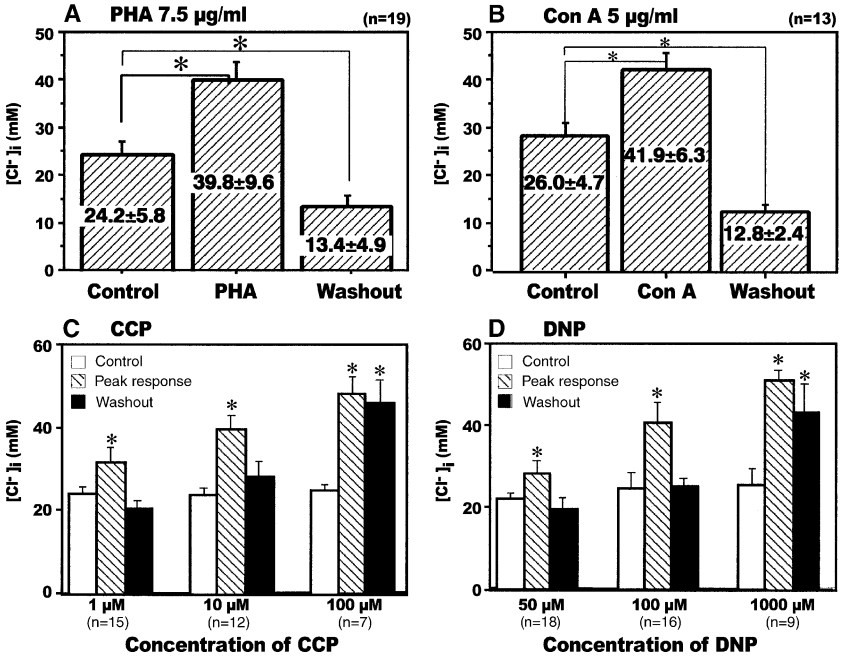

Fig. 2. Peak response of lectins – or mitochondrial metabolism inhibitors – induced increase in $[Cl^-]_i$ in human Jurkat T lymphocytes. (A and B) Effects of PHA (A) or Con A (B) in normal $NaHCO_3$-buffered Tyrone's solution on $[Cl^-]_i$ were shown. (C and D) *m*-Chlorocarbonylcyanide phenylhydrazone (CCP, C) and 2,4-dinitrophenol (DNP, D) increased $[Cl^-]_i$ in human Jurkat T lymphocytes. Numerals in parentheses indicated number of cells observed. *Significant differences from value obtained in control ($P < 0.05$).

2.4. Reagents and statistics

All salts for preparing solutions and poly-L-lysine, phytohemagglutinin, concanavalin A and other pharmacological agents were purchased from Sigma (St. Louis, MO). Sodium gluconate, potassium gluconate and calcium gluconate monohydrate were purchased from Wako (Osaka, Japan). Phytohemagglutinin, concanavalin A, CCP (*m*-chlorocarbonylcyanide phenylhydrazone) and anthracene-9-carboxylate were prepared as stock solutions with dimethylsulfoxide (DMSO). Drug stock solution was diluted in a volume of perfused solution to yield the final concentration of DMSO in used solution was $<0.5\%$, had no effect on MQAE fluorescence. The 2,4-dinitrophenol was prepared as stock solutions with ethanol and diluted with Tyrode solution to desired concentrations. Data were analyzed by basic statistical methods, including analysis of the two-tailed Student's *t*-test (unpaired), and expressed as the arithmetic means $\pm$ S.E.M. A $P<0.05$ value was considered to be statistically significant.

3. Results

3.1. Intracellular Cl^- concentrations increased during Jurkat T cell activation and its characteristics

To investigate the change of intracellular Cl^- homeostasis after lectin-stimulation in Jurkat T lymphocytes, effects of phytohemagglutinin (PHA) on $[Cl^-]_i$ were observed. As shown in Fig. 1C, in those T cells perfused with $NaHCO_3$-buffered Tyrode solution, PHA increased $[Cl^-]_i$ from a concentration at 2.5 μg/ml and showed its response on Cl^- with a dose-dependent manner. The concentration of PHA used in the present study was similar to those used to activate T cell activation in previous reports (Dupuis et al., 1989, 1993; Chang et al., 1993). After application of PHA, $[Cl^-]_i$ was significantly increased (the $[Cl^-]_i$ was 24.2 ± 5.8 mM before PHA, and 39.8 ± 9.6 mM after application PHA at a concentration of 7.5 μg/ml), then $[Cl^-]_i$ gradually returned to control or maintained at a low level. After washout of PHA, $[Cl^-]_i$ was a 13.4 ± 4.9 mM (see Fig. 2A, $n=19$, $P<0.05$).

