J. Membrane Biol. 120, 185–190 (1991)
0022263191000456

The Journal of
Membrane Biology

Voltage-Gated K^+ Channels in the Mouse Interleukin 3-Dependent Cell Line, FDC-P2

Hiroshi Tanaka†*, Yumiko Ohta†, Tsuneaki Sugimoto‡, and Yoshihisa Kurachi‡§

†The Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247, Japan, ‡The 2nd Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113, Japan, and §Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic, Mayo Foundation, Rochester, Minnesota

Summary. The electrical properties of a mouse interleukin (IL)-3-dependent cell line, FDC-P2, were examined using the tight-seal whole-cell clamp technique. Under current clamp conditions with 140 mM K^+ in the pipette, the cells had a resting potential of ~ -30 mV. Under voltage-clamp conditions, a transient outward current was elicited upon depolarization from a holding potential of -80 mV. The current was activated at potentials more positive than -10 mV and had a delayed-rectifying property. It showed rapid activation and slow inactivation during command steps. The current was abolished by Cs^+ in the pipette, indicating that K^+ is the charge carrier. The K^+ current was suppressed by tetraethylammonium with K_i of <0.1 mM and was not affected by scorpion toxin. Recovery from inactivation was steeply voltage dependent: As the holding potential was more hyperpolarized, the recovery became faster. Thus, with a holding potential of -80 mV, the current showed slight use-dependent inactivation, while the current decreased prominently by repetitive depolarization at a holding potential of -40 mV. These properties of the K^+ current are similar to those of the *l*-type K^+ channel current in mature T lymphocytes. The K^+ current in FDC-P2 cells was dramatically reduced after culture in the IL-3-free medium for 1–2 days. When IL-3 was re-added to the medium, the current was re-expressed. These observations suggest that expression of the K^+ current depends on extracellular IL-3, and that the current may play some roles in proliferation of these cells.

Key Words FDC-P2 · interleukin 3 · voltage-dependent K^+ channel

Introduction

Recent studies using the patch-clamp technique have revealed that various ionic channels are expressed in cells of the immune system: The ensemble of K^+ channels expressed by T lymphocytes changes in a stereotyped way during development. Even after the cells have matured, their electrical activity changes as mature cells interact with mitogens (Decoursey et al., 1984; McKinnon & Ceredig, 1986; Lewis & Cahalan, 1988*a*,*b*). It has also been reported that adhesion to a solid substrate alters the electrical properties of macrophages (Ypey & Clapham, 1984). These studies indicate that, during both the primary phase of differentiation and during subsequent responses to environmental stimuli, the electrical phenotype of cells in the immune system undergoes changes that may be related to cellular behavior.

Interleukin 3 (IL-3) is a lymphokine produced by activated T cells which has been shown in vitro to support the survival, growth, and development of multipotent stem cells and progenitor cells of multiple lineages, including mast cells, granulocytes, macrophages, erythrocytes, and megakaryocytes (Ihle et al., 1983; Rennick et al., 1985; Ihle & Weinstein, 1986). Several IL-3-dependent myeloid cell lines which require IL-3 for their survival and growth have been established in vitro from bone marrow, spleen, or fetal liver culture (Ihle & Weinstein, 1986; Koyasu et al., 1987). These cell lines have been used for the study of IL-3 receptor structure and its signal transduction mechanism. However, the characteristics and functional importance of IL3-induced currents in these cells have not been defined.

FDC-P2 is an IL-3-dependent cell line, a promyelocyte-like cell derived from mouse bone marrow (Dexter et al., 1980). In the present work, we studied the ionic current of FDC-P2 and FDC-P2(-) cells. We found a transient outward K^+ current in FDC-P2 cells which has characteristics similar to the *l*-type K^+ current in T-lymphocytes. The density of the K^+ current in FDC-P2 cells varied, depending on whether or not IL-3 was in the culture medium, which suggests that the K^+ current may play some role in proliferation of the cells.

