Mitogen Induction of Ion Channels in Murine T Lymphocytes

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ABSTRACT Using gigohm-seal recording, we studied ion channel expression in resting and activated T lymphocytes from mice. Both the number of channels per cell and the predominant type of K^+ channel depend upon the state of activation of the cell. Unstimulated T cells express small numbers of K^+ channels, typically a dozen per cell, and are heterogeneous, usually expressing either type n or type l K^+ channels (see DeCoursey, T. E., K. G. Chandy, S. Gupta, and M. D. Cahalan. 1987. *Journal of General Physiology.* 89:379-404). 1 d after stimulation by the murine T cell mitogen concanavalin A, large numbers of type n K^+ channels appear in enlarged, activated cells. Type n channels appear in activated cells with a time course consistent with that reported for mitogen-induced enhancement of protein synthesis. Voltage-gated tetrodo-toxin-sensitive Na^+ channels present in about one-third of unstimulated cells from the MRL-n strain are increased ~10-fold after activation.

INTRODUCTION

Recent studies using the gigohm-seal technique have revealed that the predominant ion channel in T lymphocytes and T lymphocyte–derived cell lines is a K^+-selective channel that opens and subsequently inactivates when the membrane potential is depolarized (Fukushima et al., 1984; DeCoursey et al., 1984a, b, 1985c; Matteson and Deutsch, 1984; Cahalan et al., 1985; Chandy et al., 1986). Possible physiological functions for K^+ channels in T lymphocytes include maintaining the resting potential and mediating volume-regulatory responses (DeCoursey et al., 1985c; Deutsch et al., 1986b). Evidence that K^+ channels are involved in the mitogenesis of T lymphocytes has recently been reviewed (Chandy et al., 1985).

Long-term changes in the maximum K^+ conductance ($g_{K,max}$) of activated human T lymphocytes are relatively subtle and vary with different stimuli. Phorbol myristic acid at mitogenic concentrations or allogeneic cells increase the $g_{K,max}$ of human T cells after 1–2 d by less than twofold; phytohemagglutinin reduces $g_{K,max}$ after 1–3 d; and succinyl concanavalin A has little or no effect

(Matteson and Deutsch, 1984; Lee et al., 1985; Deutsch et al., 1986a; Chandy, DeCoursey, Sharma, Cahalan, and Gupta, manuscript in preparation). Human T lymphocytes activated by allogeneic cells express on average 70% more K^+ channels per cell; the cells are enlarged, however, and the density of K^+ channels is the same as in unstimulated T cells (Chandy et al., manuscript in preparation). In contrast to the relatively small alterations of $g_{K,max}$ in human T lymphocytes, dramatic changes take place in activated murine T lymphocytes. In this article, we describe ion channel expression in several "normal" murine strains, before and after incubation for various times with the mitogen concanavalin A (Con A). Normal resting murine T cells express only a few K^+ channels, but the number of channels and the channel density increase by at least an order of magnitude within 1 d after activation. This increase in $g_{K,max}$ is dramatic compared with human T cells, since resting human T lymphocytes already express several hundred K^+ channels (DeCoursey et al., 1984b; Cahalan et al., 1985).

After activation by mitogens, lymphocytes increase in size before dividing (Biberfeld, 1971; Douglas, 1971). We studied the increase in murine T cell diameter after activation with Con A to evaluate the validity of the selection of individual cells for patch-clamp analysis, and also because cell size is a readily measurable parameter that probably reflects the degree of activation of a particular population of cells. A close correlation is found between T cell enlargement and the characteristic expression in activated T cells of large numbers of type n K^+ channels.

Preliminary accounts of some aspects of this work have appeared (DeCoursey et al., 1984a, 1985a–c).

METHODS

The mice and materials used, the procedures for isolating T cells, and the voltage-clamp techniques employed are described in the preceding article (DeCoursey et al., 1987). Hamilton's R is used to compare the goodness of fit of data to different models (Hamilton, 1964).

Enlargement and Selection for Patch-Clamping of Con A–activated T cells

Lymphocytes enlarge detectably within a few hours of activation and continue to enlarge for 24–36 h. Since responses to mitogen within a population of cells may be heterogeneous, it is helpful to consider the use of cell size in the selection of individual cells for patch-clamping. In general, cells were selected that appeared to be viable and to typify the population of cells present. To evaluate the selection of cells, it is useful to know the distribution of cell sizes at various times after activation.

The cell diameters measured in some of the cell populations used in the present study are illustrated in Fig. 1. Histograms of the diameters of C57BL/6J mouse cells after incubation with Con A for various times are compared with cells incubated for 2 d without mitogen. Some enlargement was apparent 6 h after the addition of Con A, the mean diameter increasing significantly ($p < 0.005$) from 6.5 to 6.9 μm. The fraction of cells 7–8 μm in diameter increased from 9 to 44%, whereas there was no increase in the fraction of cells >8 μm (4%), even after 12 h. By 28 h, a generalized enlargement was obvious, and there was a substantial fraction (36%) of dramatically enlarged cells (>8 μm). After 2 d of activation, there was further enlargement, and the majority of cells were >8 μm in diameter (57% by 49 h and 73% by 54 h). A similar study of cell size in MRL-n mice yielded qualitatively similar results, except that a somewhat smaller fraction of cells enlarged.

