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Ion Channels in Leukocytes

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I. INTRODUCTION

The purpose of this article is to review the ionic channels that have been characterized in leukocytes and, whenever possible, to discuss their functional significance. This review makes no attempt to provide a comprehensive view of ionic transport mechanisms in leukocytes, nor does it attempt to provide an inclusive summary of the numerous studies done using ion-flux techniques and fluorescence measurements that have examined the role of ionic transport in leukocyte function. Rather, it focuses on the electrophysiological evidence for the existence of specific ionic channels in leukocytes, with the exception of the basophil, which is not covered in this review. (Information on the basophil is more appropriately included in a review of mast cells.) Several recent reviews are available for those interested in a discussion of other transport mechanisms in leukocytes (47, 69, 73, 75, 87).

Because significant progress in this area has depended on the development of the patch-clamp technique by Neher and Sakmann in 1976 (181), the area of investigation is relatively young; the studies covered in this review are just a beginning. Undoubtedly, many channels are yet to be described in leukocytes, and much more will be learned about the relevance of ionic channels to leukocyte function. The observations that the gating of ionic channels can be modulated by phosphorylation and dephosphorylation reactions (217, 246), as well as by a variety of second messengers (132, 217), already have provided an important regulatory link between biochemical events inside leukocytes and ionic channels (72, 73; e.g., see sect. IV*A3*).

II. TERMINOLOGY

Ionic channels are integral membrane proteins that provide low-energy pathways for ions to cross cellular membranes, allowing ions to flow passively down their electrochemical gradient at rates exceeding 10^6 ions/s. The high rate of flow of ions through channels and their discrete transitions between open and closed states facilitates the measurement of these transport events (currents) through individual macromolecules. It should be noted that for small currents that are not voltage gated and that have single-channel current amplitudes too small to detect (<1 pA), discriminating between a current through an ionic channel and one produced by carrier-mediated transport can be difficult (151, 164; e.g., see sect. IV*A3*).

If an ion channel is open and the membrane potential (V_m) differs from the electrochemical potential for ion x (E_x, the potential difference at equilibrium), then current will flow into or out of the cell depending on the driving force on x. When V_m is equal to E_x, no current (I) will flow. The current flowing across an ion channel divided by the net electrochemical driving force (V) across the channel is equal to its ion conductance (G), which is expressed in siemens (S) or in reciprocal ohms ($G = I/V$), and is a measure of the ease with which ions flow across the channel. Single-ion channels have conductances in the range of picosiemens (10^{-12} S). Some channels allow current to flow more easily in one direction than in the other direction, a property called rectification. Thus an inwardly rectifying cation channel is one in which cations flow more easily into the cell than out of the cell (conversely, an inwardly rectifying anion channel would allow anions to flow out of the cell more easily than into the cell). In these cases, a plot of the relationship between current and voltage (I-V) will be nonlinear (nonohmic).

Ion channels can be characterized by their conductances, gating properties (factors controlling channel opening and closing), kinetics (rates at which channels open and close), ionic selectivity (differential permeability), and pharmacology (the action of specific agents in

blocking or changing the flow of ions). Furthermore, channel activity can be modified by the presence of competing ions or other molecules, such as GTP-binding proteins or inositol phosphates. Ionic channels have been best characterized in excitable cells where they have been studied for the past 50 years (107). With the advent of the patch-clamp technique, much progress has been made on other cell types, including leukocytes, where ionic channel openings in response to specific chemical ligands (ligand gated), voltage (voltage gated), or both have been described.

Most of the studies discussed in this review used the patch-clamp technique. This technique is extremely versatile because it can be used in a number of recording configurations (103). *1*) the cell-attached patch mode in which single-channel currents are recorded from patches of membrane in intact cells, *2*) two excised-patch modes in which patches of membrane are pulled away from the cell and single-channel currents are recorded with the inside surface of the membrane facing either the bath solution (inside-out patch) or the pipette solution (outside-out patch), and *3*) the whole cell configuration in which currents representing an average of the single-channel currents across the whole cell are measured. In the whole cell configuration, the inside of the cell is perfused with the solution in the patch electrode; this allows the addition of second messengers and other substances to the inside of the cell but also has the disadvantage of washing out intracellular constituents that might modulate the ionic channels being studied (103). This disadvantage has been recently eliminated by a modification of the whole cell configuration, the "nystatin-permeabilized patch" (111). The antibiotic nystatin is added to the pipette solution, reducing the resistance between pipette and cytoplasm. Although this is analogous to whole cell recording, large molecules and even divalent ions do not leave the cell, and second messenger-mediated responses can be observed that do not remain functional in conventional whole cell recording.

III. PHAGOCYTIC LEUKOCYTES

Macrophages, neutrophils, and eosinophils are phagocytic leukocytes that are capable of migrating toward invading microorganisms and/or tumor cells, engulfing them, and ultimately killing them. During these events a number of enzymes, cytokines, toxic oxygen products, and other factors having widespread actions are released. In recent years much has been learned about the physiology of phagocytic cells, including the role of phosphoinositide metabolism, GTP-binding proteins, and protein kinase C in phagocyte activation (10, 256, 269). In addition to these agents, interest in the possible role of ions in stimulus-response coupling in phagocytic cells has resulted in a steady increase in electrophysiological studies that have characterized a number of conductances in these cells. Table 1 contains a list of the ionic conductances in phagocytes. These studies examined neutrophils, macrophages, and related tumor cell lines. Unfortunately, no data exist on the ionic channels in the eosinophil.

A. Macrophages

Macrophages, found in virtually every tissue, originate from bone marrow cells that are released into the blood as monocytes (272). Monocytes circulate in the blood for up to several days until they emigrate into the tissues and mature into macrophages. Macrophages can survive in tissues for months and even possibly years, playing pivotal roles in numerous aspects of host defenses, including processing antigens, killing parasites and tumor cells, ingesting dead or dying cells, and secreting cytokines.

Electrophysiological studies at the whole cell or single-channel level have demonstrated that macrophages exhibit both voltage-gated and Ca-gated ionic currents. Four K currents, three Cl currents, and nonselective cation currents have been identified and are described in detail next. Although one laboratory reported action potentials in human monocyte-derived macrophages (165, 283), those events were poorly characterized, and they have not been noted by other investigators. Furthermore, no voltage-dependent Na or Ca currents have been described in macrophages. Both Fc immunoglobulin and ATP receptor-gated ionic conductances have been described in macrophages and are also discussed.

1. Potassium conductances

1) VOLTAGE-DEPENDENT INWARDLY RECTIFYING POTASSIUM CONDUCTANCE. An inwardly rectifying K (K_i) current that activates at voltages negative to −50 mV was first described in intracellular microelectrode studies of mouse spleen and thioglycolate-induced macrophages that had been cultured for several weeks (61, 65). The K_i current has since been characterized in cultured human monocyte-derived macrophages (68, 183), in the murine macrophage-like cell line J774.1 (70), in mouse peritoneal macrophages (212), and in phorbol ester-induced differentiated HL60 cells (a human promyelocytic leukemia cell line) (278). This current is similar to the inwardly rectifying K current characterized in several other cell types, including starfish egg cells (100), frog skeletal muscle (142), heart muscle (80), bovine pulmonary artery endothelial cells (251), and rat basophilic leukemia cells (154).

In macrophages, the K_i current has been best characterized in J774.1 cells, where it has a steep voltage dependence (fractional activation decreased from 66% at −90 mV to 26% at −70 mV) and a time-dependent inactivation (70). Inactivation, which was evident for voltage steps to potentials more negative than −100 mV, followed first-order kinetics and had a rate that increased with membrane hyperpolarization. Whole cell

TABLE 1. *Ion channels in phagocytes*

Channel	Gating	SCG, pS	Blockers	Present In	Reference
			Macrophage		
K channels					
K_o (outward inactivating)	Voltage	16	D 600, TEA, 4-AP, Cs_i, Ba_i	Mouse peritoneal, human blood-derived, human alveolar macrophages; J774.1, P388D1, HL60 cells	70, 183, 290
K_{o2} (outward poorly inactivating)	Voltage		TEA, 4-AP, Cs_i	Human blood-derived macrophages	183
K_i (inwardly rectifying)	Voltage	30*	Ba, Cs, Rb	Mouse peritoneal and spleen, human blood-derived macrophages; J774.1, HL60 cells	68, 79, 211, 278
K_{LCa} (large, Ca and voltage activated)	Ca_i and voltage	240*	CTX, TEA, Cs_i	Human blood derived, human alveolar macrophages	62, 120a, 121, 166
K_{ICa} (inwardly rectifying, Ca activated)	Ca_i	36*	Ba	Human blood-derived, mouse peritoneal macrophages; U937 cells	63, 104, 120a, 121
Cl channels					
Cl_L (large)	Voltage	340	DIDS	U937 cells; mouse peritoneal macrophages	121, 212, 236
Cl_i (intermediate)	Voltage	28	DIDS	U937 cells	121
Cl_s (small)	Voltage	16		U937 cells	121
Cation channels					
Nonselective	Ca_i	Variable	Zn	P388D1 cells; human blood-derived macrophages	155, 183
Fc	Fc-ligand	60		Mouse macrophages	287, 288
			Neutrophil		
K channels					
Outward				Human neutrophils	128
Ca activated	Ca_i			Human neutrophils	128
Cl channels					
Ca activated	Ca_i			Human neutrophils	128
Cation channels					
Nonselective	Ca_i	18-25 4-6		Human neutrophils	276

SCG, single-channel conductance under physiological ionic gradients or *145 mM external K (for rectifying channels, largest conductance is given). Ca_i, Cs_i, Ba_i, internal Ca, Cs, and Ba, 4-AP, 4-aminopyridine, CTX, charybdotoxin, DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; TEA, tetraethylammonium.

K_i currents showing activation and inactivation are shown in Figure 1*A*. Removal of external Na reduced (by >50%) inactivation (Fig. 1*A*, *bottom*) but did not abolish it, suggesting that some of the inactivation was due to the intrinsic voltage dependence of the channel (176). Voltage-dependent inactivation was verified in single-channel records in the absence of external Na (Fig. 1*B*). As in other cells that display this type of inwardly rectifying K conductance (100, 101), raising extracellular K concentration ($[K]_o$) increased the slope conductance for the inward currents and shifted the voltage dependence to the right, indicating that the activation of the K_i conductance depended on $[K]_o$ (70). Similar results were reported in mouse peritoneal macrophages, where increasing $[K]_o$ from 5 to 110 mM increased the maximum slope of the K_i conductance by a factor of 5.1 (212). External Ba blocked the K_i current in a voltage-dependent manner (170), with complete block occurring at 2.5 mM Ba (70). The K_i current was also reduced by the addition of 1 mM Cs (70).

Single-channel currents, the properties of which correspond to the macroscopic K_i current measured in whole cells, have been described in both J774.1 cells (170) and in human peripheral blood-derived macrophages (68). In both these cell types, single-channel currents were evident in cell-attached patches (145 mM KCl in electrode and normal saline in bath) at zero holding potential (the resting V_m of the cells). Under these conditions the single-channel conductance in cell-attached patches was 29 pS for inward currents, and the extrapolated reversal potential was near E_K. No outward currents were noted at potentials above E_K, indicating either an extreme rectification at the single-channel level or an absence of detectable channel openings positive to E_K. In ventricular heart cells, inward rectification through K_i channels was abolished by removing internal Mg (172). In contrast, the inward rectifier in bovine pulmonary endothelial cells has a voltage- and $[K]_o$-dependent gating mechanism that is distinct from Mg block (251). At potentials more negative than

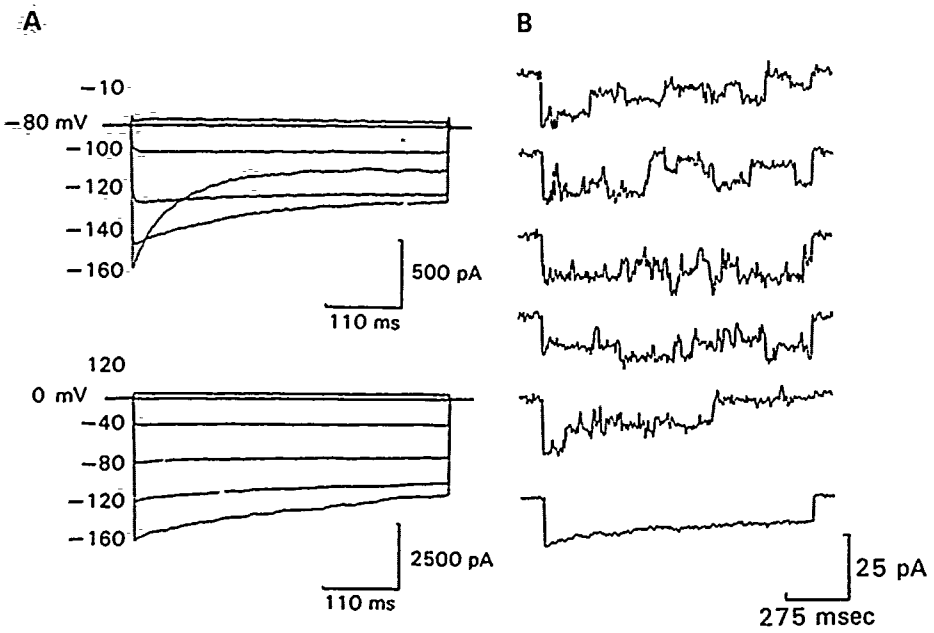

FIG. 1. Inwardly rectifying K (K_i) currents in J774.1 cell. Patch electrode contained (in mM) 145 KCl, 1 $MgCl_2$, 1.1 EGTA, 0.1 $CaCl_2$, and 10 HEPES (pH 7.3). *A*: whole cell currents in response to 440-ms test pulses given every 8 s to potentials shown. *Top*: tracings from cell held at −80 mV and bathed in 150 mM NaCl Hanks' solution. *Bottom*: tracings from cell held at 0 mV and bathed in 150 mM KCl Hanks'. *B*: first 5 current tracings are single-channel currents recorded in cell-attached patch configuration recorded in response to voltage step to −190 mV from holding potential of −30 mV. Bottom tracing is averaged current record for above tracings, comprised of 40 individual tracings. Cell bath is 150 mM KCl Hanks' solution. [From McKinney and Gallin (170).]

−120 mV (and in the absence of external Na), averaged single-channel currents showed time-dependent inactivation. The single-channel activity had complex kinetics, manifesting closures of short and long duration that indicated the presence of more than one closed state (170). Single K_i channel currents were blocked by external Ba (2.5 mM) and, like whole cell currents, the single-channel K_i conductance was proportional to the square root of $[K]_o$ (170). The density of K_i channels in J774.1 cells was estimated to be 47 channels/pF or 0.47 channels/μm^2, assuming a specific capacitance of 1 $\mu F/cm^2$ (170).

A) Expression. Macrophages do not always express the K_i conductance. Furthermore, channel expression can be modified by external factors, such as culture conditions. For example, Ypey and Clapham (290) reported that inward rectification was absent in mouse peritoneal macrophages cultured for up to 4 days, whereas previous (65) and subsequent (211) studies on mouse peritoneal macrophages cultured for 5 days or more demonstrated *I-V* relationships with prominent inward rectification. Gallin and Sheehy (70) reported that J774.1 cells that adhered to a glass or plastic surface for >18 h had a prominent K_i conductance, whereas this conductance was either absent or quite small in cells that adhered for only a few hours. Randriamampita and Trautmann (212) noted that fluid perfusion during whole cell patch-clamp recordings of J774.1 cells reduced the K_i currents. More recently it was demonstrated that the specific K_i conductance (whole cell conductance corrected for leak and normalized to membrane capacitance) of J774.1 cells allowed to adhere for 15 min to 1 h was one-half the specific K_i conductance of long-term (>18 h) adherent cells (171). The increase in specific K_i conductance was associated with a shift in V_m of the cells to more negative potentials, indicating that the K_i conductance participates in setting the cells' resting V_m. These findings are consistent with the earlier observation that block of K_i depolarizes macrophages (70). In J774.1 cells, treatment with the protein synthesis inhibitor cycloheximide abolished the adherence-induced augmentation of the specific K_i conductance, suggesting that the synthesis of new channel protein was required for the upregulation of these channels after adherence (171).

In addition to being affected by culture conditions, two reports indicate that the K_i conductance may be modified by specific agonists. A brief report by Moody-Corbett and Brehm (178) on rat thymus-derived macrophages revealed that the inwardly rectifying current was reduced by acetylcholine and muscarine. Wieland et al. (278) reported that this conductance, present in HL60 cells differentiated to macrophage-like cells with phorbol esters (but absent from HL60 cells that were differentiated to granulocyte-like cells with retinoic acid), was inhibited by the addition of recombinant human colony-stimulating factor I. Further studies are needed to determine the functional relevance of this interesting observation. It should be noted that a recent study by McCloskey and Cahalan (169) demonstrated that in rat basophilic leukemia cells the K_i conductance is inhibited by GTPγS (100 μM) and GppNHp (100 μM), two GTP analogues that activate G proteins. Thus it is possible that colony-stimulating factor I is blocking the K_i conductance in HL60 cells by activating G proteins.

II) LARGE CALCIUM- AND VOLTAGE-ACTIVATED POTASSIUM CONDUCTANCE. Single-channel patch-clamp recordings from human monocyte-derived macrophages that have been grown in culture for 1–6 wk have demonstrated a large-conductance K channel (240 pS in symmetrical K, 110 pS in 150 mM $[Na]_o$/5 mM $[K]_o$) (62, 166). Similar channels are also present in human alveolar macrophages (121) but are absent in J774.1 cells (E. K. Gallin and L. C. McKinney, unpublished observations)

and in the promonocyte cell line U937 (121). Furthermore, activation of U937 cells with recombinant interferon-γ (1,000 U/ml), recombinant interferon-αA (1,000 U/ml), or 12-*O*-tetradecanoylphorbol-13-acetate (TPA, 10 ng/ml) before recording from cells failed to induce the expression of the large-conductance Ca-activated K ($K_{L\,Ca}$) channels in excised patches (121).

In cell-attached patches from human monocyte-derived macrophages, the $K_{L\,Ca}$ channel was active only when the patch potential was stepped to very depolarized levels (>80 mV). Excised patch recordings indicated that channel open time increased with both membrane depolarization and increased intracellular Ca concentration ($[Ca]_i$) (62). However, this channel was relatively insensitive to $[Ca]_i$, because at 3×10^{-6} M $[Ca]_i$ the open-state probability of the channel at +60 mV was only 0.03–0.24 (68). Thus, in the macrophage, large increases in $[Ca]_i$ (>10^{-6} M) are required to activate $K_{L\,Ca}$ channels at negative membrane potentials. Exposing the extracellular surface of the membrane to 25 nM charybdotoxin [CTX; a proteinaceous component of toxin from *Leiurus quinquestriatus* known to block Ca-activated K channels in other cells (175, 263)] or tetraethylammonium ions (TEACl; 15 mM) abolished $K_{L\,Ca}$ channel activity (68).

