Increased Voltage-Gated Potassium Conductance during Interleukin 2–stimulated Proliferation of a Mouse Helper T Lymphocyte Clone

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Abstract. Recent work has demonstrated the presence of voltage-gated potassium channels in human peripheral blood T lymphocytes (Matteson, R., and C. Deutsch, 1984, *Nature (Lond.)*, 307:468–471; DeCoursey T. E., T. G. Chandy, S. Gupta, and M. D. Cahalan, 1984, *Nature (Lond.)*, 307:465–468) and a murine cytolytic T-cell clone (Fukushima, Y., S. Hagiwara, and M. Henkart, 1984, *J. Physiol.*, 351:645–656). Using the whole cell patch clamp, we have found a potassium conductance with similar properties in a murine noncytolytic T lymphocyte clone, L2. Under voltage clamp, a step from a holding potential of −70 mV to +50 mV produces an average outward current of 100–150 pA in "quiescent" L2 cells at the end of their weekly maintenance cycle. When these cells are stimulated with human recombinant interleukin 2 (rIL2, 100 U/ml), they grow in size and initiate DNA synthesis at ~24 h. Potassium conductance is increased as early as 8 h after stimulation with rIL2 and rises to a level 3–4 times that of excipient controls by 24 h. The level remains elevated through 72 h, but as the cells begin to leave the cell cycle at 72–96 h, the conductance decreases quickly to a value only slightly higher than the initial one. Quinine, a blocker of this conductance, markedly reduces the rate at which L2 cells traverse the cell cycle, while also reducing the rate of stimulated protein synthesis. The regulation of potassium conductance in L2 cells during rIL2-stimulated proliferation suggests that potassium channel function may play a role in support of the proliferative response.

THE process by which resting or naive T lymphocytes are activated involves cell–cell interactions, including T cells and antigen-presenting cells, and the action of soluble protein factors (40). Stimulation of T lymphocytes with antigen results in the synthesis and secretion of several of these factors or lymphokines (e.g., interleukin 2, IL2)[1] (33, 34). In addition, antigenic stimulation causes the expression of surface receptors for IL2 on T lymphocytes, which enables these cells to proliferate in response to IL2 (36). Cloned T lymphocytes possess many of the characteristics of normal activated T lymphocytes in that they maintain their specific antigen reactivity, produce lymphokines in response to stimulation with antigen, and proliferate in response to IL2 alone (11). Since cloned T lymphocytes represent a homogeneous population of T cells, they provide an excellent model system for studying T lymphocyte function.

The recent application of the whole cell patch clamp (21) to voltage clamp studies of T lymphocytes (2, 7, 8a, 15, 30) led to the discovery and characterization of a voltage-gated potassium conductance that has been found in both freshly isolated cells of human peripheral blood and a mouse cytotoxic T cell clone. Potassium channel blockers such as quinine, 4-aminopyridine, and tetraethylammonium not only block the voltage-gated current of the lymphocyte, but also inhibit mitogen-stimulated proliferation in long-term cell culture (4, 7). It has therefore been suggested that K channel function is important in the triggering and/or support of mitogen-induced proliferation.

In this study we have used a mouse T helper clone, L2, to study the effect of IL2 stimulation on the characteristics of the voltage-gated K conductance. Using the whole cell patch clamp, we have found that the average K current per cell in response to a voltage step from −70 mV to +50 mV increases within 8 h after stimulation with IL2. The voltage-gated current then continues to increase over the next 24–40 h as the cells increase in size and proliferate. When the cells stop cycling, ~96 h after IL2 addition, both average current and average cell size begin to decrease, but the decrease in current occurs more quickly. Quinine, a K channel blocker, inhibits IL2-stimulated protein synthesis and delays IL2-stimulated initiation of DNA synthesis. A preliminary report of this work has appeared (26).

[1] *Abbreviations used in this paper:* IL2, interleukin 2; rIL2, purified human recombinant interleukin 2.

Materials and Methods

Culture Conditions

The derivation and maintenance of the murine, Mls-reactive, T lymphocyte clone L2 have been described previously (18). At weekly intervals L2 cells (5×10^4) are stimulated with irradiated allogenic spleen cells (6×10^6) and secondary mixed lymphocyte culture supernatant (33%, vol/vol). For all experiments, L2 cells at day 7 of the maintenance culture cycle were separated with the use of Ficoll/Hypaque density gradient centrifugation (5). Cloned T cells ($1-3 \times 10^5$/ml) were cultured with either human recombinant IL2 at 100 U/ml (rIL2, Cetus Corp., Emeryville, CA) or excipient control.

Cells to be used for electrophysiology were either transferred to a 35-mm tissue culture grade plastic petri dish (Corning Science Products, Corning, NY; or Costar, Cambridge, MA) shortly before use or were cultured continuously in such dishes from the initiation of the experiment. When cells were transferred between dishes, they were allowed at least 1 h in full culture medium to reattach. Cells cultured in these dishes grew very well with rIL2 stimulation, even at low seeding densities ($1-2 \times 10^5$ cells/dish).

Electrophysiology

We used the whole cell variation of the patch clamp method (21) as described previously (8a, 30). Voltage clamp and current measurement were accomplished with a model EPC-7 patch clamp (List-Medical-Electronic, Damstadt, West Germany). Pulse protocols were sent to the EPC-7 and currents were digitized with the aid of specialized hardware and software developed by Dr. Clay Armstrong and colleagues (29) for use in conjunction with an LSI 11/23 microcomputer (Digital Equipment Corp., Marlboro, MA). Linear components of capacitance and ionic current were subtracted out with a P/2 procedure (1), which uses the computer to remove non-voltage-gated current. Series resistance compensation was not used in most cases; when applied it was of limited effect because of the small size and slow time-course of the voltage-gated current (see also 8a, 15). EPC-7 output was filtered at 3.3 kHz with the 3-pole Bessel filter built into the clamp.

Cell capacitance was determined by integrating the area under uncompensated capacitive spikes produced by repetitive 1-mV steps. Capacitance was determined as the difference between the total system capacitance measured after initial gigaseal formation and that measured immediately after breaking into the cell. These difference measurements of cell capacity were accurate to ±0.21 pF, as determined by calibration against a known capacitance. Electrodes were fabricated of Kimax-51 capillary tubing (Kimble 34500 Kimble Div., Toledo, OH) or Corning 8161 (a kind gift of Dr. Armstrong). When filled with our normal internal solution, pipette resistance was 3.9 ± 0.9 Megohms (n = 149) for the Kimax-51 tubing and 3.0 ± 1.1 Megohms (n = 61) for the 8161.

