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POTASSIUM AND CALCIUM CHANNELS IN LYMPHOCYTES

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ABSTRACT

Over the past decade, a variety of ion channels have been identified and characterized in lymphocytes by use of the patch-clamp technique. This review discusses biophysical and regulatory aspects of lymphocyte potassium and calcium channels with the aim of understanding the role of these channels in lymphocyte functions. Lymphocytes express both voltage-dependent potassium [K(V)] channels and calcium-activated potassium [K(Ca)] channels, and each is upregulated as cells progress toward division following mitogenic stimulation. The genes encoding two K(V) channels, Kv1.3 (type *n*) and Kv3.1 (type *l*), have been cloned. Mutational analysis is revealing functionally important regions of these channel proteins. Exogenous expression studies and the use of highly specific channel blockers have helped to establish the roles of type *n* K(V) channels in sustaining the resting membrane potential, in regulating cell volume, and in enabling lymphocyte activation. Blockade of K(V) and K(Ca) channels effectively inhibits the antigen-driven activation of lymphocytes, probably by inducing membrane depolarization and thereby diminishing calcium influx. A prolonged rise in intracellular calcium ($[Ca^{2+}]_i$) is a required signal for lymphocyte activation by antigen or mitogens. Single-cell fluorescence measurements have revealed underlying $[Ca^{2+}]_i$ oscillations that are linked closely to the opening and closing of Ca^{2+} and K^+ channels. Sustained Ca^{2+} signaling and oscillations depend absolutely on plasma-membrane Ca^{2+} channels that are activated by the depletion of intracellular calcium stores. Under physiological conditions these channels

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open as a consequence of store depletion induced by inositol 1,4,5-trisphosphate (IP_3), but they can also be activated experimentally by several agents that empty the stores without generating IP_3, such as the microsomal Ca^{2+}-ATPase inhibitor thapsigargin. The intricate causal relationships among ion channels, membrane potential, $[Ca^{2+}]_i$, and lymphokine gene expression can now be pursued at the single-cell level with patch-clamp recording, calcium-dependent dyes, reporter genes, and fluorescence video techniques. These approaches will help to clarify the essential roles of ion channels in the molecular pathways subserving activation and other lymphocyte behaviors.

INTRODUCTION

Ion channels and fluxes have long been suspected to play a role in lymphocyte signal transduction, but only relatively recently have specific channel types been defined at biophysical or molecular levels. Unlike nerve and muscle cells, lymphocytes lack electrical excitability, and yet patch-clamp studies have revealed a surprisingly complex biophysical phenotype, with multiple K^+, Ca^{2+}, and Cl^- channels expressed in patterns that are regulated differentially according to cell subset and state of activation. By controlling ion fluxes across the plasma membrane, channels mediate changes in intracellular ion concentrations and membrane potential in response to a variety of stimuli. The properties and possible functions of many of these channels have been reviewed (1–10). This review focuses on recent developments in understanding the properties and functional roles of K^+ and Ca^{2+} channels in lymphocytes. Substantial progress has been made in this area by the application of molecular biological and patch-clamp techniques together with single-cell fluorescence measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and reporter gene expression. We specifically address several outstanding issues concerning the functional roles of channels in lymphocytes, including 1) the requirement of K^+ channels for T cell activation; 2) the identity of the mitogen-stimulated Ca^{2+} channel and its mode of regulation; and 3) the means by which K^+ channels and Ca^{2+} channels may interact to influence cell-activation events.

POTASSIUM CHANNELS IN LYMPHOCYTES

Molecular Cloning of Voltage-Gated K^+ Channels in Lymphocytes

Several types of voltage-gated K^+ [K(V)] channels have been intensively studied in lymphocytes through patch-clamp studies of a variety of immature and mature T cells, B cells, and lymphoid cell lines. For convenience, these have been named types *n* (for prevalence in *normal* human T cells) (11, 12), *n'* (similar to type *n*) (13), and *l* (*large* conductance and abundance in T cells from autoimmune *lpr* mice) (14). The three channel types share two fundamental

properties: they are opened by membrane depolarization, and they are K^+-selective. However, they differ in their voltage sensitivity, their tendency to inactivate during prolonged depolarization, their kinetic behavior, their single-channel conductance, and their sensitivities to a range of pharmacological agents. The distinguishing features of these channels have been reviewed previously in detail (7, 8); the characteristics most relevant to this review are summarized in Table 1.

The molecular cloning of ion-channel genes from different cell types and species has revealed an ever-increasing complexity of the K(V) channel superfamily. Sequence analysis provides a convenient and logical means of grouping the channels into families according to their similarity to several K^+-channel genes originally identified in *Drosophila: Shaker* (Kv1), *Shab* (Kv2), *Shaw* (Kv3), and *Shal* (Kv4) (for reviews, see 8, 15). Below we summarize the results of cloning and expression studies that have identified the genes encoding the type *n* (Kv1.3) and *l* (Kv3.1) channels in lymphocytes (for review, see 9). These results establish a basis for identifying residues that are

Table 1 Potassium and calcium channels in lymphocytes[a]

Name	Conductance (p^S)	Activation (midpoint)	Blockers	Expression (resting)	Expression (proliferating)
K(V) *n* (Kv1.3)	10–18	voltage (−40 mV)	TEA (mM) CTX, NTX, MTX, KTX (nM)	human T (++) mouse T, B (+)	human T (+++) mouse T, B (+++) Jurkat (++)
K (V) *n'*	18	voltage (−30 mV)	TEA (mM) CTX (nM)	mouse T (++)	
K(V) *l* (Kv 3.1)	27	voltage (0 mV)	TEA (μM)	mouse T (++)	Loucks (++)
K(Ca) mini	2–8	Ca^{2+}_i (400 nM)	TEA (mM) apamin (pM)	mouse T (?)	Jurkat (+++)
K(Ca) midi	11–35	Ca^{2+}_i (300 nM)	TEA (mM) CTX (nM)	human T (+) mouse B (+)	human T (+++) mouse B (++) Jurkat (+)
Ca^{2+}	0.01–0.03	Ca^{2+} store depletion	Ni^{2+} (mM) econazole, SKF 96365 (μM)	human T (+++)	Jurkat (+++)

[a]Summary of characteristics, including the channel gene (if known), single-channel conductance, mode of activation, selected pharmacological blockers, and expression levels in resting and proliferating cells. Approximate potencies of blockers are indicated in ranges from mM to pM. Expression levels are approximate: +, 5–50 channels per cell; ++, 100–500 per cell; + + +, > 500 per cell; and ?, expression level unknown. See text for references and abbreviations.

critical in determining biophysical properties and pharmacological profiles. For clarity, the terms *n* and *l* are used below to refer to studies of native channels, while the Kv nomenclature is used in reference to exogenously expressed K^+ channels.

Kv1.3 ENCODES THE *n*-TYPE CHANNEL IN LYMPHOCYTES Genes encoding the *n* channel were isolated by low-stringency screening of rat or mouse genomic DNA libraries with probes derived from homospecific homologs of the *Shaker* gene (Kv1.1) (16, 17), or by screening a human Jurkat T cell cDNA library with a probe made from the rat brain *Shaker* homolog, RCK1 (18). The resulting clones, classified as Kv1.3, are all very similar. Northern analysis shows that the Kv1.3 transcript is highly expressed in rat thymus (16), and the polymerase chain reaction (PCR) has been used to detect Kv1.3 mRNA in the EL4 T cell line (19). Furthermore, mRNA derived in vitro from the rat, mouse, or human Kv1.3 genes, when expressed in *Xenopus* oocytes, encodes K^+ channels whose biophysical and pharmacological signature closely resembles that of native type *n* channels in lymphocytes (16, 18–20). Similar results were obtained after transient transfection of CTLL-2 cells (a murine cytotoxic T cell line that does not normally express either detectable K^+ currents or Kv1.3 mRNA) with a Kv1.3-containing plasmid (21). Their voltage dependence, rates of opening and closing, single-channel conductance, inactivation properties, and blockade by different compounds including tetraethylammonium (TEA) and charybdotoxin (CTX) were indistinguishable from native channels (Table 1).

Site-directed mutagenesis of the Kv1.3 gene provides a powerful tool to explore structure-function relationships for the type *n* channel. Thus far, the channel's pharmacological sensitivity and inactivation behavior have been studied most extensively. A relationship between the sites that govern blockade by TEA and inactivation was first inferred from the ability of partially blocking doses of TEA to slow the rate of inactivation (22). Similarly, protonation of his401 in the Kv1.3 channel decreases both TEA sensitivity (23) and the inactivation rate (24), and mutation of this histidine to a tyrosine reduces the extent of inactivation approximately fivefold (24). These results suggest that a histidine located near the outer pore region determines how the *n* channel inactivates and binds TEA. One speculation is that each of the four channel subunits contributes a histidine to create a Ca^{2+} binding site (24), which is consistent with biophysical evidence that Ca^{2+} binds to a site near the extracellular mouth of the *n* channel pore to cause or enhance inactivation (25).

Kv3.1 ENCODES THE TYPE *l* K^+ CHANNEL The *Shaw*-related Kv3.1 gene was first cloned by homology from rat and mouse libraries (26, 27). Heterologous expression in oocytes produced a K^+ current with similarities to type *l* channels in terms of the voltage dependence of opening, single-channel conductance, sensitivity to TEA, and insensitivity to CTX (26, 27; see Table 1). Further

studies showed that the kinetics of inactivation and deactivation (channel closing following the end of a depolarizing stimulus) were also indistinguishable from native *l* channels (28). The extremely close similarities between native *l* channels and Kv3.1 expressed in oocytes suggest that the *l* channel exists in lymphocytes as a homomultimer of four identical Kv3.1 subunits rather than as a heteromultimer containing another Kv3 subfamily member (28). Kv3.1 has also been expressed through transient transfection of CTLL-2 cells, in which it produces a conductance with *l*-type characteristics (21). Finally, Kv3.1 coding sequences have been detected using PCR in two cell types that express functional type *l* K^+ channels: a human B-lymphoma line, Louckes, and $CD4^-CD8^-$ T cells from *lpr* mice (28).

ADDITIONAL VOLTAGE-GATED K^+ CHANNELS IN LYMPHOCYTES Electrophysiological and molecular biological evidence exists for additional K(V) channels in T cells, but these are less abundant than *n*- and *l*-type channels and hence have not received as much attention. A channel classified as type *n'*, similar to type *n* but non-inactivating, is expressed by murine $CD4^-CD8^+$ thymocytes (13, 29); its molecular basis is not yet understood (Table 1). Indirect evidence for a CTX-insensitive K^+ channel in rat thymocytes is based on the inability of CTX to fully inhibit the regulatory volume decrease (RVD) induced by hypoosmotic solutions, a process that requires K^+ efflux and is completely inhibited by less selective K^+-channel blockers (30; for reviews, see 1, 31, 32). Interestingly, one study of single K^+ channels in membrane patches revealed at least two types of K^+ channels in human T cells that appear to be distinct from types *n* and *l* (33), one of which has properties that could explain the CTX-insensitive RVD activity observed in rat thymocytes (30). Several hypotheses have been advanced to explain the molecular origins of additional K^+ channels in lymphocytes, including alternative splicing (although alternative splicing of mammalian Kv1.3 mRNA is not believed to occur—17), expression of additional K^+ channel genes such as IsK (18), heteromultimer formation, and modulation of channel properties by another channel subunit (34), by channel clustering (35, but see 9), or by posttranslational modifications.

Acute Mechanisms of K(V) Channel Modulation

The functioning of K(V) channels in lymphocytes is subject to both acute and long-term modulation. Acute mechanisms are of particular interest, as physiological stimuli including antigen, neurotransmitters, and hormones act through lymphocyte receptors both to generate increases in second messengers such as Ca^{2+} and cAMP and to activate protein kinases (36–39). Results thus far have varied widely among different groups, due in part to the use of different lymphocyte subtypes and protocols, and to the fact that the behavior of K(V) channels is strongly influenced by such experimental factors as temperature and intracellular dialysis during whole-cell recording (11, 12, 40–42). Studies

of modulation using cloned and modified Kv1.3 gene products may ultimately provide a more uniform basis for comparisons.