Then, we investigated effects of concanavalin A (Con A), another T cell-activator, on $[Cl^-]_i$ in human Jurkat T lymphocytes. The results were similar to those cells after stimulation with PHA. Con A at a concentration 1.5 μM induced an elevation of intracellular Cl^- and this elevation of $[Cl^-]_i$ was concentration dependent. The peak response was obtained at concentration about 5 μg/ml and IC_{50} was about ~ 3.65 μg/ml. After application of Con A at a concentration 5 μg/ml, $[Cl^-]_i$ became to 41.9 ± 6.3 mM from 24.0 ± 4.7 mM art control level ($n=13$, Fig. 2B). There is a significant difference in the values obtained from application of Con A and pre-drug ($P<0.05$).

Similarly, mitochondrial metabolism inhibitors, such as *m*-chlorocarbonylcyanide phenylhydrazone (CCP) and 2,4-dinitrophenol, also increased $[Cl^-]_I$ in Jurkat T lymphocytes (Fig. 2C and D). The CCP and 2,4-dinitrophenol-induced increase in $[Cl^-]_i$ has a dose-dependent characteristic, and at

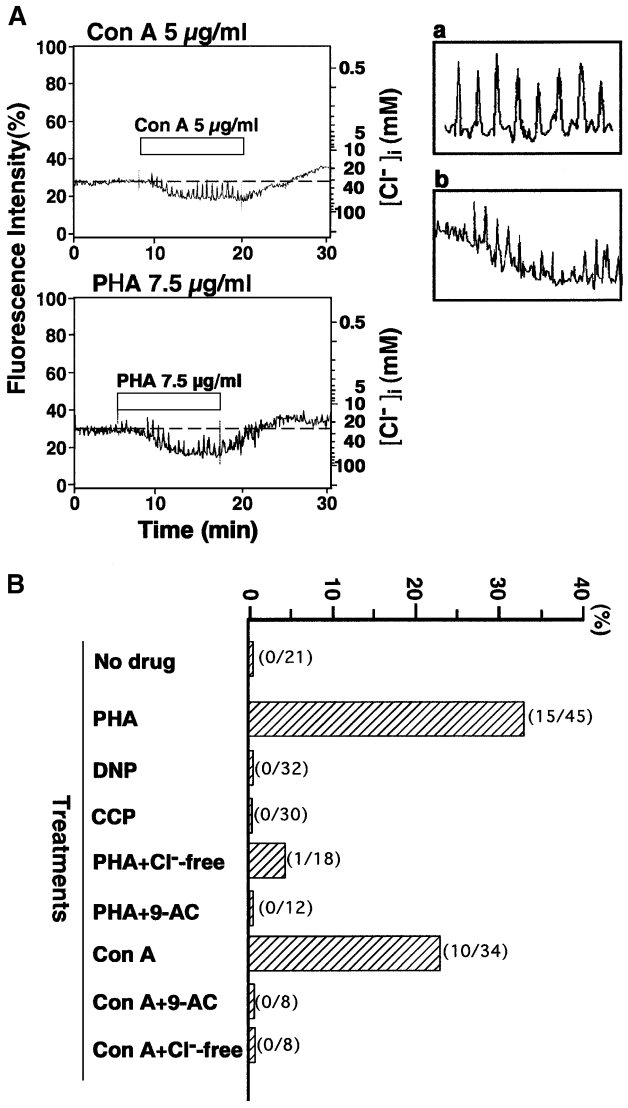

Fig. 3. Lectin-stimulation increased incidence of Cl^- oscillation in human Jurkat T lymphocytes. (A) Representative Cl^- oscillation waves recorded after application of lectins, including concanavalin A (Con A) and phytohemagglutinin (PHA). Con A (upper) or PHA (lower) triggered Cl^- oscillation in a HCO_3-buffered Tyrone solution and the enlarged oscillation waves were showed in inserted figures a and b. The frequency of Cl^- oscillation was ~ 1 Hz and the peak amplitude of oscillation arrived 10–15 mM. (B) Percentage of Jurkat T cells with occurrence of Cl^- oscillation after various treatments. The treatments included lectins, mitochondrial metabolism inhibitors, and lectins plus Cl^- blocker or lectins in a Cl^--free condition. The hitch bars are percentage of Jurkat T cells with occurrence of Cl^- oscillation. Numerals in parentheses indicated number of cells with Cl^- oscillation/numbers of cells observed in each treatment. PHA, phytohemagglutinin; DNP, 2,4-dinitrophenol; CCP, *m*-chlorocarbonylcyanide phenylhydrazone; Cl^--free, treatment in a Cl^--free condition; 9-AC, anthracene-9-carboxylate; Con A, concanavalin A.