* *Present address:* Biological Research Laboratories, Lederle (Japan), LTD., 1-6-34, Kashiwa-cho, Shiki-shi, Saitama 353, Japan.

Materials and Methods

Cell Lines

The IL-3-dependent cell line, FDC-P2, was maintained in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (FCS) (Flow Laboratories, McLean, VA) and 10% conditioned medium (CM) or WEHI-3 cells. The FDC-P2 cell line was donated by Dr. K. Kumagai (Tohoku University, Sendai, Miyagi, Japan). All cells were cultured in an incubator with 5% CO_2 and 95% air. Log-phase cultures were used. When cells were incubated in IL-3-free culture medium, 10 μg/ml of F9 antibody (Ohta et al., 1988; Sugawara et al., 1988) was added to the medium to maintain the cells.

Current Measurements

Membrane current and membrane potential were recorded with glass pipettes in the tight-seal whole-cell recording configuration (Hamill et al., 1981) through a patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, FRG). A heat-polished patch pipette, filled with an artificial internal solution (for composition, *see below*), had a tip resistance of 5–7 MΩ. Membrane current and membrane potential were monitored with a high-gain storage oscilloscope (COS5020-ST, Kikusui Electronic, Tokyo, Japan). The series resistance was compensated at the start of each experiment.

Solutions

The control bathing solution contained (in mM): NaCl 136.5, NaH_2PO_4 0.33, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES/NaOH buffer 5.5 (pH 7.4). The pipette solution contained (in mM): KCl 140, $MgCl_2$ 0.5, EGTA 5, and HEPES-KOH buffer 5 (pH 7.2). In the Cs^+-rich pipette solution, KCl in the pipette solution was replaced with equimolar CsCl. The pH of the solution was adjusted to 7.2 with CsOH.

Drugs

Mouse IL-3 was purchased from Genzyme (Boston). Scorpion toxin and tetraethylammonium were from Sigma (St. Louis).

Data Analysis

The data were stored on video tape using the PCM converter system (RP-880, NF electronic circuit design, Tokyo, Japan). The data were reproduced, low-pass filtered at 1 kHz (−3 dB) by a Bessel filter (FV-625A, NF, 48 dB/octave attenuation), sampled at 5 kHz, and analyzed with a computer (PC-9801 VM2, NEC, Tokyo, Japan). Linear leakage and capacitive transient currents were subtracted for most current traces during experiments. Statistical data were expressed as mean ± SD.

Results

Membrane Currents of FDC-P2 Cells

Under current clamp conditions with 140 mM K^+ in the pipette, the resting membrane potential of the cells was -27 ± 8 mV ($n = 7$). Action potentials could not be elicited with either depolarizing or hyperpolarizing current pulses. The capacitance of FDC-P2 cells and the input resistance of the cell/pipette assembly (the sum of the input resistance, the seal resistance, and the uncompensated series resistance) measured at −80 mV under voltage-clamp conditions were 9.3 ± 1.3 pF ($n = 38$) and 1.6 ± 0.7 GΩ ($n = 38$), respectively.

Under the voltage-clamp condition, voltage steps from a holding potential of −80 mV elicited a component of outward current when the pipette was filled with 140 mM K^+ (Fig. 1*A*). Upon depolarization, an outward current was activated and reached its peak within 10 msec during the command pulses to potentials more positive than −10 mV. The outward current gradually declined to a steady level. The decay time of the transient component of the outward current to $1/e \times$ peak value was 100–200 msec at potentials between 0 and +60 mV. Figure 1*B* shows the current-voltage relationship of the outward current. Filled circles show the initial peak current-voltage relationship measured at the peak of the outward current at each potential. Open circles are the points measured 1 sec after the onset of each voltage jump. The peak current and the steady-state current both increased with depolarization (Fig. 1*B*). The transient outward current was observed in all FDC-P2 cells examined (more than 150 cells). The current density at +30 mV was 14.5 ± 3.7 μA/cm^2 ($n = 42$) (*see also* Fig. 5).