EFFECTS OF CALCIUM There is widespread agreement that intracellular Ca^{2+} exerts an inhibitory effect on *n*-type K^+ channels in both T and B lymphocytes, although multiple mechanisms appear to exist. Bregestovski et al (43) were the first to describe Ca^{2+}-dependent inhibition of K^+ channels in excised patches and in whole-cell recordings from human T cells. Similar inhibition has been observed in cell-attached patch recordings from intact T cells treated with the Ca^{2+} ionophore ionomycin, demonstrating that the effect does not require the dilution of cellular constituents that occurs during excised-patch and whole-cell experiments (44). The *n*-type K^+ current in murine B cells and their precursors is inhibited by micromolar levels of Ca^{2+} in the recording pipette, primarily through a several-fold increase in the rate of inactivation (45). A subsequent detailed study of divalent ion interactions with the type *n* K^+ channel in Jurkat T cells found comparable effects and concluded that Ca^{2+} causes or accelerates inactivation by binding to a site located within the channel pore (25). Taken together, these results suggest that the inhibition by Ca^{2+} is unlikely to involve a kinase, phosphatase, or other diffusible cytosolic molecule.

A different type of Ca^{2+}-dependent inhibition has been observed in oocytes coinjected with mRNA for Kv1.3 and 5-HT_2 receptors (46–48). In these cells, 5-HT elicits a rise in $[Ca^{2+}]_i$ through the activation of phospholipase C (PLC) and production of inositol 1,4,5-trisphosphate (IP_3), and inhibits the K^+ current by 70–80%. Similar results were produced by directly injecting IP_3 or Ca^{2+}, and the 5-HT effect was slowed or blocked by injection of Ca^{2+} chelators, suggesting that it is mediated through a rise in $[Ca^{2+}]_i$. However, unlike the Ca^{2+}-dependent inhibition described above for lymphocytes, 5-HT-induced inhibition in oocytes is nearly irreversible and does not involve a change in the rate of channel inactivation. A similar long-lasting inhibition of type *n* channels by Ca^{2+} has not been reported in lymphocytes, although 5-HT binding to 5-HT_1 and 5-HT_3 receptors increases the maximum K^+ conductance and accelerates inactivation in a pre-B cell line (49). The different effects of Ca^{2+} on K^+ channels in oocytes as opposed to lymphocytes may reflect differences in the biochemical microenvironment or in the expression of auxiliary channel subunits. In this regard, a 41-kDa β subunit of Kv1 channels has been described that appears to play an important role in the inactivation process (50), and a protein of this size is associated with the type *n* channel in T cells (34).

EFFECTS OF PHORBOL ESTERS Phorbol esters, potent activators of protein kinase C (PKC), lack acute effects on type *n* K^+ current in human peripheral blood T cells (12), but they do appear to inhibit *n* channels in Jurkat T cells (51) and Kv1.3 channels expressed in oocytes (47, 48). The primary sequence of Kv1.3 contains a highly conserved intracellular consensus site for PKC

phosphorylation (34). A biochemical study has shown that the native type *n* channel from unstimulated Jurkat cells is phosphorylated exclusively on serines, and that cell extracts contain a relatively high PKC activity that can phosphorylate the channel in vitro (34). That PKC may be responsible for the resting phosphorylation of *n* channels in vivo is consistent with evidence that the K^+ current in Jurkat cells increases gradually during whole-cell recordings made with anti-PKC antibody or alkaline phosphatase in the recording pipette (51). The discrepancy between phorbol ester effects on Jurkat cells and human peripheral blood T cells remains unexplained. Nevertheless, these results raise the possibility that antigenic stimulation may inhibit *n* channels by generating diacylglycerol (DG) and activating PKC; thus, two downstream messengers of the antigen receptor, Ca^{2+} and DG, may act in concert to limit the number of open K(V) channels during lymphocyte activation.

EFFECTS OF cAMP The inhibitory effects of cAMP on T cell activation (37) have prompted several groups to investigate its actions on K^+ channels. A full spectrum of effects has been reported, ranging from channel inhibition, to no effect, to activation. Choquet et al (45) found that intracellular application of cAMP almost completely eliminated the type *n* K^+ current in murine B cell blasts and pre-B cell lines, and similar findings were later reported in Jurkat T cells treated with 8-Br-cAMP or the cAMP agonist PGE_2 (51). The effect in Jurkat cells was blocked by inhibitors of protein kinase A (PKA), suggesting a phosphorylation-dependent mechanism (51). As in the case of phorbol esters, the effects of cAMP in Jurkat cells do not extend in a consistent way to *n* channels in human peripheral T cells. Several studies found no effect of cAMP applied through the recording pipette or of bath-applied permeant cAMP analogs (43, 44, 52, but see 53), even at 37°C (40). Pahapill & Schlichter (44) addressed this discrepancy in a study of K^+ channels in cell-attached patches. Several agents that elevate cAMP levels induced a slow rise in K^+ channel activity at the resting potential. It is unknown whether these channels are modified *n* channels or are previously "silent" channels activated by cAMP. It should be noted that in another study PGE_2 failed to activate K^+ channels in cell-attached patches (40). The question remains why cAMP appears to inhibit K^+ channels in some cases and to activate them in others. Recent biochemical studies suggest that the actions of cAMP on the type *n* channel may be complex. In vitro PKA phosphorylates not only the Jurkat *n* channel, but also a 40-kDa protein with which it coprecipitates (34). This protein is similar in size to the recently characterized β subunit believed to accelerate the inactivation of Kv1 channels (50), and by analogy it may have a similar action on *n* channels in lymphocytes. Thus, a more complete biochemical characterization of native *n* channels and their associated subunits in different lymphocyte subtypes may shed light on the diversity of cAMP effects that have been described.

EFFECTS OF TEMPERATURE Several properties of type *n* channels are profoundly temperature-dependent, implying that caution must be exercised when extrapolating from results obtained at room temperature to predict K^+-channel behavior under physiological conditions (40, 41). Increased temperature affects the overall activity of *n* channels in several ways: 1) It increases the single-channel conductance; 2) it accelerates activation, inactivation, and deactivation kinetics; 3) it shifts the voltage dependence of inactivation and activation in opposite directions, thereby increasing by two- to threefold the number of channels active at the resting potential; and 4) it speeds the recovery from inactivation. The net effect of these changes is to increase significantly the activity of K^+ channels at 37°C relative to room temperature (40, 41). This information will be essential in developing a realistic quantitative model of K^+-channel activity in lymphocytes under physiological conditions. Such modeling may provide the ultimate test of hypotheses regarding the functional roles of K^+ channels and their modulation in the intact lymphocyte.

Ca^{2+}-Activated K^+ Channels

The existence of Ca^{2+}-activated K^+ [K(Ca)] channels in lymphocytes was first inferred from observations that Ca^{2+} ionophores increased the membrane K^+ permeability (measured with radioactive isotopes) and evoked membrane hyperpolarization (30, 54–56; for review, see 1, 57). Subsequent patch-clamp studies identified K(Ca) channels in rat thymocytes (58, 59), human and mouse B cells (58, 60, 61), and human T cells (62, 63). The current consensus is that at least two types of K(Ca) channels, readily distinguished by their different conductances and pharmacological profiles, are expressed in a lineage-specific pattern in lymphocytes (Table 1). A small-conductance (2-8 pS) "mini" channel appears to be absent from human T cells (63, but see 64), is rare in B cells (60), but is quite abundant in Jurkat cells (several hundred/cell) (62). This channel is effectively blocked by apamin but is insensitive to CTX (62). A larger, 10-35 pS "midi" channel is relatively more abundant in B cells (60), thymocytes (58), and peripheral blood T cells (63) and is rare in Jurkat (62). In contrast to the mini-K(Ca) channel, this larger channel is insensitive to apamin but is half-blocked by 3 nM CTX (60, 62, 63). Neither channel exhibits significant voltage dependence, unlike the large-conductance "maxi"-K(Ca) channel found in other cell types (65); instead, each is highly sensitive to intracellular Ca^{2+}. The calcium dependence of both channels has been measured in whole-cell recordings by intracellular perfusion with buffered Ca^{2+} solutions and by simultaneous patch-clamp and $[Ca^{2+}]_i$ measurements. Both channels are activated by $[Ca^{2+}]_i > 200$–300 nM, and activation by Ca^{2+} is cooperative, with a Hill coefficient of ≈ 4 (62, 63), suggesting that multiple Ca^{2+}-binding sites are involved in channel opening. The genes for these channels have not yet been cloned.

K^+ Channel Expression During Development and Activation

T LYMPHOCYTES The surface density of K^+ channels in the plasma membrane of T lymphocytes is regulated in intriguing ways during development and during activation of mature cells by mitogens (for reviews, see 8, 29). Developmental regulation of K(V) channels is most pronounced in murine lymphocytes. Immature thymocytes ($CD4^-$ $CD8^-$ or $CD4^+$ $CD8^+$) express hundreds of type *n* channels per cell. As the developmental lineage diverges, *n* channels are either downregulated ~10-fold in $CD4^+$ $CD8^-$ cells (helper phenotype) or supplanted with a mixture of ≈20–200 *n'* and *l* channels in most $CD4^-$ $CD8^+$ cells (cytotoxic/suppressor subset) (13). Mitogenic activation causes both classes of mature murine T cells to revert to the immature pattern of channel expression, i.e. hundreds of *n* channels per cell (66, 67). In contrast, resting human T cells express *n* channels at a level similar to that of immature or activated T cells of mice, and activation increases channel abundance only by a factor of ≈2–3 (12, 63, 68). However, the expression of K(Ca) channels in human T cells is profoundly upregulated by mitogens, increasing from ≈20 per resting cell to >500 channels per cell in T cell blasts treated with phytohemagglutinin (PHA) (63). Thus, the relative contribution of K(Ca) channels to maintaining the membrane potential may be greatly enhanced in activated cells and perhaps in memory cells. Type *l* K(V) channels have been found only in $CD4^-CD8^+$ thymocytes and peripheral T cells from mice (at low levels) and at high levels in $CD4^-CD8^-$ T cells from a variety of autoimmune mice such as *lpr, gld,* NOD, and EAE (for review, see 8). Type *l* channels have not been detected in human T cells but are present in a human Burkitt's lymphoma cell line, Louckes (69).

Recent evidence suggests that the expression of K(V) channels during T cell activation is regulated through a posttranscriptional mechanism. Following mitogenic stimulation, Kv1.3 mRNA levels remain constant or decrease in murine and human T cells as the number of channels increases (18, 20). A number of posttranscriptional mechanisms could explain the increase in K^+-channel density, including an increased rate of translation, stimulated insertion of presynthesized channels, or unmasking of previously "silent" channels. Transcriptional control mechanisms cannot be ruled out, however, as several Kv1.3 transcripts have been detected [≈9.9, 4.4, and ≈3 kb (18, 20)], and the expression of the smaller two transcripts increases with time in activated Jurkat cells (18). The relative translational efficiency of the different transcripts is not known.

B LYMPHOCYTES Independent regulation of K(V) and K(Ca) channels via different activation pathways has been demonstrated in B cells (61). Stimulation through the antigen receptor increases expression of both channels, while lipopolysaccharide (LPS) affects only K(V) channels. In each case, the surface density of the channels increases roughly 5–10-fold to hundreds per cell. Inhibitors of RNA synthesis block the induction of K(Ca) channels by antigen,

indicating a requirement for gene transcription. The functional implications of differential regulation are unknown, but the induction of K(Ca) channels appears to be correlated with an inability to differentiate (61). LPS elevates the levels of K(V) channels and triggers differentiation into plasma cells, while anti-μ induces K(Ca) channels in LPS-pretreated cells and inhibits their ability to differentiate. Identification of a specific blocker for midi-K(Ca) channels in B cells would offer a means of testing this hypothesis.