high concentrations, their response sustained even though the cells were washed with normal Tyrode solution for 10 min.

3.2. Lectins induced an oscillation change in intracellular Cl^- concentration in human Jurkat T lymphocytes

In ~ 30% (15/45 in PHA and 10/34 in Con A, see Fig. 3) cells, the Cl^- increase induced by Con A (Fig. 3A, upper trace) and PHA (Fig. 3A, lower trace) showed an oscillation characteristic, and the frequency of Cl^- oscillation was ~ 1 Hz and the peak amplitude of oscillation arrived 10–15 mM. Interestingly, this frequency of Cl^- oscillation was similar to the ligand-induced and temperature-dependent Cl^--current oscillation reported in *Xenopus* oocytes (Hulsmann et al., 1998; Miledi et al., 1987; Parker et al., 1985). The Cl^- oscillation in the present study disappeared when $[Cl^-]_i$ returned to the control levels. Furthermore, in two conditions, the Cl^- oscillation did not appear (see Fig. 3B). First, in an extracellular Cl^--free condition made by replacement of Cl^- with gluconate, the $[Cl^-]_i$-elevation was significantly suppressed and the Cl^- oscillation was not observed ($n = 18$). Second, we observed effects of metabolism inhibitors, CCP or 2,4-dinitrophenol, on changes in $[Cl^-]_i$ and results showed that CCP or 2,4-dinitrophenol increased $[Cl^-]_i$ in a concentration-dependent manner. CCP began to increase $[Cl^-]_i$ at a concentration of 1 μM and got a peak response at 100 μM (Fig. 2C). The 2,4-dinitrophenol began to increase $[Cl^-]_i$ at a concentration of 50 μM and got a peak response at 1 mM (Fig. 2D). These concentrations of CCP and 2,4-dinitrophenol were similar to those concentrations used to inhibit the metabolism of mitochondria in cardiac myocytes and other tissues. However, as shown in Fig. 3B, there was no Cl^- oscillation observed in those T cells which mitochondrial metabolism was inhibited by CCP or DNP, even though they increased $[Cl^-]_i$ in Jurkat T lymphocytes as previous reported in rat lactotrophs (Garcia et al., 1997).

3.3. The lectins-induced elevation of intracellular Cl^- in T lymphocytes does not only occur in HCO_3^--rich solution but also in a HCO_3^--free condition

To investigate whether the activation of some HCO_3^--dependent mechanisms are involved in the lectins-induced elevation of $[Cl^-]_i$ in Jurkat T lymphocytes, we observed the effects of PHA and Con A on $[Cl^-]_i$ in both HEPES (nominally HCO_3^--free) and $NaHCO_3^-$-buffered (HCO_3^--rich) conditions. As shown in Fig. 4, both PHA and Con A increased $[Cl^-]_i$ in a HEPES-buffered or in a $NaHCO_3^-$-buffered solution. However, the recovery of $[Cl^-]_i$ after washout the drug was different. In those T cells perfused with a $NaHCO_3$-buffered solution, the $[Cl^-]_i$ level gradually decreased and finally became to a range lower than that at control condition. In the T cells perfused with a HEPES-buffered solution, the peak response of $[Cl^-]_i$ was smaller than that in those T cells perfused with a $NaHCO_3$-buffered solution (Fig. 4).