For populations of resting cells or cells activated for ~10 h or less, most cells selected for patch-clamp recording were judged to be representative of the sizes present. Populations of cells activated for longer times included a substantial fraction of obviously enlarged cells, so cells were selected for study that were representative of the sizes of the enlarged
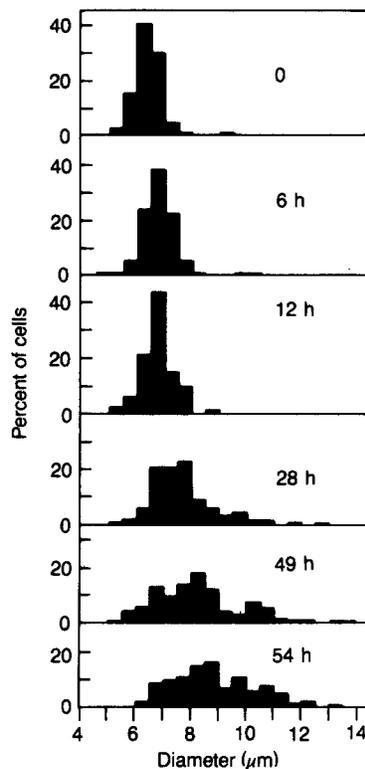
FIGURE 1. Histograms of diameters of T lymphocytes after incubation with 2 μg/ml Con A for the indicated times. T cells were isolated, using a nylon wool column, from lymph nodes from a 4-mo-old C57BL/6J mouse. The size measurement was undertaken in a manner corresponding as closely as possible to the way diameters were estimated during patch-clamp recording. Tubes containing cells in medium were removed from the incubator, shaken, and aspirated, and a few drops of medium were placed onto the glass recording chamber. The cells were allowed to settle for a few minutes and were then photographed at an image size of 100X. Transparencies were projected and cell diameters were measured, excluding cells that appeared to be damaged ("fried-egg" cells). Usually 100–200 cells were measured for each condition. Unstimulated cells ("0") were incubated for 2 d as a control for possible effects of incubation on cell size. The mean ($\pm$ SD) diameter was 6.5 $\pm$ 0.8 μm (0 h incubation with Con A), 6.9 $\pm$ 0.7 μ (6 h), 6.8 $\pm$ 0.6 μm (12 h), 7.7 $\pm$ 1.2 μm (28 h), 8.3 $\pm$ 1.6 μm (49 h), and 8.9 $\pm$ 1.5 μm (54 h), all increases significant ($p < 0.005$) compared with the 0-h control.

cells present, rather than being extreme examples. The rationale was to study activated cells, rather than cells that for some reason might not have responded to the mitogen. Thus, the diameter of resting cells studied ranged from 4.0 to 7.5 μm: cells studied within 10 h after the addition of Con A were 6.0–7.5 μm; those studied within 15–29 h were

6.5–9.0 μm; and those studied within 49–54 h were 8.0–10.0 μm. Comparison of these values with Fig. 1 shows that the diameters of the cells studied are representative.

RESULTS

K^+ Channels in Resting T Lymphocytes

Whole-cell currents recorded in T lymphocytes from several strains of mice are illustrated in Fig. 2. The predominant ionic currents in most quiescent cells were K^+ currents of variable magnitude activated by depolarization of the membrane. A striking difference between these K^+ currents, compared with those in human T lymphocytes (DeCoursey et al., 1984b; Matteson and Deutsch, 1984; Cahalan et al., 1985), is that those in murine cells are much smaller. Small K^+ currents were found in all strains of mice studied (Table I), with an average maximum K^+ conductance, $g_{K,max}$, of ≤ 0.4 nS. The average $g_{K,max}$ in human peripheral blood T lymphocytes is 4.2 nS (Cahalan et al., 1985). Because of the small amplitude of K^+ currents in most cells and the high input resistance when no channels were open, single K^+ channel currents can be clearly distinguished in many cells, as in Fig. 2, B and C.