Whole cell currents corresponding to the activity of $K_{L\,Ca}$ channels have been described in human monocyte-derived macrophages that were perfused intracellularly with a saline solution containing 3×10^{-6} M Ca (68, 183). These currents, activated during voltage steps to potentials >40 mV, were characterized by a noisy baseline (consistent with a large single-channel conductance) and had tail currents that reversed at E_K (60). In addition, either CTX (60) or TEA (68, 183) blocked this current, suggesting that whole cell outward currents represented the activation of $K_{L\,Ca}$ channels. Randriamampita and Trautmann (212) demonstrated that increasing $[Ca]_i$ from 0.1 to 1 μM increased whole cell currents in both J774.1 cells and mouse peritoneal macrophages and that quinine (0.1–1 mM) markedly reduced these currents. However, unlike the $K_{L\,Ca}$ conductance in human monocyte-derived macrophages, the whole cell currents they described in J774.1 cells and mouse peritoneal macrophages showed no voltage sensitivity, making it unlikely that they were due to the activation of $K_{L\,Ca}$ channels.

Membrane hyperpolarizations, reflecting the activation of a Ca-activated K conductance, were first described in macrophages in 1975 (71, see sect. IIIA5ii). A cell-attached patch-clamp study by Ince et al. (117) reported that, during membrane hyperpolarizations induced by microelectrode impalement, the voltage range of activation of the $K_{L\,Ca}$ channels shifted so that they were open 80% of the time at potentials of 0 to −20 mV. Nevertheless, studies using Ca-indicator dyes have reported $[Ca]_i$ increases after physiological stimulations that are too low (in the range of 0.2–1 μM) (32, 129) to activate $K_{L\,Ca}$ channels at negative V_m. Therefore it is not clear whether $K_{L\,Ca}$ channels are normally activated under conditions of physiological stimulation, either the $[Ca]_i$ sensitivity of the $K_{L\,Ca}$ channels is different in situ from that of the excised patch or these channels open rarely during stimulation. Alternatively, the $K_{L\,Ca}$ conductance may function in intracellular compartments where $[Ca]_i$ levels may be high.

iii) CALCIUM-ACTIVATED INWARDLY RECTIFYING POTASSIUM CONDUCTANCE. Gallin (63) has demonstrated in cell-attached patches from cultured human macrophages that both ionomycin (Fig. 2) and platelet-activating factor, two substances known to transiently increase $[Ca]_i$, induced bursting channel activity that was very different from $K_{L\,Ca}$ channel activity. In these studies, the induced currents, which were permeable to K and poorly permeable to Na and Cl, had single-channel conductances (with 150 mM KCl in the pipette) for inward currents of 37 pS, channel activation was independent of voltage. Similar channels have been described in human alveolar macrophages (120a).

The Ca-activated inwardly rectifying K ($K_{i\,Ca}$) channel in cultured human macrophages can be differentiated from the K_i channel on the basis of its Ca sensitivity, its conductance (37 vs. 29 pS for inward currents), its kinetics (bursting vs. nonbursting), its lack of voltage dependence, and its differing sensitivity to block by external Ba. Three millimolar Ba, a concentration that completely blocked the voltage-dependent K_i channel (170), did not significantly block the $K_{i\,Ca}$ channel at the resting V_m and produced only a partial block when the patch was hyperpolarized (63).

An inwardly rectifying K channel, the open probability of which was independent of voltage but dependent on $[Ca]_i$, also has been reported in excised inside-out patches from U937 cells (121) and in cell-attached patches from mouse peritoneal macrophages after exposure to 100 μM ATP (104). In contrast to the findings in human macrophages, the single-channel conductance in patches from U937 cells and from mouse peritoneal macrophages was only 25–28 pS at voltages between −40 and −100 mV. Similar Ca-activated inwardly rectifying K channels with single-channel conductances for inward current ranging from 50 to 25 pS have been described in lymphocytes (160), erythrocytes (98), and HeLa cells (227, 228).

Both spontaneous and Ca ionophore-induced oscillatory membrane hyperpolarizations have been recorded in macrophages using intracellular microelectrodes (71, 201), and it is likely that $K_{i\,Ca}$ channels (rather than $K_{L\,Ca}$ channels) are responsible for these events because *1)* the $K_{i\,Ca}$ channel is active at the resting V_m after exposure to ionomycin, whereas the $K_{L\,Ca}$ channel is not, *2)* the bursting pattern of the $K_{i\,Ca}$ channel is oscillatory, and *3)* the activity of the $K_{i\,Ca}$ channel is associated with the oscillatory changes in V_m induced by ionomycin.

As noted in the previous section, Randriamampita and Trautmann (212) reported a linear increase in membrane conductance in whole cell recordings of J774.1 cells and murine peritoneal macrophages obtained under conditions of high $[Ca]_i$, which they concluded was due to a voltage-insensitive Ca-activated K

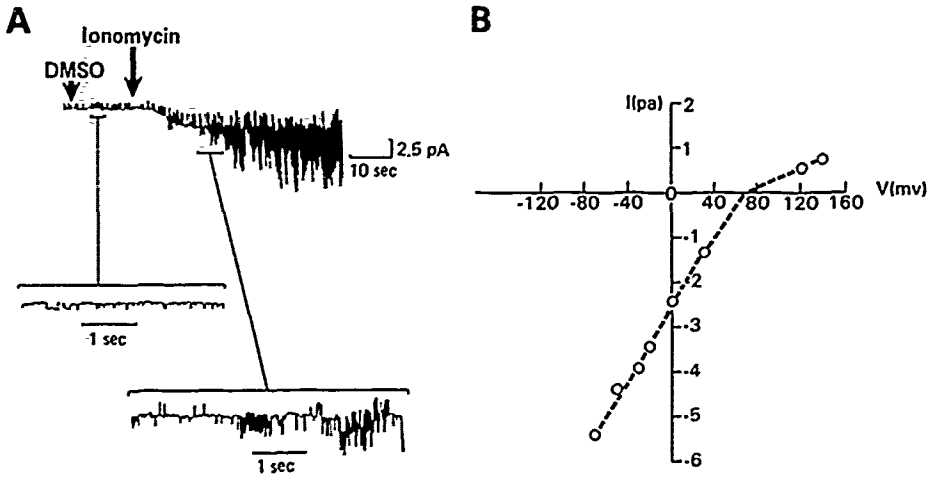

FIG. 2. Ionomycin-induced K channels in human macrophages. *A*. chart recorder tracing of current recorded from cell-attached patch in which cell was exposed first to 0.1% dimethyl sulfoxide (DMSO) and then 10^{-6} M ionomycin from perfusion pipette. Potential across patch was equal to resting membrane potential, cell was bathed in 150 mM NaCl Hanks' solution. Patch electrode contained 150 mM KCl Hanks' solution. K_i channel activity was evident before exposure of cell to either agent and can be seen in expanded time and voltage scale in middle current tracing. After ionomycin addition, Ca-activated inwardly rectifying K ($K_{i,Ca}$) channels were activated and could be seen in expanded time and voltage scale in bottom tracing. *B*. current-voltage (*I V*) relationship showing inward rectification of ionomycin-induced channels. Single-channel conductance for inward currents = 37 pS. [From Gallin (63).]

conductance. Although it is possible that this conductance was due to the activation of $K_{i,Ca}$ channels, the fact that the conductance reported by Randriamampita and Trautmann (212) showed no rectification argues against this possibility.

IV) INACTIVATING OUTWARD POTASSIUM CHANNEL. An inactivating outward K (K_o) conductance has been described at the whole cell current level in resident mouse peritoneal macrophages (290), cultured human blood-derived monocytes (183, 185), cultured human alveolar macrophages (184), and two macrophage-like cell lines, J774.1 (70, 212) and P388D1 (245). This conductance activated at potentials positive to −50 mV. Current activation had a time course that fit first-order kinetics with a time constant that decreased for steps to more depolarized potentials (183). Inactivation of the K_o current also could be fit by a single exponential with a time constant (~540 ms) that was insensitive to voltage for potentials positive to −20 mV (70). The K_o currents were blocked by extracellular 4-aminopyridine (4-AP, ~5 mM) and by intracellular Ba, Cs, and TEA (70, 183, 290). External TEA (10 mM) also partially blocked the current (290). Similar outward currents have been described in detail in T lymphocytes (17) and are discussed in section IV*A1*. Ypey and Clapham (290) recorded a 16-pS channel in outside-out excised patches under conditions of asymmetric K (140 mM in pipette, 2.8 mM in bath) that, during depolarizing voltage steps, was activated in a time-dependent manner similar to the whole cell K_o currents.

There is no consistent pattern of K_o channel expression across different types of macrophages, it was reported in only 5% of the recordings from cultured blood-derived human monocytes, whereas it was noted in 50% of the recordings from cultured human alveolar macrophages (184). Furthermore, for a given type of macrophage, the K_o conductance appears to be variably expressed with time in culture. For example, in J774.1 cells, K_o currents were described in a percentage of cells recorded from 1–8 h after adherence but were rarely present in cells from long-term adherent cultures (70). Ypey and Clapham (290), using resident mouse peritoneal macrophages, reported that K_o conductance was absent during the 1st day after isolation but was present in 96% of cells cultured 1–4 days. Randriamampita and Trautmann (212) also recorded outward currents in mouse peritoneal macrophages cultured for 1–2 days but found that the currents decreased after 5–6 days in culture. Interestingly, in those cells, fluid movement caused by perfusing the bathing medium increased the outward K current (212), whereas the addition of 2 mM *N*-formyl-methionine-leucine-phenylalanine (FMLP, a chemotactic peptide), histamine (20 mM), bradykinin (20 μM), and acetylcholine (50 μM) had no effect on the K_o current (290). (However, it should be noted that mouse macrophages do not respond to FMLP.) Finally, Nelson and colleagues reported that in human blood-derived monocytes, phorbol esters decrease the amplitude of the K_o current (185) and that treating cells for 24 h with bacterial lipolysaccharide (LPS) increased the percentage of cells expressing K_o current from near 0% to 30% (120).

V) POORLY INACTIVATING OUTWARD POTASSIUM CHANNEL. A second outward K conductance has been reported in a whole cell patch-clamp study of cultured human blood-derived monocytes (183). This conductance, noted in the majority of the cells studied, activated at voltages more positive than −10 mV and exhibited no steady-state inactivation for holding potentials of −60 to 0 mV. Inactivation, present for voltage steps

>0 mV, could be fit by a single exponential with a time constant of 951 ms at 40 mV. However, unlike the inactivating K_o conductance, little cumulative inactivation of the poorly inactivating outward K (K_{o2}) conductance was noted. Intracellular Cs blocked the current, as did extracellular TEA (4 mM). This current is similar to the slowly inactivating K_i conductance that activates at voltages of >0 mV, which has been described in murine T lymphocytes (37; see sect. IV*A1*).

2. *Chloride conductances*

Three different Cl conductances have been described at the single-channel level in excised patch-clamp studies of macrophages. At the whole cell level, Nelson et al. (183) reported an outward current in human monocyte-derived macrophages under conditions where the patch electrode contained either Cs or Na instead of K. The authors concluded that this current was probably a Cl current because *1*) its amplitude was reduced, and its reversal potential shifted when Cl was replaced with the anion aspartate; and *2*) it was blocked by the anion channel blocker 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS; 1 mM). However, it is not clear whether this whole cell current corresponds to any of the three Cl channels described in single-channel studies.

I) LARGE-CONDUCTANCE CHLORIDE CHANNEL. A very large-conductance Cl (Cl_L) (180–390 pS) channel was first reported in mouse peritoneal macrophages by Schwarze and Kolb (236) and has been described more recently in excised patches from two macrophage-like cell lines, J774.1 (212) and U937 (121). This channel is very similar to the large Cl channel described in rat skeletal myotubes (9), lymphocytes (12, 167, 195), and other cells. In mouse peritoneal macrophages, Cl_L channel activity was absent in cell-attached patches but became activated when cells were exposed to the Ca ionophore A23187 or when the patch was excised (236). Similar findings were reported by Randriamampita and Trautmann (212) in both mouse peritoneal macrophages and J774.1 cells, where the channel, rarely active in cell-attached patches, was frequently seen in excised patches. In both mouse peritoneal macrophages (236) and U937 cells (121), after excision of the patch a lag occurred before the Cl_L channels were observed. This observation has led Kanno and Takishima (121) to propose the existence of an in situ factor that inhibits these channels.

The Cl_L channels in mouse peritoneal macrophages had a selectivity ratio for Cl over Na of 5.1 (236) and a Cl-to-cation permeability ratio of between 14 and 5 (212). Multiple subconductance states of the Cl_L channel were noted in both mouse peritoneal macrophages (236) and U937 cells (121). In U937 cells the subconductance states were unaffected by the pH buffers *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), tris(hydroxymethyl)aminomethane (Tris), or *N*,*N*-bis-(hydroxyethyl)-2-aminoethanesulfonic acid (BES), and neither intracellular pH (pH_i) nor Ca affected the open probability of the channel or the frequency of appearance of the subconductance states (121). Kanno and Takishima (121) reported that in U937 cells, the anion transport blocker 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS, 100 μM) produced a flickery block of channel activity, whereas increasing DIDS to 1 mM irreversibly blocked the channel.

The Cl_L channel exhibited complex bursting behavior with at least three kinetically distinguishable nonconducting states (236). Channel activity (in symmetrical saline solution) could be induced by stepping to holding potentials on either side of 0 mV but were inactivated subsequently with the rate of inactivation increasing as the magnitude of the voltage jumps increased (236). Randriamampita and Trautmann (212) reported a somewhat different behavior for the Cl_L channel in both mouse peritoneal macrophages and J774.1 cells in that the probability of channel opening was high at positive potentials (up to 40 mV) and declined at hyperpolarized potentials or when the patch was depolarized beyond 40 mV.

The Cl_L channel has been modeled using two voltage-sensitive gates in series to describe the voltage-dependence of the burst kinetics for the channel (236). Schwarze and Kolb (236) suggested that the voltage-dependent gating properties of the Cl_L channel resemble the properties of voltage-dependent gap junctions and that the Cl_L channel may play a role in intercellular communication. However, octanol, a blocker of gap junction channels, does not block these channels, suggesting that they are not related to gap junction channels (212).

Although cell-attached patch experiments indicated that this channel is quiescent in resting cells, Cl_L channels were elicited by perfusing mouse peritoneal macrophages with zymosan (a particulate fraction of yeast cell wall that macrophages can ingest) during cell-attached patch recordings (125, 258).

II) INTERMEDIATE-CONDUCTANCE CHLORIDE CHANNEL. In addition to the Cl_L channel, two other smaller conductance Cl channels were noted 10 s to several minutes after excising patches from U937 cells (121). In symmetrical 150 mM NaCl, an intermediate Cl channel (Cl_i) that exhibited outward rectification and had a chord conductance of 17 pS between 0 and 100 mV was observed in excise patches. The channel had a Cl-to-Na or K permeability ratio of 1.8, which is similar to the cation/anion selectivity of the Cl_L channel, and although the permeability sequence for anions was not examined in this study, the authors reported that the Cl_i channel was less permeable to CH_3SO_3 than to Cl (121). Channel activity, which appeared in bursts, decreased with increasing membrane depolarization or hyperpolarization, but channels were generally more active at positive potentials than at negative potentials. Stability plots of channel activity suggested that at least two modes of channel behavior were present. Neither changes in $[Ca]_i$ nor pH_i affected open probability or moding behavior.

In addition, DIDS (10-100 μM) blocked this channel in a dose-dependent reversible manner.

III) SMALL-CONDUCTANCE CHLORIDE CHANNEL. A small Cl channel with a slope conductance of 15 pS at 0 mV in symmetrical 150 mM NaCl was also noted in excised patches from U937 cells (121). The channel Cl-to-Na permeability ratio was 15:6, thus the small-conductance Cl (Cl_s) channel had a greater anion-to-cation selectivity than the other Cl channels described in macrophages. It also was insensitive to $[Ca]_i$ and was less voltage sensitive than the Cl, channel.

3. Nonselective cation conductance(s)

Two studies, one measuring single-channel currents and the other measuring whole cell currents, suggested that macrophages possess nonselective cation channels. Lipton (155) described an increase in single-channel activity in cell-attached patches from the murine macrophage cell line P388D1 after exposure to immunoglobulin G2b (IgG2b) or to immune complexes but not after exposure to ascites fluid with IgG2a. Excised patches from cells that had been exposed to antibody revealed similar channel activity that was unaffected by ionic substitutions of Na, K, or Cs. Channel activity with several different conductances was noted, including one ranging from 35 to 45 pS and another ranging from 120 to 150 pS. The reversal potential of the channels under conditions of a fivefold salt gradient across the patch indicated that these channels were permeable to cations but not to anions. Channel activity increased significantly when $[Ca]_i$ was raised, supporting the view that the channels were Ca gated. These observations are quite interesting, but unfortunately, they have not been confirmed or extended.

Slowly activating outward currents were reported in whole cell patch recordings from cultured human blood-derived monocytes during voltage steps to >20 mV (183). These currents were inhibited by external Zn (4-100 μM) and were present when the patch pipette contained Cs, K, or Na. In addition, substitution of Cl for aspartate or gluconate did not alter the current amplitude, leading the authors to conclude that it was a nonselective cation current. Because the currents activated at very positive potentials, it seems unlikely that they represent activation of the channels described by Lipton (155).

4. Conductances induced by specific ligands or cell functions

I) ADENOSINE TRIPHOSPHATE. Extracellular ATP (100-1,000 μM) permeabilizes the membrane of murine macrophages and J774.1 cells to cations (261) or to small (<961 Da) membrane-impermeant molecules, such as Lucifer yellow (262). The enhanced permeability does not involve hydrolysis of ATP by an ecto-ATPase, because addition of Mg (which is required for ATPase activity) inhibited permeabilization, and the poorly hydrolyzable ATP analogue ATPγS (in the presence of 1 mM EDTA) also increased cation efflux (261, 264). Furthermore, by growing J774.1 cells in the presence of ATP, an ATP clone of J774.1 cells was obtained that had normal ecto-ATPase activity but failed to respond to ATP with an increase in permeability (262). Similar ATP-induced permeability changes have also been demonstrated in rat mast cells (30) and in chronic lymphocyte leukemic cells (279).

Whole cell patch-clamp techniques have demonstrated that ATP permeabilization is associated with a rapid membrane depolarization and an increase in membrane conductance (16). Unfortunately, cell-attached patches with ATP in the patch electrode did not reveal single-channel currents (16). Therefore further studies are required to determine whether ATP directly activates a membrane channel or whether it secondarily releases an intracellular signal that opens a channel.

The physiological significance of the ATP-induced conductance is unknown. It is likely that macrophages are exposed to exogenous ATP, because they are often present at sites of cell injury and with cells, such as platelets, the secretory granules of which contain ATP. The ATP-induced permeabilization, therefore, is likely to occur under physiological circumstances and may be important in regulating the subsequent responses of the macrophage.

II) PHAGOCYTOSIS. Phagocytes ingest particles through receptors for the Fc domain of IgG, as well as through complement receptors or through non-receptor-mediated mechanisms (252). The ionic requirements or signaling events that underlie different kinds of phagocytosis can differ. For example, C3bi[1]-mediated phagocytosis occurs at very low levels of free $[Ca]_i$, whereas Fc-mediated phagocytosis is inhibited by those conditions (144). The role of ionic conductances in phagocytosis has best been studied during Fc-mediated phagocytosis, where evidence exists that ionic conductances are activated during phagocytosis and that the Fc receptor itself may be an ionic channel. However, as discussed next, these results are somewhat controversial.