K(V) Channels as a Pharmacological Target

A prospective therapeutic intervention in immune disease is based on pharmacological inhibition of type *n* K^+ channels. These channels are blocked by a remarkable variety of agents, including classical K^+ channel blockers [TEA and 4-aminopyridine (4-AP)], Ca^{2+}-channel antagonists (verapamil and dihydropyridines), K(Ca) channel blockers (quinine and cetiedil), calmodulin antagonists (trifluoperazine and chlorpromazine), and the steroid hormone progesterone (for reviews, see 2, 7, 8). Many of these compounds also inhibit lymphocyte activation (below); however, their rather low affinity ($K_i = 10^{-5}$ to 10^{-2} M) and specificity undermine their usefulness either as research probes for K(V) channel function or as therapeutic tools. The more recent identification of peptide toxins from scorpion venoms that block K(V) and K(Ca) channels with high affinity and specificity offers great promise. CTX blocks type *n* K(V) as well as K(Ca) channels with a K_i of $\approx 10^{-9}$ M (60, 70, 71). Noxius toxin (NTX) (64, 70), margatoxin (MTX) (72), and kaliotoxin (KTX) (61) are even more potent and are also selective for K(V) over K(Ca) channels. It is encouraging to note that low, therapeutic concentrations of calcium antagonists that also block *n* channels (verapamil, nifedipine, nimodipine, and diltiazem) significantly enhance the immunosuppressive effects of cyclosporin A (CsA) in vitro (73). Thus, K^+-channel blockers may synergize with low doses of CsA in vivo to increase the survival of organ grafts while minimizing the nephrotoxicity and hypertension associated with conventional CsA therapy. To this end, several pharmaceutical companies are currently attempting to develop K(V) blocking agents of high affinity and specificity using both toxins and organic compounds. The success of this approach may be aided by the restricted tissue distribution of Kv1.3 mRNA, which is expressed abundantly in thymus and spleen, at a low level in fibroblasts, brain, and kidney, and not detectably in liver or heart (16, 19).

Functional Roles for K^+ Channels in Lymphocytes

CONTROL OF MEMBRANE POTENTIAL The membrane potential (V_m) exerts powerful effects on lymphocyte activation (see below). Based on measurements of radiolabeled and fluorescent membrane potential probes, the resting potential of quiescent T cells from humans and mice is -50 to -70 mV (56, 64, 74, 75; for review, see 1). In human T cells, the resting potential results

primarily from a K^+-diffusion potential contributed by *n*-type K^+ channels. It has been estimated that on average only several *n* channels are open in resting T cells, and that this can account for the resting K^+ efflux (11, 41). This conclusion agrees well with the observation that the resting potential of T cells slowly fluctuates by ≈8 mV, presumably due to the opening and closing of single K^+ channels (76). The extremely high input resistance of resting T cells makes it possible for such a small number of open channels to determine the membrane potential and creates a substantial reserve of dormant K^+ channels to "protect" against depolarization. High-affinity blockers provide definitive pharmacological evidence for the role of *n* channels in setting the resting potential. CTX, margatoxin, and NTX depolarize resting human T cells (30, 64), and although CTX also blocks K(Ca) channels, its effect on V_m is no greater than that of the more selective K(V) blockers (64).

In murine T cells, the contribution of K(V) channels to establishing the resting potential correlates well with their level of expression (75). Type *n* channels play a dominant role in immature thymocytes or activated peripheral T cells, both of which express hundreds of channels per cell. In contrast, V_m in mature resting T cells (with ≈10 *n* channels/cell) is determined largely by the electrogenic activity of the Na^+/K^+ ATPase. Blocking the pump with ouabain, low temperature, or removal of K_o^+ depolarizes these cells, whereas moderate changes in the transmembrane K^+ gradient have little effect (75). The Na^+/K^+ pump's contribution to V_m may explain why CTX does not depolarize rat thymocytes (55), cells that express low numbers of type *n* channels (M Cahalan, unpublished observation).

K(Ca) channels do not appear to contribute significantly to V_m in resting lymphocytes but exert a powerful influence when $[Ca^{2+}]_i$ is elevated. $[Ca^{2+}]_i$ in resting lymphocytes is 50–100 nM (1), well below the measured threshold for activating K(Ca) channels (200–300 nM) (58, 62, 63). K(Ca) channels are silent in cell-attached recordings from intact resting lymphocytes except during a brief period immediately after establishing the membrane-pipette seal, when a $[Ca^{2+}]_i$ spike often occurs (58, 59). The rise in $[Ca^{2+}]_i$ elicited by mitogens or Ca^{2+} ionophores is sufficient to activate K(Ca) channels in both whole-cell and cell-attached configurations (58, 59, 62, 63). Thus activated, K(Ca) channels cause a membrane hyperpolarization that is prevented or reversed by CTX (55, 59, 77) or quinine (56), both of which block K(Ca) channels in patch-clamp studies (60, 63).

REGULATION OF CELL VOLUME When exposed to hypoosmotic solutions, T cells swell rapidly, then shrink over several minutes back to approximately their normal size. This regulatory volume decrease (RVD) response is mediated by the stimulated efflux of K^+ and Cl^-, which drives osmotically obligated water from the cell (1). A model for RVD in T cells has been proposed in which cell swelling activates Cl^- channels (78), eliciting an efflux of Cl^- that depolarizes

the membrane and thereby opens type *n* K(V) channels (31, 32). Several types of evidence support a role for type *n* channels in RVD. First, the density of *n* channels in several types of lymphocytes correlates with the ability to volume regulate (75, 79). Second, a series of pharmacological reagents block *n* channels and inhibit RVD with the same relative order of potency (reviewed in 31, 32). Finally, increasing the expression of type *n* channels by mitogenic stimulation of murine T cells (79) or by transfection of Kv1.3 into the K^+-channel and RVD-deficient CTLL-2 cell line (21) confers the ability to volume regulate. Significantly, *l* channels expressed by CTLL-2 cells after transfection with Kv3.1 did not fulfill this role (21). These results present compelling evidence for the roles of swelling-activated Cl^- channels and type *n* K^+ channels in RVD, as Cl^- channels can only depolarize the cell to a maximum of ≈ -35 mV (the estimated equilibrium potential for Cl^-), above the activation threshold for *n* channels (-60 mV) but well below that of type *l* channels (-10 mV). K(Ca) channels normally do not appear to be involved in RVD but can assume this function in murine T cells treated with ionomycin (75).

ACTIVATION OF LYMPHOCYTES Two types of circumstantial evidence support a role for K^+ channels in the activation of lymphocytes. First, as described above, T and B cell mitogens stimulate an increase in the density of K(V) and K(Ca) channels, and the density of K^+ channels in thymocyte subsets is positively correlated with proliferative activity. Second, pharmacological studies suggest a requirement for functional K^+ channels in the activation of T and B cells. A variety of chemically distinct K(V) channel blockers, including TEA, 4-AP, quinine, verapamil, diltiazem, cetiedil, trifluoperazine, and chlorpromazine, inhibit activation, gene expression, killing by cytotoxic T cells and NK cells, lymphokine secretion, and proliferation (4, 80–82). The discovery that K^+-channel blockers can inhibit proliferation has since been extended to a variety of cell types outside the immune system (reviewed in 83, 84).

The interpretation of results obtained with K^+-channel blockers is complicated by the fact that some of these compounds have effects on proteins other than K(V) channels. For example, TEA and quinine also block K(Ca) channels, and trifluoperazine is a potent inhibitor of calmodulin. The identification of high affinity, specific K^+-channel blockers from scorpion venoms has allowed more definitive studies of K^+-channel functions in intact cells. Deutsch and colleagues first demonstrated that nanomolar concentrations of CTX effectively inhibit PHA- or antigen-driven T cell activation in vitro (71). The inhibition appears to result from suppression of IL-2 transcription, as the level of IL-2 mRNA and secreted IL-2 are both reduced in CTX-treated cultures (85), and normal proliferation can be restored by supplying exogenous IL-2 (71). Because CTX blocks K(V) and K(Ca) channels with similar potency, it cannot be used to distinguish their functional roles. Lin et al (86) addressed this

point using the selective K(V)-channel blockers, margatoxin (MTX) and noxiustoxin (NTX); like CTX, both peptides block mitogen-induced proliferation, the mixed lymphocyte response, and the secretion of IL-2 and γ-IFN. Moreover, MTX inhibits IL-2 secretion at lower concentrations than does CTX, consistent with its higher affinity for the type *n* K^+ channel. These results provide the strongest available evidence for a role of K(V) channels in mitogenesis. However, even though CTX and NTX block activation with equal efficacy (86), it should be noted that the effects of these peptides do not rule out a role for K(Ca) channels downstream from events involving K(V) channels. At present the absence of a selective blocker for K(Ca) channels precludes a test of this possibility.

Considerable evidence suggests that functional K^+ channels are required during T cell activation to maintain a membrane potential that promotes Ca^{2+} influx. A negative membrane potential enhances Ca^{2+} entry by optimizing the electrochemical driving force for Ca^{2+} movement through Ca^{2+} channels; in addition, indirect effects of V_m on the activity of the Ca^{2+} channels themselves have also been suggested (87–89). Several types of evidence support a V_m-mediated link between K^+ channels, Ca^{2+} influx, and cell activation. High affinity K^+-channel antagonists like CTX, NTX, and MTX depolarize human T cells (30, 64) and inhibit T cell activation induced by Ca^{2+}-dependent pathways (anti-CD2 or anti-CD3) but not Ca^{2+}-independent ones (anti-CD28+ PMA, or IL-2) (86). The immunosuppressive effects of CTX in vitro can be mimicked by an equivalent depolarization invoked directly by elevated $[K^+]_o$ (85). Furthermore, depolarization inhibits the $[Ca^{2+}]_i$ rise induced by T cell mitogens (88, 90–93), whereas hyperpolarization by the potassium ionophore valinomycin reverses the effect of K^+-channel blockade on $[Ca^{2+}]_i$ (87).

While these studies suggest that K(V) channels are needed to enhance Ca^{2+} entry, several unresolved discrepancies remain. First, Gelfand & Or (77) found that CTX had no effect on either the mitogen-induced $[Ca^{2+}]_i$ rise or activation of human lymphocytes, even though it effectively blocked RVD. Second, even doses of CTX more than tenfold greater than the K_i for K^+-channel blockade inhibit activation by only 50–60% (71, 85, 86). This may be due to the fact that CTX evokes a maximal depolarization of only 20–25 mV (30, 64), implying that CTX-insensitive K^+ or Cl^- channels also contribute to the resting potential, particularly when *n* channels are blocked. Contributions of CTX-insensitive mechanisms to membrane potential might also explain the lack of CTX effects observed by Gelfand & Or (77). Finally, in the study by Lin et al (86), although CTX did not affect the long-lasting plateau level of $[Ca^{2+}]_i$, it inhibited activation significantly even when added hours after the mitogen, i.e. well into the plateau phase. In this regard, it should be noted that $[Ca^{2+}]_i$ oscillations may be important in controlling lymphocyte activation (94), and

these would not be detected in the population $[Ca^{2+}]_i$ measurements discussed above. Blockade of K(V) and K(Ca) channels with CTX and apamin effectively inhibits PHA-triggered $[Ca^{2+}]_i$ oscillations in Jurkat cells, but it has only a small effect (≈25% decrease) on the average $[Ca^{2+}]_i$ of the cell population (62). Thus, further studies of the effects of channel antagonists on $[Ca^{2+}]_i$ at the single-cell level may better define the role of K(V) channels in Ca^{2+} signaling and lymphocyte activation.