The properties of two types of K^+ channels in murine T lymphocytes are described in the preceding article (DeCoursey et al., 1987). We designated the two types of K^+ channels type l (for lpr, or "large") and type n ("normal"). Type n K^+ channels closely resemble voltage-gated K^+ channels in human T cells (DeCoursey et al., 1984b; Cahalan et al., 1985) and in murine macrophages (Ypey and Clapham, 1984; Gallin and Sheehy, 1985). We used several approaches to determine which types of channels were present in individual murine T cells. In cells with larger K^+ currents, TEA sensitivity and K^+ current kinetics and voltage dependence were studied. In most of these cells, one or the other channel type predominated, accounting for at least 80–90% of the total g_K. Therefore, the cells were classified according to the predominant type of K^+ channel present, although both type l and type n K^+ channels can be present in individual T cells (Chandy et al., 1986; DeCoursey et al., 1987). The g_K of 13 MRL-n lymph node cells was categorized on the basis of macroscopic properties. MRL-n cells with a higher $g_{K,max}$ tend to be mainly type l, while the type n cells tend to have a smaller $g_{K,max}$, the largest being 0.56 nS. Given this tendency of MRL-n cells with a high $g_{K,max}$ to express type l channels, the population of cells categorizable on the basis of macroscopic current behavior is clearly biased, since cells with sizable currents are more readily categorized. For this reason, we classified six other cells with $g_{K,max}$ values ranging from 0.01 to 0.26 nS, on the basis of the predominant unitary K^+ current amplitude seen in repeated ramp-clamp runs; three appeared to express mainly type l and three expressed type n channels. Thus, of 19 unstimulated MRL-n cells categorized, 8 were mainly type n and 11 were type l, which suggests that roughly half of the MRL-n cells are of each type. Resting T lymphocytes from murine strains other than MRL-n also express both types of K^+ channels. In summary, based on average whole cell and single channel conductances, an average of 12 K^+ channels were found, with both type n and l K^+ channels present, in individual resting T lymphocytes from several strains of mice.
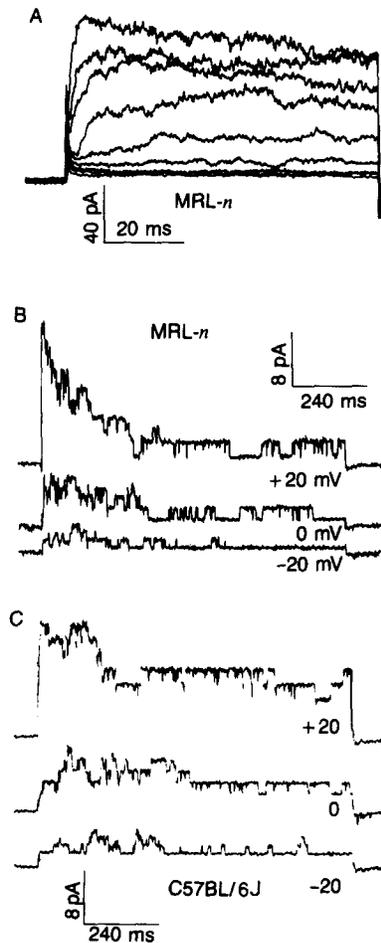
FIGURE 2. Whole-cell currents, reflecting the presence of small numbers of K^+ channels in murine T lymphocytes. (A) Currents during steps to −60 through +20 mV in 10-mV increments, from a holding potential of −80 mV in a lymph node cell from a 4-mo-old MRL-n mouse. Recorded 75 min after the whole-cell configuration was attained. (B) Current in an MRL-n cell, held at −80 mV and stepped to the potentials shown. Inactivation of the K^+ currents is apparent even in these records in which the peak currents correspond to fewer than 10 K^+ channels. In other records from this cell, single K^+ channel currents of distinctly smaller amplitude were also present. (C) Currents in a C57BL/6J mouse cell, held at −80 mV and stepped to the potentials indicated. Both this cell and the one in B had mainly type *l* K^+ channels.

The expression of ion channels in murine macrophages varies markedly at different times after plating of suspended cells (Ypey and Clapham, 1984; Gallin and Sheehy, 1985); this is possibly related to the activation of these cells during the process of adherence to the substrate. To test whether in vitro cell culture might alter the expression of ion channels in murine T cells, we compared MRL-

n cells cultured for 1, 2, or 3 d after separation. No significant changes were detected in $g_{K,max}$.

Increased Number of Type n K^+ Channels in Activated T Cells

The $g_{K,max}$ values in individual cells incubated for various times in the presence of the mitogen Con A are plotted in Fig. 3. The geometric means and SD for resting (unstimulated by mitogen) cells from three strains are shown on the left. Little change was detectable during the first 10 h. After ~15 h, $g_{K,max}$ clearly increased in most cells. After 1 d of incubation with Con A, the typical cell had a $g_{K,max}$ ~10-fold larger than that in resting cells. In several cells incubated with Con A for 2 d, $g_{K,max}$ appeared to have further increased. The mean $g_{K,max}$ in seven BALB/c cells activated with Con A for 2–3 d was 4.1 ± 2.6 nS (± SD) (not shown). When cells from all control strains were pooled, the $g_{K,max}$ for all cells

TABLE I

Maximum K^+ Conductance in Resting Murine T Lymphocytes

Strain	Source	Number	$g_{K,max}$	SD	Range
			nS	nS	nS
MRL-n	LN*	28	0.44	0.71	0–2.8
	SPL‡	10	0.17	0.11	0.01–0.34
	All	38	0.37	0.62	0–2.8
CBA/J	SPL	5	0.14	0.23	0–0.54
	SPL§	12	0.16	0.16	0–0.54
C57BL/6J	LN	10	0.16	0.13	0–0.33
BALB/c	SPL	2	0.32	—	0.31–0.32
C3H/HeJ	SPL	2	0.01	—	0–0.01

The mean value for $g_{K,max}$ for C57BL/6J cells was reported in DeCoursey et al. (1985c). The maximum K^+ conductance ($g_{K,max}$) was calculated from the largest K^+ current recorded in each cell, after subtraction of a linear leak current, using the measured reversal potential, or assuming that the reversal potential was −80 mV. Means are arithmetic (cf. Fig. 3).