In the first of a series of studies on the Fc receptor, Young et al. (286), using tetraphenylphosphonium ions (TPP^+) to indirectly monitor V_m, demonstrated that the binding and cross-linking of the γ2b/γ1-Fc receptor by IgG or immune complexes depolarized J774.1 cells. The depolarization required a multivalent Fc ligand and was dependent on external Na. In a related study, in which purified γ2b/γ1-Fc receptors were inserted into lipid vesicles, ligand binding to an Fc receptor containing proteoliposomes increased cation permeability (287). Finally, Young et al. (288) demonstrated that adding ligand to bilayers containing the Fc receptor induced cation-selective ion channels that had a conductance of 60

[1] C3bi is a cleavage product of the third component of complement that binds to the surface of particles as a consequence of complement activation and renders particles recognizable by phagocytic leukocytes, thereby serving as an opsonin.

pS in symmetrical 1 M KCl and that inactivated several minutes after the addition of ligand.

If the Fc receptor complex is an ionic channel, then it follows that ionic currents should be evident during electrophysiological recordings from cells that are internalizing IgG-coated particles or aggregated IgG (aIgG) Several studies directly monitored channel activity in intact macrophages or macrophage membranes before and after addition of aIgG. Nelson et al. (182) recorded whole cell currents as well as single channels in human alveolar macrophages exposed to aIgG. The application of aIgG to cells during whole cell recordings produced an inward current that diminished with successive applications of aIgG, indicating that the response desensitized. In cell-attached patches, channel activity was noted only when the electrode contained aIgG and not when aIgG was applied to the bath. The channels had a unitary conductance of 350 pS in symmetrical 140 mM NaCl Hanks' solution. Changing the permeant cation from Na to K did not affect the reversal potential, indicating that if the channel was a cation channel, it was nonselective. Unfortunately, similar responses have not been noted in patch-clamp studies of J774.1 cells exposed to ligands that bind to and cross-link the Fc receptor (D. J. Nelson, personal communication). In addition, there is a significant difference between the value of conductance obtained in this study (350 pS in physiological saline) and that obtained by Young et al. (288) on the isolated Fc receptor (60 pS in symmetrical 1 M KCl).

The Fc receptor-ligand complex may indirectly activate ionic channels through a second messenger (114, 155). As discussed in section III*A3*, when IgG2b was added to the bath during a cell-attached recording from P388D1 cells, multiple single-channel current amplitudes were evident, representing either several different types of channels or a single-channel type with different subconductance states; the smallest channels had conductances of 35–45 pS and were cation selective (155). Channel activity could be maintained after excision of the patch, and activity was modulated by changes in $[Ca]_i$. Lipton (155) suggested that these channels are activated by $[Ca]_i$ increases that occur after binding and cross-linking of the Fc receptor. Using a similar experimental protocol in patch-clamp recordings from cultured human macrophages, Ince et al. (114) demonstrated transient changes in background current along with the activation of several types of channels with conductances ranging from 26 to 163 pS after ingestion of either IgG-coated or unopsonized latex beads. In this study some of these channels reversed near E_K, but the ionic selectivity of these channels was not investigated.

In contrast to these studies, Randriamampita and Trautmann (212) reported that ion-channel activation does not necessarily occur during Fc-mediated phagocytosis; during whole cell patch-clamp recordings, exposure of murine macrophages to aIgG or to the monoclonal antibody 2.4G2 did not induce membrane currents. In addition, resting V_m values obtained from whole cell recordings immediately after macrophages ingested opsonized erythrocytes were identical to those obtained before phagocytosis. Thus they concluded that Fc-mediated phagocytosis can occur under conditions where no detectable conductances are activated. These findings agree with the observations of Gallin (63) in which intracellular recordings from mouse peritoneal macrophages before and during ingestion of opsonized erythrocytes indicated that phagocytosis occurred without any changes in V_m or input resistance. The discrepancies between these observations and those already discussed indicate that the ionic events associated with Fc-mediated phagocytosis are still unresolved.

5. *Physiological role of ionic conductances*

1) SETTING MEMBRANE POTENTIAL. The resting V_m of the macrophage or of any other cell influences cell function by affecting the gating of voltage-dependent ion channels, the diffusion of ions through non-voltage-gated channels, and the transport of ions and/or substances that use ions as cotransporters. The relationship between resting V_m and ionic permeability has been studied most thoroughly by Ince et al. (116) in human monocytes. Using ion-substitution experiments, they demonstrated that the intracellular content of Na, K, and Cl in human monocytes is 21, 122, and 103 mM, respectively, and that resting V_m is dependent on external K for $[K] > 10$ mM. [It should be noted that the value of 103 mM for $[Cl]_i$ is surprisingly high and differs considerably from the values of 44 and 36 mM for J774.1 and HL60 cells, respectively (173, 216a).[2]] Below 10 mM K the Cl permeability also affected V_m, whereas changing $[Na]_o$ had no effect on resting V_m. Thus in human monocytes and in monocyte-derived macrophages where resting V_m values ranging from −30 mV to −56 mV have been reported (68, 116, 118, 183), both Cl and K conductances/transporters participate in setting V_m. In addition, the Na-K pump contributes between −7 and −11 mV to the resting V_m of macrophages (66).

When present in macrophages, the K_i conductance plays a role in maintaining the V_m close to E_K. Support for this conclusion comes from the finding that macrophages exhibiting this conductance had resting V_m values closer to E_K than macrophages that did not express this conductance (65, 66, 70, 212) and that Ba (2.5 mM), which blocks the K_i conductance, depolarized J774.1 cells by ≥20 mV (70). Furthermore, in J774.1 cells, the presence of this conductance was associated with a shift in the resting V_m to more hyperpolarized levels (171). In macrophages in which the K_i conductance sets the resting V_m, a small inward current that might be produced by the activation of a specific conductance or a nonspecific leak conductance can result in two stable states of resting membrane (−28 and −80 mV) (65). This phenomenon, which is related to the steep voltage dependence of

[2] This discrepancy may be due to the fact that the $[Cl]_i$ in human monocytes was measured in HCO_3-free medium (171a).

the K_i conductance, has also been reported in rat basophilic leukemia cells (154) and in cardiac Purkinje fibers (60) and may be functionally important.

Although the K_i conductance, if present, plays a significant role in setting the resting V_m of the cells, it should be noted that other K conductances may also contribute to the resting V_m. For example, Ypey and Clapham (290) reported that mouse peritoneal macrophages cultured for 24 h displayed only a K_o conductance, not a K_i conductance, and these cells had resting V_m values of −80 to −90 mV (equal to E_K). Because the K_o conductance was reported to activate at potentials positive to −60 mV, it is not clear which K conductance established the resting V_m in these cells.

In macrophages, as in other cell types, V_m affects voltage-dependent ionic conductances and other transport processes that depend on ionic gradients, such as Na-dependent amino acid transport. Several studies have examined the phagocytic ability and the NADPH oxidase activity of macrophages depolarized by high-K medium to determine if a negative V_m is required for these processes. Pfefferkorn (203) reported that J774.1 cells ingest the opsonized protozoan parasite *T. gondii* normally in 120 mM K medium (which depolarizes macrophages to near 0 mV, unpublished observations). Phagocytosis of unopsonized zymosan by murine peritoneal macrophages also occurs normally in high-K medium, although high-K medium does prevent the induction of phospolipase activity that normally occurs after ingestion of zymosan (1). Depolarization by high K does not induce superoxide release in mouse peritoneal macrophages, nor does it interfere with the release of superoxide induced by phorbol myristate acetate (PMA) (123). Similar results were noted when FMLP-induced superoxide release was measured in guinea pig alveolar macrophages depolarized by incubation in 110 mM K/35 mM Na medium. In these cells, increasing $[K]_o$ to 142 mM (and decreasing $[Na]_o$ to 1 mM) decreased superoxide production by 25%, but this decrease was due to the $[Na]_o$ decrease rather than to the increase in $[K]_o$ (110).

II) OSCILLATIONS IN MEMBRANE POTENTIAL. Intracellular microelectrode studies have shown that both human and murine macrophages exhibit spontaneous and electrically or mechanically induced oscillations in V_m from a level of −30 or −10 mV to potentials near E_K (approximately −80 mV) (48, 71). In addition, hyperpolarizations that sometimes oscillate can be induced by addition of Ca ionophores or chemotactic factors (64, 71). Because none of the above treatments dependably induced oscillations in V_m, these events have been difficult to study. More recently, Soldati and Persechini (257) reported that, in the absence of Na, large depolarizing voltage steps reliably induced V_m oscillations in mouse macrophage polykaryons.

Hyperpolarizing membrane oscillations have been ascribed to the activation of a Ca-dependent K conductance, because they involved an increase in conductance, reversed near E_K, were blocked by ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), and were induced by either ionomycin (48, 71) or the intracellular injection of Ca (202). The V_m oscillations were not blocked by TEACl (50 mM), but they were blocked by the addition of either quinine (0.2–1.5 mM) or Ba (20 mM) (4, 117). As discussed in section III.A.1.iii, it is likely that the $K_{i,Ca}$ conductance underlies the V_m oscillations, although it should be noted that Ince et al. (117) reported activation of large-conductance channels (presumably $K_{L,Ca}$ channels) during mechanical microelectrode-induced hyperpolarizations in human macrophages. Further studies on the effects of pharmacological blockers on both $K_{i,Ca}$ and $K_{L,Ca}$ conductances and the V_m oscillations are needed to determine if both these channels play a role in V_m oscillations.

Ince et al. (115) recorded rapid transients immediately after microelectrode impalement and concluded that spontaneous oscillations in V_m were an artifact of recording induced by a leak of external Ca into the cell after impalement by microelectrodes. However, experiments recording currents with patch-clamp electrodes in the cell-attached patch and whole cell configurations (where electrode-induced leak current was negligible) confirmed the presence of spontaneous V_m oscillations (63; see section III.A.1.iii). Interestingly, Kruskal and Maxfeld (129) have demonstrated that spontaneous oscillations in $[Ca]_i$ occur in macrophages after adherence. It is likely that the oscillations in $[Ca]_i$ are linked to the activation of K conductance and that V_m oscillations occur under physiological conditions. However, the functional relevance of these oscillations is not known.

III) CHANGES DURING MATURATION OR AFTER ACTIVATION. Macrophages orginate from bone marrow promonocyte cells that are released as monocytes into the blood where they circulate, leave the circulation (with a half time of 17 h), emigrate into tissues, and mature into resident tissue macrophages (272). Tissue macrophages exposed to microorganisms, LPS, and a variety of cytokines can be activated to exhibit enhanced tumor cell killing and increased secretory responses, and it has been well documented that after activation some of their surface antigens differ from those of monocytes or of resident tissue macrophages (272).

Several studies have indicated that maturation and/or activation can modulate the expression of ionic conductances. In human peripheral blood monocytes the expression of the $K_{L,Ca}$ channel increased during the first 7 days in culture, a time period during which monocytes mature into macrophages. <5% of cell-attached patches obtained from cells 24 h after plating exhibited this channel, whereas >80% of the patches obtained after 5 days in culture did exhibit this channel (68). If it is true that the $K_{L,Ca}$ conductance is absent from freshly isolated blood monocytes, then the presence of these channels (in the plasma membrane) must not be required for phagocytosis, chemotaxis, and other functions that are normally carried out by peripheral blood monocytes. However, this observation has not been confirmed in a recent study of whole cell currents in cultured human blood-derived monocytes by Nelson et al. (183). In J774.1 cells, adherence is the trigger for increased channel expression. As discussed in section

IIIA II, a twofold increase in the density of K_i channels occurred during the first 18 h after adherence (171). It is well known that adherence itself can "activate" macrophages to increase responsiveness to a variety of stimuli (31).

The activating stimuli LPS also modulates the expression of ionic currents in macrophages. Jow and Nelson (120; see sect. IIIA IIV) showed that treating cultured human peripheral blood-derived macrophages for 24 h with LPS increased the percentage of cells expressing the K_o current from ~0 to ~30%. In contrast, the same treatment in J774.1 cells did not increase the K_o current but decreased the density of K_i channels compared with untreated cells (171). This was due to the fact that LPS-treated J774.1 cells increased their membrane area (as measured by membrane capacitance) more than they increased K_i channel expression.

IV) OTHER POSSIBLE FUNCTIONS. With the exception of the role of the K_i conductance in setting V_m, the role of ionic conductances in macrophage function has not been established. These conductances are likely to serve some of the same functions in phagocytes that they do in other cells. Thus the K_o conductance may be important for restoring V_m to negative values after depolarization. The Ca-activated K conductances may have a similar role after the transient increases in $[Ca]_i$ that occur during phagocytosis (144, 285) and after activation with chemotactic peptides (102). It is also possible that Cl and K conductances play a role in volume regulation in the macrophage, as they do in other cell types (190). Although ion-sensitive microelectrodes used in studies of mouse macrophage polykaryons have demonstrated that $[K]_i$ does not change during spontaneous membrane hyperpolarizations (202), Holian and Daniele (110) demonstrated that there was a 17% decrease in $[K]_i$ in human alveolar macrophages 20 min after stimulation with the chemotactic factor *N*-formyl-methionyl-phenylalanine (FMP). Changes in $[K]_i$ could influence synthetic processes (135, 247, 274) and receptor-mediated endocytosis (134). Intracellular K levels might also modulate the contractile machinery of the macrophage, because the macrophage contains an actin-modulating protein, acumentin, the activity of which is modified by changes in $[K]_i$ (100–200 mM) (259).

Only a few preliminary studies have investigated the effect of pharmacological blockers of ionic conductances on phagocyte functions. In J774.1 cells, Ba (2 mM), which blocks the K_i channel, does not block chemotaxis in response to endotoxin-activated mouse serum, release of hydrogen peroxide after stimulation with PMA, or phagocytosis of opsonized erythrocytes (unpublished observations). Therefore it is unlikely that the K_i conductance plays a crucial role during these events. In alveolar macrophages the extracellular Ca-dependent component of the K efflux stimulated by FMP is blocked by quinine (1 mM), a well-known inhibitor of Ca-activated K channels (110). Quinine also blocked the FMP-induced release of superoxide in these cells, but the inhibitory effect of quinine also occurred under conditions in which the FMP induced K efflux was absent (low extracellular Ca), indicating that these two effects of quinine were not directly related (110). In human peripheral blood-derived macrophages, TEA (10 mM), which blocks K_{Ca} channels, did not inhibit chemotaxis toward FMLP (unpublished observations). Additional studies examining the effects of blockers should help to clarify the role of ionic conductances in macrophage function.

The observation that buffering $[Ca]_i$ to 1–10 nM in J774.1 cells has no effect on cell spreading or the ingestion of IgG-coated erythrocyte ghosts (46) suggests that Ca-activated ion channels are not required for these events. This is consistent with the lack of K_{Ca} channels in the plasma membrane of freshly isolated human peripheral blood monocytes (68), even though they are capable of carrying out phagocytosis.

B. Neutrophils

Neutrophils, also called polymorphonuclear leukocytes because of their multilobed nucleus, are the most prevalent blood phagocyte. These cells contain numerous secretory granules. After phagocytosis or stimulation with a variety of factors, including leukotriene B_4 and platelet-activating factor (PAF), the contents of these granules are released along with superoxide and other free radicals formed during the oxidative burst. Neutrophils are very motile cells and are often the first cells found at sites of infection. Thus they are very important in phagocytosing and killing invading bacteria.

Although only two electrophysiological studies have been performed on neutrophils, these studies indicate that neutrophils exhibit at least four different ionic conductances. Future studies are needed to further characterize each of these conductances, to determine what additional conductances are present in neutrophils, and to understand their relevance to the large body of information that has accumulated from biochemical, flux, and fluorescence measurements.

1. Potassium conductances

I) OUTWARD POTASSIUM CONDUCTANCE. Whole cell patch-clamp experiments in human neutrophils have demonstrated a current that activated at positive potentials and reversed around −25 mV when NaCl was the predominant salt in the bath and the pipette contained KCl/K-aspartate (128). This current, which had a threshold of activation of −60 mV, was reduced when the pipette concentration of K was reduced but was unaffected by changes in the Cl concentration. Unlike the inactivating K_v current described in macrophages (290), this current showed no inactivation during depolarizing voltage steps up to 4 s. Furthermore, pharmacological studies indicated that the current was not blocked by CTX (1,000 μM), apamin (20 nM), quinine (200 μM), or 4-AP (10 mM). The authors speculated that this channel may be responsible for maintaining the resting V_m (128).

II) CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE. Krause and Welsh (128) also observed that ionomycin produced an increase in outward current that was twofold larger when the pipette contained KCl than when it contained NaCl. When the pipette contained NaCl, changing the bath solution to Na-isethionate abolished the ionomycin-induced current, whereas it only partially decreased the ionomycin-induced current when KCl was in the pipette. These data suggest that human neutrophils have a Ca-activated K conductance (in addition to a Ca-activated Cl conductance described in sect. III*B3*). The presence of a Ca-activated K current was further corroborated by the observation that ionomycin induced outward currents in whole cell patch-clamp recordings done on human neutrophils in symmetric K-aspartate solutions (128).

2. *Calcium-activated cation conductance*

Von Tscharner et al. (276), using patch-clamp techniques to examine ionic channels during stimulation with the chemotactic peptide FMLP, demonstrated that adding FMLP to the bath during cell-attached patch recordings induced two different Ca-activated cation-nonselective channels. The presence of FMLP in the patch pipette did not increase the probability of channel opening, indicating that the activated channels were not directly coupled to the FMLP receptor. Depleting $[Ca]_i$ by loading cells with fura-2 prevented FMLP-induced channel activation, and treating cells with saponin to increase $[Ca]_i$ activated channels in the absence of FMLP. Thus FMLP-activated channels appeared to be Ca activated. Two types of single-channel currents with conductances of 18-25 and 4-6 pS were identified. Ion-substitution experiments indicated that they were equally permeable to K, Na, and Ca. Inositol trisphosphate, which releases Ca from intracellular stores in neutrophils (260), failed to induce the activity of these channels when added to the inside surface of the membrane. (The possible relevance of these channels to FMLP-induced increases in $[Ca]_i$ are discussed in section III*B4*II.)

3. *Chloride conductances*

In whole cell patch-clamp recordings from human neutrophils exposed to ionomycin, Krause and Welsh (128) reported that substituting isethionate for Cl in the bath decreased (by 80%) the currents in response to positive voltage steps and shifted the reversal potential for the currents to more positive potentials, suggesting that neutrophils also display a Ca-activated Cl conductance. The current that was sensitive to removal of Cl had no apparent voltage sensitivity. Whole cell Cl currents were also described in a preliminary study of human neutrophils by Schumann et al. (235).

4. *Physiological role of ionic conductances*

I) SETTING RESTING MEMBRANE POTENTIAL. There are no direct electrophysiological measurements of the resting V_m in neutrophils, but measurements with the indirect probes triphenylmethylphosphonium ($TPMP^+$) (240), TPP^+ (179), and the fluorescent dye 3,3'-dipropylthiadicarboxycyanine [$diSC_3(5)$] (255) have yielded resting V_m values for suspended neutrophils of −54, −67, and −53 mV, respectively. From the resting V_m value and ion fluxes, Simchowitz et al. (255) calculated the relative ionic permeability of the neutrophil membrane to K, Na, and Cl to be 10.1.1. They concluded that the small permeability to Na accounts for the deviation of the resting V_m from E_K at physiological $[K]_o$ (4.5 mM), while above 10 mM $[K]_o$, V_m follows E_K. Unfortunately, no data exist about the particular ionic channels that underly resting V_m.

Although it is clear from experiments measuring V_m with voltage-sensitive fluorescent probes that various substances that activate neutrophils produce changes in V_m (238, 268), the events underlying these potential changes are poorly understood, and their relationship to signal transduction is unclear. For example, neutrophils depolarized by high K can still migrate in response to FMLP (179, 248). Although Roberts et al. (221) demonstrated that the number of neutrophils migrating in high-K/low-Na medium is increased, this increase was due to the reduction in extracellular Na and not to the increase in K. Furthermore, data on the relationship between depolarization induced by high K and the oxidative burst have demonstrated both decreases (126) and increases (156) in superoxide generation, and these changes have been attributed to the effects of Na removal rather than to membrane depolarization.