CALCIUM CHANNELS IN LYMPHOCYTES

A rise in $[Ca^{2+}]_i$ is an essential triggering signal for many of the events associated with lymphocyte activation. These events include changes in motility and cell shape occurring soon after T cells contact antigen-presenting cells (95, 96), transcription of genes for lymphokines such as IL-2 (97–99), cell killing by cytotoxic T cells (100, 101), apoptosis of self-reactive T cells during development in the thymus (102, 103), and induction of tolerance to self-antigens in mature peripheral T cells (104, 105). In the past several years, substantial progress has been made in understanding many of the key molecular events upstream and downstream of the Ca^{2+} signal. Binding of antigen (or mitogens) to the TCR activates multiple tyrosine kinases and leads to the phosphorylation and activation of phospholipase Cγ1 (for reviews, see 39, 106). Once activated, the enzyme cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the second messengers IP_3 and diacylglycerol (DG). IP_3 evokes a rise in $[Ca^{2+}]_i$, which in combination with DG activates PKC. The elevation of $[Ca^{2+}]_i$ appears to be necessary and, together with PKC, sufficient to drive many events in T cell activation (for reviews, see 36, 97). Ca^{2+} exerts much of its influence through the activation of transcription factors that control the production of lymphokines such as IL-2 (99, 107). Additional signaling molecules such as ras operate within parallel pathways to play critical roles in T cell activation, but these are beyond the scope of this review (107, 108).

Because of its central role in lymphocyte activation, an intense effort has been focused on defining the underlying basis of the Ca^{2+} signal. The first phase of the $[Ca^{2+}]_i$ rise is generally understood to be mediated by IP_3 binding to its receptor in the endoplasmic reticulum (ER) membrane and opening a Ca^{2+} channel that releases stored Ca^{2+} into the cytosol (109). The second phase, considerably less well understood, consists of a prolonged influx of Ca^{2+} across the plasma membrane. Because of the finite capacity of the ER Ca^{2+} store, intracellular release generates a transient signal that is neither necessary nor sufficient to drive activation (110, 111). Ca^{2+} influx and elevation of $[Ca^{2+}]_i$ must be sustained for >30 min to commit T cells to become activated and express the IL-2 gene (94, 107, 111). Considerable effort has been spent over the past few years to identify the Ca^{2+} channels that carry out this signaling function and to understand the mechanisms that regulate their activity.

Identification of Mitogen-Regulated Calcium Channels

A great variety of channels have been proposed to mediate mitogenic Ca^{2+} influx in lymphocytes, an indication of both the intense interest in elucidating this pathway and the technical difficulties inherent in doing so. Below, we consider many of the proposed mechanisms in the context of the characteristics of the Ca^{2+} influx pathway, as inferred from $[Ca^{2+}]_i$ measurements in intact cells (90, 93, 109, 112, 113). Based on these studies, any potential candidate for the mitogen-activated Ca^{2+} channel should exhibit the following properties: 1) activation by mitogens or downstream messengers (e.g. IP_3); 2) significant Ca^{2+} permeability and an ability to carry Mn^{2+}; 3) activity that is temporally correlated with increases in $[Ca^{2+}]_i$; 4) decreased Ca^{2+} conductance upon depolarization; 5) feedback inhibition by intracellular Ca^{2+}; and 6) inhibition by Ni^{2+} and imidazole antimycotics such as SKF 96365. So far, these six criteria have been met only for depletion-activated Ca^{2+} channels.

DEPLETION-ACTIVATED CALCIUM CHANNELS These channels were first described by Lewis & Cahalan (93) in Jurkat T cells using whole-cell recording and simultaneous $[Ca^{2+}]_i$ imaging with fura-2. A small, highly selective Ca^{2+} current appeared spontaneously during intracellular dialysis with solutions containing EGTA. The current displayed many of the properties expected for mitogenic Ca^{2+} channels, including high permeability to Ca^{2+}, a tight temporal link to changes in $[Ca^{2+}]_i$, inhibition by depolarization, evidence of Ca^{2+}-dependent feedback inhibition, and blockade by Ni^{2+} and Cd^{2+}. In perforated-patch recordings, the current did not develop spontaneously but could be activated by PHA in an oscillatory manner. A calculation based on the lymphocyte volume demonstrated that the observed 1- to 10-pA Ca^{2+} current was sufficiently large to account for the observed rate at which $[Ca^{2+}]_i$ rises during oscillations in PHA-stimulated cells ($\approx$100 nM/sec maximum) (93), even allowing for substantial binding of Ca^{2+} by cytoplasmic buffers (114). The mode of regulation for these channels was unclear at the time; although activation by PHA could be explained through the action of IP_3, activation by intracellular dialysis alone was unlikely to involve the same messenger. The ability of IP_3 to activate a similar Ca^{2+} current in Jurkat cells was later shown directly by flash photolysis of intracellular caged IP_3 (115).

The key to understanding this Ca^{2+} channel came from the capacitative Ca^{2+} entry hypothesis originally proposed by Putney to explain receptor-mediated Ca^{2+} influx in exocrine cells (116). According to this hypothesis, depletion of the ER Ca^{2+} store generates a signal that activates Ca^{2+} influx across the plasma membrane. Supporting evidence has accumulated rapidly since the isolation of compounds that block Ca^{2+}-ATPases in the ER membrane and thereby unmask a passive Ca^{2+} leak that empties the stores without generating IP_3. Store depletion by several of these compounds, including thapsigargin (TG), tert-butylhydroquinone (tBHQ), and cyclopiazonic acid (CPA), activates

Ca^{2+} influx in rat thymocytes and human T cells (113, 117–119), as does store depletion induced simply by prolonged incubation of cells in Ca^{2+}-free media (113, 120). Overlap between the depletion-activated and mitogen-activated Ca^{2+} entry mechanisms was first indicated by observations that TG depletes the same Ca^{2+} store that is mobilized by TCR ligation (112, 113, 117, 119), and that stimulation of the TCR fails to evoke an additional $[Ca^{2+}]_i$ increase in cells pretreated with a maximal dose of TG (113, 119). In view of these results, Zweifach & Lewis (121) used perforated-patch recording to test whether the mitogen-regulated Ca^{2+} current is activated by store depletion, by comparing the Ca^{2+} currents activated by TG and PHA in Jurkat T cells. The two currents were identical in every property that was tested, including divalent ion selectivity, voltage-independence of gating, sensitivity to blockade by Ni^{2+}, and single-channel conductance. The single-channel conductance was estimated from noise analysis to be ≈24 fS in 110 mM $Ca^{2+}{}_o$; this extremely small value and the channel's high Ca^{2+} selectivity readily distinguish it from IP_3-gated Ca^{2+} permeable channels previously described in T cells (see below; 122, 123). The simplest conclusion consistent with these results is that mitogen-activated Ca^{2+} channels in T cells are regulated by the depletion of Ca^{2+} stores, and that under physiological conditions of TCR stimulation, IP_3 activates Ca^{2+} channels through store depletion rather than by a direct action on the Ca^{2+} channels themselves. This conclusion was later confirmed by Gardner and colleagues (124) in a comparison of whole-cell Ca^{2+} currents in Jurkat cells treated with TG, tBHQ, CPA, and intracellular IP_3; in particular, the TG-induced current was not increased further by IP_3-mobilizing agents and vice versa, consistent with previous evidence that the two stimulus pathways converge upon the same channel (113, 119). Choquet and coworkers have recently described an apparently identical depletion-activated Ca^{2+} current in human peripheral blood T cells (125). Thus, present evidence favors the idea that stimulation of the TCR triggers an influx of Ca^{2+} through low conductance, voltage-independent Ca^{2+} channels that are controlled indirectly by IP_3-driven depletion of the intracellular Ca^{2+} stores.

Although additional Ca^{2+} entry pathways activated by the TCR may exist, most evidence argues otherwise. First, stimulation through the TCR fails to elevate $[Ca^{2+}]_i$ or the Ca^{2+} current when added after an optimal dose of TG (113, 119, 124; but see 126). Second, antigen-unresponsive T cells from patients with a primary immunodeficiency lack the sustained mitogen-driven increase in $[Ca^{2+}]_i$ in conjunction with an absence of depletion-activated Ca^{2+} current (125). Taken together, these findings suggest that depletion-activated Ca^{2+} channels play the major and perhaps the sole role in mediating the influx of Ca^{2+} essential for T cell activation.

IP$_3$-GATED CHANNELS Kuno et al (122) were the first to report PHA-activated single-channel Ca^{2+} and Ba^{2+} currents in cell-attached patches from human

T cells. Similar currents were observed in excised patches exposed to IP_3 on the intracellular face, leading to the conclusion that the channels were directly activated by this messenger (123). These channels fulfill several of the criteria for being mitogenic Ca^{2+} channels: They are activated by cross-linking of the TCR as well as by IP_3; they conduct current in the presence of isotonic Ca^{2+} or Ba^{2+}; they exhibit voltage-independent gating, and the current amplitude decreases with depolarization; and they are inhibited by micromolar $[Ca^{2+}]_i$ and by Cd^{2+} (122, 123, 128). Moreover, Khan et al (129) have detected IP_3R-like immunoreactivity on the surface of human T cells by means of an antiserum generated against the cerebellar microsomal IP_3 receptor. Concanavalin A caps the immunoreactivity as well as Ca^{2+} influx sites as inferred from elevated $[Ca^{2+}]_i$ near the cap (129). However, several necessary criteria supporting a role of the IP_3 receptor in the influx of Ca^{2+} remain untested. First, the single-channel currents appeared to have a low selectivity for Ca^{2+} over monovalent ions (123), raising the question as to whether they have the capacity to conduct significant amounts of Ca^{2+} under physiological conditions. A correlation of their activity with the increase in $[Ca^{2+}]_i$ has not been demonstrated. Second, activity of IP_3-gated channels has not been observed in whole-cell recordings, suggesting that the density of these channels is quite low. Even in the relatively noninvasive perforated-patch mode, electrical noise expected from the opening and closing of 8-pS IP_3-gated channels was undetectable in PHA-treated cells (121). Although IP_3 activates a Ca^{2+} current in whole-cell recordings from Jurkat cells (115), this appears to be identical to the depletion-activated Ca^{2+} current (121, 124). Third, the correlation of IP_3R-like immunoreactivity and gradients of $[Ca^{2+}]_i$ in capped cells may be misleading. Apparent gradients often result from the uptake of fura-2 by organelles, which can be significant in human T cells (130) and could underlie the spatial non-uniformity of the total fura-2 concentration that was observed (129). Furthermore, con A caps a large number of membrane glycoproteins in T cells, including perhaps the depletion-activated Ca^{2+} channel. For these reasons, it appears unlikely that plasma-membrane IP_3 receptors mediate significant Ca^{2+} entry, and their physiological function in T cells remains unclear (124).

OTHER POSSIBLE CALCIUM CHANNELS IN LYMPHOCYTES Two lines of evidence suggest that voltage-dependent Ca^{2+} channels are not expressed at a significant level in T cells. First, depolarization of resting Jurkat cells or human T cells with high $[K^+]$ (87, 88, 91–93, 131) or by current injection (132) fails to raise $[Ca^{2+}]_i$ significantly; and second, several groups have failed to observe voltage-gated Ca^{2+} currents under whole-cell recording conditions optimal for their detection in excitable cells (122, 133, 134). However, there are two reports of voltage-gated Ca^{2+} currents in whole-cell recordings from T cell lines. In one case, a large, slowly inactivating inward current was activated at potentials above −60 mV in a subset of Jurkat 77 T cells (135), while in another a small,

rapidly inactivating inward current was reported in Jurkat, Molt-4, and HSB T cell lines (136). The second current was active transiently (duration < 10 msec) at potentials above −20 mV, while it was inactivated at potentials more positive than −90 mV; these properties appear to be inconsistent with a role in mediating a sustained rise in $[Ca^{2+}]_i$ at physiological potentials of −50 to −80 mV. There is no direct evidence to show that either current is carried by Ca^{2+}, and although the size of both of these currents appears to be enhanced by stimulation of TCR, significant activity of the channels at physiological membrane potentials has not been demonstrated. Several experiments could clarify the existence and role of these channels in T cells: if they are to mediate mitogenic Ca^{2+} influx, then Ca^{2+}_o removal should eliminate the current, depolarization should increase $[Ca^{2+}]_i$, and holding the cell at potentials below the channel's threshold for activation should prevent the $[Ca^{2+}]_i$ increase triggered by mitogens.