Since this current deactivated rapidly (<1 msec), the reversal potential could not be accurately measured. When KCl was substituted for CsCl in the pipette solution, the outward current was completely abolished. With Cs^+ in the pipette solution, no voltage-dependent inward current, e.g. Na^+ and Ca^{2+} current, was detected. It was also observed that the outward current was not affected by substituting Cl with aspartate in the pipette solution (*not shown*). These observations indicate that the main charge carrier of the outward current is K^+.

Effects of Tetraethylammonium and Use Dependency of the K^+ Current

K^+ channels in T lymphocytes have been classified into three different types, i.e., n, n', and l, based on their differences in sensitivity to TEA and in the use-dependent inactivation (Lewis & Cahalan, 1988*a*,*b*). We examined the effects of extracellular TEA and repetitive depolarizing stimulation on the K^+ current in FDC-P2 cells (Figs. 2 and 3).

As shown in Fig. 2, 0.1 mM TEA effectively inhibited both the peak and the steady level of the K^+ current; the peak current at +30 mV was re-

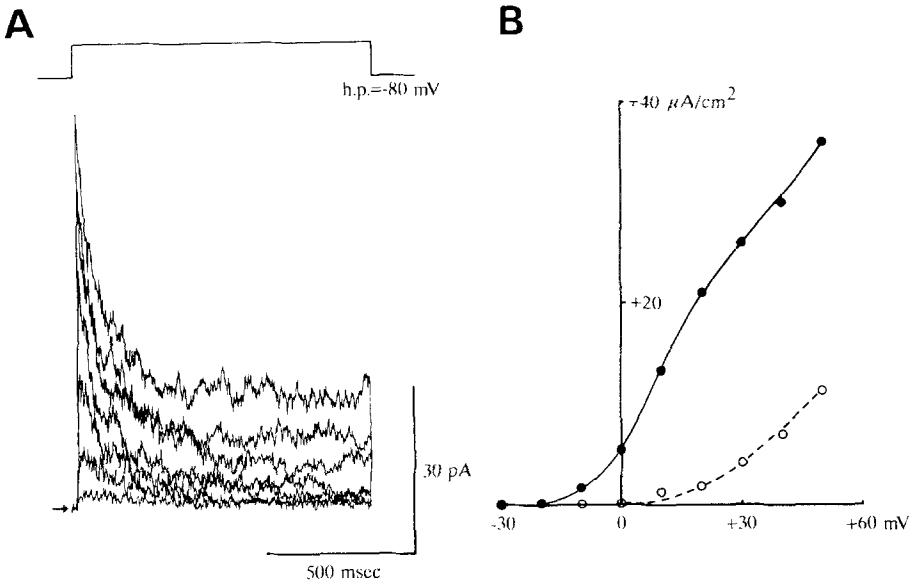

Fig. 1. Voltage-gated K^+ current in FDC-P2 cells. (*A*) Original current traces. The holding potential (*h.p.*) was −80 mV. Depolarization voltage steps (duration 1 sec) were applied to the cell by 10-mV steps from −70 to +50 mV. The elicited currents between −10 and +50 mV were superimposed. The interval of the command pulses was 20 sec. The capacitive and leak currents were subtracted. The voltage protocol is indicated above the current traces. The arrow indicates the zero current level. (*B*) The current-voltage relation of the outward current. Filled circles are the peak current at each command pulse. Open circles are the current levels at the end of the command pulses. The densities of the outward current were calculated from the cell capacitance, assuming that the cell membrane capacitance is 1 $\mu F/cm^2$