II) OTHER POSSIBLE FUNCTIONS. The Ca-activated conductances described are likely to be activated by the biphasic rise in $[Ca]_i$ that occurs in neutrophils after stimulation by a variety of factors, including FMLP, PAF, and leukotriene B_4 (47, 145, 209). This increase in $[Ca]_i$ has been linked to differential secretion from the three distinct granule populations that are present in neutrophils (145). The early transient $[Ca]_i$ rise is due to a release of $[Ca]_i$ stores, whereas the more sustained $[Ca]_i$ increase requires extracellular Ca and has been attributed to an influx of Ca (3). A stimulus-induced influx of Ca was further corroborated by the observation that extracellular Mn (presumably influxing through Ca-permeable channels) was able to quench the increase in fura-2 fluorescence induced by FMLP, leukotriene B_4, and PAF and that La, Co, and Ni inhibit the influx of Mn (174).

Von Tscharner et al. (276) suggested that intracellular release of $[Ca]_i$ from stores caused the transient activation of Ca-gated cation channels, allowing Ca to flow into the cell. Nasmith and Grinstein (180) tested this possibility by examining FMLP-induced Ca changes under conditions where neutrophils had been loaded with the Ca chelator bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid and demonstrated that

the rise in $[Ca]_i$ that depended on extracellular Ca could still be stimulated by FMLP when intracellular free Ca levels were maintained at or below resting levels. However, Pittet et al. (205) found that the FMLP-stimulated influx of Ca into HL60 cells differentiated into neutrophil-like cells was closely correlated with the rise in $[Ca]_i$ as well as an intracellular accumulation of inositol 1,3,4,5-tetrakisphosphate [Ins-(1,3,4,5)P_4]. They concluded that an elevation of $[Ca]_i$ could activate Ca influx by acting directly on Ca-activated channels, as suggested by von Tscharner et al. (276), or by increasing the production of Ins(1,3,4,5)P_4.

It is possible that the FMLP-induced Ca influx does not occur through ionic channels but occurs through other ion-transport mechanisms. Simchowitz and Cragoe (254) have characterized an electrogenic Na-Ca exchanger in neutrophils that can transport one Ca ion into the cell in exchange for three Na ions. This exchanger is activated by FMLP and may account for some of the observed increase in $[Ca]_i$.[3] Further studies are needed to examine this possibility and to delineate the events that underly the Ca influx.

The early events that follow the binding of FMLP to the membrane include (in addition to increases in $[Ca]_i$) changes in V_m, pH, and the transport of other ions. Studies with fluorescent dyes have shown that FMLP induces an initial depolarization followed by a repolarization that is completed within 8-10 min (141, 240). The FMLP-induced depolarization required a stimulus concentration of at least 10^{-8} M, whereas lower concentrations induced either no change (45) or a slight hyperpolarization (136).

Despite many studies, it is not clear which ionic conductances, if any, are involved in these responses. Decreasing $[Na]_o$ to 20 mM and varying $[K]_o$ from 1 to 100 mM did not change the amplitude of the membrane depolarization, indicating that the depolarization does not involve a Na conductance (238). As noted, von Tscharner et al. (276) proposed that the transient depolarization is caused by an influx of cations through the Ca-dependent cation-nonselective ion channels induced by FMLP. It is plausible that Ca provides a signal leading to depolarization, because it has been demonstrated that an increase in $[Ca]_i$ precedes the depolarization (136). However, Di Virgilio et al. (45) showed that even in Ca-depleted cells where no increase in free-$[Ca]_i$ occurs FMLP can induce a depolarizing response, although it is diminished. Alternatively, it is possible that the membrane depolarization induced by FMLP is due to the FMLP-induced increase in a Cl conductance reported by Schumann et al. (235). It is also possible that the repolarization phase of the FMLP-induced V_m changes involves an additional membrane permeability and that it may be dependent on external Ca (268). Furthermore, FMLP and other activating agents directly stimulate Na-H exchange (89, 253). Although this transport system is not electrogenic, the changes in intracellular Na or pH, which are substantial, could affect ion conductances and/or the Na-K pump (which is electrogenic), thereby producing changes in V_m.

The purpose of the FMLP-induced V_m changes is unclear, because cells can migrate (248) and produce an oxidative burst (110) when depolarized by high K. Nevertheless, the observation that FMLP fails to induce V_m changes in neutrophils from patients with chronic granulomatous disease, a condition in which phagocytes are incapable of producing an oxidative burst (239), supports the view that the membrane depolarization (or events leading to it) is associated with the oxidative burst, even if depolarization is not required for activation of the oxidase. A close association between membrane depolarization and the oxidative burst was also demonstrated in a study that measured FMLP- and PMA-induced V_m changes and superoxide release in HL-60 cells at varying stages of differentiation (124). Similarly, Di Virgilio et al. (45) demonstrated that the dose-response relationship for the FMLP-induced depolarization in neutrophils was identical to the dose-response relationship for FMLP-induced activation of the NADPH oxidase. This study also showed that the increase in $[Ca]_i$, which occurs during FMLP stimulation, is reduced when neutrophils are depolarized and enhanced when they are hyperpolarized during FMLP stimulation. Part of the increase in $[Ca]_i$ requires extracellular Ca and has been attributed to the influx of Ca (3). Therefore the FMLP-induced depolarization may serve to limit the influx of Ca into the cell after stimulation as it does in other cells (208). In addition to limiting the influx of Ca, a recent report by Pittet et al. (214) indicates that membrane depolarization (in the absence of extracellular Ca) diminishes both the release of intracellular Ca and the rise in inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] that is produced after stimulation of neutrophil-like HL60 cells with FMLP or leukotriene B_4.

IV. LYMPHOCYTES

T, B, and natural killer (NK) lymphocytes participate in a complex series of interactions that underlies the function of the immune system. These include recognition of antigens, cytotoxicity, and lymphokine and antibody secretion (186). Advances in recent years have helped explain how these functions are carried out at the single-cell level (262a). Although questions still remain about lymphocyte ion-transport mechanisms and their functional relevance, considerable progress has been made in identifying the ionic conductances in these cells and characterizing the changes in ionic conductances that occur during lymphocyte activation (18, 20, 72, 73, 87, 152). The conductances described in lymphocytes are listed in Table 2 and are summarized next.

[3] If the Na-Ca exchanger is the major pathway for Ca influx in stimulated neutrophils, then depolarizing the neutrophil should increase the agonist-induced Ca influx. However, Di Virgilio et al. (45) demonstrated that depolarization of FMLP-stimulated neutrophils reduced the Ca influx.

TABLE 2. *Ion channels in lymphocytes*

Channel	Gating	SCG, pS	Blockers	Present In	Reference
K channels					
K_n (n type)	Voltage, Ca	12–13	4-AP, TEA, CTX, quinine, verapamil, D 600, Ni, Hg, La, nifedipine, diltiazem, chlorpromazine, forskolin, trifluorperazine, noxiustoxin	Human and murine T-cells, T-cell lines, cytotoxic T-cells, NK cells, human and murine B-cells	29, 35, 36, 58, 163, 172, 231, 252, 267
K_l (l type)	Voltage	21–27	TEA, Co	Murine T-cells	24
$K_{n'}$ (n' type)	Voltage	18	CTX	Murine T-cells	148
K_{Ca} (Ca activated)	Ca	25 7 93?	Apamin	Rat thymocytes, human B-cells, human T-cells?, murine B-cells?	20, 95, 159, 160, 168
Na channels	Voltage	20	TTX	Human thymocytes, T-cell lines, murine T-cells	36, 139, 231
Ca channels					
Ca_v (voltage activated)	Voltage			Jurkat 77 6.8 cells, hybridoma cell lines*	52, 57, 59
Ca_{Ins} ($InsP_3$ activated)	$InsP_3$	7	Ca	Human T-cells, Jurkat E6-1 cells	72, 150
Ca_s (small)		<1	Ni, Cd	Jurkat cells	151, 152
Cl channels					
Cl_L (large)	Voltage, PKa	365	Ni, Zn	Thymocytes, T-cells, murine B-cells	12, 19, 167, 230
Cl_s (small)†		~2.6		Murine spleen cells, Jurkat E6-1 cells, human T-cells	19, 147
Cl_A (cAMP activated)	PKa	40		Jurkat E6-1 cells, human T- and B-cells, murine B-cells?	15, 25

SCG, single-channel conductance under physiological ionic gradients (for rectifying channels, largest conductance is given). $InsP_3$, inositol trisphosphate, NK, natural killer, PKa, catalytic subunit of protein kinase A. * Hybridoma cell lines constructed from fusion of S194 cells and splenic B lymphocytes. † ATP and hypotonic medium required in patch electrode.

A. T Lymphocytes and Natural Killer Cells

T lymphocytes develop in the thymus and have both effector and regulator functions (262a). As effector cells they participate in graft versus host reactions, cytotoxicity, and delayed hypersensitivity, whereas as cell regulators they either help or suppress the activity of other lymphocytes. Cytotoxic T-cells kill in an antibody-dependent manner a variety of target cells, including those bearing foreign histocompatibility antigens as well as host tumor cells and virally infected cells, which share the same major histocompatibility antigens. In contrast, NK cells are large granular lymphocytes that can kill tumor and virus-infected cells in culture in the absence of antibody. Variations in the surface glycoproteins (i.e., CD_4 and CD_8) of these different lymphocytes correlate with their functional heterogeneity and therefore provide useful phenotypic markers for the different cell types. In vivo T-cell activation is initiated by the binding of specific antigens to the T-cell receptor, whereas in vitro activation can be accomplished using lectins and phorbol esters or monoclonal antibodies against specific surface antigens. The activation of T-cells results in a series of well-studied integrated events that ultimately leads to an increase in DNA synthesis and cell division.

1. Potassium conductances

1) OUTWARD VOLTAGE-GATED POTASSIUM CONDUCTANCE. Outward voltage-dependent K currents were first described in human peripheral blood T lymphocytes by Matteson and Deutsch (163) and by DeCoursey et al. (35) and in murine cytotoxic T-cell clones by Fukushima et al. (58). Similar currents have since been reported in a wide variety of lymphocytes, including immature human thymocytes (230), human helper-inducer T-cells ($T4^+$), suppressor cytotoxic T-cells ($T8^+$), alloreactive-cytotoxic T-cells (36), human NK cells (231), subsets of murine thymocytes (172), a murine noncytolytic T-cell clone (141), and a variety of murine cell lines (36). Only one type of outward voltage-dependent K conductance has been described in human T lymphocytes, whereas three voltage-dependent K conductances have been delineated in murine T lymphocytes. These are *1)* the n (for normal)-type K (K_n) conductance, which is the only voltage-dependent K conductance described in human T lymphocytes (17, 35) and is also present in murine

TABLE 3. *Three types of voltage-dependent K channels in murine T lymphocytes*

	Type n (normal)	Type n' (n-ish)	Type l (large)
Conductance	12-18 pS	18 pS	21-27 pS
Gating			
$V_{1/2}$	−30 mV	−10 mV	0 mV
Use-dependent inactivation	Yes	No	No
Closing rate τ (−60 mV)	30 ms	30 ms	1 ms
Pharmacology			
TEA (K_i)	~10 mM	~100 mM	~0.1 mM
CTX (K_i)	300 pM	<5 nM	Not blocked

$V_{1/2}$, membrane potential at which one-half of the channels are activated, K_i, inhibitory constant. [From Lewis and Cahalan (149).]

lymphocytes (37); *2*) the l-type K (K_l) conductance, which is present in large numbers in lymphocytes from the MRL/lpr mouse strain but is also found in T lymphocytes from normal strains of mice (24, 34); and *3*) the type n' (K_n) conductance, which is found in subsets of murine thymocytes (148). A mixture of these channels can be expressed in a given murine T lymphocyte, although one type of channel often predominates (19). Table 3 and Figure 3 summarize some of the characteristics of these voltage-gated K channels.

A) N-type potassium conductance. The K_n conductance is the best characterized ionic conductance in leukocytes. It was first described in whole cell patch-clamp studies of peripheral human blood T lymphocytes (35, 163) and in murine clonal cytotoxic T lymphocytes (58). Under whole cell patch-clamp conditions, K_n currents have a threshold of activation in the −50- to −60-mV range, and the conductance is fully activated above 0 mV. [The threshold for activation for this current may differ in cell-attached recordings (S. C. Lee and C. Deutsch, personal communication).] Similar to delayed rectifying K currents in vertebrate nerve and muscle cells, K_n currents in both human and murine T lymphocytes exhibit sigmoidal voltage-dependent activation kinetics (35, 38, 58, 163) and could be fitted to a Hodgkin-Huxley type n^4j model (17, 38). However, the rate of K_n current deactivation determined from relaxation of tail currents was an order of magnitude slower in lymphocytes than in skeletal muscle (17). When $[K]_o$ was varied, the K_n reversal potential followed the Nernst equation for K, indicating that the current is K selective (17, 58, 165).

The K_n current decreases or inactivates during voltage steps lasting >20 ms. During prolonged depolarization the K_n current inactivates to a steady-state level that, in human T-cells, was half maximal at −70 mV and was complete at almost all potentials that elicited the K_n currents (17), whereas in the murine clonal cytotoxic T-cell line inactivation was absent at −75 mV (58). If the inactivation of K_n currents is state dependent and not voltage dependent, as has been suggested recently for similar channels in rat type II alveolar epithelial cells (34), then these apparent differences in "steady-state" inactivation may be ascribable to a difference in the experimental protocol rather than to actual differences. In addition, recovery from inactivation was much slower than the onset of inactivation during depolarization (17, 38, 58). When pairs of identical pulses separated by different time intervals were used to examine recovery from inactivation, short (80 ms) depolarizing pulses produced a peak current for the second pulse that was smaller than the current at the end of the first pulse (35, 138). This phenomenon of cumulative inactivation has been described in other cells (5, 290). For longer pulses (500-600 ms), recovery time increased and, in the case of human T lymphocytes, was fit by two exponentials with time constants of 10 and 420 s (17). The kinetics of K_n channel inactivation suggest the existence of more than one inactivated state of the channel (17).

Several changes in the K_n current occur during the first 10-15 min after establishing the whole cell patch configuration (17, 40, 58). These include increases in the peak current, the rate of activation, and the rate of inactivation and a −10- to −20-mV shift in the voltage dependence of activation. Similar shifts have been noted in other currents studied using the whole cell patch technique, and it has been postulated that they are due to dissipation of a Donnon potential due to the slow diffusion of large cytoplasmic anions into the pipette (161), although in some cases the voltage shifts are larger than can be accounted for by this mechanism (53). Although junctional potential shifts probably account for some of these changes, several additional factors also may underlie these changes. These include the likely effect of intracellular fluoride on augmenting the rate of K_n inactivation (17) and the removal of inactivation of the K_n conductance when cells are held at −80 or −90 mV. That is, if the resting V_m of intact lymphocytes is −70 mV or less negative (estimates of V_m in unstimulated T lymphocytes obtained using indirect fluorescent probes range from −50 to −70 mV) (87), then the K_n conductance would be partially inactivated at rest.

1) Effects of univalent cations. Ion-substitution studies revealed the following permeability ratio for the K_n conductance. K (1.0) > Rb (0.77) > NH_4 (0.10) > Cs (0.02) > Na (<0.01) (17), which is similar to those reported for other delayed rectifying K channels (106, 218). In high-K medium, the K_n conductance increased, the instantaneous I-V relation became inwardly rectifying, and the rate of channel closing (deactivation) slowed (17). Furthermore, peak G-V relations indicated that the K_n conductance was activated at potentials 10-20 mV more negative in high-K or high-Rb Ringer than in NH_4-containing or normal Na-containing Ringer, suggesting that permeant cations interact with the gating mechanism of the K_n channel (17).

2) Effects of calcium. Although Cahalan et al. (17) originally reported that changing the $[Ca]_i$ from 10^{-8} to 10^{-6} M (with glutamate or aspartate as the primary internal anion) had no effect on the magnitude of the K_n current, several more recent observations have demon-

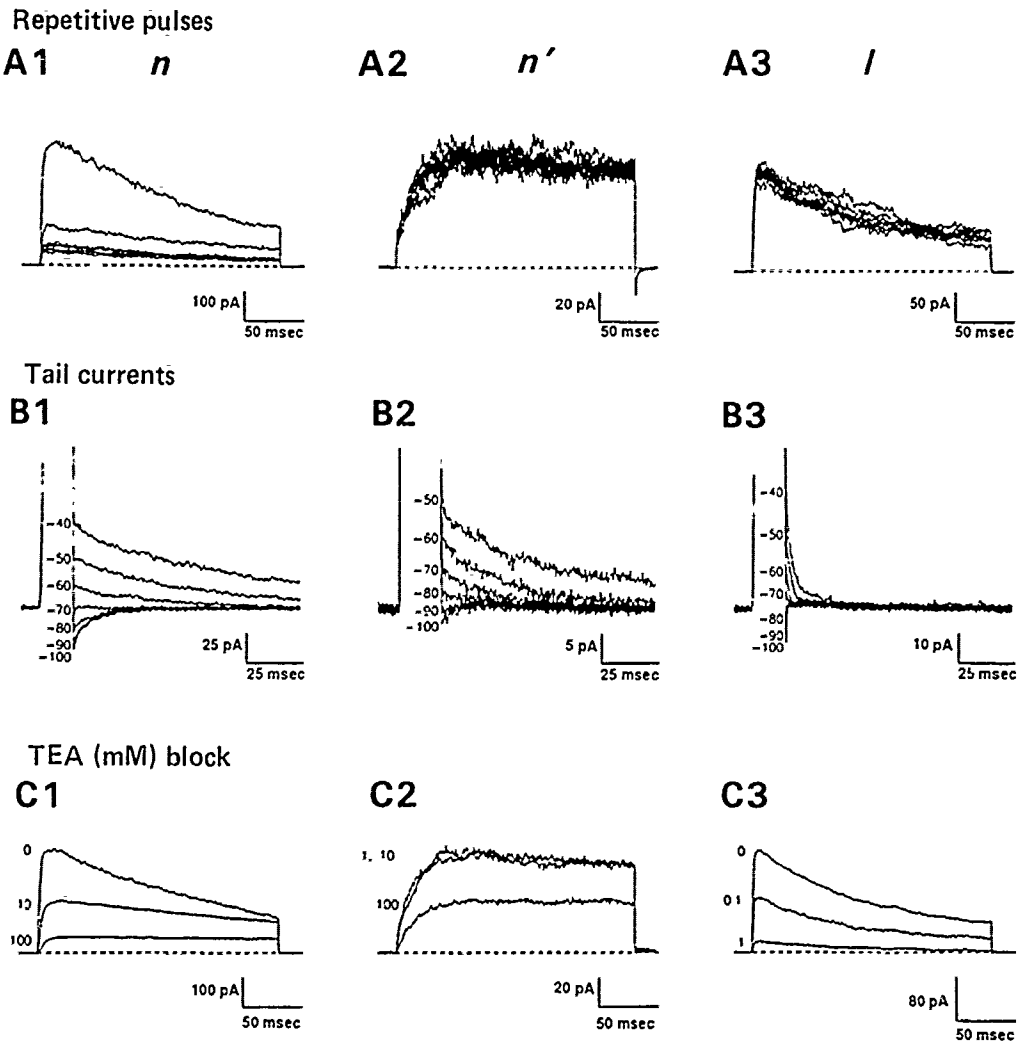

FIG. 3. Characterization of 3 types of thymocyte K currents. *A-C, columns 1-3* represent n, n' and I K currents, respectively. *A*, cumulative inactivation of K current during repetitive depolarization. Voltage stimuli (200-ms pulses from −80 to +30 mV) were delivered at rate of 1/s from holding potential of −80 mV. Seven responses are superimposed. *B*, voltage dependence of K-channel closing rates. Activating pulses of 10-20 ms from holding potential (−80 mV) to +30 mV (currents not shown) were followed by pulses of −100 to −40 mV. *C*, K-channel blockade by tetraethylammonium (TEA). K currents were elicited by pulses from −80 to +30 mV in presence of 0.1-100 mM TEACl. In *C1*, TEA slows apparent inactivation rate of K current, not by unmasking TEA-resistant current component but through direct effect on kinetics of type n channels. Cells were bathed in Ringer solution containing (in mM) 160 NaCl, 4.5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, and 5 Na-HEPES (pH 7.4). Pipette contained (in mM) 134 KF, 11 K_2-EGTA, 1.1 $CaCl_2$, 2 $MgCl_2$, and 10 mM K-HEPES (pH 7.2). [From Lewis and Cahalan (148).]

strated that the K_n conductance can be modulated by $[Ca]_i$. First, when the patch pipette contained 10^{-6} M Ca, whole cell currents decreased during the first 5-10 min of recording from human T lymphocytes, whereas they increased during the first 5-10 min of recording when the pipette contained 10^{-8} M Ca (14). Second, adding 1 μM A23187 increased the rate of K_n inactivation and reduced current amplitude by 56%. A similar effect of A23187 on K_n currents was reported in the human Jurkat T-cell line (52). Finally, single-channel recordings of human T-cells obtained in excised inside-out patches indicated that increasing the $[Ca]_i$ from 10^{-8} to 10^{-6} M almost completely blocked channel activity in a reversible manner (14).