Cells on dishes were washed extensively with a warm protein-free recording solution. This solution was (in mM) 140 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 5.5 glucose, 10 Hepes, pH 7.3 with NaOH, 290–300 mOsM as measured with a freezing point osmometer (Precision Systems, Inc., Natick, MA). The bath was grounded via a salt bridge of 3–4% agar (wt/vol) dissolved in the above saline without glucose. The bridge led to a small reservoir that contained pipette filling solution. Electrical connections were made with Ag-AgCl wire. All experiments were done at room temperature (18–22°C).

In the majority of the experiments, the filling solution of the patch electrodes consisted of (in mM) 90 KCl, 40 KF, 2.0 $MgCl_2$, 11.0 dipotassium EGTA, 1.0 $CaCl_2$, 10 Hepes, pH 7.2 with KOH. In other instances the solution contained only 1–3 mM EGTA with no Ca, and KCl at 100 mM, but with no observable differences in the characteristics of the recording. Final osmolarity of all filling solutions was 270–285 mOsM.

Cells were clamped at a holding potential of −70 mV. This is near the experimentally estimated value for the resting membrane potential of lymphocytes from various species as determined by equilibrium distribution and fluorescent probe techniques (8, 9, 12, 24, 35). Zero current (i.e., resting membrane) potentials were not determined in our experiments; the high intrinsic resistance of lymphoid cells and the consequently large contribution to the leak current from the patch electrode seal render such measurements inaccurate, as discussed in reference 15. We used the peak current exhibited when cells were stepped from −70 mV to +50 mV for 25 ms as a standard measure of conductance.

Cell Cycle Analysis

Cultures of L2 cells, initiated at the same time as the cultures for electrophysiological studies, were stimulated with rIL2 (100 U/ml) in the presence or absence of quinine (100 μM). Cells were collected at various times after stimulation and separated using Ficoll/Hypaque density gradient centrifugation. Cells at the interface were washed and pelleted. The pellets were washed and resuspended in 0.1% sodium citrate (wt/vol) at pH 7.0 containing 50 μg/ml of propidium iodide (25). Using a FACS IV (Becton Dickinson, Mountain View, CA), DNA content of individual cells was determined from the fluorescence of bound dye. FACS-produced histograms of linear fluorescence intensity vs. cell number were deconvoluted into ten gaussian curves by the method of Fried (14), and curve fitting was performed using a simplex algorithm (31). The calculated percentage of cells in each of the curve-defined areas yields a detailed picture of the progression through the G_1, S, G_2, and M phases of the cell cycle. The integration of the first curve gives the G_1 percentage. The integration of the last curve gives the G_2 + M percentage, and the sequence of intervening eight curves gives the early, middle, and late S phases. To estimate the error in making these assignments, the variance-covariance matrix of the multiplying factors of the guassians was constructed and used to estimate the variances of the calculated G_1, S, and G_2 + M by standard procedures (42).

Protein Gels

L2 cells (10^6/ml) were stimulated with rIL2 (100 U/ml) in the presence or absence of quinine (100 μM) or cycloheximide (15 μg/ml). At various times the cells were harvested, pelleted, and 10^6 cells were resuspended in 1 ml of methionine-free Dulbecco's modified Eagle's medium that contained 50 μCi of [^{35}S]methionine (specific activity > 800 Ci/mmol; New England Nuclear, Boston, MA), and incubated at 37°C. At the end of 1 h, the cells were harvested, washed twice in cold phosphate-buffered saline, and lysed with 100 μl of extraction buffer (0.01 M Tris pH 7.4, 0.15 M NaCl, 0.5% Nonidet-40). The lysates were incubated on ice for 20 min, and 40 μl of the cell lysates were combined with 10 μl of 5× sample buffer (0.25 M Tris pH 6.8, 10% sodium dodecyl sulfate [SDS], 25% sucrose, 0.005% bromphenol blue, 25% 2-mercaptoethanol). Proteins were separated on a 12% polyacrylamide gel containing 0.1% SDS. Proteins were detected with a sensitive silver-staining technique (39). In addition, the gels were treated with Enlightening (New England Nuclear), dried, and exposed to x-ray film for 1–7 d at −70°C to determine *de novo* protein synthesis. A 5-μl aliquot of the cell lysates was assayed for radioactivity to determine the level of total protein synthesis. 85% of the [^{35}S]-methionine in cell lysates is precipitable with trichloroacetic acid regardless of the time after stimulation with rIL2.

The autoradiographs were scanned with a Hoefer model GS 300 densitometer (Hoefer Scientific Instruments, San Francisco, CA) that was connected to a Waters M840 data reduction system (Waters Instruments, Inc., Rochester, MN) consisting of an analog-digital converter, a DEC Professional 380 microcomputer, and software for data analysis and graphics.

Reagents

Purified human recombinant IL2 (lot No. LP230b) was a gift of Cetus Corp. This substance, produced by recombinant DNA technology in *E. coli* (41), is 98% pure by SDS PAGE analysis and contains 0.02 ng of endotoxin per 2×10^5 U of IL2 as determined by the Limulus assay. An excipient control was supplied by the manufacturer. Specific activity of rIL2 was assessed by Cetus Corp. using a standard bioassay (17) and is nearly identical to that of native human IL2 that had been purified to homogeneity from culture supernatants of mitogen-activated T cells (37, Koths, K., et al., manuscript submitted for publication).

Cycloheximide, EGTA, and Hepes were from Sigma Chemical Co. (St. Louis, MO). KF and quinine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Propidium iodide was obtained from Calbiochem-Behring Corp. (La Jolla, CA).