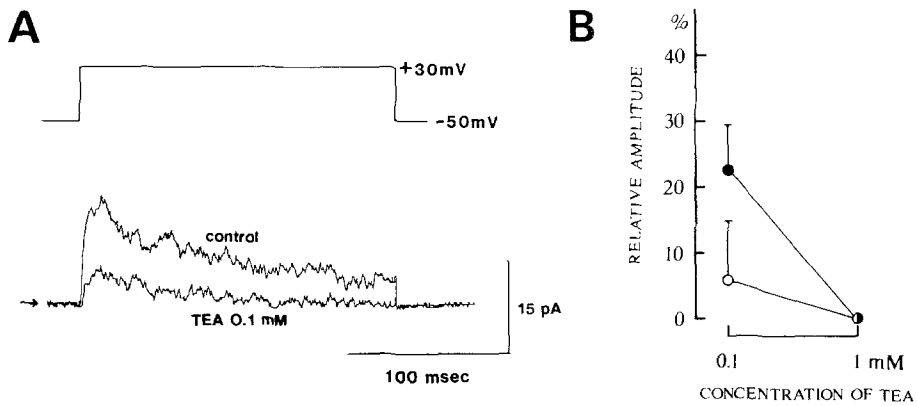

Fig. 2. Effects of TEA on the K^+ current in FDC-P2 cell. (*A*) The control outward K^+ current and the current under perfusion of 0.1 mM TEA elicited by depolarizing voltage steps to +30 mV from the holding potential of −50 mV were superimposed. The voltage protocol was indicated above the current traces. The arrow indicates the zero current level. The capacitive and leak currents were subtracted. (*B*) Relative amplitudes of the peak current and the current at 200 msec after the onset of the command steps to +30 mV while perfusing 0.1 or 1 mM TEA-containing solution. The relative amplitudes of the currents were expressed in percentages with reference to the values in the control bathing solution. Mean ± SD (n = 4)

duced to 22.6 ± 7.0% (n = 4) of the control and the current level at the end of the command pulse (200 msec) was reduced to 6.0 ± 9.0% (n = 4). The K^+ current was completely suppressed by 1 mM TEA in these cells. We also observed that scorpion toxin (100 μg/ml) which contains charybdotoxin did not affect the K^+ current in three cells (*not shown*).

Figure 3 shows use-dependent inactivation of the K^+ current in FDC-P2 cells. The upper traces are typical examples of the currents evoked by repetitive voltage steps to +30 mV with a holding potential of −80 mV (left) and −40 mV (right). A train of nine sequential voltage steps (200 msec in duration, 800 msec interval between the steps) was applied to a cell at each holding potential. At 30–40 sec after the holding potential was set at −80 or −40 mV, each train was started. In this example, with a holding potential of −80 mV, the reduction of the out-