3.4. Cl^--free solution inhibited the increase in intracellular Cl^- induced by PHA

To investigate whether influx of extracellular Cl^- involved the lectin-induced elevation of $[Cl^-]_i$, we chose gluconate as a Cl^- replacement in perfused solution and then observed effects of PHA on $[Cl^-]_i$ in a Cl^--free condition. After perfusion with a Cl^--free solution, the lectin-induced Cl^- elevation was significantly suppressed and $[Cl^-]_i$ gradually decreased to a low level at 3.5 ± 1.2

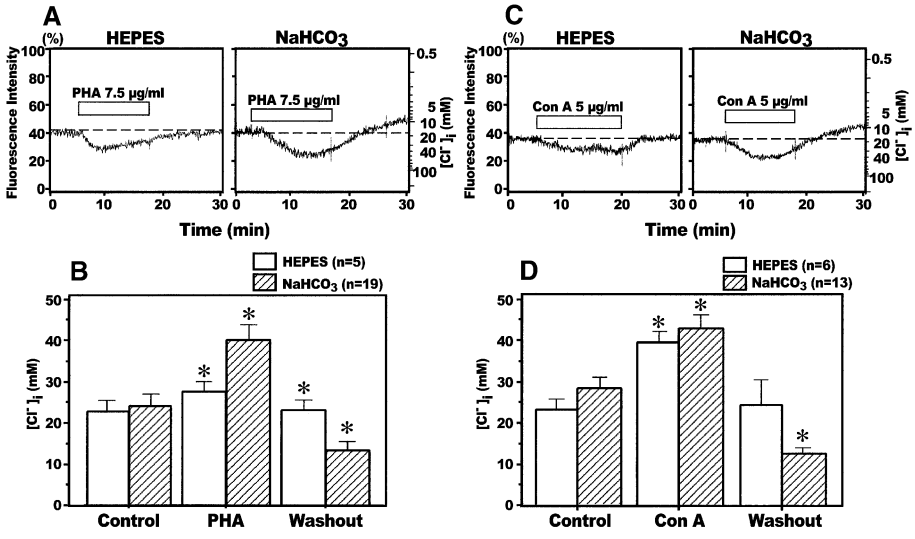

Fig. 4. The lectin-stimulated increase in $[Cl^-]_i$ was observed in both HEPES- and $NaHCO_3$-buffered solution after application of PHA or Con A. (A and B) Effects of PHA (7.5 μg/ml) on $[Cl^-]_i$. PHA increased $[Cl^-]_i$ in both HCO_3^--free and HCO_3^--rich solution. However, in HCO_3^--rich solution, the Cl^- gradually decreased and became to a level lower than that in control level. (C and D) Effects of Con A (5 μg/ml) on $[Cl^-]_i$ in HCO_3^--free and HCO_3^--rich solution. Similar result was obtained after application of Con A.

mM ($n=6$). There was a significant difference between control of cells with a normal Cl^- condition and the cells treated with a Cl^--free solution (compared with the value obtained before application of Cl^--free solution, $P<0.05$). However, under the Cl^--free condition, PHA still triggered an increase in $[Cl^-]_i$, even if this increase was slight and transient (Fig. 5B). Similar results were obtained from other six experiments.

3.5. Lectin-induced T cell proliferation was inhibited in a Cl^--free condition

As shown in Fig. 6A, the PHA (10 μg/ml)-induced increase in T cell proliferation was significantly inhibited when T cells were perfused with a Cl^--free solution. And the proliferative response almost did not occur in a Cl^--free only (no drug) condition (Fig. 6A). Furthermore, similar results were also obtained in the Con A-stimulated Jurkat T lymphocytes. The inhibiting rate was ~66.32% and 67.21%, respectively (data not shown).