Results

Cell Cycle Analysis

At day 7 of the maintenance culture cycle, L2 cells are relatively small, and 95% have a G_1 content of DNA (Fig. 1). These L2 cells contain more RNA than primary splenic lymphocytes (G. Otten, University of Chicago, personal communication), express receptors for IL2, and will proliferate in response to exogenously added IL2 (22). As shown in Fig. 1, this subclone of L2 begins to enter the S phase of the cell cycle 22 h after stimulation with rIL2 (G_1 = 53%, S = 46%, G_2 + M = 1%). At 28 h after stimulation, cells can be found in all phases of the cell cycle (G_1 = 26%, S = 60%, G_2 + M = 14%), and by 34 h at least 27% of the cells have completed

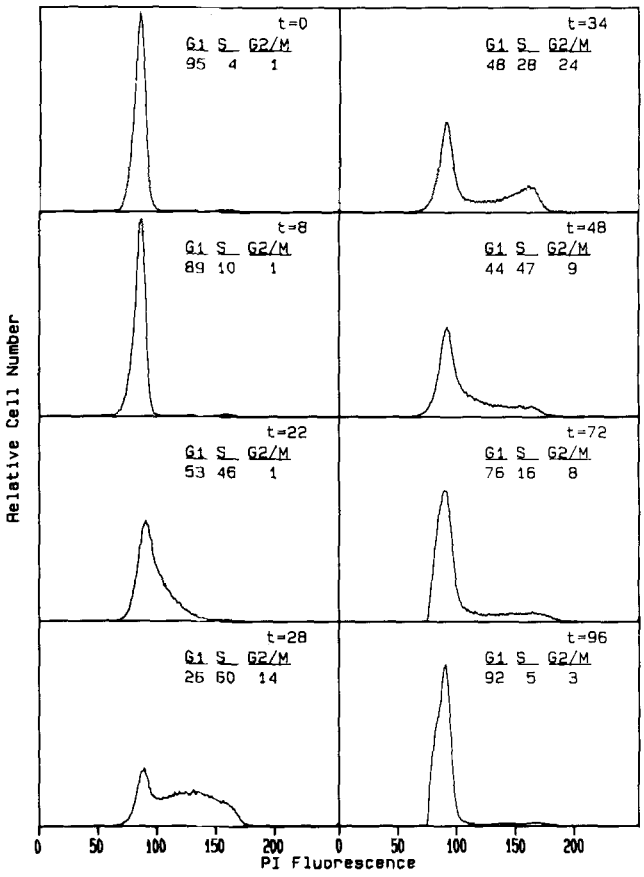

Figure 1. Cell cycle analysis of rIL2-stimulated L2 cells. L2 cells were stimulated with 100 U/ml of human recombinant IL2. At the indicated times cells were harvested from culture, washed, and stained with the DNA-binding fluorescent dye propidium iodide. The fluorescence intensity of individual cells was determined in a fluorescence activated cell sorter (FACS IV) and plotted on a linear scale vs. cell number. Each panel represents ~50,000 cells. Each time point was carried out in duplicate. At zero time, <5% of the population exhibited the higher DNA content of cells in S or G_2 + M, and this is minimally changed at 8 h after rIL2 addition. At 22 h, entry into early S phase is apparent; and by 28 h, most of the cells are in some phase of DNA replication. At 34 h many cells have progressed through a complete replication, divided, and returned to the diploid state, while many others are in G_2 + M. At 48 h, in a more heterogeneous population, about the same number of cells are cycling as at 34 h. However, by 72 h a smaller fraction of the population is in S or G_2 + M; and at 96 h, the population profile has returned to the original seen at time zero, indicative of very few cells in cycle. An estimation of the error in our assignments of the percent of the population in each phase of the cell cycle was done as described in Materials and Methods. Our ability to make these assignments is most unambiguous for G_1 and G_2 + M, for which the standard deviations were consistently <±1%. The standard deviations for S phase averaged ±3–4%, with a high of ±6% (t = 8 h) and a low of ±1% (t = 28 h).

one cell division cycle. The cells continue to divide for the next 36 h, although the percentage of dividing cells appears to be decreasing by 72 h. At 96 h after rIL2 stimulation, L2 cells are no longer dividing. This pattern of cycling has been seen consistently with rIL2-stimulated L2 cells. In addition, cell cycle histograms obtained after stimulation of L2 cells in the presence of the mitosis-arresting drug colchicine show that at least 85% of the cells traverse the cell cycle under these conditions (34a).

L2 Cells Have a Voltage-Gated Potassium Conductance

L2 cells in whole cell voltage clamp show a voltage-gated outward K^+ current that is very similar to that previously observed in human peripheral blood T-lymphocytes (2, 7, 30) and a mouse cytotoxic T-cell clone (15). Voltage-gated current as a function of activating voltage is shown in Fig. 2. Peak current is very nearly a linear function of voltage from −10 to +70 mV (Fig. 2*B*), indicating that maximum channel gating is achieved by ~−10 mV. This pattern was not noticeably different in activated cells.

The reversal potential of the current can be determined from an instantaneous I-V curve such as that shown in Fig. 3. The reversal potential in the example cell is ~−75 mV, consistent with a channel highly selective for K^+. Similar to its effect on the K current in human peripheral blood T cells (7, 30) and a murine T cell clone (15), 0.1 mM quinine added to the bath completely blocked the voltage-gated current of L2 cells; 0.05 mM quinine produced about a 65% block.

From a holding potential of −70 mV, the threshold for initiation of current in the L2 cells was −24 ± 5 mV (n = 25) when measured within 3 min of breaking into the cell and drifted ~10 mV to more negative potentials over the next 10–20 min. This drift has been attributed to dissipation of a junction potential between the cytoplasm and pipette filling solution (28) and has been reported in lymphocytes before (2, 8a, 15). The full current record could be fit with a Hodgkin-Huxley (23) type equation of the form

$$I = k(1 - e^{-t/\tau_1})^x e^{-t/\tau_2},$$

in which k is an arbitrary scaling factor, and τ_1 and τ_2 are the activation and inactivation time constants, respectively. A reasonable fit of the rising phase of the current could be obtained with x = 3. In cells maintained in the whole cell configuration at least 5 min, the outward current in response to a step from −70 to +50 mV was characterized by activation and inactivation time constants of 1.9 ± 0.8 ms and 220 ± 39 ms (n = 14), respectively. For comparison with reference 15, the activation time constant fit as a simple exponential (x = 1) was 3.1 ± 1.5 ms. Neither the current initiation threshold nor the current kinetics were significantly changed by cell activation. We did not make a detailed study of the voltage-dependence of these time constants.

Cell Conductance after IL2 Stimulation

Cell current measured in response to a voltage step from −70 mV to +50 mV increased as the cells grew and entered the cell cycle in response to stimulation with rIL2. For example, typical current traces are shown in Fig. 4 for L2 cells that have been stimulated with rIL2 for 1–7 d. All of the traces exhibit similar activation and inactivation kinetics. Measured peak current ranges from 120 pA ("C", control cell) to 790 pA (day 2 after rIL2 stimulation) and back to 155 pA (at day 7). Considerable overlap in the magnitude of cell current was observed for the different days, especially days 1–3 for larger voltage-gated currents, and days 4–8 for generally smaller currents.