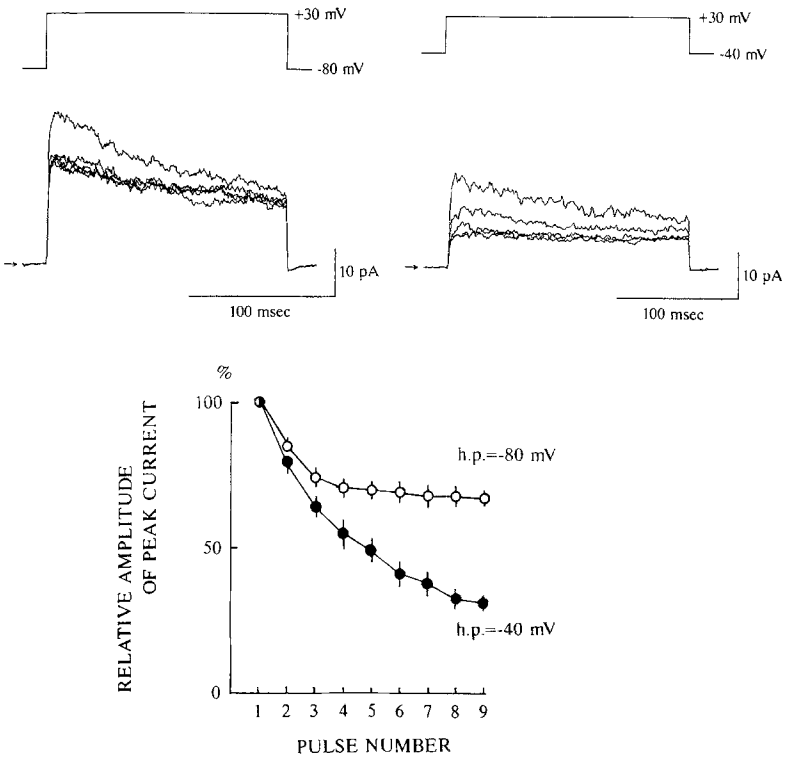

Fig. 3. Voltage-dependence of use-dependent inactivation. *Upper traces:* Trains of 9 voltage steps to +30 mV (200 msec in duration, 800 msec between the pulses) were applied to a FDC-P2 cell at intervals of 30 sec with a holding potential of −80 mV (left traces) and −40 mV (right). The currents elicited by the first, third, fifth, seventh, and ninth voltage pulses in each set were superimposed. The voltage protocols were indicated above the current traces. The arrows indicate the zero current level. *Lower graph:* The relative amplitude (%) of peak current of each pulse in a set with reference to that of the first step in each set was plotted at each pulse. The holding potential is indicated. Mean ± SD (n = 10)

ward current during the repetitive depolarizing pulses was not prominent. When the holding potential was changed to −40 mV, the peak current and the current at the end of the pulse decreased to 60 and 50% of those at −80 mV, respectively. At −40 mV, the repetitive voltage steps to +30 mV caused prominent reduction of the outward current.

In the lower graph of Fig. 3, alterations of the relative amplitude of the initial peak current at +30 mV with a holding potential of −80 mV (open circles) and −40 mV (filled circles) were shown. The data were obtained from 10 different FDC-P2 cells. As indicated in Fig. 3, with a holding potential of −80 mV, the peak current decreased only to 70% at the ninth step, while it decreased to 30% with a holding potential of −40 mV. These results indicate that the use-dependent inactivation of the K^+ current is voltage-dependent in FDC-P2 cells: the use-dependent inactivation became more prominent as the holding potential was more depolarized.

To elucidate the mechanisms underlying the voltage-dependence of use-dependent inactivation of the K^+ current, the recovery kinetics of the K^+ current from inactivation was examined at various holding potentials (−60, −80, and −100 mV) in three cells (Fig. 4). Two voltage steps to +30 mV (*P1* and *P2* in the inset of Fig. 4) were applied to the cells at various intervals (ΔT) from 0.2 to 15 sec. The interval of the sets of two voltage steps (*P1* and *P2*) was 30 sec. The relative amplitude (%) of the peak outward current during the second voltage steps to +30 mV (*P2*) with reference to the peak during the conditioning pulse (*P1*) was plotted at each duration of the intervals (ΔT). The recovery of the peak current amplitude was composed of two phases, i.e., the initial rapid exponential recovery and the late slow recovery, irrespective of the holding potential. The time constant of the initial rapid recovery phase was fairly constant among different holding potentials and was around 200–300 msec. On the other hand, the slow recovery phase differed prominently with different holding potentials: the recovery was complete within 2 sec at −100 mV, whereas recovery required ~5 sec at −80 mV and ~15 sec at −60 mV.

Effects of IL-3 in the Culture Medium on the K^+ Current in FDC-P2 Cells

Figure 5 shows the density of the K^+ current at +30 mV with a holding potential of −80 mV in the same batch of FDC-P2 cells cultured in IL-3-containing medium and IL-3-free medium. The FDC-P2 cells cultured in the control IL-3-containing medium had a mean K^+ current of 10.6 ± 3.5 $\mu A/cm^2$ (n = 18). All cells examined exhibited K^+ current. When the same population of cells was cultured in IL-3-free,