Increasing $[Ca]_o$ 10-fold from ~2 to ~20 mM did not increase K_n current amplitude but shifted the *G-V* relationship to more positive potentials (17, 58) and increased the rate of inactivation (36, 94). In Jurkat E6-1 cells the rate of K_n channel inactivation was increased as both internal and external Ca increased, suggesting that Ca can enter open K_n channels from either the inside or the outside. The effects of Ca on K_n channel inactivation are consistent with a model that proposed that Ca binding to a site inside the channel induces a conformational change that inactivates the channel rather than inactivation produced by a direct block by Ca (94).

3) pH effects. Because changes in pH_i occur in lymphocytes after mitogen stimulation (44, 78), clarifying the role of K_n conductance in mitogenesis necessitates examining the effects of pH_i on the K_n conductance. In

whole cell recordings, Deutsch and Lee (42) reported that the conductance is insensitive to external pH (pH_e) until pH_e is lowered below 6.6. In contrast, changing the pH_i from 5.2 to 8.2 increased the average peak K_n current in human T lymphocytes threefold but had no effect on the threshold of activation or on inactivation. Deutsch and Lee (42) described the pH_i dependence of the K_n conductance by a model with two strongly cooperative proton-binding sites with an acidic dissociation constant of 7.15. The threefold increase in whole cell currents was accompanied by an increase in single-channel conductance. However, the increase in single-channel conductance was not sufficient to account for the increase in whole cell peak current, indicating that the number of open channels may also be affected by pH_i. Thus it is possible that alterations in the K_n conductance induced by pH_i changes that occur during the cell cycle may play an important role in proliferation.

4) Temperature effects. Most of the patch-clamp studies in leukocytes have been done at room temperature (~22°C). However, two recent studies on human peripheral T lymphocytes examined the effect of temperature on K_n conductance (138, 194). Changing the temperature from 5 to 42°C increased the K_n current amplitude and the rates of activation and inactivation and shifted the threshold of activation to more negative potentials (194). Both deactivation (the rate of decay of the current on repolarization) and recovery from inactivation were quite temperature sensitive (138). At 37°C, cumulative inactivation did not occur if the interpulse interval was >1-2 s, whereas at 22°C, inactivation was observed with an interpulse interval of 20 s. These changes indicated that increasing the temperature augmented the number of open K_n channels. From the whole cell conductance and Boltzmann fits of the activation and inactivation curves, Pahapill and Schlicter (194) estimated that from −50 to −70 mV (the estimated resting V_m), 5-20 K_n channels would be open at 37°C compared with 3-7 channels at 20°C. The single-channel conductance also increased as temperature increased (138, 194) so that the increase in whole cell conductance at 37°C probably results from increases in both the single-channel conductance and open channel probability (194).

5) Single channels. Because T lymphocytes have a very high input resistance (on the order of 20 GΩ), it is possible to record single-channel currents in the whole cell recording configuration (14, 17, 138). In human T lymphocytes, single-channel currents obtained in this manner had conductances of 14-16 and 6-9 pS in normal Ringer solution and 40 and 24 pS in high-K medium (14, 17). In murine T lymphocytes, K_n channels have a conductance of 12 pS (38). Single-channel currents displayed flicker or rapid closures within bursts, indicating at least two nonconducting states of the channel. In excised patches exposed to symmetrical high-K solutions, *I-V* relationships of single K_n channels displayed inward rectification, similar to the inward rectification noted in instantaneous *I-V* relations of whole cell currents in high-K Ringer (17). Unitary current amplitude increased in size as temperature was raised from 22 to 37°C, the most prevalent channel had a conductance of 26 pS at 37°C compared with 14 pS at 22°C (138). The number of K_n channels estimated from the conductance of the single-channel currents and the whole cell conductance is 300-500 and 10-15 channels in human T-cells (17) and resting murine T lymphocytes (38), respectively.

6) Antagonists. Antagonists of the K_n conductance are numerous. They include classic K channel blockers, classic Ca channel blockers, polyvalent cations, and other compounds. The K channel blockers quinine (58, 163), TEA, and 4-AP all reversibly block this conductance when added to the bath with an inhibitory constant (K_i) in human T lymphocytes for quinine, 4-AP, and TEA of 14 μM, 190 μM, and 8 mM, respectively (35). Of these, channel block by TEA has been the best characterized (38, 93). In murine thymocytes, TEA reduced the apparent single-channel amplitude, probably by producing a fast block, the kinetics of which were above the frequency range explored (38). The dose-response relationship for the single-channel block in murine thymocytes was similar to that for blocking macroscopic currents (38). In the human lymphoma line Jurkat E6-1, TEA both reduced the peak K_n current and slowed the time course of decay so that the K_n current integral in the presence of TEA was unchanged (93). These data fit a kinetic scheme in which open K channels blocked by TEA cannot inactivate (93).

The K_n channels also are blocked by a number of agents that inhibit Ca channels. These include polyvalent cations and the Ca channel antagonists diltiazem, verapamil, and nifedipine (23, 36, 38, 250). In human T lymphocytes, the potency sequence of half block for these agents was verapamil (6 μM) > nifedipine (24 μM) > diltiazem (60 μM) (23). Although the potency sequence has not been reported in murine T-cells, these agents also produced half block in concentrations ranging from 4 to 40 mM (38). At intermediate concentrations of verapamil, K_n channels inactivated more rapidly and once inactivated recovered more slowly. Block by verapamil was use dependent, increasing with frequency of channel opening (36, 250). This is similar to the use-dependent block of Ca channels previously described for Ca channel antagonists (137).

Inorganic polyvalent cations reduce the K_n conductance and shift the *G-V* curve to more positive potentials (36, 38). Divalent cations have been shown to induce similar shifts in *G-V* relationships of other K conductances (54, 81). In human T lymphocytes the potency sequence for these blockers is Hg > La > Zn > Co > Ba, Cd > Mn, Ca > Sr > Mg (36). In addition, Ni (1 mM) also reduces the K_n current in human T lymphocytes (163). Both Co and La have similar blocking effects on K_n channels in murine T-cells, although murine cells may be somewhat less sensitive to polycation block (38). The interaction of Ba with the K_n channel has been studied in Jurkat E6-1 cells, where it was demonstrated that external Ba enters the open channel, producing a use-dependent block, and is trapped inside the channel when it closes (94).

Other compounds that decrease the K_n conductance are the calmodulin antagonists chlorpromazine and trifluoperazine (19), forskolin (127), retinoic acid (249), and toxins isolated from scorpion venom (210, 226). In human T lymphocytes, forskolin (20 μM) decreased the K_n conductance without changing the voltage dependence and kinetics of inactivation. Surprisingly, the effects of forskolin were not mediated by a rise in adenosine 3',5'-cyclic monophosphate (cAMP), since raising cAMP levels with isoproterenol plus phosphodiesterase or with dibutyryl cAMP had no effect on the K_n conductance (127) and neither did 1,9-dideoxyforskolin, an analogue of forskolin that does not stimulate adenylate cyclase in human lymphocytes (127). There is precedent for a direct block of K channels by forskolin from studies in invertebrate neurons (33).

Charybdotoxin blocked K_n channels in human and murine T lymphocytes with a K_i of ~0.5 nM (210, 226). Because it inhibits the K_n conductance at much lower concentrations than other antagonists, it may be a promising tool for examining the functional relevance of these channels (210). Block by CTX appears to occur when the channel is in either the closed or open state, because it blocked at a holding potential of −80 mV and because there was no indication of recovery during depolarization. Noxiustoxin, purified from *Centruroides noxius* also blocks K_n channels with a K_i of 0.2 nM (226).

Schumann and Gardner (234) reported that the sensory neuropeptide substance P, which stimulates T-lymphocyte proliferation (198) and lymphokine secrection (193), decreases the peak amplitude of the K_n current and accelerates the rate at which the current inactivates in Jurkat E6-1 cells. The effects of substance P could be mimicked by the addition of GTPγS to the pipette. Intracellular application of GDPβS (100-500 μM) blocked the action of substance P, suggesting that GTP-binding proteins may modulate the K_n current (234). However, GTPγS did not have this effect in normal human T-cells (137).

7) Expression in resting cells. With the exception of the T-cell lines CCRF HSB 2 and P12-Ichikawa, K_n channels are present in all T lymphocytes that have been examined (36). Identification of subsets of human T lymphocytes with antibodies indicated that resting T4/Leu3$^+$, T8/Leu2$^+$, and alloactivated cytotoxic T-lymphocytes all expressed K_n channels, although cytotoxic T-cells appear to have more channels (36). Schlicter et al. (232), comparing the expression of K_n channels in immature human thymocytes (negative for the T3 receptor) with channel expression in mature T3$^+$ T cells, found that channel expression in these two groups of cells was similar and concluded that K_n channels are expressed very early in the differentiation process, possibly before thymic processing. The largest number of K_n channels (1,500 channels/cell) has been reported in CCRF CEM 3A cells. Despite the variation in K_n channel numbers in different cell lines, no correlation has been made between the function of different cell lines and K_n channel number (36).

This is not the case in primary murine T lymphocytes, which have only 10–15 K_n channels in the resting mature cell (36). In murine T-cells, there also was a correlation between the type of K channel present and T-cell function, K_n channels predominate in mature thymocyte subsets that are $CD_4^+CD_8^-$ (precursors to helper T-cells), whereas in the mature $CD_4^-CD_8^+$ thymocyte subset that contains precursors to cytotoxic and supressor T-cells, K_i and K_n channels predominate (148, 149). Functionally immature $CD_4^+CD_8^+$ or $CD_4^-CD_8^-$ thymocytes expressed 5- to 10-fold more K_n channels than mature resting thymocytes (148). There also was a correlation between the number of K_n channels and the ability of thymocytes to proceed through the cell cycle (148, 172).

8) Effects of activation. The T-cell activation after in vitro exposure to polyclonal mitogens and cytokines occurs over a period of hours or days and often results in amplification of the K_n conductance. In human T-cells, a 20- to 24-h exposure to the tumor promoter TPA or concanavalin A (ConA) increased K_n conductance 1.7- (40) and 2-fold (163), respectively. In contrast to the slow effects of TPA and ConA, DeCoursey et al. (35) reported that 1 min after exposure of human T lymphocytes to phytohemagglutinin (PHA), the kinetics of K_n channel gating were modified so that the G-V curve was shifted by −15 mV, thus the number of channels open at rest would double. However, subsequent studies on human immature thymocytes and human T lymphocytes have failed to demonstrate these rapid PHA-induced changes in the K_n conductance (138, 232). No effects on the K_n current were noted when isoproterenol, prostaglandin E_2, cAMP analogues, or nucleotides were added to the bath or pipette in human T lymphocytes maintained at 37°C (138).

Although murine resting T lymphocytes have many fewer K_n channels than human T-cells, after stimulation with the mitogen ConA, actively dividing cells of either the helper or suppressor cell variety exhibited a large augmentation in channels (19, 38). This augmentation of the K_n channels is a likely cause for 10- to 15-mV hyperpolarization in murine cells exposed to ConA (122, 271). In the murine noncytolytic T-cell clone L2, exposure to interleukin 2 (IL-2) increased the amplitude of the K_n current threefold at 24 h (141). The increased current was maintained for 72 h and paralleled the IL-2-induced augmentation in cell size and DNA synthesis.

9) Cloning. Molecular characterization of the K_n channel has been carried out using probes from Shaker-related K channel genes to screen cDNA libraries from both rats (19) and mice (97). Injection of mRNA encoded by either of the intron-less genes MK3 (97) or RGK5 (49) into *Xenopus* oocytes resulted in the expression of ionic channels with similar biophysical and pharmacological properties to the K_n channel. The amino acid sequence of RGK5 is 60–70% homologous with the Shaker core sequence (49). Using the polymerase chain reaction, Grissmer et al. (97) demonstrated that MK3 is transcribed and expressed in mouse T-cells. In addition, probes prepared from the unique 5'-noncoding region of

MK3 were used to localize MK3 to human chromosome 13 (97).

B) L-type potassium conductance. The K_l channels were first described in T-cells from mice containing two different autosomal recessive mutations, lpr/lpr and gld/gld (24, 34, 96). Both of these mutations result in a lymphoproliferative systemic lupus erythematous (SLE)-like disease that develops early in life. The K_l channels also are found in small numbers in T lymphocytes from control mice (24). The K_l conductance can be distinguished from the K_n conductance in that it activates 30 mV more positive than K_n channels, closes much more rapidly on repolarization, and inactivates more slowly and less completely (37). [Concanavalin A shifted the voltage dependence of K_l channels by about −10 mV, but these channels still opened at more positive potentials than K_n channels (24).] The K_l channels also recover from inactivation more rapidly so that little inactivation accumulates with repetitive pulses. The pharmacological block of K_l channels also differs from K_n channels; they are less sensitive to Co block, are 100 times more sensitive to TEA [mean inhibitory constant ($K_{1/2}$) = 50–100 μM] (37), and they are not blocked by CTX (225). Consistent with the whole cell data, single-channel K_l currents are open at more positive potentials and have a larger (21 pS) conductance (37) than K_n channels. Addition of 0.1 mM TEA to the bath reduces single-channel conductance by 50% (37, 149), which is compatible with a rapid open-channel block mechanism like that for K_n channels.

Shapiro (241) has reported that K_l channels are similar to one of the "fast" K conductances called g_{f2} channels in node of Ranvier (50). Unlike K_n currents, but like g_{f2} currents, K_l current activation could not be fitted by Hodgkin-Huxley n^4 kinetics but was well-fitted by a delay followed by a single exponential. Besides similarities in kinetics and voltage dependence, pharmacological agents known to block g_{f2} channels, such as capsaicin (51) and naloxone (112), also block K_l channels (241).

The ionic selectivity of K_l channels to monovalent cations is roughly similar to that for K_n channels, except for a higher Cs permeability (243). For K_l channels, the permeability sequence based on biionic reversal potentials is K > Rb > NH_4, Cs >> Na. The species of permeant ion strongly modulates deactivation kinetics of K_l channels (243). This phenomenon is similar to permeant ion effects on K_n channels (17). The inward NH_4 current was over 10 times that for Cs, even though the permeabilities were nearly identical. This large discrepancy between selectivity and conductance indicates that K_l channels, like most other K channels (108), are multiion pores.

Monoclonal antibodies against specific subsets of T-cells were used to characterize K_l channel expression in both T-cells from healthy mice and T-cells from murine models of SLE, type-1 diabetes mellitus, and experimental allergic encephalomyelitis (21, 96). The T-cells from these diseased mice, which were phenotypically Thy1,2$^+$, CD_4^-, CD_8^-, B220$^+$, and F23.1sp$^+$, displayed a large number of K_l channels, whereas phenotypically similar T-cells from control mice or mice before the onset of the disease did not. Other T-cell subsets from these diseased mice expressed a normal pattern of K channels (21). Thus the abundant expression of the K_l channel may be a marker for the onset of autoimmunity. In developing thymocytes from normal mice, K_l channels were rarely found in $CD_4^-CD_8^-$ or $CD_4^+CD_8^+$ thymocytes but were found on $CD_4^+CD_8^-$ thymocytes, which are destined to become major histocompatibility class I-restricted cytotoxic or supressor T-cells (149). There is no evidence for the existence of K_l channels in human T-cells. However, K_l channels have been reported in the human Burkitt's lymphoma cell line Loucks (242).

C) N′-type potassium conductance. Examination of subsets of developing T-cells from the murine thymus has revealed a K conductance that has the same voltage dependence of activation, closing kinetics, and sensitivity to CTX as the K_n conductance but displayed little cumulative inactivation and was less sensitive to block by TEA ($K_{1/2}$ = 100 mM) (148). However, it was blocked by nanomolar concentrations of CTX (226). Corresponding single-channel currents having a conductance of 17 pS have been identified (148). Cell surface-staining techniques revealed that $CD_4^-CD_8^+$ cells destined to become MHC class I-restricted cytotoxic or supressor T-cells most often expressed as K_n channels.

II) CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE. Radioisotope flux and potentiometric dye studies have provided substantial indirect evidence that T lymphocytes possess a Ca-activated K conductance (87, 91, 119, 219, 271). For example, in rat thymic lymphocytes and human peripheral blood mononuclear cells, elevation of $[Ca]_i$ in the submicromolar range by exposure to ionomycin induces a membrane hyperpolarization (measured with fluorescent dyes) that parallels the increase in $[Ca]_i$, depends on the K gradient, and is inhibited by 25 nM CTX (91).

Despite such observations, patch-clamp studies by several different investigators in a variety of T-cells failed to demonstrate Ca-activated K conductances in T-cells until Mahaut-Smith and Schlichter (160) demonstrated the presence of a Ca-activated K conductance in rat thymocytes and human B lymphocytes. In that study, exposing cells to ionomycin induced channel activity in 90% of the cell-attached patches. Two amplitude channels were noted. The smaller (7 pS) channel was not well characterized, but the larger channel had little voltage dependence in the range from 20 to −120 mV applied to the pipette and exhibited inward rectification. Its single-channel conductance was 25 pS for inward currents and 15 pS for outward currents. Channel activity could be retained in a percentage of the patches after excision and increased when $[Ca]_i$ was increased from 100 to 300 nM. However, excision of the patch altered the kinetic behavior and the conductance of the channel, and channel activity frequently disappeared. Similar channel activation has been reported after exposure of rat thymocytes to the mitogen ConA (159).