The average peak current, capacitance, and current density of cells during the 8-d time course of the experiment are shown in Fig. 5*A*. We could not detect a change in average current at 4 h after adding rIL2. A small, but significant

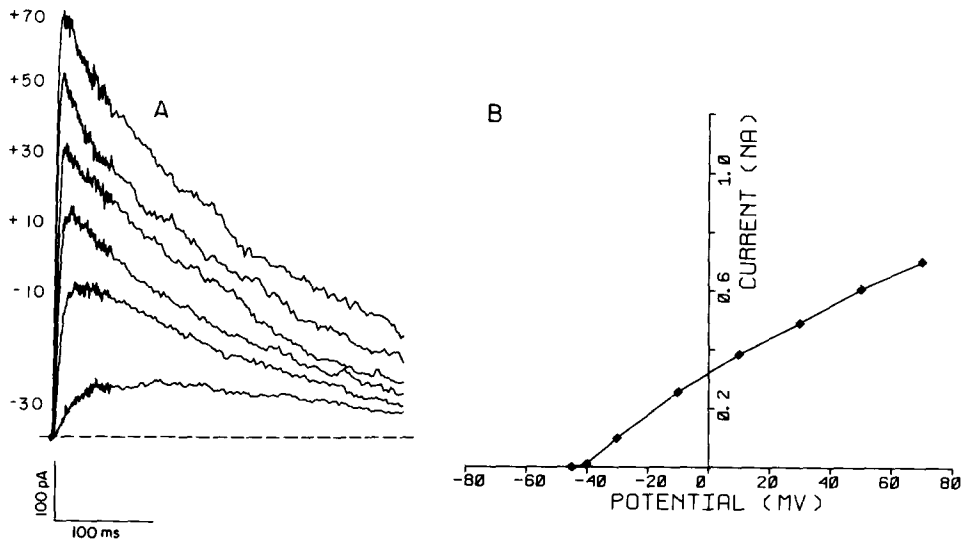

Figure 2. Peak current as a function of voltage. (*A*) A representative example of the peak current vs. voltage relationship. An L2 cell was prepared for electrophysiology as described in the text and placed in whole-cell patch clamp. The membrane potential was held at −70 mV and then stepped for 358 ms to the indicated voltages. Digitization rate was 0.5 ms/pt. This particular cell was stimulated with rIL2 for 3 d. Measured cell capacitance was 9.29 pF. Electrode resistance was 2.7 Megohm, and the filling solution contained 3 mM EGTA and no Ca^{2+}. These traces were made 29–34 min after beginning whole cell recording. (*B*) Peak current vs. voltage derived from the current records in *A*. Peak current is a nearly linear function of voltage positive from −10 mV.

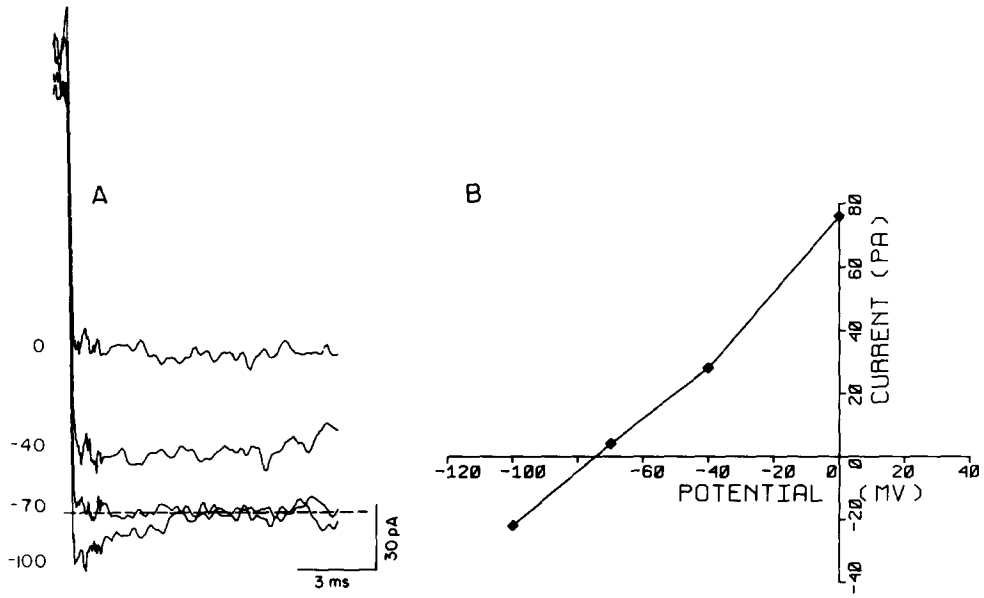

Figure 3. Instantaneous current as a function of voltage. (*A*) A representative example of "instantaneous" current vs. voltage. An L2 cell in whole cell patch clamp was stepped from −70 mV to +100 mV for 15 ms to maximally activate the K channels (only the very end of this is shown). Then the voltage was stepped back to various intermediate voltages as indicated to measure the current flow, especially at membrane potentials where channels normally would not be open. The dotted line represents the level of zero current. Digitization rate was 0.02 ms/pt. This particular cell was a 24-h control (i.e., t = 24 h, *NO IL2* in Fig. 5). Measured cell capacitance was 1.7 pF. The intracellular solution contained 1 mM EGTA and no Ca^{2+}. Electrode resistance was 3.9 Megohm. These data were collected 8–9 min after beginning whole cell recording. (*B*) Instantaneous current vs. voltage from the data in *A*. The reversal potential of the current under these conditions (i.e., where current crosses zero) was ~−75 mV.

increase could be seen at 8 h (Student's t-test with matched cultures containing excipient control, $P < 0.05$). The magnitude of the current reached an average value 3–4 times that of the original unstimulated controls by 24 h and remained high through the 72-h time point. Cell capacitance, a measure of cell surface area, also increased to about the same extent. In the 24-h period between 72 and 96 h, average cell current dropped ~50% and then declined slowly back to the initial level on succeeding days. Cell capacitance also decreased beginning with day 4, but its decline was more gradual than that of the conductance. These changes were reflected in the calculated current density (I at +50 mV/capacitance, I/C). The current density increased after 24 h and remained elevated for another 24 h, the period corresponding to peak DNA synthesis. By Student's t-test, the increase in current density was significant in stimulated vs. unstimulated cells at