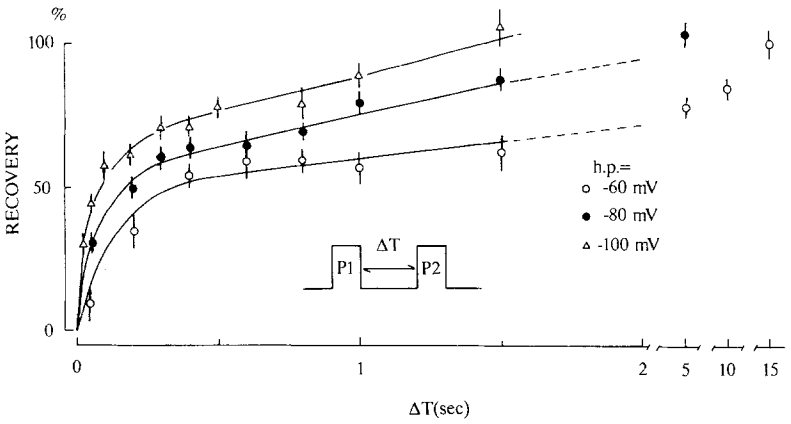

Fig. 4. Recovery from inactivation of the K^+ current. The two successive voltage steps to +30 mV were applied at intervals of 30 sec. The relative amplitude of the peak current during the second voltage step (*P2*) was expressed in percentage with reference to the amplitude of the peak current in the first step (*P1*). The relative amplitudes of the peak current in the second steps were plotted at each interval between the two voltage pulses (ΔT). The holding potential of the cell was −100 mV (△), −80 mV (●), and −60 mV (○). Mean ± SD (n = 3)

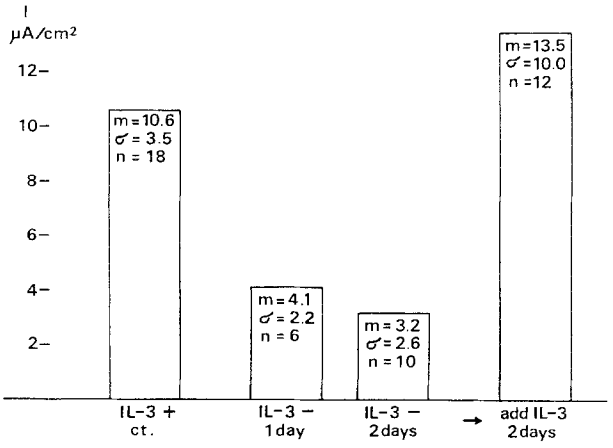

Fig. 5. Current density of the K^4 current in IL-3-containing medium and IL-3-free medium. The density of the K^+ current was obtained from the peak amplitude of the outward current during voltage steps to +30 mV from a holding potential of −80 mV. It was assumed that the capacitance of the cell membrane was 1 $\mu F/cm^2$. After having obtained the control values (*ct.*) from the FDC-P2 cells cultured in IL-3-containing medium, the cells were cultured for two days in the IL-3-free, F9 antibody-containing medium. The medium was changed again to IL-3-containing medium, and the cells were cultured for two days. F9 antibody was added to the IL-3-free medium to support growth of the cells (Ohta et al., 1988; Sugawara et al., 1988)

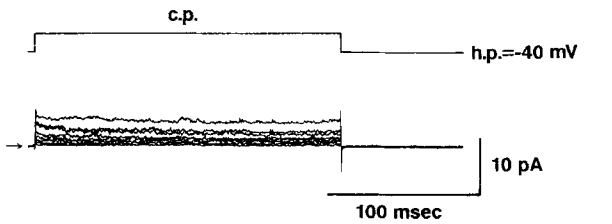

Fig. 6. The membrane currents of FDC-P2(-) cell. The pipette contained K^+-rich solution. The cell was held at −80 mV. The membrane currents elicited by voltage steps to −60, −40, −20, 0, +10, +20, +30, and +40 mV were superimposed. The cell had a small time-independent outward current. The voltage protocol was indicated above the current traces. The arrow indicates the zero current level. Capacitive and leak currents were subtracted

F9 antibody-containing medium, the density of the K^+ current of the cells decreased dramatically to 4.1 ± 2.2 $\mu A/cm^2$ (n = 6) after culturing for 24 hr in IL3-free medium and to 3.2 ± 2.6 $\mu A/cm^2$ (n = 10) after 48 hr. The density of the K^+ current recovered to 13.5 ± 10.0 $\mu A/cm^2$ (n = 12) at 48 hr after IL-3 was readded to the medium. When we cultured the cells in the IL-3-free, F9 antibody-free medium, the cells could not be maintained. F9 antibody, when added to the IL3-containing medium, did not affect the K^+ current within 72 hr of culturing (*not shown*).