* LN, lymph node.
‡ SPL, spleen.
§ Includes seven cells incubated with Con A for ≤5 h.

activated for 1 d with Con A was 2.0 ± 1.2 nS (mean ± SD, $n = 16$), and the mean for cells activated with Con A for 2–3 d was 5.2 ± 3.6 nS ($n = 12$). The increase in $g_{K,max}$ between cells activated for 1 d and those activated for 2–3 d is significant ($p < 0.005$). The data in Fig. 3 may not strictly reflect the time course of the increase in $g_{K,max}$ in individual cells; cell-to-cell heterogeneity might result in nonsynchronous activation of cells.

Since activated cells are larger than resting cells, some of the increase in $g_{K,max}$ could reflect the increased surface area, if the K^+ channel density in the membrane remained constant. In Fig. 4, the $g_{K,max}$ in murine T cells incubated with Con A (filled symbols) is plotted as a function of cell diameter. The curve shows the $g_{K,max}$ expected if the density of K^+ channels is the same in activated cells as in resting MRL-n cells. Most cells incubated with Con A for only a few hours had not yet enlarged and had a small $g_{K,max}$. In the cells that were clearly

enlarged, the density of K^+ channels was increased by about an order of magnitude. Similar results are obtained if the membrane area is estimated by the input capacity (data not shown). One large, quiescent MRL-n cell, 9 μm in diameter, had a $g_{K,max}$ of 3.5 nS, larger than that of any other resting MRL-n cell studied, and the channels were type n on the basis of low TEA sensitivity. The K^+ channels in this (presumably) spontaneously activated cell thus resembled those in mitogen-activated cells. Inspection of Fig. 1 suggests that T cells >8 μm in diameter, after incubation for <12 h with Con A, are likely to be spontaneously activated cells, since the majority of cells enlarge more slowly. The cell in Fig. 3, with a high $g_{K,max}$ (2.2 nS) after only 7 h of incubation with Con A, probably falls
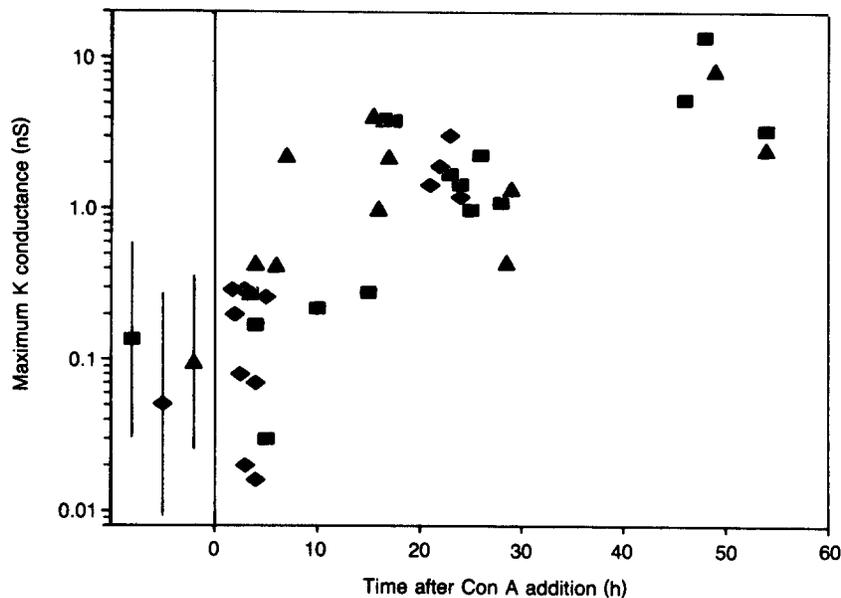
FIGURE 3. Maximum K^+ conductance of murine T cells, plotted against time of incubation with 2 μg/ml Con A. Mouse strains are MRL-n (■), CBA/J (◆), and C57BL/6J (▲). The geometric means and SD of unstimulated cells from the three strains studied are plotted on the left. As described in the Methods, the cells selected for study were within the range of typical sizes for the respective time of incubation with Con A.

into this category, since it was 9 μm in diameter. On the other hand, large cells studied after 1 d of activation are likely to represent mitogen-activated cells.

The close correlation found between T cell enlargement and the characteristic expression in activated cells of large numbers of type n K^+ channels is consistent with the interpretation that unstimulated cells that are enlarged and express large numbers of K^+ channels reflect the small subset of cells that are spontaneously activated. In general, then, murine T cell diameter appears to be a good determinant of activation, particularly as manifested by an increased $g_{K,max}$. However, even cells that were not clearly enlarged after 2 d of incubation with Con A were apt to have a much larger $g_{K,max}$ than unstimulated cells of similar

size. Conversely, unstimulated cells within the range of typical cell sizes exhibited no clear correlation between diameter and $g_{K,max}$ (Fig. 4), as observed previously for unstimulated human peripheral T cells (Cahalan et al., 1985; Deutsch et al., 1986a).