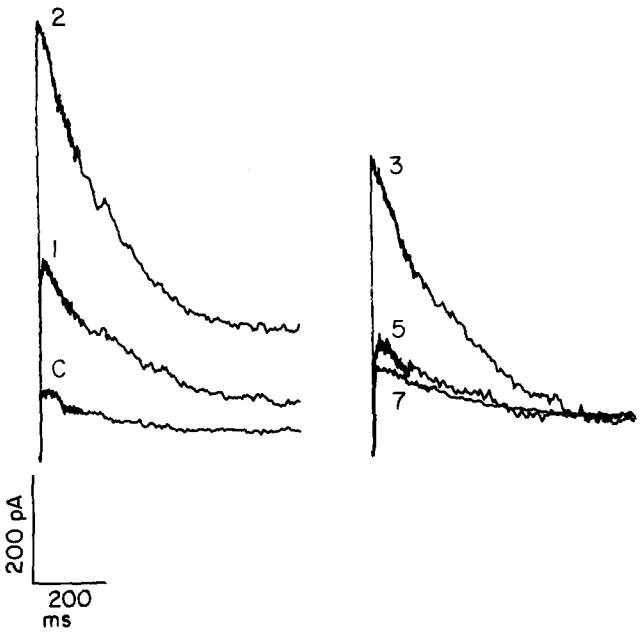

Figure 4. Current as a function of time after rIL2 stimulation. Representative current traces from individual cells as a function of length of exposure to rIL2. Each record is the response of a cell to a voltage step from −70 to +50 mV for 710 ms. Digitization rate was 1.0 ms/pt. The number next to each peak is the number of days exposure to rIL2. The "C" cell is the same control cell as in Fig. 3; the day 3 cell is the same as in Fig. 2. Cell capacitance of the other cells was: day 1, 3.01 pF; day 2, 10.95 pF; day 5, 2.61 pF; day 7, 6.81 pF. The level of non-inactivating current was not consistent.

24 h ($P < 0.01$). At 72 h, I/C is decreased and is significantly less than I/C at 24 h after rIL2 stimulation ($P < 0.05$). All current densities 72–192 h after IL2 stimulation were also significantly ($P < 0.02$ or better) less than I/C at 24 h.

The distribution of individual cell capacitance vs. current, represented as average values in Fig. 5*A*, are shown in Fig. 5*B*. In the panels depicting 0 h and 24 h (*NO IL2*), the current and capacitance of unstimulated cells were relatively homogeneous. With increasing time after rIL2 stimulation (panels 8 h–72 h), there was a progressive increase in cell current and capacitance. In addition, as cells began to be distributed throughout all phases of the cell cycle (24–72 h, Fig. 1), there was also an increase in population heterogeneity as reflected by the distribution in the current vs. capacitance plots (Fig. 5*B*). The standard deviations in the average current and capacitance values were quite large, reflecting the wide range of values seen in the population. Both in absolute terms and as a percent of the mean, the standard deviations increased after rIL2 stimulation. The standard deviations in the current increased from ±58 pA for 24-h (*NO IL2*) controls (still ±50% of the mean) to ±282 pA for day 3 stimulated cells (±79% of the mean for day 3). Standard deviation in peak current of activated cells returned to the level of unstimulated cells by day 6. Standard deviation in measured capacitance increased from a low of ±0.86 pF for 24-h (*NO IL2*) controls (±33% of the mean) to a high of ±2.49 pF on day 2 (±46% of the mean), and returned to ±1.27 pF by day 8 (±34% of the mean). The statistical spread in current as a percentage of the mean was always larger than that for the capacitance, as previously observed for lymphocytes (2, 8a, 15). Large standard deviations in the average current and capacitance at 48–72 h are consistent with the heterogeneity in cell cycle stage and DNA content of the population observed at this time (Fig. 1). Also at 72 h, we began to find individual cells that were large (capacitance > 5 pF) but had small current (<200 pA at +50 mV), as illustrated in Fig. 6 for three cells of equal size.

Effect of Quinine on Cell Cycle Progression

Previous studies using phytohaemagglutinin-stimulated human peripheral blood mononuclear cells have shown that the K channel blocker quinine inhibits mitogen-induced DNA synthesis as measured by thymidine incorporation (4, 7). Therefore, we investigated the effects of quinine on IL2-driven L2 cell proliferation. L2 cells were harvested at 24, 48, and 69 h after IL2 stimulation, and analyzed for DNA content. As shown in Figs. 1 and 7, L2 cells that have been stimulated with rIL2 began to enter the S phase of the cell cycle at 24 h after stimulation, were distributed in all phases of the cycle at 48 h, and were returning to a predominantly G_1 population by 69–72 h. The addition of quinine at the initiation of the cultures caused a marked inhibition of cell cycle progression at 24 h, 48 h, and 69 h after stimulation with rIL2 (Fig. 7). At least a portion of the cells that were treated with quinine entered the cell division cycle but their time of entry appears to be ~24 h later than that for cultures that contained rIL2 alone. At this time it is not known whether quinine slows cell cycle progression by blocking K conductance or by an alternative mechanism(s). Drugs that inhibit DNA, RNA, and protein synthesis also block cell cycle progression. Similarly, it is possible that quinine slows cycle progression by direct inhibition of DNA, RNA, and/or protein synthesis. To determine if quinine inhibited rIL2-stimulated protein synthesis in L2 cells, we studied the effect of quinine on IL2-induced protein synthesis and compared its effects to cycloheximide, an inhibitor of protein synthesis.

Effect of Quinine and Cycloheximide on Protein Synthesis

In previous experiments we have observed that the rate of protein synthesis in L2 cells stimulated with rIL2 increases 5–10-fold within 24 h. In particular, a 52-kD protein that is not synthesized at an appreciable rate in resting cells is synthesized at increased rates in the middle of the G_1 phase and throughout the proliferative response (38). As expected, this band was observed in the rIL2-stimulated cells at 24 and 48 h, but had diminished markedly by 72 h after stimulation (Fig. 8). At 24 h, the rate of total protein synthesis, as measured by the amount of [^{35}S]methionine incorporation into L2 cells in a 1-h pulse, was 12-fold higher in L2 cells stimulated with rIL2 than in cells without rIL2. In addition, there was a 26-fold increase in the rate of synthesis of the 52-kD protein. In the culture that contained quinine, total protein synthesis was 35% of the culture that contained rIL2 alone and the rate of synthesis of the 52-kD protein was 13% of the control culture that contained IL2 alone. Cycloheximide inhibited protein synthesis by 95% with all bands showing a marked decrease. As shown for the 48-h time point, total protein synthesis in the presence of quinine was 53% of the control (IL2 alone) culture and expression of the 52-kD protein was 35% of the control culture. At 72 h, total protein synthesis in the culture that contained quinine was 130% of the control culture, although expression of the 52-kD protein was equal in both