Figure 6 shows the membrane currents of FDC-P2(-), a mutant cell from FDC-P2, which proliferates in the IL-3-free medium (Sugawara et al., 1988). The holding potential was −80 mV and command voltage steps to various potentials were applied to the cell. It is evident that FDC-P2(-) cells did not have the transient outward K^+ current observed in FDC-P2 cells, but a small time-independent outward current was elicited in 10/10 cells examined.

Discussion

The present study showed that an IL-3-dependent cell line, FDC-P2 cells, have a voltage-gated K^+ current. The K^+ current showed the following properties: (i) The deactivation of the K^+ current was very rapid (<1 msec). (ii) The K^+ current was inhibited by TEA with K_i of less than 0.1 mM. Scorpion toxin did not affect the K^+ current. (iii) The use-dependent inactivation was not prominent when the cell was held at −80 mV. These properties of the

K^+ current in FDC-P2 cells are very similar to those of the *l*-type K^+ current identified in mature T lymphocytes (CD4-$CD8^+$ cells and cytotoxic or suppressor T cells) (Lewis & Cahalan, 1988*a,b*). Thus, it is suggested that the major component of the K^+ current in FDC-P2 cells is composed of the *l*-type K^+ channel, although we cannot completely exclude the possibility that different types of K^+ channels similar to *n* and *n'* types are also expressed in the cells. This observation is consistent with the previous report that the cells have a specific marker for mature T lymphocytes, Thy-1,2 (Dexter et al., 1980), although FDC-P2 cells have various properties similar to those of promyelocytes; the cells contain large irregular granules and proliferate to granulocytic cells.

The effects of IL-3-free medium on the K^+ current of FDC-P2 cells suggest that IL-3 is essential for the cells to express the K^+ channel. Phosphorylation by a tyrosine kinase is involved in IL-3-dependent signal transduction (Pierce et al., 1985; Kipreos & Wang, 1988; Koyasu et al., 1987; Wang et al., 1989). Such mechanisms may underlie the expression of the K^+ channel.

Although it is unclear whether the K^+ current plays a functional role in proliferating the cells in the present study, the following observations are consistent with the idea: (i) The density of the K^+ current decreased in the IL-3-free culture medium, and the cell proliferation was greatly disturbed. It recovered by addition of IL-3 to the medium, when the cells started to proliferate. (ii) The IL-3-dependent mutant of FDC-P2 cells, FDC-P2(-), did not have the K^+ current. Further studies are also necessary to elucidate the functional roles of the K^+ channel in FDC-P2 cells (Schell et al., 1987; *see also* Schlichter, Sidell, & Hagiwara, 1986).

In the previous studies (Ohta et al., 1988; Sugawara et al., 1988), it was shown that F9 antibody may have an effect similar to IL-3 in proliferating FDC-P2 cells. Since density of the transient outward K^+ current in FDC-P2 cells decreased during culturing in the IL-3-free, F9-containing medium, it is evident that the action of F9 antibody and IL-3 differ in expression of the K^+ channel in the cells.

The authors thank Dr. David Clapham (Mayo Foundation) for his kind comments on the manuscript, and Monica Zien for typing the manuscript.