A Ca^{2+} entry pathway independent of the phosphatidylinositol cascade has been proposed based on the ability of mitogens to evoke Ca^{2+} influx in HPB-ALL cells without measurable production of inositol phosphates or release of intracellular Ca^{2+} (137). In lipid vesicles reconstituted from HPB-ALL plasma membrane, anti-CD3 mAb induced Ca^{2+} uptake to a greater extent than nonmitogenic control antibodies to CD2 or Thy 1.2; this was specifically inhibited by several conditions that reduce influx in intact HPB-ALL cells. It is not known whether this pathway can account for Ca^{2+} influx in intact HPB-ALL cells, nor whether it also exists in nontransformed cells. Another Ca^{2+} entry mechanism distinct from the TCR-triggered pathway has been reported in T cells from patients with multiple sclerosis. γ-interferon activates Ca^{2+} entry in these cells apparently through activation of protein kinase C (138). Patch-clamp studies may help to clarify the nature of these additional routes for Ca^{2+} entry.

CD20, a B cell plasma membrane phosphoprotein thought to play a role in B cell activation and cell-cycle progression, has been proposed to be a Ca^{2+} channel (139). The evidence for this is indirect, based largely on the appearance of an inwardly rectifying Ca^{2+} current and an increased resting Ca^{2+} permeability in cells transfected with CD20, and an acute increase in whole-cell conductance induced by an anti-CD20 mAb. Several key questions about the nature and function of CD20 remain. First, the increase in conductance induced by cross-linking CD20 is nonselective and thus distinct from the resting CD20-associated conductance. Second, the authors do not exclude the possibility that CD20 is a channel modulator rather than a channel itself. In fact, other work has shown that mAb binding to CD20 initiates tyrosine kinase activity and oncogene expression (140, 141), actions suggestive of a biochemical modulator. Finally, the mechanisms that control CD20-linked Ca^{2+} entry under physiological conditions are unknown, although a link to depletion-activated Ca^{2+} channels has been proposed (139).

Properties of Depletion-Activated Calcium Channels

The evidence reviewed above strongly supports the conclusion that depletion-activated Ca^{2+} channels underlie mitogenic Ca^{2+} influx in T cells. Although their properties differ from those of voltage-gated Ca^{2+} channels in electrically excitable cells, T cell Ca^{2+} channels closely resemble Ca^{2+} release-activated Ca^{2+} (CRAC) channels originally described in rat mast cells by Hoth & Penner (142, 143). Patch-clamp studies indicate that these channels are present in a wide variety of cell types, including RBL cells, fibroblasts, thyrocytes, hepatocytes, HL-60 cells, and *Xenopus* oocytes (144–146); indirect evidence based on Ca^{2+}-sensitive dyes suggests an even wider distribution (116). A complete understanding of the capacitative Ca^{2+} entry pathway in lymphocytes and other cells will require cloning of the CRAC channel and other elements involved in its regulation. The unique nature of the channel and its mode of activation, a frustrating lack of high-affinity ligands, and an extremely widespread tissue distribution make its cloning a challenging goal. The defining properties of CRAC channels are reviewed below with particular emphasis on T cells and mast cells, in which they have been studied most extensively. This channel "fingerprint" may prove to be useful for the identification of these channels in other immune cells.

ION SELECTIVITY CRAC channels are extremely selective for Ca^{2+} over monovalent cations, having a relative Ca^{2+} permeability comparable to that of voltage-gated Ca^{2+} channels (121, 124, 143). The divalent conductance sequence for the channel is $Ca^{2+} > Ba^{2+} \simeq Sr^{2+} >> Mn^{2+}$, with Ba^{2+} and Sr^{2+} carrying current about 50% as well as Ca^{2+}. This sequence is unusual, as most voltage-gated Ca^{2+} channels conduct Ba^{2+} and Sr^{2+} better than Ca^{2+} (147).

PHARMACOLOGY No specific, high-affinity blockers of I_{CRAC} (the current through CRAC channels) have been identified. A variety of organic antagonists of Ca(V) channels do not affect the current (124, 148), but imidazole antimycotics such as econazole and SKF 96365 inhibit capacitative Ca^{2+} influx (149, 150) and I_{CRAC} (148, 150). These compounds block with relatively low affinity (K_i values of 0.6 μM for econazole and 4–12 μM for SKF 96365) and in mast cells block nonselective cation channels and chloride channels with similar potency (148). Divalent (Ni^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}) and trivalent (La^{3+}) ions also inhibit I_{CRAC} in the range of 0.1–5 mM (93, 121, 124, 143), but they block type *n* K(V) channels at similar concentrations (134, 135, 151) and therefore cannot be used to elucidate the function of CRAC channels in intact cells.

UNITARY CONDUCTANCE AND LEVEL OF EXPRESSION One hallmark of the CRAC channel is its extremely small single-channel conductance [10–24 fS in T cells (121) and $<$1 pS in mast cells (143)]. This conductance is $\approx$100-fold smaller than that of voltage-gated Ca^{2+} channels, far too small for the single-

channel currents to be detected in membrane patches or even for whole-cell current noise to be visible by eye. In principle, the low transport rate predicted by the unitary conductance could be achieved by a single-ion transport mechanism like an ion exchanger. Na^+/Ca^{2+} exchange has in fact been proposed to underlie Ca^{2+} entry in T cells (152), but such a mechanism cannot explain observations that Ca^{2+} influx decreases with depolarization (93) and is insensitive to the removal of $Na^{2+}{}_o$ (114, 153). The shape of its noise spectrum suggests that I_{CRAC} is carried by channels rather than by a single-ion transport mechanism, and based on the size of the unitary and whole-cell currents, a typical Jurkat cell may express > 10,000 CRAC channels (121).

ACTIVATION AND INACTIVATION The gating of depletion-activated Ca^{2+} channels is their least well-understood feature and perhaps the most important from a physiological viewpoint. CRAC channels are not opened directly by depolarization (93, 115, 121, 124, 142), IP_3 (121, 124, 142), or IP_4 (124, 142). Most attention has been focussed on the possibility of activation by a diffusible messenger released from intracellular Ca^{2+} stores, although activation by physical contact between proteins in the ER and plasma membranes or by stimulated insertion of new channels is also possible. One hypothesis is that Ca^{2+} entry in T cells is directly driven by Ca^{2+} released from intracellular stores (154–156), based in large part on the ability of high concentrations of intracellular Ca^{2+} buffers to abolish capacitative Ca^{2+} entry (154–156; but see 110). The strongest evidence against Ca^{2+}-dependent activation is that I_{CRAC} is induced in whole-cell recordings by intracellular dialysis with Ca^{2+} buffers that clamp $[Ca^{2+}]_i$ to <10 nM, well below the resting level (93, 124, 142). In addition, TG or IP_3 activates I_{CRAC} in advance of the $[Ca^{2+}]_i$ rise in buffer-loaded cells (93, 115, 121, 124, 142). Thus, it appears that CRAC channels are opened by a signal other than Ca^{2+} itself, although modulatory roles for Ca^{2+} should not be excluded (see below).

The ability of several imidazole inhibitors of cytochrome P450 to inhibit capacitative Ca^{2+} entry originally led to the suggestion that a P450 metabolite is the activating messenger (157). However, a subsequent study has shown that chemical modification of these compounds can greatly reduce the anti-P450 activity without altering the inhibition of Ca^{2+} influx, suggesting that the imidazoles may block CRAC channels directly (158). A more recent candidate for the CRAC channel messenger is CIF (Ca^{2+} Influx Factor), a low-mol-wt phosphate-containing molecule present in Jurkat cell extracts (159). CIF activity is released from the microsomal fraction into the cytosol following depletion of Ca^{2+} stores, and when applied to several nonlymphoid cell types elicits a small, fluctuating $[Ca^{2+}]_i$ rise without triggering intracellular Ca^{2+} release. These results are consistent with a role for CIF as the messenger that couples ER depletion to CRAC channel activation. Confirmation will require the demonstration that purified CIF activates I_{CRAC} when applied

intracellularly, for example through a patch pipette. The molecular identity of CIF is not known, although present data argue against a wide variety of known messengers (159). Other mechanisms involving small G proteins (160, 161), phosphatases (144), tyrosine kinases (162, 162a, 162b), nitric oxide (163), and cGMP (163, 164) have also been proposed to evoke capacitative Ca^{2+} entry in various cells, but further experiments are needed to assign a specific action to these in lymphocytes.

Ca^{2+} entering the cell through CRAC channels feeds back to inhibit the channel's activity in several ways. Rapid inactivation of the channels occurs over tens of msec (143, 165) and is driven by local intracellular Ca^{2+} accumulation and binding to sites located probably on the channel itself (165). In addition, increased $[Ca^{2+}]_i$ slowly inhibits I_{CRAC} over tens of seconds (93, 115, 121, 166). Part of this slow inactivation process is due to uptake of Ca^{2+} by stores, as it is blocked by TG (166). A second type of slow Ca^{2+}-dependent inhibition appears to involve a protein phosphatase, as it is sensitive to okadaic acid, a phosphatase inhibitor (166; A Zweifach, R Lewis, unpublished observations). Slow activation and inactivation of I_{CRAC} is likely to play a major role in generating oscillations of $[Ca^{2+}]_i$ (93, 114, 130). In addition, negative feedback by Ca^{2+} may act as an essential brake that prevents inappropriate or excessive increases in $[Ca^{2+}]_i$. These regulatory mechanisms may suggest useful therapeutic strategies for controlling lymphocyte responsiveness in vivo.

Receptor-mediated inhibition of capacitative Ca^{2+} entry has been described in B cells. Cross-linking of Fcγ type II receptors (FcγRII) to membrane immunoglobulin (mIg) inhibits anti-μ-induced influx of Ca^{2+} without preventing its release (167, 168). Because this treatment does not affect TG-induced Ca^{2+} entry (168), it probably does not inactivate the CRAC channel or preempt its activation signal. It is possible that stimulation of FcγRII enhances pumping and reuptake of Ca^{2+} by the stores and thereby prevents the activation of I_{CRAC} by anti-μ (D Choquet, personal communication). Cross-linking of FcγRII to mIg in vivo may contribute to the inhibition of B cell activity during the late phases of the humoral immune response, when concentrations of circulating antibody are high (167).

Lymphocyte Signaling by Calcium Oscillations

Single-cell measurements of $[Ca^{2+}]_i$ with fluorescent dyes have revealed a remarkable degree of dynamic behavior that is undetectable in cell populations. A number of studies have shown that stimulation of the TCR by mitogens or antigen elicits pronounced $[Ca^{2+}]_i$ oscillations in T cells and related cell lines (93, 95, 114, 169). In many cells, including antigen-stimulated B cells (170), $[Ca^{2+}]_i$ oscillations appear to result from the repetitive release and reuptake of Ca^{2+} by intracellular stores (171, 172). T cell oscillations are unusual in that they absolutely require Ca^{2+} influx, which is believed to occur through CRAC channels (93, 114). In single Jurkat cells stimulated with PHA, I_{CRAC} oscillates

in tandem with periodic changes in $[Ca^{2+}]_i$ (93). A recent study suggests how the properties of CRAC channels may explain this behavior (130). Moderate release of stored Ca^{2+} by low doses of Ca^{2+}-ATPase inhibitors or a Ca^{2+} ionophore effectively induces $[Ca^{2+}]_i$ oscillations in human T cells similar to those triggered by mitogens. Both Ca^{2+} influx and the content of intracellular stores fluctuate in oscillating cells, supporting an oscillation model based on cross-communication between Ca^{2+} stores and CRAC channels in the plasma membrane (130). According to this model, a small Ca^{2+} leak from the ER, e.g. induced by IP_3, causes partial depletion that activates a fraction of the cell's CRAC channels. The ensuing influx elevates $[Ca^{2+}]_i$, promoting store refilling and subsequent channel closure. Ca^{2+} pumps in the plasma membrane then reduce $[Ca^{2+}]_i$ once more, enhancing efflux from the ER and triggering the next cycle. An essential element of any oscillation model is the presence of strong positive and negative feedback. K(Ca) channels may contribute feedback by generating membrane potential oscillations that would enhance Ca^{2+} influx during the rising phase (through hyperpolarization) and would diminish Ca^{2+} influx during the falling phase [as K(Ca) channels close and the membrane depolarizes]. Membrane-potential oscillations of this kind have been seen to accompany $[Ca^{2+}]_i$ oscillations in PHA-stimulated Jurkat cells (173).