At this time, it is not clear how widely distributed the Ca-activated K conductance is among T-cells. A pre-

liminary report in Jurkat E6-1 lymphocytes, in which the perforated patch technique (111) was used, demonstrated that ionomycin induces a current that may be a Ca-activated K conductance (95). Further work is needed to determine if this channel is found in most T-cells and whether the previous failure to detect Ca-activated K channels was due simply to washout of the current in the whole cell and excised patch configuration.

2. Sodium conductance

Sodium channels with gating properties similar to Na channels in excitable cells have been described in human and murine T lymphocytes (36), in human thymocytes (231), and in several T-cell lines, including Jurkat E6-1, MOLT-4, MOLT-17, CCRF-CEM, CEM-1-3, and K562 cells (36, 139, 232, 284). The Na currents were abolished by replacement of external Na with Cs or TEA; tetrodotoxin (TTX; 100 nM) also reversibly blocked the channels. The Na channels from murine T-cells were less sensitive than human T-cells to TTX block (17, 38). Single-channel currents evident in some whole cell records indicated that the single-channel conductance was 20 pS for human T-cells (17) and 16–18 pS for murine T-cells (38).

Sodium channels are present in only a small percentage (3 of 90 cells) of human resting T lymphocytes (17) but are in as many as one-third of the unstimulated mouse MRL-n strain of T lymphocytes (38). Activation of murine T-cells with ConA did not increase the percentage of cells expressing Na currents but did produce a 10-fold increase in the number of Na channels per cell. However, T-cells from other strains of mice had fewer Na channels. With the exception of murine NK clonal cells and K563 leukemic cells that express a high density of channels, T-cells do not usually contain enough Na channels to generate action potentials (18).

3. Calcium conductances

The T-cell receptor/CD_3 ligands or mitogenic lectins induce an increase in $[Ca]_i$ that is essential for subsequent activation events (72, 87, 271). The rise in $[Ca]_i$ has two components, a release of Ca from intracellular stores (2, 113) and an influx of extracellular Ca that is blocked by Ca channel antagonists, such as La (2, 188). Although it has been postulated that the influx of Ca occurs through classic voltage-dependent Ca channels, such channels have only been reported in Jurkat 77 6.8 cells [(52), studies of other Jurkat T-cell lines have failed to confirm this report (36, T. E. DeCoursey, personal communication)]. In those cells, a voltage-gated Ca conductance with slow kinetics, consistent with the behavior of L-type channels (187), was enhanced after exposure to PHA and may mediate the PHA-stimulated rise in $[Ca]_i$ that occurs after PHA stimulation. Patch-clamp studies have failed to demonstrate classic voltage-gated Ca channels in other types of T-cells (17, 58, 163). Thus Ca influx during T-cell activation must be mediated by a non-voltage-gated Ca transport mechanism(s) (72, 152).

Voltage-insensitive inward Ca (or Ba) currents were first described in cell-attached and whole cell recordings from a human cloned helper T-cell line (131). In cell-attached patches with 110 mM Ba in the patch electrode, single-channel currents had a linear I-V curve with an extrapolated reversal potential of ~60 mV and a ~7 pS conductance. They were infrequently open in cell-attached recordings of quiescent cells. However, the probability of opening was increased by both application of PHA, indicating that PHA was exerting its effect through a second messenger (131). A PHA-stimulated voltage-insensitive current that was blocked by Cd was also noted in whole cell records (131), however, there was little evidence that it was a Ca current. Similar single-channel activity was recorded in human cloned helper T-cells using monoclonal antibodies against the antigen/major histocompatibility complex receptor T3-Ti (CD_3 specific) or the sheep erythrocyte-binding protein T11 (CD_2 specific), demonstrating that a variety of stimuli that increase $[Ca]_i$ activate these channels (72). Although little is known about the pharmacology of this channel, in Jurkat E6-1 cells it could be activated by the Ca agonist BAY K 8644 (289). Moreover, as shown in Figure 4, exposure of the cytoplasmic membrane surface to micromolar concentrations of $Ins(1,4,5)P_3$ activated similar channels in a dose-dependent fashion during excised inside-out patch recordings from human Jurkat E6-1 T-cells (130), suggesting that this Ca-permeable channel is part of a relatively new class of inositol phosphate-sensitive Ca channels (200, 270). In addition, excised patch studies indicate that the channel may be autoregulated by Ca, since elevating $[Ca]_i$ suppressed channel activity (72; Fig. 4).

Lewis and Cahalan (151) have demonstrated that exposure of Jurkat T-cells to PHA-induced oscillations in $[Ca]_i$ depended on $[Ca]_o$ influx and were suppressed by depolarizing the cells with high-K medium. Whole cell patch-clamp recordings from those cells revealed a small (~7 pA in 10 mM intracellular EGTA) voltage-independent Ca current that was activated within seconds of obtaining a whole cell recording. The current was inward at potentials up to 20 mV, did not reverse at voltages up to 100 mV, was diminished by decreasing $[Ca]_o$, and was blocked by 5 mM Ni or 1 mM Cd. Interestingly, this current developed without any notable single-channel activity or increase in baseline noise. Thus the single-channel conductance must be quite small (<1 pS) and may even be due to the activation of an electrogenic pump or exchange mechanism rather than an ion channel. In perforated patch-clamp recordings (obtained by placing nystatin, a pore-forming antibiotic, in the pipette), an oscillating Ca current, the rise and fall of which preceded the oscillations in $[Ca]_i$, could be activated by adding PHA (151). The temporal correlation between the oscillations in $[Ca]_i$ and current activation supports the view that this current was causally related to the $[Ca]_i$ oscillations.

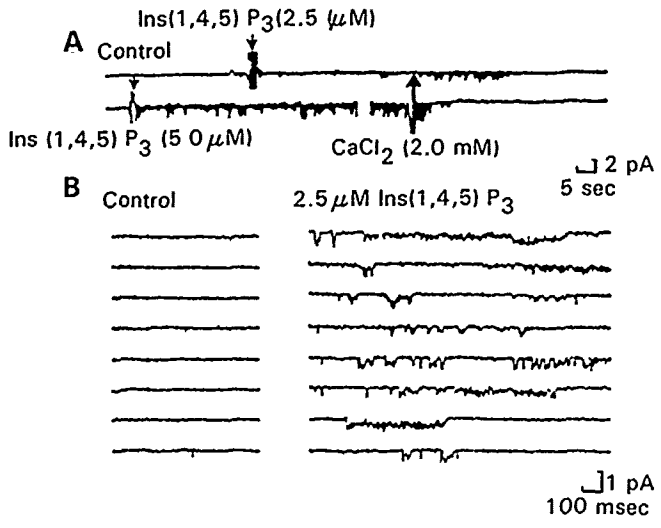

FIG. 4. Single inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]-induced inward currents recorded from inside-out membrane patch excised from Jurkat cell. Patch pipette contained 110 mM $BaCl_2$, 200 nM tetrodotoxin (TTX), and 10 mM HEPES-KOH (pH 7.3). Membrane patch cytoplasmic surface was bathed in solution containing (in mM) 150 KCl, 0.55 $CaCl_2$, 2 $MgCl_2$, 1.1 EGTA, and 10 HEPES-KOH (pH 7.0). Holding potential was 0 mV. Inward currents are seen as downward deflections from baseline. All records were filtered at 1 kHz. *A*: Ins(1,4,5)P_3-induced inward currents from 1 excised inside-out patch at slow sweep speed. Arrows, addition of Ins(1,4,5)P_3 and $CaCl_2$ to bath. No inward currents were observed in patch for >5 min before addition of Ins(1,4,5)P_3. First break in recording indicates interruption of 1 min during which potential was changed. First bath addition of Ins(1,4,5)P_3 resulted in inward current activity within 10 s of addition to bath. Inward currents appeared for <60 s and then disappeared for period of >4 min. Top and bottom traces were interrupted during this interval. Second application of Ins(1,4,5)P_3 (final concentration 2.0 μM) immediately elicited inward currents that were sustained for 2 min. Interruption in trace indicates a 2-min interval during which membrane potential was changed. Inward currents were abolished by application of $CaCl_2$ (final concentration 2 mM) to internal bathing solution. *B*: Ins(1,4,5)P_3-induced unitary inward currents at higher time resolution with sampling rate of 4 kHz. *Left*, control recordings before application of Ins(1,4,5)P_3. *Right*, inward current traces in presence of 2.5 μM Ins(1,4,5)P_3. [Reprinted by permission from Kuno and Gardner (130) Copyright® 1987 Macmillan Magazines Limited.]

Both the small Ca (Ca_s) current described by Lewis and Cahalan (151) and the Ins(1,4,5)P_3-induced Ca/Ba (Ca_{Ins})-permeable current described by Kuno and Gardner (130) were voltage insensitive and were inhibited by increases in $[Ca]_i$. However, these currents differed in the following ways. *1)* from reversal potential measurements, the Ca_s current is more selective for Ca than the Ca_{Ins} current; *2)* the amplitude of the whole cell Ca_s current was 100-fold smaller than the whole cell Ca_{Ins} current, and *3)* no single-channel current fluctuations accompanied Ca_s currents. It is unlikely that these differences reflect differences in experimental protocol, although the study of Lewis and Cahalan (151) was performed in 2 mM extracellular Ca and most of the recordings of Kuno and Gardner (130) were obtained in 110 mM Ba. Further studies are needed to determine if these are related currents or if two different voltage-insensitive Ca transporters exist in T lymphocytes.

A single study on bilayers containing plasma membrane from the human T-cell line REX has shown small (2–3 pS under conditions of symmetrical 100 mM Ca) single-channel Ca or Ba currents that were inhibited by La (199). These currents were induced by adding three different monoclonal antibodies that interacted with the T3-Ti receptors on REX cells but that were not activated by monoclonal antibodies directed against the Ti receptor of different T-cell lines or against the T4 antigen. Because channel activity was present in isolated bilayers, the data suggest a physical link between the T3-Ti receptor and the ionophore and, in contrast to the findings of Gardner (72), argue against the indirect activation of the channels by a second messenger (199). Unfortunately, no data were presented on channel selectivity or voltage dependence. Furthermore, the relationship of these currents to the Ca_{Ins} channels described by Gardner (72) is uncertain.

4. Chloride conductances

I) LARGE CHLORIDE CONDUCTANCE. Single-channel currents of a large-conductance voltage-dependent Cl channel, similar to the Cl_L channels described in macrophages, myotubes (236), and B-cells (12), have been described in whole cell (19) and excised patch (230) recordings from T lymphocytes and thymocytes. Single-channel conductance was ~365 pS. The channel exhibited several subconductance states that had the same voltage sensitivity, ionic selectivity, and block by Zn as the fully conducting channel. The most frequently noted subconductance states were in multiples of 45 pS. Unlike the macrophage, where the anion/cation selectivity of the Cl_L channel was 5:1 (236), in the T-cell this channel was 30 times more selective for Cl than for Na or K (230). Anion selectivity sequences determined from either reversal potentials or conductance ratios produced similar results in contrast to the findings in B lymphocytes, where different selectivity sequences were determined using these two types of measurements (12). The selectivity sequence for the T-cell Cl_L channel was $I > NO_3 > Br, Cl > F$, isethionate, $HCO_3 > SO_4 >$ gluconate, propionate > aspartate (230). As in macrophages (see sect. III,4,2), these channels opened and then closed in a time-dependent manner with excursions of ±20 mV, and the steady-state probability of channel opening fit a bell-shaped curve, fitting a model of a channel with one gate that closes at negative potentials and a second gate that closes at positive potentials (230).

Interestingly, recent observations by Pahapill and Schlichter (195) indicated that channel activity changes dramatically when cells are maintained at 37°C rather than at room temperature. It should be emphasized that all but a few of the patch-clamp studies in leukocytes have been performed at room temperature and that the activity of ionic channels under these conditions may differ from their activity at 37°C. For example, at 37°C, Cl_L channels were active at rest, and activity increased with hyperpolarization.

In excised patches, the Cl_L channel can be activated by the catalytic subunit of protein kinase A plus ATP, indicating that it may be regulated by second messengers (194). The channel was reversibly blocked by Zn or Ni (1 mM) added to the cytoplasmic side of the membrane. Block by Zn was decreased by hyperpolarizing the membrane, suggesting that the cation plugged the channel (230).

II) SMALL CHLORIDE CONDUCTANCE. One study reported that when the patch electrode contained a hypertonic K-aspartate/ATP-containing solution, T lymphocytes exhibited a small Cl (Cl_s) conductance that is absent immediately after establishing the whole cell configuration but then slowly develops (147). This conductance was present in mouse splenic T-cells, Jurkat E6-1 cells, and human T-cells, had an anionic permeability sequence of NO_3 > Br, Cl, F > methanesulfonate > ascorbate > aspartate, and could be reversibly blocked by 2 mM SITS (19, 147). The Cl_s channels were too small to resolve at the single-channel level, but noise analysis resulted in an estimate of 2.6 pS for the single-channel conductance. From this estimate and the measured whole cell conductance, the number of Cl_s channels was estimated to be quite high, on the order of 1,000 (19). Hydrolysis of ATP appears to be required for activation of this conductance, since the current was induced by internal ATP and ATPγS (a nonhydrolyzable ATP analogue) but not by ADP, AMP, or AMP-PCP (147). It was hypothesized that the Cl_s conductance participates in the regulatory volume decrease (RVD) that occurs in lymphocytes exposed to hypotonic conditions. Unfortunately, no subsequent studies have examined this channel or its role in RVD.

III) ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE-DEPENDENT CHLORIDE CONDUCTANCE. Chloride channel activity similar to that described in epithelial cells (277) was reported in excised inside-out patches from Jurkat E6-1 cells (25). These single-channel currents were outwardly rectifying in symmetrical solutions and had a slope conductance at 0 mV of 40 pS. Reversal potential shifts produced by changing extracellular Na and Cl concentrations indicated that the Cl-to-Na permeability ratio was 10:1. In excised patches the kinetic behavior of the channel was complex, with prolonged bursts interrupted by brief flickers and long closures, channel activation required 10^{-7} M $[Ca]_i$. However, once the channel was activated, lowering $[Ca]_i$ to 10^{-9} M had no affect on channel gating. Similar channel activity could be induced in cell-attached patches after exposure to 8-bromo-cAMP (25). Furthermore, channel activity was induced when the cytoplasmic surface of excised patches was exposed to the catalytic subunit of cAMP-dependent protein kinase plus ATP. Thus this channel appeared to be a cAMP-dependent Cl (Cl_A) channel similar to that described in airway epithelial cells (277). In epithelial cells, regulation of the Cl_A channel was reported to be defective in cystic fibrosis (55, 277), and Chen et al. (25) have shown that it is also defective in lymphocytes from cystic fibrosis patients (see sect. IV*B4*).

More recently, Bubien et al. (15), using fluorescence digital imaging of the halide-sensitive fluorophore 6-methoxy-*N*-(3-sulfopropyl)quinolinium and whole cell patch clamping, confirmed that both T and B lymphocytes from cystic fibrosis patients lack a Cl current that can be activated by the addition of either cAMP or ionomycin to the cells. They also reported that lymphocytes in G_1 had a significant spontaneous Cl permeability, whereas G_0 and S phase cells had a low Cl permeability (15).

5. *Physiological relevance*

I) SETTING RESTING MEMBRANE POTENTIAL. Estimates of V_m in populations of normal resting mammalian lymphocytes obtained using potentiometric-sensitive dyes and $TPMP^+$ range from −50 to −70 mV (39, 122, 271, 280). These studies indicated that the V_m of lymphocytes was primarily a K diffusion potential (87). From patch-clamp studies, Cahalan et al. (17) estimated in human T-cells that 0.1–2 (of the 300–400) K_n channels are open at the resting V_m. Using a $[K]_i$ of 130 mM (39) and the unitary K_n conductance, Cahalan et al. (17) calculated that the net K efflux through open K_n channels would be 10–400 × 10^{-18} mol/min and concluded that this flux could account for the resting K fluxes measured in lymphocytes by Segel and Lichtman (237). Further support for the involvement of K_n channels in setting the resting V_m comes from the observation that CTX, which blocks K_n channels (226), depolarizes human peripheral blood monocytes under conditions (depleted $[Ca]_i$) in which a Ca-activated K conductance should not be active (91). However, this observation differs from that of Wilson et al. (281), who reported that CTX had no effect on the resting V_m of unstimulated human T-cells. In addition, K_n single-channel currents should be evident in cell-attached patches if they participate in setting the resting V_m, but Deutsch (personal communication) reports a failure to record K_n channels at −70 mV in cell-attached patch experiments. Furthermore, even though K_n channels may contribute to the resting V_m in T-cells that have high numbers of K_n channels, this is less likely for cells that exhibit fewer K_n channels, such as resting mature murine T lymphocytes. The observations that CTX has little effect on the resting V_m of rat thymocytes (91) or murine spleen and thymus cell (281) may reflect this possibility.

Because the V_m of quiescent T-cells is significantly less negative than the E_K (which is approximately −90 mV), other permeabilities, such as the Cl or Ca conductances described, must contribute to the V_m. In human cloned T lymphocytes, a voltage-independent Ca-permeable channel has been shown to be open, albeit infrequently, at rest (131) and therefore would tend to depolarize the cells. In addition, the preliminary report by Pahapill and Schlichter (195), indicating that at 37°C human T-cell Cl_L channels are active at rest, implies that these channels contribute to the resting V_m. This seems somewhat surprising for several reasons. First, the basal Cl permeability in human lymphocytes is very low,

and it is unaffected by changing V_m (which would not be the case if Cl transport occurred via a voltage-dependent conductive pathway) (86). Second, Cl_L channels have such a large conductance that they would be expected to overwhelm the K_n conductance, effectively clamping the cell at E_{Cl}. Further studies are required to determine which conductances play a role in setting the resting V_m of the different types of T-cells.

II) RESPONSE TO MITOGENS. Numerous studies have implicated both K and Ca conductances in T lymphocyte proliferation (for reviews see Refs. 72, 87). In contrast, Na transport also is stimulated during mitogenesis, but the main route for Na entry is via the Na-H exchanger (87). This conclusion fits with the observation that TTX had no detectable effect on mitogenic response of T-cells (17). At present, no direct data exist about the function of Cl channels in T-cell proliferation, although DIDS, an anion transport antagonist, inhibited the ability of monoclonal anti-CD_3 complex antibodies to stimulate Ca influx in Jurkat T-cells without affecting Ca release from intracellular stores (222).

A) Potassium channels. Because patch-clamp studies have only recently described Ca-activated K channels in thymocytes, little electrophysiological data exist supporting their role in the proliferative response of T-cells. In contrast, substantial evidence supports a role for voltage-dependent K channels. Three types of evidence indicate that voltage-gated K channels, and in particular K_n channels, play a role in mitogenesis. First, a variety of mitogens increase the K_n current density (14, 38, 40, 141, 163). The current amplification is consistent with reports of mitogen-induced increases in K efflux and membrane hyperpolarization (237, 270). Moreover, the time courses of the augmentation of K fluxes and the K_n conductance are similar. Thus in human cells, both the K_n conductance and K fluxes were increased within minutes of adding mitogen (17, 36), whereas in murine splenic lymphocytes augmentation of the K_n conductance and K fluxes occurred 15 h after ConA addition (38). However, the lack of voltage-gated K channels in the T-cell line CTLL-2 that responds normally to mitogens (36) suggests that these channels are not absolutely required for proliferation. That is, there are different pathways of activation for a given type of T-cell, as well as different types of T-cells, and it is unlikely that the K_n conductance is required for activation in each of these instances.