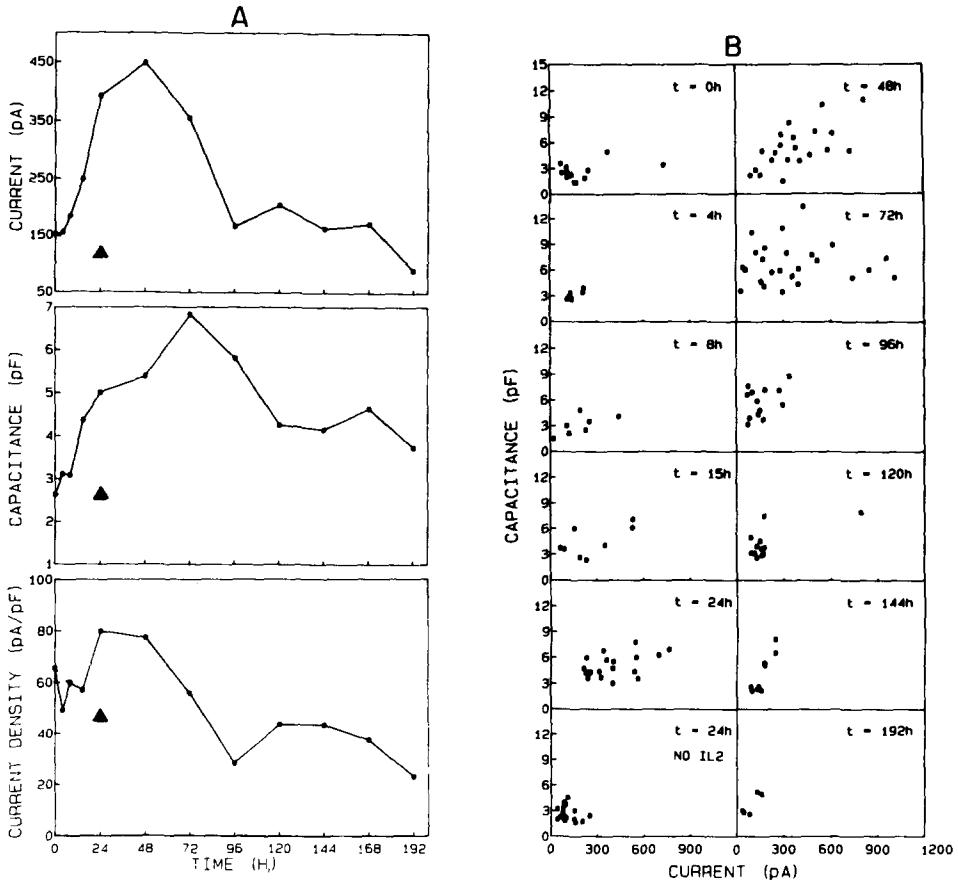

Figure 5. Peak current and capacitance after rIL2-stimulation of L2 cells. (*A*) At various times after rIL2 stimulation, cells were prepared for electrophysiology as described in the text. Peak current was the magnitude of outward current measured in response to a voltage step from the holding potential of −70 mV to +50 mV, usually within a few minutes of establishing the whole-cell configuration. Average peak current rose quickly in the first 24 h and remained elevated through 72 h. However, by 96 h average current had dropped to a value only slightly higher than the starting value. Capacitance, a measure of cell surface area, was determined as the difference of system capacitance before and then after breaking into the cell. Capacitance also increased quickly after rIL2 stimulation. It peaked later than the current and stayed elevated longer. Its decline as the cells left the cell cycle was more gradual. Current density is calculated from the raw data as peak current divided by capacitance, and then averaged. The separate point (▲) at lower values in each panel represent the current and capacitance of unstimulated cells of the same age. Note that the Y-axes of the current and capacitance panels do not begin at zero. (*B*) The standard deviations of these current and capacitance time points are very large and are indicative of a real population diversity, especially at 48–96 h after rIL2 stimulation. Fig. 5 *B* is a raw data plot of capacitance vs. current in individual cells as a function of time after rIL2-stimulation as described in *A*. As average peak current and capacitance increased, the population became more heterogeneous. This is especially marked at 72 h, when some large cells with small currents were found. At later times the population distribution was similar to the $t = 0$ h panel, although capacitance seemed to lag somewhat in returning to control levels.

72-h cultures. The level of expression of the 52-kD protein at 72 h was 19% of the cultures stimulated with IL2 for 24 h in the absence of quinine.

Discussion

IL2 is a T cell growth factor that is synthesized and secreted by activated T cells. It binds to high affinity receptors in the plasma membrane of activated T cells, thereby enabling in vitro proliferation of lymphocytes. The expression of these IL2 receptors is first detectable ~6 h after lectin stimulation (on human lymphocytes, reference 3). Once functional IL2 receptors are present, IL2 alone is sufficient to drive the cells to proliferate. To determine physiological changes that are specific to IL2 stimulation, we chose to study the murine IL2-dependent T cell clone, L2. IL2 alone is sufficient to drive proliferation of these cells; presumably the early events associated with antigen- or mitogen-stimulation of other lymphocytes have already occurred or are bypassed in the case of L2 cells. Thus, the L2 cell line and purified recombinant IL2 constitute a simple model system for dissecting the physiological processes that are required for, or regulate, IL2-driven proliferation. We have used this model to determine the electrical properties of T cells after mitogenic stimulation with the physiological growth factor, IL2.

Potassium Conductance and the Cell Cycle

The T lymphocyte clone L2 was found to have a voltage-gated potassium conductance that is very similar to that found previously in human peripheral blood lymphocytes (2, 7, 8a, 30) and a murine cytotoxic T cell line (15). In terms of the activation and inactivation kinetics and the threshold for initiation of the K current, we found L2 to be more similar to the human T cells than to the mouse cytotoxic T cell line. Compared with this murine line, the L2 potassium current was about twice as slow to activate, twice as fast to inactivate,