In all clearly activated murine T cells, i.e., enlarged cells with large $g_{K,max}$ values, the predominant K^+ channel present was type n. This conclusion is based on several kinds of macroscopic measurements (DeCoursey et al., 1987), and was confirmed by a TEA dose-response curve, revealing low sensitivity to TEA, in at least one cell from each strain, including MRL-n, CBA/J, BALB/c, and C57BL/6J.
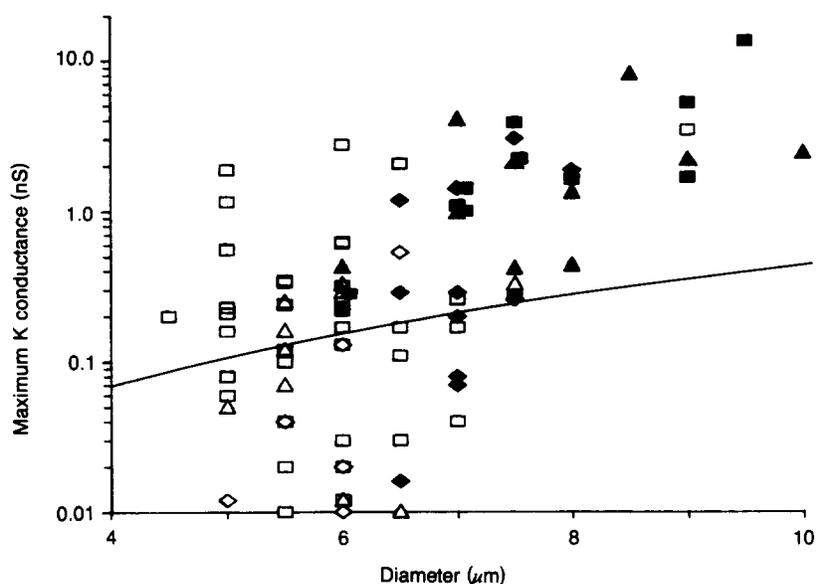
FIGURE 4.   Maximum K^+ conductance in T cells from three control strains of mice, plotted as a function of cell diameter. Symbols represent the same strains as in Fig. 3; open symbols represent unstimulated cells; filled symbols represent cells incubated with Con A. A number of the cells plotted were incubated with Con A for only a few hours, and their size and g_K had not yet increased. Note that one large unstimulated MRL-n (9 μm diam) had a g_K in the same range as Con A–activated cells. The curve is drawn through the mean diameter and geometric mean $g_{K,max}$ of all resting MRL-n cells, and shows the relationship expected if $g_{K,max}$ is directly proportional to membrane area.

Na$^+$ Channels

Inward current single channel events were detected in 11 of 34 resting MRL-n cells studied. Fig. 5A shows whole-cell currents at high gain in an MRL-n cell. Inward currents were observed during depolarizing steps positive to about -70 mV, and were largest at -30 to -20 mV. The voltage dependence and single channel conductance of these events resembles voltage-dependent Na^+ currents in muscle and nerve. Fig. 5B shows that the current-voltage relation of the single

open channel is linear between −60 and +30 mV. Assuming a reversal potential of +60 mV, the single channel conductance in Ringer is 16–18 pS, which is similar to that of Na^+ channels in cultured rat cells (Sigworth and Neher, 1980). In 11 cells with inward current events, the largest inward currents observed correspond to 5.7 ± 1.7 (mean ± SE) channels, with a range of 1–8 channels in 10 cells and 22 channels in the remaining cell. These inward currents are probably due to Na^+ channels, although, in some cells, inward current events that did not inactivate during 100-ms pulses were also observed. An intriguing aspect of the distribution of Na^+ channels is that they were never observed in cells expressing predominantly type *l* K^+ channels. Na^+ channels were detected
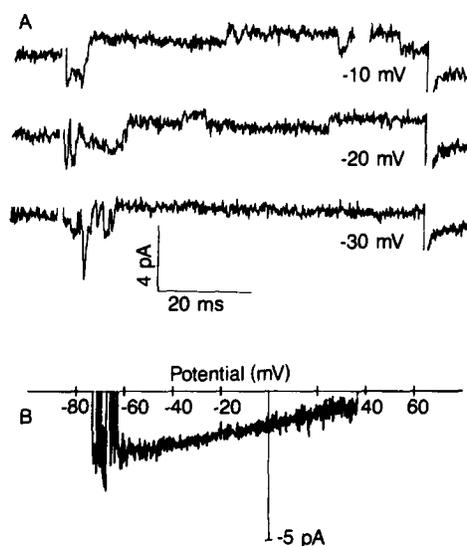
FIGURE 5. Na^+ currents in unstimulated MRL-*n* T lymphocytes. (*A*) Whole-cell currents in a resting MRL-*n* cell held at −100 mV and stepped to the potentials indicated. The downward deflections reflect Na^+ channel opening. In the records at −10 and −20 mV, single K^+ channel openings can be seen as upward deflections late in the records. (*B*) Average current-voltage relationship of single open Na^+ channels in a resting MRL-*n* cell. Whole-cell configuration; 2 kHz; 64 ramps analyzed. By linear regression, the single Na^+ channel conductance is 16.7 pS and the extrapolated reversal potential is +67 mV.

in only 1 of 41 cells studied from murine strains other than MRL: a C57BL/6J cell activated for 29 h with Con A had at least eight Na^+ channels. It appears that MRL-*n* cells are simply more likely to express Na^+ channels than are cells from other strains.