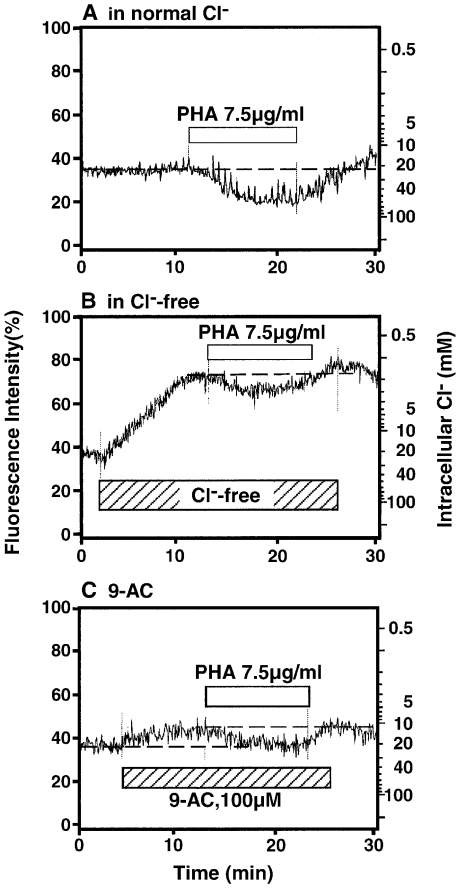

Fig. 5. Inhibition by Cl^--free solution and Cl^- blockers of the lectin-stimulated increase in $[Cl^-]_i$. (A) Application of PHA (7.5 μg/ml) in a normal Cl^- condition. Jurkat T cell was perfused with a normal Tyrode solution and the MQAE fluorescence intensity was analyzed, and then PHA was added at ~12 min for 10 min. (B) Application of PHA in a Cl^--free condition, which were made by replacing Cl^- with iso-osmotic gluconate. When MQAE intensity obtained a stable level, 7.5 μg/ml PHA was added for 10 min. (C) Pretreatment with 9-AC (100 μM), a Cl^- blocker, inhibited the PHA-induced increase in $[Cl^-]_i$ in a Jurkat T cell perfused with normal Cl^- Tyrode solution. Data were obtained from different Jurkat T cells and showed that both Cl^--free solution and 9-AC suppressed the PHA-induced increase in $[Cl^-]_i$ in Jurkat T cells.

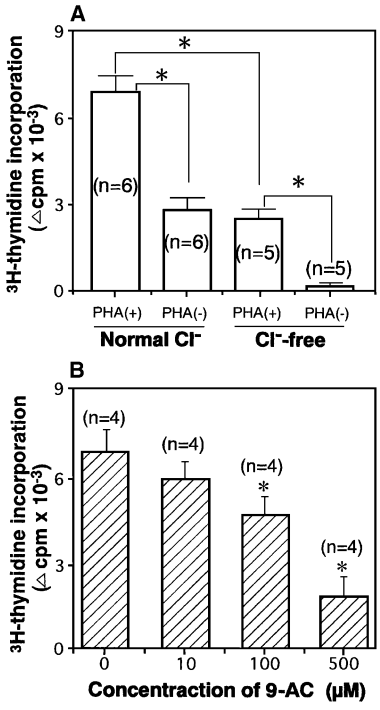

Fig. 6. Effects of a Cl^--free solution or 9-AC, a Cl^- blocker, on the lectin-stimulated increase of proliferation in Jurkat T lymphocytes. (A) Inhibition by Cl^--free solution of the lectin (PHA)-stimulated proliferation. Numerals in parentheses indicated number of experiments. PHA(+), treatment with phytohemagglutinin 10 μg/ml; PHA(−), without phytohemagglutinin. (B) Effects of anthracene-9-carboxylate on lectin (PHA)-stimulated proliferation. Data showed that application of anthracene-9-carboxylate inhibited the [^{3}H]-thymidine incorporation. *Significant differences from values obtained in PHA-stimulated cells only or between indicated bars ($P<0.05$). Similar results were obtained from four different experiments.

3.6. Effects of 9-AC on PHA-induced changes in $[Cl^-]_i$ and in [^{3}H]-thymidine incorporation

Since previous reports showed that anthracene-9-carboxylate (9-AC) suppressed the PHA-activated Cl^- currents in guinea pig ventricular myocytes (Shuba et al., 1996), we investigated whether 9-AC had any effects on lectin-induced changes in $[Cl^-]_i$ and proliferative response in the T cells. Data showed that 9-AC inhibited PHA-induced increase in $[Cl^-]_i$ (Fig. 5C) and suppressed PHA-stimulated proliferation (Fig. 6B) in Jurkat T lymphocytes with a concentration-dependent manner. At a concentration of 9-AC (500 μM), the proliferation response become 28.9% of that in PHA only (Fig. 6B, $P<0.05$).

4. Discussion

In the present study, we described changes in intracellular Cl^- homeostasis after application of PHA or Con A to stimuli T cell activation in human Jurkat T lymphocytes and our findings included: (1) $[Cl^-]_i$ and incidence of Cl^- oscillation increased during lectin-stimulation in Jurkat T lymphocytes. (2) both removal of extracellular Cl^- in medium and application of 9-AC inhibited lectins-induced Cl^- increase and T lymphocyte proliferation. Our study suggested that this change in Cl^- homeostasis would in partly respond to Jurkat T lymphocyte activation and intracellular Cl^- would play an important role in Jurkat T lymphocyte activation and proliferation.