What is the physiological function of $[Ca^{2+}]_i$ oscillations in T cells? A recent study addressed this question using an IL-2 reporter gene to determine the calcium dependence of IL-2 gene expression in single murine T hybridoma cells (94). IL-2 transcriptional activity was monitored with a *lacZ* gene driven by the NF-AT (Nuclear Factor of Activated T cells) enhancer, and $[Ca^{2+}]_i$ was elevated to steady levels using high doses of TG or allowed to fluctuate by stimulating with anti-CD3. In the presence of PMA, transcription was very sensitive to $[Ca^{2+}]_i$; a threefold increase in steady state $[Ca^{2+}]_i$ activated transcription in half the cells. In response to stimulation through the TCR, cells expressing *lacZ* tended to have larger and more frequent $[Ca^{2+}]_i$ spikes than *lacZ*$^-$ cells. These results show at a single-cell level the dependence of gene expression on $[Ca^{2+}]_i$ and suggest that $[Ca^{2+}]_i$ oscillations may serve to enhance the efficiency of signaling from the TCR to the nucleus.

TOWARD A MOLECULAR MECHANISM OF ANTIGEN-TRIGGERED CALCIUM SIGNALING

Progress over the past several years in understanding ion channels, Ca^{2+} regulation, and gene transcription in T cells suggests a model that links antigen recognition at the cell surface to the expression of specific genes in the nucleus through changes in $[Ca^{2+}]_i$ and PKC activity (Figure 1). Ca^{2+}-dependent signaling in lymphocytes may be considered to occur in three phases. First, molecular events at the cell surface commence with the binding of the peptide-MHC complex to the antigen receptor and include the subsequent stimulation

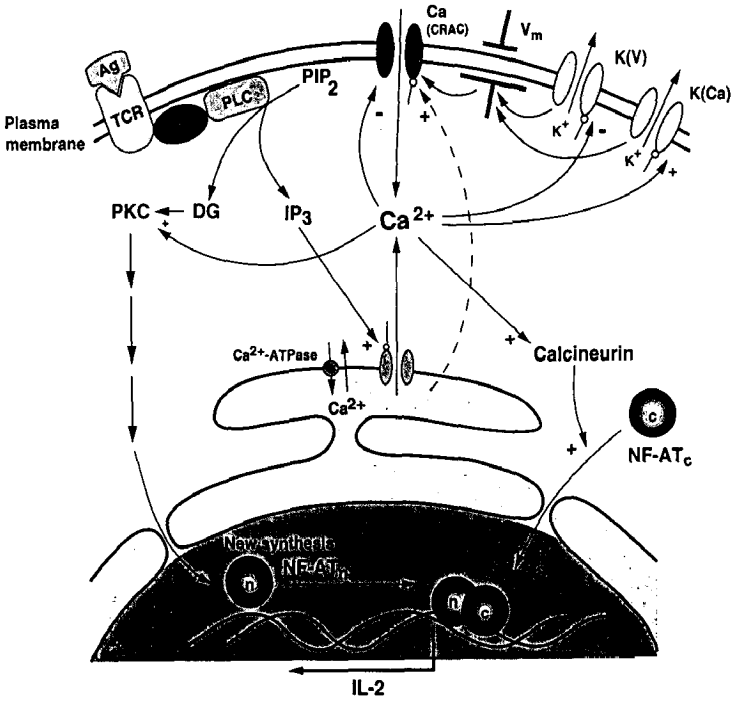

Figure 1 A cartoon of early events in T cell activation. Selected events from antigen binding to changes in ion channel activity to IL-2 gene expression are shown. Arrows labeled with + or − indicate stimulatory or inhibitory interactions, respectively.

of tyrosine kinases, phosphorylation and activation of PLCγ1, and hydrolysis of PIP_2 to produce IP_3 and diacylglycerol (reviewed in 106, 107). During the second phase, IP_3 carries the activation signal to the ER, where it releases stored Ca^{2+} through IP_3-activated channels. Depletion of stores triggers the opening of Ca^{2+} channels in the plasma membrane, leading to Ca^{2+} entry. Type *n* K(V) channels ensure the proper initiation of Ca^{2+} entry by maintaining the electrical gradient that helps drive Ca^{2+} into the cell. As $[Ca^{2+}]_i$ rises, K(Ca) channels open and may assume control of the membrane potential as K(V) channels undergo Ca^{2+}-dependent inactivation. Delayed communication between the ER and plasma-membrane CRAC channels, together with positive and negative feedback provided by K(Ca) channels and Ca^{2+}, may generate $[Ca^{2+}]_i$ oscillations under moderate stimulation conditions. The third phase of signal transduction connects these cytoplasmic events with gene transcription in the nucleus and probably occurs concomitantly with the first and second phases described above (98, 99, 107). Activated by diacylglycerol in concert with increased $[Ca^{2+}]_i$, PKC stimulates de novo synthesis of the nuclear-targeted component of NF-AT ($NF\text{-}AT_n$). In addition, the rise in $[Ca^{2+}]_i$ drives the

Ca^{2+}/calmodulin-dependent activation of the phosphatase calcineurin. Dephosphorylation of one or more substrates by calcineurin in turn promotes the translocation of a preexisting cytosolic NF-AT_c subunit across the nuclear membrane, where it combines with NF-AT_n to help drive expression of the IL-2 gene (for review, see 99, 107).

This model is certainly an oversimplification of the finely coordinated and complex sequence of parallel pathways engaged during T cell activation; it may, however, provide a useful framework for further studies of the roles of ion channels in shaping these events. As we attempt to describe the ionic signaling events that couple antigen recognition to the control of gene expression, important gaps in our understanding become apparent. The specific functions of K^+ channels in lymphocytes are not yet fully understood, nor have the ramifications of K^+-channel diversity, modulation, and developmental regulation been explained. Basic features of depletion-activated Ca^{2+} channels are obscure; how are these channels controlled at a molecular level, and how does feedback regulation of their activity create $[Ca^{2+}]_i$ oscillations? Finally, the meaning of $[Ca^{2+}]_i$ oscillations is a mystery. Do oscillations encode specificity by selecting certain response pathways over others, or are they simply a means of enhancing the efficiency of signal transduction under conditions of weak antigenic stimulation? Application of increasingly sensitive techniques of molecular physiology will help us answer these questions and unravel the intricate web that connects ion channels, intracellular messengers, and gene transcription in lymphocytes.

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Literature Cited

1. Grinstein S, Dixon SJ. 1989. Ion transport, membrane potential, and cytoplasmic pH in lymphocytes: changes during activation. *Physiol. Rev.* 69:417–81
2. Lewis RS, Cahalan MD. 1990. Ion channels and signal transduction in lymphocytes. *Annu. Rev. Physiol.* 52:413–28
3. Cahalan MD, Lewis RS. 1990. Functional roles of ion channels in lymphocytes. *Semin. Immunol.* 2:107–17
4. Deutsch C. 1990. K^+ channels and mitogenesis. *Prog. Clin. Biol. Res.* 334: 251–71
5. Gardner P. 1990. Patch clamp studies of lymphocyte activation. *Annu. Rev. Immunol.* 8:231–52
6. Premack BA, Gardner P. 1991. Role of ion channels in lymphocytes. *J. Clin. Immunol.* 11:225–38
7. Gallin EK. 1991. Ion channels in leuko-

cytes. *Physiol. Rev.* 71:775–811
8. Cahalan MD, Chandy KG, Grissmer S. 1991. Potassium channels in development, activation, and disease in T lymphocytes. *Curr. Top. Membr.* 39:357–94
9. Chandy KG, Gutman GA, Grissmer S. 1993. Physiological role, molecular structure and evolutionary relationships of voltage-gated potassium channels in T lymphocytes. *Sem. Neurosci.* 5:125–34
10. Cahalan MD, Lewis RS. 1994. Regulation of chloride channels in lymphocytes. *Curr. Top. Membr.* 42:103–29
11. Cahalan MD, Chandy KG, DeCoursey TE, Gupta S. 1985. A voltage-gated potassium channel in human T lymphocytes. *J. Physiol.* 358:197–237
12. Deutsch C, Krause D, Lee SC. 1986. Voltage-gated potassium conductance in human T lymphocytes stimulated with phorbol ester. *J. Physiol.* 372:405–23
13. Lewis RS, Cahalan MD. 1988. Subset-specific expression of potassium channels in developing murine T lymphocytes. *Science* 239:771–75
14. DeCoursey TE, Chandy KG, Gupta S, Cahalan MD. 1987. Two types of potassium channels in murine T lymphocytes. *J. Gen. Physiol.* 89:379–404
15. Chandy KG, Gutman GA. 1995. Voltage-gated potassium channel genes. In Ligand- and Voltage-gated Ion Channels, ed. RA North, pp. 1–71. Boca Raton, FL:CRC. In press
16. Douglass J, Osborne PB, Cai YC, Wilkinson M, Christie MJ, Adelman JP. 1990. Characterization and functional expression of a rat genomic DNA clone encoding a lymphocyte potassium channel. *J. Immunol.* 144:4841–50
17. Chandy KG, Williams CB, Spencer RH, Aguilar BA, Ghanshani S, Tempel BL, Gutman GA. 1990. A family of three mouse potassium channel genes with intronless coding regions. *Science* 247:973–75
18. Attali B, Romey G, Honore E, Schmid-Alliana A, Mattei MG, Lesage F, Ricard P, Barhanin J, Lazdunski M. 1992. Cloning, functional expression, and regulation of two K^+ channels in human T lymphocytes. *J. Biol. Chem.* 267:8650–57
19. Grissmer S, Dethlefs B, Wasmuth JJ, Goldin AL, Gutman GA, Cahalan MD, Chandy KG. 1990. Expression and chromosomal localization of a lymphocyte K^+ channel gene. *Proc. Natl. Acad. Sci. USA* 87:9411–15
20. Cai YC, Osborne PB, North RA, Dooley DC, Douglass J. 1992. Characterization and functional expression of genomic DNA encoding the human lymphocyte type *n* potassium channel. *DNA Cell Biol.* 11:163–72
21. Deutsch C, Chen LQ. 1993. Heterologous expression of specific K^+ channels in T lymphocytes: functional consequences for volume regulation. *Proc. Natl. Acad. Sci. USA* 90:10036–40
22. Grissmer S, Cahalan M. 1989. TEA prevents inactivation while blocking open K^+ channels in human T lymphocytes. *Biophys. J.* 55:203–6
23. Kavanaugh MP, Varnum MD, Osborne PB, Christie MJ, Busch AE, Adelman JP, North RA. 1991. Interaction between tetraethylammonium and amino acid residues in the pore of cloned voltage-dependent potassium channels. *J. Biol. Chem.* 266:7583–87
24. Busch AE, Hurst RS, North RA, Adelman JP, Kavanaugh MP. 1991. Current inactivation involves a histidine residue in the pore of the rat lymphocyte potassium channel RGK5. *Biochem. Biophys. Res. Commun.* 179:1384–90
25. Grissmer S, Cahalan MD. 1989. Divalent ion trapping inside potassium channels of human T lymphocytes. *J. Gen. Physiol.* 93:609–30
26. Luneau CJ, Williams JB, Marshall J, Levitan ES, Oliva C, Smith JS, Antanavage J, Folander K, Stein RB, Swanson R, Kaczmarek LK, Buhrow SA. 1991. Alternative splicing contributes to K^+ channel diversity in the mammalian central nervous system. *Proc. Natl. Acad. Sci. USA* 88:3932–36
27. Yokoyama S, Imoto K, Kawamura T, Higashida H, Iwabe N, Miyata T, Numa S. 1989. Potassium channels from NG108-15 neuroblastoma hybrid cells: primary structure and functional expression from cDNAs. *FEBS Lett.* 259:37–43
28. Grissmer S, Ghanshani S, Dethlefs B, McPherson JD, Wasmuth JJ, Gutman GA, Cahalan MD, Chandy KG. 1992. The Shaw-related potassium channel gene, Kv3.1, on human chromosome 11, encodes the type *l* K^+ channel in T cells. *J. Biol. Chem.* 267:20971–79
29. Lewis RS, Cahalan MD. 1988. The plasticity of ion channels: parallels between the nervous and immune systems. *Trends Neurosci.* 11:214–18
30. Grinstein S, Smith JD. 1990. Calcium-independent cell volume regulation in human lymphocytes. Inhibition by charybdotoxin. *J. Gen. Physiol.* 95:97–120
31. Cahalan MD, Lewis RS. 1988. Role of potassium and chloride channels in volume regulation by T lymphocytes. In *Cell Physiology of Blood. Soc. Gen. Physiol. Ser.*, ed. RB Gunn, JC Parker,