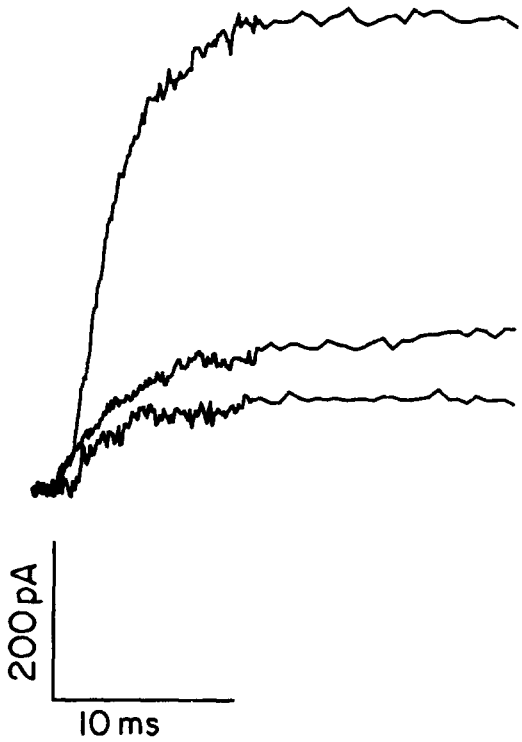

Figure 6. Peak current in 3-d activated L2 cells can be very heterogeneous. An example of the current in response to a step from −70 to +50 mV for 25 ms in three co-cultured cells exposed to rIL2 for 3 d. From largest current to smallest, measured peak current and cell capacitance of these cells was 583 pA/8.94 pF, 199 pA/8.01 pF, and 118 pA/10.43 pF, respectively. Day 3 cell currents were the least predictable. On some occasions all cell currents were large; at other times, almost all were small; and at other times, it was mixed, as in this case.

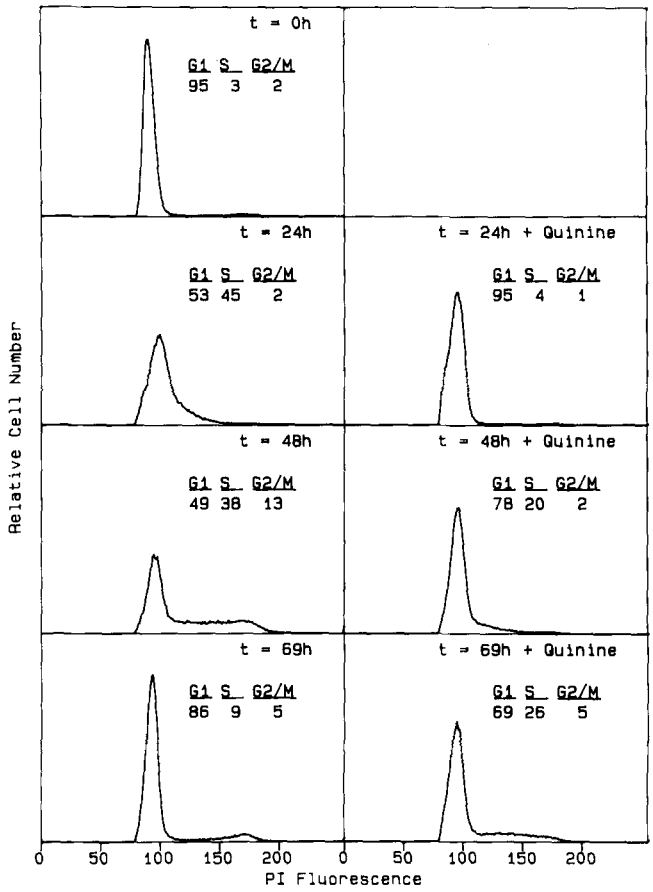

Figure 7. Effect of quinine on cell cycle progression. L2 cells were stimulated with 100 U/ml rIL2. At the same time some cultures also received 0.1 mM quinine. The cells were then harvested at 24, 48, and 69 h after stimulation, stained with propidium iodide, and analyzed for DNA content with a FACS IV as described for Fig. 1. Entry into S phase was markedly inhibited by quinine, but "breakthrough" can be seen at later times. Each panel represents the profile of 50,000–60,000 cells.

and had an activation threshold ~15 mV more positive. These differences in electrical properties could be related to the differences in the functional nature of these two cell lines; that is, one is cytotoxic, whereas the L2 line is noncytolytic and has "helper" characteristics. However, we have observed considerable, albeit somewhat less, variation in these parameters in human peripheral blood T lymphocytes from different donors (Lee, S., D. Krause, and C. Deutsch, unpublished observations); thus, clonal variation between the two mouse lines is also possible. There was little statistical variation in the activation threshold or activation and inactivation rate constants in the L2 cells themselves.

We found that the voltage-gated potassium conductance of the L2 line increases after stimulation with rIL2. The increase correlates well with the increase in cell size and entry into the cell cycle. At 72–96 h after stimulation, fewer cells are proliferating and the average conductance decreases towards the level of unstimulated control cells. Cell capacitance also decreases, but more slowly. As a consequence of a small phase difference in the rates of change in voltage-gated cell current and capacitance, the calculated average current density increased significantly by day 1 and then fell by day 4 to a value at or somewhat less than that of the unstimulated control. It is clear that the K conductance is a dynamic parameter that is correlated with cell growth and proliferation. Whether the increase in K conductance plays a regulatory role in stimulated proliferation remains to be determined. The decrease as cells leave the cycle might suggest a reduced need for this conductance, or even a specific requirement for its down-regulation in order for the cells to leave the cycle and readjust cell size. The voltage sensitivity and activation and inactivation rates did not appear to vary significantly over the growth period.

T cells from lymph nodes or spleens of MRL +/+ mice exhibit a time-dependent increase of 10-fold in voltage-gated K conductance within 16–54 h in response to the mitogen concanavalin A (6). This increase is even more dramatic than that which we observed in the L2 line, consistent with the fact that the quiescent primary MRL +/+ T cells have very low initial K conductance. The maximal average conductance of the IL2-stimulated L2 cells was ~3.7 ± 2.3 nS (day 2 conductance). This is very close to values reported by Fukushima et al. (15) for the conductance of a cytotoxic T cell clone 3 d or later after stimulation with irradiated spleen cells (3.6 ± 2.9 nS) and by DeCoursey et al. (6) for these concanavalin A-stimulated T cells of MRL +/+ mice (3.8 ± 1.2 nS). In all cases, the maximal conductance of the stimulated murine T cells is about the same or somewhat less than that observed in unstimulated human peripheral blood T lymphocytes (2, 8a, 30). Mitogen stimulation of human T cells induces less