Oscillatory signals in numerous biological systems have long been recognized and the most striking examples are oscillation in membrane potentials seen in neuronal and cardiac cells (Fewtrell, 1993). In the present study, it is interesting that Cl^- oscillations occurred in ~ 30% T cells after application of lectins and the frequency or amplitude was similar to some Cl^--current oscillations in previous reports which showed ligand-induced (Hulsmann et al., 1998) or temperature-dependent (Miledi et al., 1987; Parker et al., 1985) in *Xenopus* oocytes. Because our experiments were done under a constant temperature condition, the mechanism for Cl^- oscillation in Jurkat T lymphocytes may be not a temperature-dependent one but involved in some ligand-induced changes. One possible mechanism maybe similar to the mechanism in the oscillation Cl^- currents that occurred in *Xenopus* oocytes (Hulsmann et al., 1998; Miledi et al., 1987; Parker et al., 1985), which was known induction by elevations of intracellular inositol 1.4.5-triphosphate (IP_3) content, and intracellular injection of inositol 1,4,5-trisphosphate evoked an oscillatory membrane current. The receptor activation may result in the increase hydrolysis of phosphatidylinositol 4,5-bisphophate to produce inositol 1.4.5-triphosphate, which then causes mobilization of Ca^{2+} from internal stores. This released Ca^{2+}, in turn, activates the membrane Cl^- channels. Another possibility is that the internal massager system has some controlling mechanisms, including a feedback element, to control the Cl^- channels. Therefore, the system settles to a new equilibrium level following an oscillatory time course when perturbed. In the present study, we have not investigated whether modulation of inositol 1.4.5-triphosphate signalling was involved in controlling of Cl^- oscillation in Jurkat T lymphocytes or not because some reports that showed spontaneous activities in Jurkat T lymphocytes were not mediated by the usual TCR signaling or T cell activation pathways. However, if change of Cl^- oscillation in Jurkat T cell tumor line is a modulator on intracellular Ca^{2+} response, it also implied that $[Cl^-]_i$ would be taken as an important step for regulation of T cell activation and proliferation in other normal mature human T lymphocytes.

Indeed, at current moment, it is still not known what mechanisms are involved in lectin-induced Cl^- oscillation in Jurkat T lymphocytes. Alternatively, our data showed that cell metabolism inhibitors, 2,4-dinitrophenol and CCP significantly increased $[Cl^-]_i$ in Jurkat T lymphocytes and the results were similar with previous reports in cardiac guinea pig ventricular cells (our recent data, unpublished) or in rat lactotrophs (Garcia et al., 1997). However, the oscillation of intracellular Cl^- did not appear in CCP and 2,4-dinitrophenol-induced increase in $[Cl^-]_i$ (see Fig. 3B). Based on this fact, it is possible that formation of oscillation of intracellular Cl^- would depend on some influxes of extracellular Cl^- but not on reuptake of Cl^- to mitochondria. Furthermore, our data showed that the peak of $[Cl^-]_i$ after washout of lectins in a $NaHCO_3$-buffered condition was different from that in a HEPES-buffered condition (see Fig. 4). This result implied that some HCO_3^--dependent Cl^- transporter may be activated during Jurkat T cell activation and resulted in Cl^- efflux in $NaHCO_3$-buffered condition while this activation of Cl^- transporter is inactivated in HEPES-buffered condition.

The fact that a Cl^--free solution and application of 9-AC blocked the T cell proliferation, the Cl^- elevation indicates that intracellular Cl^- homeostasis plays an important role in T cell activation. This hypothesis is also supported by many previous studies which showed that Cl^- removal inhibited thapsigargin-stimulated Ca^{2+} response in antigen-specific mouse T cells (Kerschbaum et al., 1997) or suppressed the hypoxia and reoxygenation-induced Ca^{2+} overload in rat cardiac myocytes (Kawasaki et al., 2001). In conclusion, our findings suggest that $[Cl^-]_i$ increased during T cell activation and removal of extracellular Cl^- from cultured medium or application of some Cl^- blockers, such as 9-AC, decreased the elevation of $[Cl^-]_i$ and suppressed the Jurkat T cell proliferation. To understand the mechanisms involved in modulation of intracellular Cl^- homeostasis in T cell activation and proliferation, further investigations in mature human T lymphocyte isolated from peripheral blood or in cultured T cells need to be undertaken.

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