43:281–301. New York: Rockefeller Univ. Press
32. Deutsch C, Lee SC. 1988. Cell volume regulation in lymphocytes. *Renal Physiol. Biochem.* 3–5:260–76
33. Lee SC, Levy DI, Deutsch C. 1992. Diverse K^+ channels in primary human T lymphocytes. *J. Gen. Physiol.* 99:771–93
34. Cai YC, Douglass J. 1993. In vivo and in vitro phosphorylation of the T lymphocyte type *n* (Kv1.3) potassium channel. *J. Biol. Chem.* 268:23720–27
35. Honoré E, Attali B, Romey G, Lesage F, Barhanin J, Lazdunski M. 1992. Different types of K^+ channel current are generated by different levels of a single mRNA. *EMBO J.* 11:2465–71
36. Weiss A, Imboden JB. 1987. Cell surface molecules and early events involved in human T lymphocyte activation. *Adv. Immunol.* 41:1–38
37. Coffey RG, Hadden JW. 1985. Neurotransmitters, hormones, and cyclic nucleotides in lymphocyte regulation. *Fed. Proc.* 44:112–17
38. Plaut M. 1987. Lymphocyte hormone receptors. *Annu. Rev. Immunol.* 5:621–69
39. Premack BA, Gardner P. 1992. Signal transduction by T-cell receptors: mobilization of Ca and regulation of Ca-dependent effector molecules. *Am. J. Physiol.* 263:C1119–40
40. Lee SC, Deutsch C. 1990. Temperature dependence of K(+)-channel properties in human T lymphocytes. *Biophys. J.* 57:49–62
41. Pahapill PA, Schlichter LC. 1990. Modulation of potassium channels in human T lymphocytes: effects of temperature. *J. Physiol.* 422:103–26
42. Oleson DR, DeFelice LJ, Donahoe RM. 1993. A comparison of K^+ channel characteristics in human T cells: perforated-patch versus whole-cell recording techniques. *J. Membr. Biol.* 132:229–41
43. Bregestovski P, Redkozubov A, Alexeev A. 1986. Elevation of intracellular calcium reduces voltage-dependent potassium conductance in human T cells. *Nature* 319:776–78
44. Pahapill PA, Schlichter LC. 1992. Modulation of potassium channels in intact human T lymphocytes. *J. Physiol.* 445:407–30
45. Choquet D, Sarthou P, Primi D, Cazenave P-A, Korn H. 1987. Cyclic AMP-modulated potassium channels in murine B cells and their precursors. *Science* 235:1211–14
46. Kavanaugh MP, Christie MJ, Osborne PB, Busch AE, Shen K-Z, Wu Y-N, Seeburg PH, Adelman JP, North RA. 1991. Transmitter regulation of voltage-dependent K^+ channels expressed in *Xenopus* oocytes. *Biochem. J.* 277:899–902
47. Attali B, Honoré E, Lesage F, Lazdunski M, Barhanin J. 1992. Regulation of a major cloned voltage-gated K^+ channel from human T lymphocytes. *FEBS Lett.* 303:229–32
48. Aiyar J, Grissmer S, Chandy KG. 1993. Full-length and truncated Kv1.3 K^+ channels are modulated by 5-HT1c receptor activation and independently by PKC. *Am. J. Physiol.* 265:C1571–78
49. Choquet D, Korn H. 1988. Dual effects of serotonin on a voltage-gated conductance in lymphocytes. *Proc. Natl. Acad. Sci. USA* 85:4557–61
50. Rettig J, Heinemann SH, Wunder F, Lorra C, Parcej DN, Dolly JO, Pongs O. 1994. Inactivation properties of voltage-gated K^+ channels altered by presence of β subunit. *Nature* 369:289–94
51. Payet MD, Dupuis G. 1992. Dual regulation of the *n* type K^+ channel in Jurkat T lymphocytes by protein kinases A and C. *J. Biol. Chem.* 267:18270–73
52. Krause D, Lee SC, Deutsch C. 1988. Forskolin effects on the voltage-gated K^+ conductance of human T cells. *Pflügers Arch.* 412:133–40
53. Soliven B, Nelson DJ. 1990. Beta-adrenergic modulation of K^+ current in human T lymphocytes. *J. Membr. Biol.* 117:263–74
54. Tsien RY, Pozzan T, Rink TJ. 1982. T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. *Nature* 295:68–71
55. Grinstein S, Smith JD. 1989. Ca^{2+} induces charybdotoxin-sensitive membrane potential changes in rat lymphocytes. *Am. J. Physiol.* 257:C197–C206
56. Wilson HA, Chused TM. 1985. Lymphocyte membrane potential and Ca^{2+}-sensitive potassium channels described by oxonol dye fluorescence measurements. *J. Cell. Physiol.* 125:72–81
57. Rink TJ, Deutsch C. 1983. Calcium-activated potassium channels in lymphocytes. *Cell Calcium* 4:463–73
58. Mahaut-Smith MP, Schlichter LC. 1989. Ca(2+)-activated K^+ channels in human B lymphocytes and rat thymocytes. *J. Physiol.* 415:69–83
59. Mahaut-Smith MP, Mason MJ. 1991. Ca(2+)-activated K^+ channels in rat thymic lymphocytes: activation by concanavalin A. *J. Physiol.* 439:513–28
60. Partiseti M, Choquet D, Diu A, Korn H. 1992. Differential regulation of

voltage- and calcium-activated potassium channels in human B lymphocytes. *J. Immunol.* 148:3361–68
61. Partiseti M, Korn H, Choquet D. 1993. Pattern of potassium channel expression in proliferating B lymphocytes depends upon the mode of activation. *J. Immunol.* 151:2462–70
62. Grissmer S, Lewis RS, Cahalan MD. 1992. Ca^{2+}-activated K^+ channels in human leukemic T cells. *J. Gen. Physiol.* 99:63–84
63. Grissmer S, Nguyen AN, Cahalan MD. 1993. Calcium-activated potassium channels in resting and activated human T lymphocytes. *J. Gen. Physiol.* 102:601–30
64. Leonard RJ, Garcia ML, Slaughter RS, Reuben JP. 1992. Selective blockers of voltage-gated K^+ channels depolarize human T lymphocytes: mechanism of the antiproliferative effect of charybdotoxin. *Proc. Natl. Acad. Sci. USA* 89:10094–98
65. Latorre R, Oberhauser A, Labarca P, Alvarez O. 1989. Varieties of calcium-activated potassium channels. *Annu. Rev. Physiol.* 51:385–99
66. McKinnon D, Ceredig R. 1986. Changes in the expression of potassium channels during mouse T cell development. *J. Exp. Med.* 164:1846–61
67. DeCoursey TE, Chandy KG, Gupta S, Cahalan MD. 1987. Mitogen induction of ion channels in murine T lymphocytes. *J. Gen. Physiol.* 89:405–20
68. Deutsch C, Price M, Lee S, King VF, Garcia ML. 1991. Characterization of high affinity binding sites for charybdotoxin in human T lymphocytes. Evidence for association with the voltage-gated K^+ channel. *J. Biol. Chem.* 266: 3668–74
69. Shapiro MS, DeCoursey TE. 1988. Two types of potassium channels in a lymphoma cell line. *Biophys. J.* 53:a550
70. Sands SB, Lewis RS, Cahalan MD. 1989. Charybdotoxin blocks voltage-gated K^+ channels in human and murine T lymphocytes. *J. Gen. Physiol.* 93:1061–74
71. Price M, Lee SC, Deutsch C. 1989. Charybdotoxin inhibits proliferation and interleukin 2 production in human peripheral blood lymphocytes. *Proc. Natl. Acad. Sci. USA* 86:10171–75
72. Garcia-Calvo M, Leonard RJ, Novick J, Stevens SP, Schmalhofer W, Kaczorowski GJ, Garcia ML. 1993. Purification, characterization, and biosynthesis of margatoxin, a component of *Centruroides margaritatus* venom that selectively inhibits voltage-dependent potassium channels. *J. Biol. Chem.* 268:18866–74
73. Marx M, Weber M, Merkel F, Meyer zum Büschenfelde KH, Köhler H. 1990. Additive effects of calcium antagonists on cyclosporin A-induced inhibition of T-cell proliferation. *Nephrol. Dial. Transplant.* 5:1038–44
74. Deutsch CJ, Holian A, Holian SK, Daniele RP, Wilson DF. 1979. Transmembrane electrical and pH gradients across human erythrocytes and human peripheral lymphocytes. *J. Cell. Physiol.* 99:79–94
75. Ishida Y, Chused TM. 1993. Lack of voltage sensitive potassium channels and generation of membrane potential by sodium potassium ATPase in murine T lymphocytes. *J. Immunol.* 151:610–20
76. Maltsev VA. 1990. Oscillating and triggering properties of T cell membrane potential. *Immunol. Lett.* 26:277–82
77. Gelfand EW, Or R. 1991. Charybdotoxin-sensitive, Ca^{2+}-dependent membrane potential changes are not involved in human T or B cell activation and proliferation. *J. Immunol.* 147:3452–58
78. Lewis RS, Ross PE, Cahalan MD. 1993. Chloride channels activated by osmotic stress in T lymphocytes. *J. Gen. Physiol.* 101:801–26
79. Lee S, Price M, Prystowsky MB, Deutsch C. 1988. Volume response of quiescent and interleukin 2-stimulated T-lymphocytes to hypotonicity. *Am. J. Physiol.* 254:C286–96
80. Chandy KG, DeCoursey TE, Cahalan MD, McLaughlin D, Gupta S. 1984. Voltage-gated potassium channels are required for human T lymphocyte activation. *J. Exp. Med.* 160:369–85
81. Amigorena S, Choquet D, Teillaud JL, Korn H, Fridman WH. 1990. Ion channels and B cell mitogenesis. *Mol. Immunol.* 27:1259–68
82. Schlichter L, Sidell N, Hagiwara S. 1986. Potassium channels mediate killing by human natural killer cells. *Proc. Natl. Acad. Sci. USA* 83:451–55
83. Dubois J-M, Rouzaire-Dubois B. 1993. Role of potassium channels in mitogenesis. *Prog. Biophys. Mol. Biol.* 59:1–21
84. Nilius B, Droogmans G. 1994. A role for K^+ channels in cell proliferation. *News Physiol. Sci.* 9:105–10
85. Freedman BD, Price MA, Deutsch CJ. 1992. Evidence for voltage modulation of IL-2 production in mitogen-stimulated human peripheral blood lymphocytes. *J. Immunol.* 149:3784–94
86. Lin CS, Boltz RC, Blake JT, Nguyen M, Talento A, Fischer PA, Springer MS, Sigal NH, Slaughter RS, Garcia ML, Kaczorowski GJ, Koo GC.