Increased numbers of Na^+ channels were detected in some activated T cells (Fig. 6). Na^+ currents were detected in 4 of 11 MRL-*n* cells activated for 15–54 h with Con A. As in the cell illustrated in Fig. 6, Na^+ currents in activated cells were much larger than in resting cells. Assuming a unitary conductance of 18 pS, the maximum conductances correspond to 8, 19, 53, and 200 Na^+ channels. Although the number of cells with Na^+ currents was small, the simplest interpre-

tation is that the fraction of cells expressing Na^+ channels does not change upon activation (32% of resting cells, 36% of activated cells); the number of Na^+ channels per cell increases dramatically ($p < 0.025$) by about an order of magnitude, an increase comparable to that observed for K^+ channels.

Inward currents in activated MRL-n T cells were reversibly inhibited by addition of the specific Na^+ channel blocker tetrodotoxin (TTX) to the bath, and were reversibly abolished by replacement of external Na^+ by tetramethyl-ammonium$^+$ or Cs^+ (data not shown), which confirmed their identity as Na^+ currents. The sensitivity of Na^+ currents to TTX was studied in two MRL-n T cells activated for 2 d with Con A. In one cell, half-block by TTX was observed only at ~ 1 μM; in the other, half-block was at ~ 40 nM. Although more complete characterization is desirable, the TTX sensitivity of Na^+ channels in activated murine T cells appears to be much lower than in human T cells (Cahalan et al., 1985).
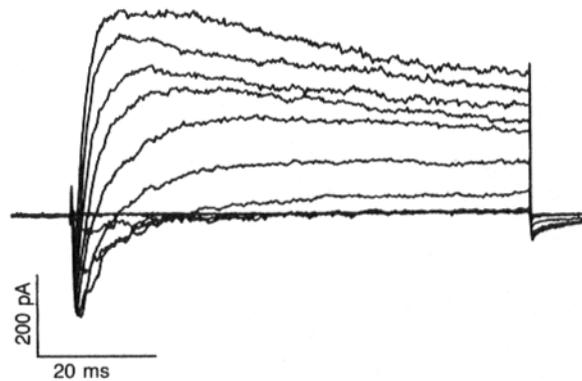
FIGURE 6.   Large whole-cell Na^+ and K^+ currents in an MRL-n cell incubated for 46 h with Con A. The cell was held at -100 mV and stepped every 20 s to potentials from -80 to $+20$ mV in increments of 10 mV. A sizable K^+ current is first apparent at -40 mV; Na^+ current starts to be activated at about -60 mV. Recorded 20 min after the transition to the whole-cell configuration.

In Con A–activated MRL-n cells, the Na^+ currents were large enough to allow macroscopic characterization. The peak Na^+ conductance was half-maximal at -48 mV in the cell shown in Fig. 7 A, and at -46 mV in another cell. MRL-n Na^+ currents display voltage-dependent inactivation, with properties like those in other excitable cells. In the cell shown in Fig. 7 B, half the Na^+ channels were inactivated at -70 mV. The small number of Na^+ channels in most resting MRL-n T cells made quantification difficult, but a roughly comparable effect of holding potential was seen on the frequency of unitary Na^+ channel events during a given test pulse.

In three separate experiments, Con A–stimulated [^{3}H]thymidine incorporation (a measure of DNA synthesis) by MRL-n T cells was not affected by TTX, at concentrations ranging from 20 nM to 2 μM (data not shown). Apparently, functional Na^+ channels are not required for mitogenic activation of murine T lymphocytes.
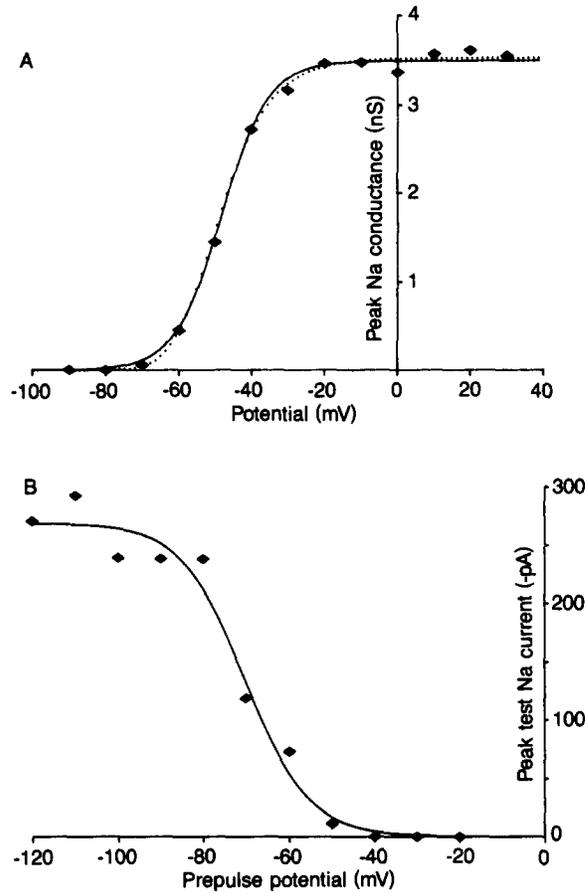
FIGURE 7. Voltage dependence of Na^+ currents in the Con A–activated MRL-n cell shown in Fig. 6. (A) Voltage dependence of peak Na^+ conductance, g_{Na}. The cell was held at -100 mV and stepped to the potentials shown, and the peak Na^+ current was measured. A reversal potential of $+50$ mV was assumed to calculate g_{Na}. The smooth curve shows the best nonlinear least-squares fit of the data points to a simple Boltzmann function:

$$g_{Na}(V)/g_{Na,max} = 1/\{1 - \exp[(V - V_m)/k_m]\}^x,$$

where $x = 1$, and midpoint $V_m = -47.7 \pm 0.5$ mV ($\pm$ SD in the fit), slope factor $k_m = -6.5 \pm 0.4$ mV, and $g_{Na,max} = 3.50 \pm 0.04$ nS (Hamilton's $R = 0.026$). The dotted curve shows the best fit to a Hodgkin-Huxley–type m^3 function (Hodgkin and Huxley, 1952), as above but with $x = 3$, with $V_m = -59.6 \pm 0.8$ mV, $k_m = -8.5 \pm 0.5$ mV, and $g_{Na,max} = 3.53 \pm 0.04$ nS ($R = 0.025$). The Hamilton R values for the two fits are too similar to distinguish between them (see Hamilton, 1964). (B) Voltage dependence of steady state Na^+ current inactivation. The cell was held at -100 mV, stepped for 60 ms to the potential on the abscissa, V_{pre}, and then stepped to the test potential, -30 mV, at which Na^+ currents were large. The peak Na^+ current during the test pulse is plotted, along with a curve showing the best fit of the data points to a simple Hodgkin-Huxley–type Boltzmann distribution:

$$I_{Na}(V_{pre})/I_{Na,max} = 1/\{1 + \exp[(V_{pre} - V_h)/k_h]\},$$

with fitted parameters ($\pm$ SD in the fit): maximum test Na^+ current ($I_{Na,max}$) = 268 ± 10 pA, midpoint $V_h = -69.8 \pm 1.5$ mV, and slope factor $k_h = 7.5 \pm 1.3$ mV.

DISCUSSION

Ion Channels in Resting Murine T Lymphocytes

Voltage-dependent K^+-selective channels are the most abundant ion channels in T lymphocytes from all "normal" mouse strains studied. The density of K^+ channels is quite low in resting lymph node or splenic T cells, on the order of 10 per cell. In striking contrast, unstimulated human peripheral blood T lymphocytes express several hundred K^+ channels (DeCoursey et al., 1984b; Cahalan et al., 1985), comparable to the numbers of K^+ channels in Con A–activated murine cells. The dramatic difference in the numbers of K^+ channels expressed by human and murine resting T lymphocytes is not due to the different methods of T cell separation in these studies, since human cells purified by nylon wool column (as were the murine cells) express roughly the same numbers of K^+ channels as human T cells separated by rosetting with sheep erythrocytes (DeCoursey et al., 1985c). There may be species differences in K^+ channel expression in T lymphocytes. Another possibility is that K^+ channel expression may be different in peripheral blood T lymphocytes compared with T cells residing in lymph node and spleen.

Roughly half of all resting T cells from MRL-n mice express predominantly type l K^+ channels; the remainder express predominantly type n channels. Although MRL-n cells with type l channels tended to have a higher $g_{K,max}$, they had a smaller $g_{K,max}$ than T lymphocytes from the mutant strain MRL-l, in which all cells had type l K^+ channels (Chandy et al., 1986). Small numbers of both type l and type n channels were also found in resting T lymphocytes from other mouse strains examined.

About one-third of all T cells from MRL-n mice expressed Na^+ channels resembling those of excitable cells. Na^+ channels were only rarely detected in other murine strains, are rarely observed in human peripheral T cells (Cahalan et al., 1985), and are occasionally present in human thymocytes (Schlichter et al., 1986b). Some human T cell–derived cell lines express Na^+ channels regularly, however (DeCoursey et al., 1985c), as do two murine natural killer cell lines (unpublished studies in collaboration with Dr. G. Dennert, University of Southern California), and the human erythroleukemic cell line K562 (Schlichter et al., 1986a).

Increased Ion Channel Expression in Activated T Lymphocytes

The number of K^+ channels increases dramatically from an average of 12 channels in a resting T cell to ~400 in cells activated with Con A for 2–3 d. Type n K^+ channels are selectively increased over type l channels. Although the diameter of Con A–activated cells from control mice increases upon activation (Fig. 1), the $g_{K,max}$ increases to a much greater extent than the surface area. When the K^+ channel density is calculated for each individual cell, the increase is still about an order of magnitude. The average K^+ channel density in resting murine cells from several control strains is ~0.1 μm^{-2}, increasing to ~0.9 μm^{-2} after 1 d of incubation with Con A, and to 2.2 μm^{-2} after 2 d. After activation, human and murine T lymphocytes have a similar $g_{K,max}$. Perhaps a large number of K^+ channels is required during proliferation of T lymphocytes in both species.