Second, pharmacological evidence using a diverse group of K_n channel antagonists on a variety of T-cells shows that these agents inhibit DNA and protein synthesis at similar (11, 19, 22, 39, 141, 209), although not always identical, concentrations (225), providing indirect evidence that K_n channels are involved in proliferation. [It should be also noted that if K-channel blockers block in a state-dependent manner (34), then the dose-response relationship of K_n current block at 20 mV (where most of the dose-response relationships have been determined) and the dose-response relationship of block at the resting V_m may differ.] Despite these findings, it has not been proven that the functional effects of the antagonists are actually mediated by K-channel block. For example, K-channel blockers have been shown to directly inhibit Ins(1,4,5)P_3 release from brain microsomes (196). Schell et al. (229) have reported that TEA and 4-AP (at concentrations that inhibit the K_n conductance) inhibit [^{3}H]thymidine and amino acid incorporation in two tumor cell lines that replicate autonomously, suggesting that these agents act on the uptake of nutrients rather than on pathways related to T-cell activation.

Third, support for the involvement of K_n channels in proliferation comes from the observation that T-cells from diseased MRL-lpr/lpr mice that fail to respond to mitogens or antigens do not upregulate the number of K_n channels on their surfaces. Rather, these cells constitutively express 20-fold more K_i channels on their surfaces than T-cells from MRL-lpr/lpr mice before the onset of the disease (18).

The mechanism by which K channels are involved in T-cell activation is unknown. Phytohemagglutinin increases K current in immature thymocytes, which do not proliferate (231), indicating that although augmentation of the K_n conductance may be necessary, it is not sufficient to induce proliferation. Before voltage-independent Ca channels were described in T-cells, Cahalan and co-workers (17, 22) proposed that Ca influx might occur through voltage-gated K channels. Although it recently has been demonstrated that Ca ions can enter open K channels and cross the membrane to the inside when the channel closes (94), it is unlikely that this mechanism provides a significant route for Ca entry, since K channels fail to conduct in isotonic Ba (232) and stepped depolarization of Jurkat T-cells to 0 mV (which activates K channels) does not detectably alter $[Ca]_i$ (150). Furthermore, indirect evidence that K_n channels are not permeable to Ca is provided by the following observations. *1)* depolarization of human T lymphocytes (thus activating K_n channels) fails to increase $[Ca]_i$ (77), and *2)* K channel antagonists only partially inhibit mitogen-induced $[Ca]_i$ increases (18, 22, 77). Alternatively, voltage-gated K channels may indirectly affect mitogenesis by changing intracellular K levels, since $[K]_i$ has been linked with protein synthesis (135), or they might set the resting V_m, which may affect the mitogen-induced rise in $[Ca]_i$ (76, 188), thereby modulating the proliferative response. Unfortunately, the relationship of V_m to T-cell activation is unclear: Gelfand et al. (76) reported that depolarization with high-K medium inhibited PHA-induced Ca uptake after short-term exposure to mitogen. However, for lymphocytes cultured in high-K medium and continuously exposed to PHA, proliferation was almost normal (43).

B) Calcium channels. It is well established that mitogens produce a rapid increase in $[Ca]_i$ that is partly due to an influx of extracellular Ca (87). The extensive evidence supporting the view that Ca influx and the subsequent rise in $[Ca]_i$ provide an important signal for proliferation has been the subject of other reviews (72, 87, 153) and therefore is not covered in depth here. Although it was originally hypothesized that voltage-

gated Ca channels were responsible for the extracellular Ca-dependent mitogen-induced $[Ca]_i$ rise, it is now believed that they do not play a role in the mitogen-dependent Ca influx, because *1*) they are not detectable in most T-cells (17, 72, 163), *2*) concentrations of Ca channel blockers known to inhibit voltage-dependent Ca channels do not consistently block mitogen-induced Ca increases (72), and *3*) depolarization by high-K medium (which would be expected to increase the influx of Ca through voltage-gated channels) has no effect on $[Ca]_i$ (72, 76. 188). This view has been confirmed by two recent descriptions of voltage-insensitive Ca currents in T lymphocytes (72, 151), supporting the hypothesis that a non-voltage-gated Ca transport process is responsible for the mitogen-induced Ca influx.

Kuno and Gardner (74, 130) suggested that T-cell activation by mitogens results in an $Ins(1,4,5)P_3$ increase that releases Ca from intracellular stores and activates a transmembrane Ca channel (see sect. IV*A3*). Previous observations that mitogens increase the phosphorylation of phosphoinositides and the generation of inositol phosphates activation fit with this hypothesis (113, 270). However, it is not clear which of the two Ca currents, the Ca_{Ins} current described by Gardner et al. (72) or the Ca_s current described by Lewis and Cahalan (151), is responsible for mitogen-induced increase in $[Ca]_i$ or, alternatively, whether the two currents are related (see sect. IV*A3*). By simultaneously measuring $[Ca]_i$ and membrane currents, Lewis and Cahalan (151) demonstrated a temporal relationship between the PHA-induced Ca currents and the oscillations in $[Ca]_i$, thus providing strong evidence that these currents underlie the mitogen-induced Ca oscillations. Agonist-induced or $Ins(1,4,5)P_3$-induced Ca currents similar to those described by Lewis and Cahalan (151) were described in whole cell recordings of rat peritoneal mast cells (164). It should be noted that non-voltage-gated Ca permeable channels have been described only in T-cell lines. Therefore the functional relevance of these channels in normal T-cells must be regarded as speculative.

It has been suggested that the step that requires Ca in mitogenesis is the production of IL-2, because IL-2 production requires both extracellular Ca and an increase in $[Ca]_i$, whereas the expression of IL-2 receptors is independent of the rise in $[Ca]_i$ (176). Further support for this suggestion comes from the observation that exogenous IL-2 can trigger proliferation in IL-2 receptor-bearing cells in the absence of an increase in $[Ca]_i$ (183). Nevertheless, the role of Ca in signal transduction is more complex, since the requirement for extracellular Ca and a rise in $[Ca]_i$ can be eliminated by treating cells with phorbol ester plus mitogens (79). Also, anti-Thy-1 antibody stimulation of IL-2 secretion in a murine T-cell variant missing the T-cell antigen receptor occurs in the absence of any increase in $[Ca]_i$ or phosphatidylinositol hydrolysis (265). Therefore although Ca channels/transporters appear to play an important role in mitogen-induced IL-2 production, Ca-independent modes of signal transduction also are present in the T-cell.

C) Cytotoxicity. Cytotoxicity mediated by either cytolytic T lymphocytes (CTL) or NK cells share several identifiable stages, including a Ca-dependent programming for a lysis stage that has an optimal temperature of 37°C (6, 109, 211). This dependence on extracellular Ca, together with the rise in $[Ca]_i$ after target cell binding to CTL and the immediate decline in $[Ca]_i$ after removal of extracellular Ca, led to the hypothesis that Ca channels play a pivotal role in cell-mediated cytotoxicity (82, 206). Furthermore, the rise in $[Ca]_i$ correlates with a shape change in the CTL cells and a reorientation of cytoplasmic granules (83). Poenie et al. (206) postulated that binding the target cell to the T-cell receptor opens Ca channels activated by secondary events that occur between receptor binding and channel opening. Voltage-dependent Ca channels have not been described in CTL or NK cells (58, 232), and depolarizing CTL with high K failed to induce a rise in $[Ca]_i$ (82). Thus it is likely that voltage-independent Ca channels/transporters similar to those described by Lewis and Cahalan, (151) or Gardner (72, 74) mediate the rise in Ca in the effector cell.

The evidence implicating voltage-gated K channels present in both mouse CTL (58) and NK cells (250) in cell-mediated cytolysis is threefold. First, the amplitude of the CTL K currents in cytotoxic T-cell-target cell conjugates was enhanced by replacing external Mg with Ca (conditions required for the lethal hit to take place) (58). Second, Rb efflux was stimulated when cloned CTL loaded with ^{86}Rb were mixed with appropriate target cells (224). Third, pretreatment of NK cells with verapamil, 4-AP, Cd, and quinidine inhibited killing at doses comparable to those that blocked voltage-gated K channels (232). Furthermore, by adding EDTA and channel blockers at various times during cell killing, Sidell et al. (250) demonstrated that K channels play a role in the Ca-dependent killing phase and particularly in the release of NK cytolytic factor. The K-channel blockers 4-AP and quinidine similarly inhibit cytolysis by lymphokine-activated killer cells (143).

The role of K channels in this phase of cytolysis is unclear. The findings that depolarizing NK cells with high K has no effect on NK-mediated cytolysis and that valinomycin, which should prevent the depolarization produced by K-channel blockers, did not reverse the block of cytolysis by quinidine, verapamil, or 4-AP (233) argue against the possibility that K channels may function indirectly through effects on V_m. It should be noted that cytolytic activity mediated by CTL cells is not inhibited by verapamil (82), demonstrating that there are distinct differences in the mechanisms of cytolysis mediated by these two cell types. In CTL cells, inhibition by 4-AP and TEA of cytotoxicity can be overcome by adding IL-2, suggesting that the importance of K channels in cytotoxicity may be related to their action on IL-2 production (244).

One report implicated Cl fluxes in cell-mediated cytotoxicity (84). In that study, CTL-mediated cytotoxicity was inhibited by isosmotic replacement of Cl with impermeant anions, and stilbene disulfonates blocked cy

tolysis, providing indirect evidence that anion transport may be involved in delivery of the lethal hit (84).

D) Volume regulation. Exposing lymphocytes to anisotonic medium results in shrinkage or swelling, followed by a regulation back to near normal size even though the cells remain in anisotonic medium (41, 90). In T-cells, the RVD that occurs within 5 min of hypotonic swelling is associated with a loss of both Cl and K and is dependent on an outwardly directed K electrochemical gradient (90). The increase in Cl permeability is at least partly conductive because it is associated with a depolarization, so that V_m approaches E_{Cl} and Cl flux is independent of the concentrations of external anions or internal cations (86). The ATP-dependent Cl_s channels described by Lewis and Cahalan (147) may account for the increase in Cl permeability during RVD because they are osmotically activated (19). On the other hand, the anion permeability sequence of the Cl_v channel in T-cells (230) is identical to the sequence for supporting volume changes in swollen lymphocytes (87) and differs slightly from the permeability sequence for the ATP-dependent Cl_s channel (147). Therefore further studies are required to determine which Cl channel(s) is involved in volume regulation and how changes in cell volume regulate this Cl conductance.

Despite proposals to the contrary (88, 90), neither a Ca conductance nor a Ca-activated K conductance appear to be involved in the RVD response, because RVD does not require extracellular Ca and because $[Ca]_i$ does not increase during RVD (92). Alternatively, it is likely that the voltage-gated K_n channels provide the route for the RVD-associated K efflux, since in the murine T lymphocyte clone L2 the RVD response is correlated with the expression of voltage-gated K channels. Quiescent L2 cells have low levels of K_n channels and show no RVD, whereas L2 cells stimulated with IL-2 to proliferate exhibit an increase in K_n channels and a RVD response (140). Furthermore, RVD is blocked by quinine, TEA, verapamil, and CTX (41, 88, 92, 140) at concentrations that block K_n conductance. It has been suggested that during RVD K_n channels are activated by depolarization induced by either the increase in Cl permeability that occurs during RVD (19) or the activation of stretch-activated nonspecific cation channels (which have been described in other cell types but not in lymphocytes) (41). Interestingly, gadolinium, which blocks stretch-activated channels, also blocks RVD (41). The increase in K permeability during RVD is present in cells depolarized by high K, indicating that the RVD-activated K conductance can be triggered in a voltage independent manner (92).

Because cells (with the exception of kidney medullary cells) are exposed to a narrow range of tonicity, the physiological significance of RVD is unclear. Deutsch and Lee (41) postulated that volume regulation supports cell cycle progression and that cells may encounter and/or generate local anisotonic conditions. Conversely, Grinstein and Dixon (87) speculated that the system for volume regulation is a vestige from a time when osmoregulation was important.

B. B Lymphocytes

As the effector cells of the humoral limb of immunity, B lymphocytes (which in mammals develop in the adult bone marrow or the fetal liver) synthesize and secrete antibody (262a). Surface immunoglobulin serves as the antigen receptor on the B-cell. The binding of antigen to its receptor initiates a complex series of events leading to cell proliferation and the production of antibody-secreting cells. The end stage of this maturation process is the large plasma cell that can secrete antibody at a rate of ~2,000 molecules/s.

1. Potassium conductances

1) OUTWARD VOLTAGE-GATED POTASSIUM CONDUCTANCE The major ionic conductance in both murine B-cells immortalized at various stages of differentiation with Abelson murine leukemia virus and in murine and human resting and LPS-stimulated B-cells is an inactivating outward K current similar in its threshold of activation (−40 mV) and block by TEA to the K_n channel described in T lymphocytes (29, 266, 267). [A preliminary study has reported K_i channels in a human B-cell-derived lymphoma cell line, Louckes (242).] Sutro et al. (267) reported that human tonsillar B-cells and murine B-cells have 60 and 23 K_n channels/cell, respectively. Their voltage for half activation ranged from −20 to −28 mV, and its time constant of inactivation was 140 ms (shorter than that of human T-cells) (29, 267). As reported for the K_n channel in human T lymphocytes, increasing $[Ca]_i$ from 10^{-8} to 10^{-6} M decreased the K-current amplitude and increased the rate of current inactivation in murine B-cells (29). In addition, in murine B-cells, like T-cells, the outward K current is blocked by a variety of pharmacological agents, including extracellular verapamil (K_i = 10 μM), cetiedil (K_i = 20 μM), quinine (K_i = 22 μM), 4-AP (K_i = 300 μM), TEA (K_i = 10 mM), and Co or Cd (K_i = 10 mM) (29, 267). The mechanism of K_n channel block in B-cells was described in only one preliminary study, which demonstrated that 4-AP blocks open channels and that the K_i of channel block by 4-AP depends on both internal and external pH (25). Thus the ionized form of 4-AP may block from inside the cell. Furthermore, data on rate of 4-AP block and washout of block suggest that two blocked states may exist (25).

A) Modulation of conductance. After treatment with LPS, B-cells increase in size, proliferate, and secrete antibodies. Sutro et al. (267) reported that LPS treatment also doubled the density of K channels in murine B-cells and that the increase in channel density was closely associated with the increase in cell size, that is, cells that failed to increase their cell size did not increase their K_n channel density. However, different results were obtained by Choquet et al. (29), who reported that the K_n conductance (unfortunately channel density was not measured) in murine LPS-treated B-cell blasts did not differ from the conductance in B-cell lines that were

immortalized at earlier stages of differentiation. Thus it is unclear whether the state of immunocompetency of the B-cell influences the presence of the K_n conductance.

Choquet et al. (29) reported that GTP caused a small initial increase in the K_n current, which may reflect the regulation of this conductance by a GTP-binding protein. When the patch pipette contained cAMP (1 mM), theophylline (1 mM, a phosphodiesterase inhibitor), and ATP (5 mM) the outward K_n current was significantly diminished during the first 5 min of recording from murine B-cells (29). Furthermore, adding the adenylate cyclase activator forskolin plus theophylline to the bath also decreased the current and increased the rate of inactivation. These effects were not due to a shift in the *G-V* relationship for the K_n current or to the induction of an inward current. Also, adding the adenylate cyclase antagonist adenosine (2 mM) suppressed the effect of forskolin. Taken together, these observations suggest that forskolin acts specifically through its action on adenylate cyclase to increase cAMP and that cAMP modulates the K_n conductance in B-cells. As described in section IV*A*1*iA*5, forskolin also reduced the K_n conductance in human T-cells, but in these cells cAMP did not appear to modulate the K_n conductance and the effects of forskolin were not mediated by its efffect on adenylate cyclase (127).

In addition to modulation by cAMP, the K_n conductance in murine B-cells can be modulated by serotonin [5-hydroxytryptamine (5-HT)] (27). That is, exposure to 5-HT (10 μM) induced a transient increase in the peak current, as well as a more maintained increase in the rate of current inactivation. After treatment with 5-HT, the cells became refractory to repeated stimulation, even after prolonged washing. The increase in the peak current was transduced by a $5HT_1$ receptor, while the acceleration of inactivation involved $5\text{-}HT_3$ receptors (27).

B) Single-channel currents. Single-channel K currents with the same conductance (17 pS) as the K_n currents in T-cells were reported in both human tonsillar B-cells and mouse splenic B-cells (266, 267). However, they have not been well characterized. McCann et al. (168), studying cell-attached and excised inside-out patches in murine spleenic B-cells, reported two different outward single-channel K currents with conductances of 18 and 30 pS. The 18-pS channel (present in 9% of the patches examined) and the 30-pS channel (present in 3% of the patches) exhibited different voltage sensitivities, the 18-pS channel opened at voltages more depolarized than −20 mV, and the 30-pS channel opened at voltages more depolarized than 60 mV. McCann et al. (168) also reported that both channels were blocked by TEA and Ba, but the blocking concentrations or the conditions of block were not specified. They suggested that the 18- and 30-pS channels correspond to the T-cell K_n or $K_{n'}$ channel and K_1 channel, respectively. However, this suggestion is equivocal because the voltage dependence reported by McCann et al. (168) for the 30-pS channel is very different from that of the T-cell K_1 channel. Further characterization of the K channels present in B-cells is required to determine their relationship to the K channels described in T-cells.

II) CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE. Simultaneous measurements of both $[Ca]_i$ and V_m with fluorescent dyes have demonstrated that either antibodies against surface immunoglobulins (anti-Ig) or ionomycin induce membrane hyperpolarizations in human tonsillar B-cells (157). The anti-Ig-induced membrane hyperpolarization was associated with an increase in $[Ca]_i$ and was abolished by placing cells in high-K medium, strongly suggesting that a Ca-activated K conductance was responsible for the membrane hyperpolarization. Although whole cell patch-clamp recording techniques have not demonstrated a Ca-activated K current in B-cells, two recent studies demonstrated Ca-activated K single-channel currents that may be responsible for the stimulus-induced hyperpolarization in B-cells (160, 168). As discussed in section IV*A*1*iii*, Mahaut-Smith and Schlicter (160) demonstrated that exposure of both human tonsillar B-cells and rat thymocytes to ionomycin (in cell-attached patch experiments) induced two single-channel current amplitude fluctuations (7 and 25 pS with 140 mM K in the pipette). The larger channel was inwardly rectifying and had an open-channel probability that was only weakly voltage dependent. The K channel activity that was sensitive to $[Ca]_i$ was also reported in excised patches from these cells (160).