References

DeCoursey, T.E., Chandy, K.G., Gupta, S., Cahalan, M.D. 1984. Voltage-gated K^+ channels in human T lymphocytes: A role in mitogenesis? *Nature* **307**:465–468

Dexter, T.M., Garland, L., Scott, D., Scolnick, E., Metcalf, D. 1980. Growth of factor-dependent hemopoietic precursor lines. *J. Exp. Med.* **152**:1036–1047

Hamill, O., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391**:85–100

Ihle, J.N., Keller, J., Oroszlan, S., Henderson, L.E., Copeland, T.D., Fitch, F., Prystowsky, M.B., Golewasser, E., Schrader, J.W., Palaszynski, E., Dy, M., Lebel, B. 1983. Biologic properties of homogenous interleukin-3: I. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, P cell-stimulating factor activity, colony-stimulating factor activity, and histamine-producing cell-stimulating factor activity. *J. Immunol.* **131**:282–287

Ihle, J.N., Weinstein, Y. 1986. Immunological regulation of hematopoietic/lymphoid stem cell differentiation by interleukin 3. *Adv. Immunol.* **39**:1–50

Kipreos, E.T., Wang, L.Y.J. 1988. Reversible dependence on growth factor interleukin-3 in myeloid cells expressing temperature sensitive v-abl oncogene. *Oncogene Res.* **2**:277–284

Koyasu, S., Tojo, A., Miyajima, A., Akiyama, T., Kasuga, M., Urabe, A., Schreurs, J., Arai, K.I., Takaku, F., Yahara, I. 1987. Interleukin-3-specific tyrosine phosphorylation of a membrane glycoprotein of M_4 150,000 in multi-factor-dependent myeloid cell lines. *EMBO J.* **6**:3979–3984

Lewis, R.S., Cahalan, M.D. 1988*a*. Subset-specific expression of potassium channels in developing murine T lymphocytes. *Science* **239**:771–775

Lewis, R.S., Cahalan, M.D. 1988*b*. The plasticity of ion channels: Parallels between the nervous and immune systems. *Trends Neuro. Sci.* **5**:214–218

McKinnon, D., Ceredig, R. 1986. Changes in the expression of potassium channels during mouse T cell development. *J. Exp. Med.* **164**:1846–1861

Ohta, Y., Tamura, S., Tezuka, E., Sugawara, M., Imai, S., Tanaka, H. 1988. Autoimmune MRL/1 pr mice sera contain IgG with interleukin-3-like activity. *J. Immunol.* **140**:520

Pierce, J.H., Flore, P.P.D., Aaronson, S.A., Potter, M., Pumphrey, J., Scott, A., Ihle, J.N. 1985. Neoplastic transformation of mast cells by Abelson-MuLV: Abrogation of IL-3 dependence by a nonautocrine mechanism. *Cell* **41**:685–693

Rennick, D.M., Lee, F.D., Yokota, T., Arai, K.I., Cantor, H., Nabel, G.J. 1985. A cloned MCGF cDNA encodes a multilineage hematopoietic growth factor: Multiple activities of interleukin-3. *J. Immunol.* **134**:910–914

Schell, S.R., Nelson, D.J., Fozzard, H.A., Fitch, F.W. 1987. The inhibitory effects of K^+ channel-blocking agents on T lymphocyte proliferation and lymphokine production are "nonspecific." *J. Immunol.* **139**:3224–3230

Schlichter, L., Sidell, N., Hagiwara, S. 1986. K channels are expressed early in human T-cell development. *Proc. Natl. Acad. Sci. USA* **83**:5625–5629

Sugawara, M., Hattori, C., Tezuka, E., Tamura, S., Ohta, Y. 1988. Monoclonal autoantibodies with interleukin-3-like activity derived from a MRL/1 pr mouse. *J. Immunol.* **140**:526–630

Wang, H.M., Collins, M., Arai, K., Miyajima, A. 1989. EGF induces differentiation of an IL-3-dependent cell line expressing the EGF receptor. *EMBO J.* **8**:3677–3684

Ypey, D.L., Clapham, D.E. 1984. Development of a delayed outward-rectifying K^+ conductance in cultured mouse peritoneal macrophages. *Proc. Natl. Acad. Sci. USA* **81**:3083–3087

Received 15 August 1990; revised 17 October 1990