The large number of K^+ (and Na^+, if present) channels that appear in Con A–activated murine T cells might be (*a*) newly synthesized, (*b*) presynthesized molecules inserted into the membrane as a result of activation of the cell, or (*c*) present in resting T cell membranes in nonfunctional (i.e., nonconducting) form, becoming functional (conducting) during cell activation, for example, by biochemical modification of the channel protein. The time course of the increase in numbers of K^+ channels in Con A–activated murine T cells (Fig. 3) is consistent with a mechanism involving synthesis and insertion of new channels into the membrane. Lee et al. (1986) recently reported that the numbers of K^+ channels increase detectably in a mouse helper T lymphocyte clone 8 h after stimulation with interleukin-2, reaching a maximum in this model system by 24 h at three to four times the resting value. An increased g_K was correlated with increased cell size and entry into the cell cycle. In human T cells, the overall rate of protein synthesis increases, starting ~2 h after addition of the mitogen phytohemagglutinin (Kay, 1968). The increased rate of protein synthesis is maximal at ~10-fold after 2 d and appears to be generalized: the synthesis of most of the several hundred most common proteins increases to roughly the same extent, with a few exceptions (Lester et al., 1981). The order-of-magnitude increase in numbers of both Na^+ and K^+ channels might therefore reflect generalized cell growth, rather than a specific functional adaptation of the cells. If newly functional channels appear in preparation for cell division, then the increase might be expected to be roughly proportional to the membrane area, as is the case for the increased g_K in human T cells activated by allogeneic cells (Chandy et al., manuscript in preparation) and interleukin 2–stimulated helper T cell clones (Lee et al., 1986). Instead, in the mouse, the density of ion channels in T cell membranes increases by at least 10-fold, which suggests that the daughter cells immediately after cell division will express many more channels than resting cells. Perhaps activated or dividing T cells are subject to larger depolarizing ion fluxes than are resting cells, and therefore require many more K^+ channels to maintain their resting potential or cell volume.

Role of K^+ Channels in Murine T Lymphocyte Activation

We have calculated (Cahalan et al., 1985) that the magnitude of K^+ efflux through K^+ channels open at the resting potential in human peripheral blood T cells is comparable to values reported in radioisotope flux studies, and that the rapid increase in K^+ efflux occurring after mitogen stimulation can be accounted for by the rapid alteration by mitogen of the voltage dependence of the K^+ channel (DeCoursey et al., 1984*b*, 1985*c*; Cahalan et al., 1985), leading to an increase in the average number of K^+ channels open at a given resting potential. One difference between human and murine T lymphocytes is that K^+ fluxes are increased in human cells within minutes of the addition of mitogen (Quastel and Kaplan, 1970; Averdunk, 1972; Segel and Lichtman, 1976), whereas in splenic T lymphocytes from BALB/c mice, K^+ fluxes are not measurably altered during the first 6 h after addition of Con A, but are significantly increased after 14 h (Owens and Kaplan, 1980). As shown in Fig. 3, the number of K^+ channels in murine T cells follows a similar time course after the addition of Con A, with no detectable change within the first several hours, but a substantial increase after

~15 h. Perhaps the number of K^+ channels is so low in resting murine cells that even if Con A were to shift the voltage dependence of the channels, any alterations in K^+ efflux would be too small to detect; K^+ fluxes might increase only after the number of K^+ channels has increased substantially.

The appearance of large numbers of type n K^+ channels in Con A–activated T cells from normal mice, together with the presence of large numbers of K^+ channels in human T cells, suggests that more K^+ channels are required during activation or cell division than in resting murine cells. This idea is supported by the observation that mouse clonal T cell lines maintained in a state of chronic activation by stimulation with the appropriate antigen or interleukin-2 express large numbers of type n K^+ channels (DeCoursey et al., 1985c; Lee et al., 1986). Finally, during thymocyte differentiation, cortical thymocytes expressing receptors for peanut agglutinin have hundreds of K^+ channels per cell (unpublished data in collaboration with Dr. R. Lewis). These are daughter cells of the most actively dividing thymocytes (Rothenberg and Lugo, 1985). Thus, most actively proliferating murine T cells, thymocytes, and clonal cells express large numbers of type n K^+ channels.

Pharmacological evidence further indicates an essential role for type n but not type l channels in mitogenesis. K^+ channel blockers inhibit mitogen-stimulated [^{3}H]thymidine incorporation and interleukin-2 production by "normal" murine T cells only at concentrations that block type n K^+ channels (Chandy et al., 1986). Moreover, in the presence of concentrations of TEA that block type l but not n K^+ channels, mitogenesis proceeds normally. Whether type l channels, abundant in functionally abnormal MRL-l T cells and also present in small numbers in control T cells, perform any physiological function remains unclear.

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