A different Ca-activated K channel was reported recently in 3% of the patches from resting murine B-cells (168). These channels had a conductance of 93 pS and, in cell-attached patches, were observed only after applying large depolarizing potentials. Similar channels were also noted in inside-out patches, where they opened at both positive and negative potentials under conditions of normal physiological gradient with 10^{-8} M $[Ca]_i$. Channel activity increased when $[Ca]_i$ was increased, and adding EGTA inactivated the channel, demonstrating that the channel was sensitive to $[Ca]_i$ (168).[4]

2. *Calcium conductance*

No ligand-dependent voltage-independent Ca channels similar to those described in T-cells (72, 73) have been reported in B-cells. Nor have any voltage-dependent Ca channels been found in murine resting or LPS-activated B-cells (29, 266). Voltage-dependent Ca channels were reported in the murine myeloma cell line S194 (which produces but does not secrete immunoglobulin) and hybridoma cell lines constructed from the fusion of S194 cell and splenic B lymphocytes (13, 56, 59). In these cells, inward Ca currents were activated at potentials greater than −50 mV and peaked at −20 mV. During long (>50 ms) voltage steps, current inactivated with a single exponential time course, the time constant of which decreased as the V_m became more positive, even

[4] A recent study by Brent et al. (14a) indicates that B-cells have yet another type of Ca-sensitive channel, a nonselective cation channel that is inhibited by $[Ca]_i$.

beyond the peak for the inward current. Substituting Na for TEA had no effect on the decay of the inward Ca current. Thus the current decay is not due to the development of an outward current or to a Ca-induced inactivation of the current but probably reflects voltage-dependent inactivation (56, 57). The channel also was permeable to Sr and Ba (permeability sequence = Sr > Ca = Ba) (56) and surprisingly was rather insensitive to block by D 600 and nifedipine (59). The Ca channels in hybridoma cells are similar to the T-type or low-threshold Ca channels in other cells that are less sensitive to organic Ca channel blockers than other Ca channels (180). More recently, Bosma and Sidell (13) demonstrated that both retinoic acid ($K_{1/2}$ = 5 × 10^{-5} M) and octanol (53 μM produced a 40% decrease) blocked the peak voltage-gated Ca currents in a similar (MHY206) hybridoma cell line. In that study, octanal also increased the rate of current inactivation.

Because no other voltage-dependent conductances were present, murine hybridoma cells provided Fukushima and Hagiwara (57) with a model for examining the permeability of monovalent cations through the hybridoma Ca channel. They demonstrated that internal monvalent cations can carry outward currents through Ca channels and that external monovalent cations carry inward currents through the channel when external divalent cations are reduced to the micromolar range. The selectivity of monovalent cations through Ca channels (in the absence of Ca) was Na > K > Rb > Cs. In the presence of Ca or other divalent cations, monovalent cation currents were blocked in a voltage-dependent manner. The data indicated that under normal conditions Ca channels are occupied by divalent cations and are impermeable to monovalent cations. However, when divalent cations are extruded from the channel, the Ca channel becomes permeable to monovalent cations (57).

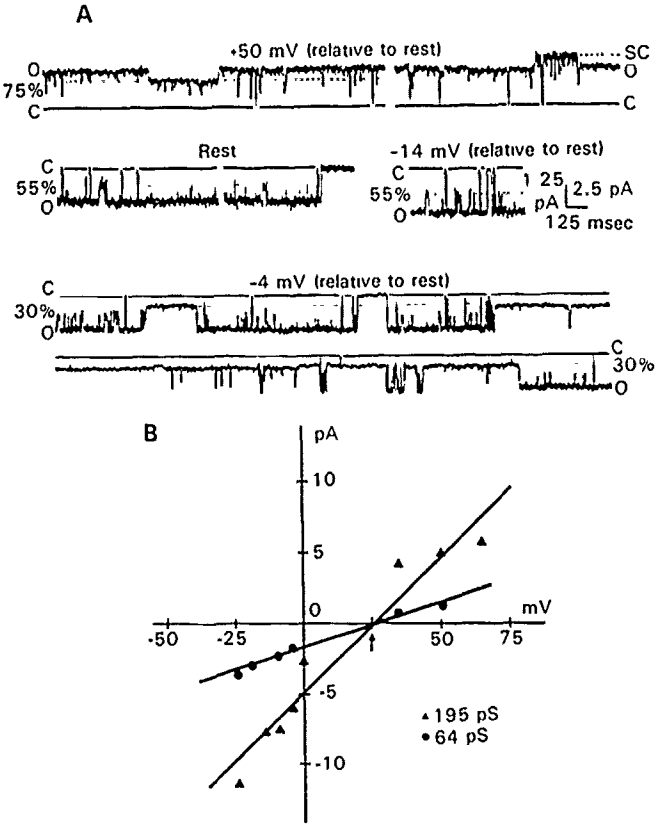

FIG 5. Cell-attached patch-clamp recording of subconductance levels of large-conductance anion channel in hybridoma cell line constructed from fusion of S194 mouse myeloma cells and mouse splenic B lymphocytes. *A*: records showing various subconductance levels indicated by dotted lines. SC, supraconductance level; C and continuous line, closed level, O, open level. Calibration of 1.25 pA is for currents recorded at rest voltage only, all other refer to 2.5-pA calibration. All voltages are relative to resting potential of cell. *B*. current-voltage relation for currents recorded from different on-cell patch for main open level (195 pS) and 30% subconductance level (64 pS). Reversal is shown at arrow at +25 mV above rest. [From Bosma (12).]

3. Sodium conductance

Inward whole cell currents that had a time course of activation and decay similar to Na currents were reported in a single recording from a human tonsillar B-cell (267). Inward single-channel currents having a 17-pS conductance were also observed. Unfortunately, these events were not characterized, so it was not possible to conclude that they represented the activation of Na channels.

4. Chloride conductance

1) LARGE-CONDUCTANCE CHLORIDE CHANNEL. A large-conductance anion-permeable channel with multiple conductance levels has been reported in murine splenic B lymphocytes (167) and in an antibody-secreting transformed hybridoma cell line made by fusing a rat myeloma with splenic B lymphocytes (12). The activity of this channel, shown in Figure 5, is similar to the Cl_L channel activity described in macrophages, T lymphocytes, and other cells (9). It was frequently seen in excised inside-out patches, where its conductance in symmetrical NaCl or in physiological solutions was 350-400 pS (12 167). It was estimated that there were 400-500 channels/hybridoma cell (12). The anion-to-cation permeability ratio was 10.1 in hybridoma cells (12) and 33.1 in murine B-cells (167), thus in B-cells, like T-cells, the Cl_L channel was more selective for anions than the Cl_L channel in macrophages (236). In hybridoma cells, the Cl_L channel permeability sequence for anions was F > I > SCN > Br > Cl > glucuronate > NO_3 > aspartate, which differs from the permeability sequence reported in T-cells (12). Moreover, the conductance sequence in hybridoma cells, which was Cl > SCN = F > Br > NO_3 > I, glucuronate > aspartate, was not the same as its permeability sequence, suggesting that the permeating ions were interacting within the channel (12). Interaction of permeant anions with the channel also was supported by the finding that in mixtures of anions there was an anomalous mole fraction dependence of channel

conductances, that is, a mixture of SCN and Cl (highly permeable anions when present individually) reduced the channel conductance. Mixtures of K and Tl produce a similar effect on the inwardly rectifying K channel in oocytes and other cells (99, 223).

As noted in macrophages and other cells, B-cell Cl_L channel activity is characterized by subconductance levels. These included states that were 10, 30, 55, and 75% of the full conductance level (12). In addition, a supraconductance level of 510 pS was noted, which has not been described in either macrophages or T-cells. The supraconductance level tended to close more frequently than the other levels, suggesting that entry to the closed state was easier from this level (12).

Spontaneous Cl_L channel activity was only noted in 10% of the cell-attached patches from hybridoma cells, but it could occasionally be activated by depolarizing steps (12). In these cases, the conductance levels of the fully opened state and the substates were lower that those in excised patches, probably because intracellular Cl was reduced. In the cell-attached patch, channel open probability increased up to 10 mV (relative to rest) and decreased for depolarizations beyond 10 mV (relative to rest). The behavior of the Cl_L channel changed in three ways after excision: *1*) open probability did not decrease for potentials positive to 10 mV, *2*) the voltage dependence of opening became less steep at negative potentials, and *3*) there was no maintained residence of the channel in the 30% subconductance state. Bosma (12) postulated that these changes may be due to modulation of the channel by cytoplasmic constituents, fitting with the observation in T-cells that the Cl_L channel can be activated by the catalytic subunit of protein kinase A plus ATP (195).

Similar to the Cl_L data in macrophages and T-cells, the gating characteristics of the channel could be modeled by two independent voltage-dependent gates (12). The Cl_L channel was reversibly blocked by cytoplasmic SITS with a K_i of 5.08 mM at 0 mV. The distance of the blocking site in the membrane was estimated (from the voltage dependence of the SITS block) to be 0.37 from the cytoplasmic side. Here DIDS was five times less effective than SITS and anthracene-9-carboxylic acid (1 mM) had no effect on the channel (12).

II) ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE-REGULATED CHLORIDE CHANNEL. Chen et al. (25) demonstrated that normal human Epstein Barr virus (EBV)-transformed (B-cell) lymphoblasts contained a cAMP-dependent Cl (Cl_A) channel similar to that present in Jurkat E6-1 cells (see sect. IV*A4*III). This channel was also present in excised patches from mutant human EBV-transformed lymphoblasts from patients with cystic fibrosis. However, in patches from those cells, regulation by cAMP was defective. A 40-pS Cl channel has also been described in 10% of excised patches from murine splenic B-cells (168). Similar to the cAMP-regulated channel, this Cl channel exhibited outward rectification in symmetrical solution but, unlike the cAMP-regulated channel, $[Ca]_i$ had no effect on channel activity. Further experiments are required to determine if these two 40-pS Cl channels represent different Cl channels.

III) OTHER CHLORIDE CHANNELS. Two other Cl conductances have been reported in patch-clamp studies of murine splenic B-cells, but neither has been well characterized. The first was described in section IV*A4*II and is a Cl_s channel present in both T-cells and B-cells that is slowly activated in whole cell recordings when the patch electrode contains hypertonic medium plus ATP (19, 147). In addition, McCann et al. (168) described a 128-pS Cl channel that was inactive in cell-attached patches but was activated in excised inside-out patches from murine splenic B-cells. The channel had a linear *I-V* relationship in a symmetrical Cl gradient and was voltage-dependent with channel openings increasing as the patch was depolarized beyond −50 mV. It was selective for Cl over cations (Cl-to-Na permeability ratio = 11, Cl-to-K permeability ratio = 8) but was permeable to aspartate (Cl-to-aspartate permeability ratio = 4).

5. *Physiological relevance of ionic conductances*

When activated by a combination of signals that include the binding of antigen and signals from accessory cells and T-cells, the B-cell enlarges, divides, and differentiates into an antibody-secreting cell (186). This process is complex, and the mechanisms of signal transduction that occur during the various stages of activation are not well understood. Moreover, when compared with T-cells, relatively few electrophysiological studies have been done on B-cells, and there are little or no data elucidating the physiological relevance of the ionic conductances that have been described. On the other hand, support for the involvement of ionic channels comes from numerous studies using indirect probes of V_m and Ca (87). A few of these studies are discussed here in terms of their relationship to the electrophysiological data on B-cells, however, the reader is encouraged to refer to other reviews for a more comprehensive discussion (75, 87).

I) SETTING RESTING MEMBRANE POTENTIAL. The resting V_m of mouse spleen B-cells as assessed by two different V_m-sensitive fluorescent dyes is approximately −60 mV and is relatively dependent on K (220). Although it is clear that K conductance(s) plays an important role in setting the resting V_m, no data exist on the relative contribution of any of the other ionic conductances that have been described in B-cells to the resting V_m.

II) B-CELL ACTIVATION. *A) Potassium channels.* Potassium (^{86}Rb) fluxes occur after stimulation of B-cells with anti-Ig (105) or LPS (191), as well as in B-cells activated in the presence of mitogen-stimulated cells (192). As noted, anti-Ig also produced a membrane hyperpolarization in B-cells, supporting the view that K fluxes occur via a conductive pathway (158). Nevertheless, there is little information about the functional importance of K conductances in B-cell activation. Vayu-

vegula et al. (273) reported that verapamil, quinine, 4 AP, and TEA block anti-IgM-induced B-cell proliferation with a potency sequence that is identical to that for K_n-type channels in T-cells. In contrast, Ransom and Cambier (214) reported that TEA (10–100 mM) did not block anti-Ig-induced IA expression in murine B-cells.[5] Furthermore, examination of the K_n conductance in several B-cell lines exhibiting different stages of immunoglobulin secretion demonstrated a lack of correlation between these two phenomena, suggesting that the presence of this conductance is independent of immunocompetency.

If the K_n conductance is important in B-cell activation, then the observations that the K_n conductance is modulated by both $[Ca]_i$ and cAMP are certain to have functional implications since both $[Ca]_i$ (8, 157, 196) and cAMP (189, 191) have been implicated in B-cell activation. The rise in $[Ca]_i$ might also activate Ca-activated K conductance(s), resulting in a membrane hyperpolarization. In addition, the actions of 5-HT on the K_n conductance may also play a role in B-cell activation.

B) Calcium channels. Indirect evidence indicates that voltage-gated Ca channels present in hybridoma cell lines obtained from the fusion of S194 and murine splenic B-cells may be related to immunoglobulin secretion and/or cell proliferation (13, 59). A comparison of the Ca currents in the S194 cell line and in two murine hybridoma cell lines during a 4-day culture period indicated that *1)* the Ca current of secreting hybridomas was larger than the Ca current of nonsecreting S194 cells, *2)* changes in Ca current correlated with the time after the cells were tranferred to fresh medium, and *3)* there were parallel changes in the density of Ca current and immunoglobulin secretion (59). However, a high concentration (100 μM) of D 600 completely blocked both proliferation and antibody production, but it only blocked Ca currents by 37%. In a different hybridoma cell line, Bosma and Sidell (13) demonstrated that retinoic acid, a biologically active metabolite of vitamin A, produces a dose-dependent block of Ca channels in the MHY206 hybridoma cell line that correlated with the ability of retinoic acid to inhibit cell proliferation. Thus the data are suggestive that the Ca current in hybridoma cells may be related to immunoglobulin secretion and/or cell proliferation.

With the exception of S194-derived hybridoma cells, neither ligand-induced or voltage-gated Ca channels have been described in murine and human resting or LPS-stimulated B-cells. However, several observations have indicated that B-cell activation by some stimuli induces a Ca influx and that this influx occurs through Ca channels (87). First, antibodies against the antigen receptor on mouse splenic B lymphocytes induce a rise in $[Ca]_i$ that precedes capping, and removing external Ca diminishes the $[Ca]_i$ increase (208). Similarly, a biphasic increase in $[Ca]_i$ was reported after exposure of human tonsillar B-cells and murine B-cells to anti-Ig antibodies, and removing extracellular Ca inhibited the second more prolonged phase of the $[Ca]_i$ increase (8, 157). {It should be noted that oscillations in $[Ca]_i$ lasting for hours have been demonstrated in single murine B-cells and that in the absence of external Ca the oscillations only persist for several minutes (282).} Second, the addition of Mn, which can permeate Ca channels but quenches the fluorescence of the Ca indicator indo-1, also demonstrated the activation of a Ca permeability after anti-IgM stimulation of human B-cells (157). Third, anti-Ig induced a membrane depolarization in both murine B lymphocytes and human tonsillar B-cells that was dependent on extracellular Ca (157, 213).

Depolarizing B-cells or B-cell tumor cell lines with high-K medium had no effect on the increase in $[Ca]_i$ produced by anti-Ig antibodies (8, 133, 157), indicating that the influx pathway does not involve voltage-gated Ca channels. Furthermore, in the WEHI-231 B-cell line derived from a lymphoma, anti-IgM, which produces a rapid rise in $[Ca]_i$, failed to induce a simultaneous membrane depolarization (which would be expected if a voltage-gated Ca channel was activated). This observation is supported by the absence of voltage-gated Ca currents in patch-clamp studies of resting and LPS-activated B-cells (29, 168). Hence, it is likely that ligand-gated voltage-independent Ca channels are involved in B-cell activation by anti-Ig antibodies. Because B-cell activation induces both phosphoinositide turnover and Ca mobilization (7, 133, 215), a likely candidate would be an $Ins(1,4,5)P_3$-sensitive Ca channel similar to that described in T lymphocytes (72, 73).

Although increasing the influx of Ca with Ca ionophores can result in an activation of B-cells (213, 216), it is not clear at what stage of B-cell activation, if any, influx of Ca (in contrast to release of intracellular Ca stores) is required. For example, although a $[Ca]_i$ increase precedes capping induced by anti-Ig antibodies, cells in Ca-free medium that were depleted of $[Ca]_i$ capped normally without a rise in $[Ca]_i$ (208). Furthermore, not all agents that activate B-cells increase $[Ca]_i$; neither LPS nor PMA, which activate B-cells, induces an increase in $[Ca]_i$ (7). Therefore at least two different activation pathways, one that requires $[Ca]_i$ and one that does not, are present in B-cells.

C) Volume regulation. As described in section IV*A*5*iv*, the RVD response that occurs after exposure to hypotonic medium involves increased permeability to both Cl and K (for reviews see Refs. 41, 87). Unlike human T-cells, RVD takes $\geq$1 h to occur in human tonsillar B-cells (26). Although swelling in B-cells induces an increase in Cl conductance that is comparable to that noted in T-cells, no augmentation of K permeability occurs (86) However, adding a cation ionophore results in a secondary RVD response in hypotonically stressed B-cells. These observations indicate that the Cl permeabil-

[5] The reason for the discrepancy between these two observations has been clarified by recent studies that demonstrate that TEA blocks B-cell proliferation during the second half of the G_1 phase of the cell cycle, whereas IA expression occurs just before stimulated B-cells enter G_1 (2a, 14a).

ity pathway and the K permeability pathway involved in the RVD response are independent and that the inability of B-cells to undergo RVD is due to a low K permeability.

The absence of an osmotically induced K conductance in human tonsillar B-cells compared with human T-cells may be related to the difference in the number of K_n channels in each cell type, both human tonsillar B-cells and B-cells from peripheral blood have ~20% of the outward K conductance reported in resting human T-cells (40, 267). This correlation between the increase in K permeability after hypotonic stress and the number of K_n channels supports the idea that K_n channels are responsible for the RVD-associated K efflux (see sect. IV*A5*IV).

No direct evidence indicates which Cl conductance is responsible for the Cl flux during RVD. Nevertheless, it is likely that similar pathways for Cl flux are used by both T- and B-cells and that this pathway involves the ATP-dependent Cl_s channels reported in both T- and B-cells (147).

V. CONCLUSION

The objective of this review is to provide readers with a summary of the electrophysiological data describing ion conductances in leukocytes and their potential physiological relevance. Leukocytes contain a diversity of both voltage-gated and/or second messenger-modulated ion channels, and the identifying features of many of these channels are known. Despite considerable progress, a myriad of questions remain, with the most relevant being the relationship of these ion channels to leukocyte function.

Some of the answers to this question are likely to involve the coupling of ion channels to intracellular signaling pathways (130). A prodigious increase in understanding of the biochemical signaling pathways in leukocytes has occurred in parallel with progress in describing ionic conductances in these cells. This increase in knowledge, together with the unique adaptability of the patch-clamp technique to different recording modes, should provide a clarification of the functional relevance of ion channels in leukocytes.

Several other points are salient to a discussion of the physiological relevance of the ion channels described here. First, activation of leukocytes can occur through multiple signaling pathways, and it is likely that ionic conductances, which may be important in one signaling pathway, are not important in others. Second, the relevance of data obtained from tumor cell lines, which vary widely, to normal leukocyte function must be interpreted with caution until it is repeated in normal leukocytes. Third, unlike ion channels in excitable cells, many of the ionic conductances that have been described in leukocytes may have only indirect effects on cell function. That is, by influencing ion homeostasis and membrane potential, ion channels may modulate protein synthesis (135), recycling of receptors (12), or influx of Ca (45, 152) without being directly involved in the signaling pathways.

Furthermore, little is known about the presence of ion channels in intracellular compartments or about the disribution of ion channels on the leukocyte surface during capping, phagocytosis, or other physiological events. The application of molecular biological techniques to the study of leukocyte ion channels will surely provide useful mechanisms to better define the importance of ion channels in leukocyte function and to elucidate how they relate to similar ion channels found in other cell types.

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