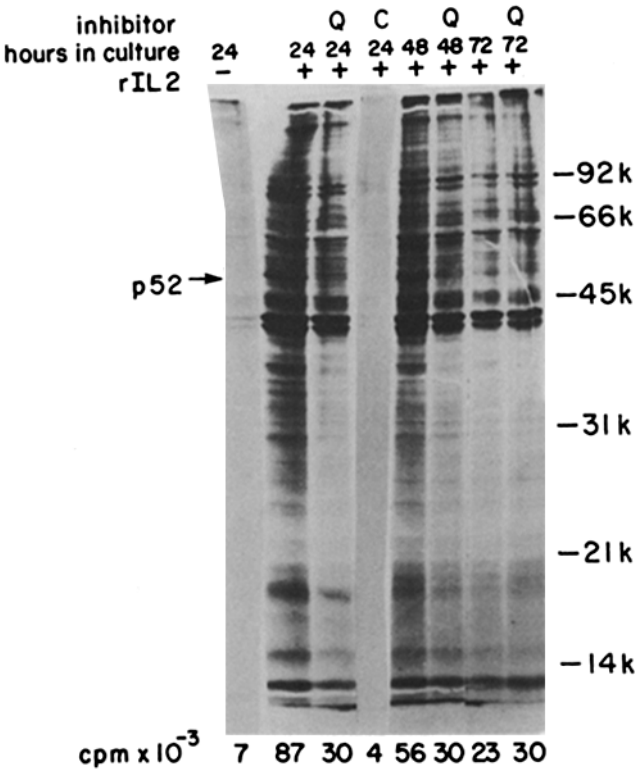

Figure 8. Effect of quinine and cycloheximide on protein synthesis. Similar cultures to those described in Fig. 7 were used to assay for the rate of protein synthesis in rIL2-stimulated cells in the absence or presence of quinine (0.1 mM; lanes marked *Q*) or cycloheximide (15 μg/ml; lane *C*). At various times 10^6 cells were removed from culture and resuspended in 1 ml of methionine-free Dulbecco's modified Eagle's medium that contained 50 μCi [^{35}S]methionine and incubated for 1 h at 37°C. Cells were then lysed, and the proteins separated by SDS PAGE electrophoresis. Newly synthesized proteins were visualized by autoradiography. Total protein synthesis in the 1-h pulse is indicated at the bottom of each lane as counts per minute of incorporated ^{35}S in a 5-μl aliquot of cell lysate. The rate of total protein synthesis increased ~12-fold in 24 h in rIL2-stimulated cells. Synthesis of a 52-kD protein (arrow) serves well as a marker of cell proliferation. Cells cultured in the presence of 0.1 mM quinine had a lower overall rate of protein synthesis and showed diminished levels of the 52-kD protein. Cycloheximide strongly inhibited protein synthesis with little if any detectable expression of the 52-kD protein. Cycloheximide also completely inhibited DNA synthesis (data not shown).

than a twofold increase in peak voltage-gated K current (8a, 30). It is possible that both the mouse and human cells have an equivalent requirement for this K conductance to support cell growth and proliferation; the difference is that the human cell starts with nearly sufficient K conductance to meet its needs, but the murine T-lymphocyte does not.

The other studies in which increased K conductance of stimulated lymphocytes has been described involved stimulation with either phorbol ester (8a) or mitogenic lectins (6, 30). The former involves direct activation of protein kinase C; whereas the latter induce both phosphatidylinositol turnover (27) and the production, release, and self-utilization of IL2 (36). Binding of IL2 has been reported to effect a rapid redistribution of protein kinase C to cellular membranes in IL2-dependent murine CT6 cells, with a similar dose-dependence as IL2-driven proliferation (10). This redistribution of kinase C is similar to that produced in the same cells with phorbol ester and may require the breakdown of phosphatidylinositol (10). Activation of protein kinase C as an integral part of the proliferative response may lead to enhanced expression of K channels and the observed increase in cellular K conductance.

A possible role for the K channel in stimulated T cell growth is to maintain a permissive membrane potential. In human T cells, the K channel has a very small, but finite probability of being open at the resting potential (2), and probably is important in setting this potential, as quinine depolarizes lymphocytes (13, 35). Depolarization or hyperpolarization of T cells from the resting potential diminishes the size of the increase in intracellular Ca^{2+} due to binding of lectin or specific activating antibody (16, 32). The diminished size of the Ca^{2+} increase is correlated with a diminished proliferative response in human peripheral blood T cells (16). However, a Ca^{2+} response to direct IL2 stimulation has not yet been demonstrated.

The K channel may also function as a modulator of cell volume. There is reason to believe that the voltage-gated potassium conductance is the source of the increased K^+ permeability seen in lymphocytes exposed to hypotonic shock (8a, 19). As such, the K channel would provide an important support function for coordinated uptake of nutrients and cell growth, but might not be directly involved in the transduction of the IL2 signal.

The Effect of Quinine

Addition of quinine simultaneously with rIL2 to L2 cells markedly inhibits protein synthesis and subsequent cell division. This is not a general cytotoxic effect since addition of quinine at 18 or 30 h after IL2 stimulation had little or no effect on DNA synthesis at 48 h (data not shown). Inhibition of stimulated protein synthesis has been reported in phytohaemagglutinin-stimulated human peripheral blood lymphocytes incubated with the K channel blockers quinine, 4-aminopyridine, and tetraethylammonium (4).

We have shown that the increased rate of expression of a 52-kD protein in L2 cells is associated with the proliferative response in these cells (38). A 51-kD and a 60-kD protein induced by phytohaemagglutinin-stimulation of human lymphocytes have also been reported to correlate with entry into S phase (20). The level of expression of the 52-kD protein appears to reflect the level of inhibition of cell cycle progression of cycloheximide- or quinine-treated cells.

At this time, it is not known whether the inhibitory effect of quinine on protein synthesis is related to its effect on the potassium conductance. If the mechanism by which quinine inhibits protein synthesis is unrelated to the K channel, then the ability of quinine to block cell cycle progression may also be unrelated to its effect on K conductance. While our own data suggest that K conductance is highly regulated during cellular proliferation, a truly specific blocker of this channel is necessary to elucidate the role of K channels in cellular proliferation.

In summary, we found a voltage-gated potassium conductance in the murine helper T cell clone, L2. The magnitude of this conductance increases in the first 24–48 h after stimulation with human recombinant IL2. As cells leave the cell cycle beginning at ~96 h, the conductance decreases. The K channel blocker quinine markedly slows cell cycle progression and inhibits protein synthesis to an extent sufficient to ac-

count for its inhibitory effect on proliferation. It is not known if the inhibition of protein synthesis is a direct consequence of blocking the K channel, or if the drug has additional effects. This simple system, consisting of cloned T cells and rIL2, provides an excellent model for studying the role of K channels in mitogenesis.

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