1993. Voltage-gated potassium channels regulate calcium-dependent pathways involved in human T lymphocyte activation. *J. Exp. Med.* 177:637–45
87. Randriamampita C, Bismuth G, Debre P, Trautmann A. 1991. Nitrendipine-induced inhibition of calcium influx in a human T-cell clone: role of cell depolarization. *Cell Calcium* 12:313–23
88. Sarkadi B, Tordai A, Gárdos G. 1990. Membrane depolarization selectively inhibits receptor-operated calcium channels in human T (Jurkat) lymphoblasts. *Biochim. Biophys. Acta* 1027:130–40
89. Sarkadi B, Tordai A, Müller M, Gárdos G. 1990. Regulation of stimulus-induced calcium transport pathways in human T (Jurkat) lymphoblasts. *Mol. Immunol.* 27:1297–306
90. Gelfand EW, Cheung RK, Grinstein S. 1984. Role of membrane potential in the regulation of lectin-induced calcium uptake. *J. Cell. Physiol.* 121:533–39
91. Gray LS, Gnarra JR, Russell JH, Engelhard VH. 1987. The role of K^+ in the regulation of the increase of intracellular Ca^{2+} mediated by the T lymphocyte antigen receptor. *Cell* 50:119–27
92. Oettgen HC, Terhorst C, Cantley LC, Rosoff PM. 1985. Stimulation of the T3-T cell receptor complex induces a membrane-potential-sensitive calcium influx. *Cell* 40:583–90
93. Lewis RS, Cahalan MD. 1989. Mitogen-induced oscillations of cytosolic Ca^{2+} and transmembrane Ca^{2+} current in human leukemic T cells. *Cell Regul.* 1:99–112
94. Negulescu PA, Shastri N, Cahalan MD. 1994. Intracellular calcium dependence of gene expression in single T lymphocytes. *Proc. Natl. Acad. Sci. USA* 91:2873–77
95. Donnadieu E, Cefai D, Tan YP, Paresys G, Bismuth G, Trautmann A. 1992. Imaging early steps of human T cell activation by antigen-presenting cells. *J. Immunol.* 148:2643–53
96. Donnadieu E, Bismuth G, Trautmann A. 1994. Antigen recognition by helper T cells elicits a sequence of distinct changes of their shape and intracellular calcium. *Curr. Biol.* 4:584–95
97. Gelfand EW. 1990. Cytosolic calcium changes during T- and B-lymphocyte activation: biological consequences and significance. *Curr. Top. Membr. Transp.* 35:153–77
98. Crabtree GR. 1989. Contingent genetic regulatory events in T lymphocyte activation. *Science* 243:355–61
99. Rao A. 1994. NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. *Immunol. Today* 15:274–81
100. Poenie M, Tsien RY, Schmitt-Verhulst A-M. 1987. Sequential activation and lethal hit measured by $[Ca^{2+}]_i$ in individual cytolytic T cells and targets. *EMBO J.* 6:2223–32
101. Haverstick DM, Engelhard VH, Gray LS. 1991. Three intracellular signals for cytotoxic T lymphocyte-mediated killing. Independent roles for protein kinase C, Ca^{2+} influx, and Ca^{2+} release from internal stores. *J. Immunol.* 146:3306–13
102. McConkey DJ, Jondal M, Orrenius S. 1992. Cellular signaling in thymocyte apoptosis. *Semin. Immunol.* 4:371–77
103. Nakayama T, Ueda Y, Yamada H, Shores EW, Singer A, June CH. 1992. In vivo calcium elevations in thymocytes with T cell receptors that are specific for self ligands. *Science* 257:96–99
104. Schwartz RH. 1990. A cell culture model for T lymphocyte clonal anergy. *Science* 248:1349–56
105. Nghiem P, Ollick T, Gardner P, Schulman H. 1994. Interleukin-2 transcriptional block by multifunctional Ca^{2+}/calmodulin kinase. *Nature* 371:347–50
106. Weiss A, Littman DR. 1994. Signal transduction by lymphocyte antigen receptors. *Cell* 76:263–74
107. Crabtree GR, Clipstone NA. 1994. Signal transmission between the plasma membrane and nucleus of T lymphocytes. *Annu. Rev. Biochem.* 63:1045–83
108. Woodrow M, Clipstone NA, Cantrell D. 1993. p21ras and calcineurin synergize to regulate the nuclear factor of activated T cells. *J. Exp. Med.* 178:1517–22
109. Imboden JB, Stobo JD. 1985. Transmembrane signalling by the T cell antigen receptor. Perturbation of the T3-antigen receptor complex generates inositol phosphates and releases calcium ions from intracellular stores. *J. Exp. Med.* 161:446–56
110. Gelfand EW, Cheung RK, Mills GB, Grinstein S. 1988. Uptake of extracellular Ca^{2+} and not recruitment from internal stores is essential for T lymphocyte proliferation. *Eur. J. Immunol.* 18:917–22
111. Goldsmith MA, Weiss A. 1988. Early signal transduction by the antigen receptor without commitment to T cell activation. *Science* 240:1029–31
112. Llopis J, Chow SB, Kass GEN, Gahm A, Orrenius S. 1991. Comparison between the effects of the microsomal Ca^{2+} translocase inhibitors thapsigargin and

2,5-di-(t-butyl)-1,4- benzohydroquinone on cellular calcium fluxes. *Biochem. J.* 277:553–56
113. Mason MJ, Mahaut-Smith MP, Grinstein S. 1991. The role of intracellular Ca^{2+} in the regulation of the plasma membrane Ca^{2+} permeability of unstimulated rat lymphocytes. *J. Biol. Chem.* 266:10872–79
114. Donnadieu E, Bismuth G, Trautmann A. 1992. Calcium fluxes in T lymphocytes. *J. Biol. Chem.* 267:25864–72
115. McDonald TV, Premack BA, Gardner P. 1993. Flash photolysis of caged inositol 1,4,5-trisphosphate activates plasma membrane calcium current in human T cells. *J. Biol. Chem.* 268:3889–96
116. Putney JW Jr. 1990. Capacitative calcium entry revisited. *Cell Calcium* 11:611–24
117. Gouy H, Cefai D, Christensen SB, Debré P, Bismuth G. 1990. Ca^{2+} influx in human T lymphocytes is induced independently of inositol phosphate production by mobilization of intracellular Ca^{2+} stores. A study with the Ca^{2+} endoplasmic reticulum-ATPase inhibitor thapsigargin. *Eur. J. Immunol.* 20:2269–75
118. Mason MJ, Garcia-Rodriguez C, Grinstein S. 1991. Coupling between intracellular Ca^{2+} stores and the Ca^{2+} permeability of the plasma membrane. Comparison of the effects of thapsigargin, 2,5-di-(tert-butyl)-1,4-hydroquinone, and cyclopiazonic acid in rat thymic lymphocytes. *J. Biol. Chem.* 266:20856–62
119. Sarkadi B, Tordai A, Homolya L, Scharff O, Gárdos G. 1991. Calcium influx and intracellular calcium release in anti-CD3 antibody-stimulated and thapsigargin-treated human T lymphoblasts. *J. Membr. Biol.* 123:9–21
120. Montero M, Alvarez J, Garcia-Sancho J. 1990. Uptake of Ca^{2+} and refilling of intracellular Ca^{2+} stores in Ehrlich-ascites-tumour cells and in rat thymocytes. *Biochem. J.* 271:535–40
121. Zweifach A, Lewis RS. 1993. Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. *Proc. Natl. Acad. Sci. USA* 90:6295–99
122. Kuno M, Goronzy J, Weyand CM, Gardner P. 1986. Single-channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper T lymphocytes. *Nature* 323:269–73
123. Kuno M, Gardner P. 1987. Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature* 326:301–4
124. Premack BA, McDonald TV, Gardner P. 1994. Activation of Ca^{2+} current in Jurkat T cells following the depletion of Ca^{2+} stores by microsomal Ca^{2+}-ATPase inhibitors. *J. Immunol.* 152:5226–40
125. Partiseti M, Le Deist F, Hivroz C, Fischer A, Korn H, Choquet D. 1994. The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. *J. Biol. Chem.* 269:32327–35
126. Chow SC, Kass GEN, Orrenius S. 1993. Two independently regulated Ca^{2+} entry mechanisms coexist in Jurkat T cells during T cell receptor antigen activation. *Biochem. J.* 293:395–98
127. Deleted in proof
128. Gardner P, Alcover A, Kuno M, Moingeon P, Weyand CM, Goronzy J, Reinherz EL. 1989. Triggering of T-lymphocytes via either T3-Ti or T11 surface structures opens a voltage-insensitive plasma membrane calcium-permeable channel: requirement for interleukin-2 gene function. *J. Biol. Chem.* 264:1068–76
129. Khan AA, Steiner JP, Klein MG, Schneider MF, Snyder SH. 1992. IP_3 receptor: localization to plasma membrane of T cells and cocapping with the T cell receptor. *Science* 257:815–18
130. Dolmetsch R, Lewis RS. 1994. Signaling between intracellular Ca^{2+} stores and depletion-activated Ca^{2+} channels generates $[Ca^{2+}]_i$ oscillations in T lymphocytes. *J. Gen. Physiol.* 103:365–88
131. Gelfand EW, Cheung RK, Grinstein S. 1986. Mitogen-induced changes in Ca^{2+} permeability are not mediated by voltage-gated K^+ channels. *J. Biol. Chem.* 261:11520–23
132. Lewis RS, Cahalan MD. 1988. Voltage-dependent calcium signalling in single T lymphocytes. *Soc. Neurosci. Abstr.* 14:298
133. DeCoursey TE, Chandy KG, Gupta S, Cahalan MD. 1984. Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis? *Nature* 307:465–68
134. Matteson DR, Deutsch C. 1984. K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. *Nature* 307:468–71
135. Dupuis G, Héroux J, Payet MD. 1989. Characterization of Ca^{2+} and K^+ currents in the human Jurkat T cell line: effects of phytohaemagglutinin. *J. Physiol.* 412:135–54

136. Densmore JJ, Szabo G, Gray LS. 1992. A voltage-gated calcium channel is linked to the antigen receptor in Jurkat T lymphocytes. *FEBS Lett.* 312:161–64
137. O'Riordan C, Rosoff PM. 1993. Reconstitution of a T cell receptor-stimulated plasma membrane calcium transporter: lack of dependence on inositol phosphates. *Cell Calcium* 14:119–33
138. Martino G, Clementi E, Brambilla E, Moiola L, Comi G, Meldolesi J, Grimaldi LM. 1994. γ interferon activates a previously undescribed Ca^{2+} influx in T lymphocytes from patients with multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 91:4825–29
139. Bubien JK, Zhou LJ, Bell P, Frizzell RA, Tedder TF. 1993. Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca^{2+} conductance found constitutively in B lymphocytes. *J. Cell Biol.* 121:1121–32
140. Smeland E, Godal T, Rudd E, Beiske K, Funderud S, Clark EA, Pfeifer-Ohlsson S, Ohlsson R. 1985. The specific induction of myc protooncogene expression in normal human B cells is not a sufficient event for acquisition of competence to proliferate. *Proc. Natl. Acad. Sci. USA* 82:6255–59
141. Kansas GS, Tedder TF. 1991. Transmembrane signals generated through MHC class II, CD19, CD20, CD39, and CD40 antigens induce LFA-1-dependent and -independent adhesion in human B cells through a tyrosine kinase-dependent pathway. *J. Immunol.* 147:4094–102
142. Hoth M, Penner R. 1992. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 355:353–56
143. Hoth M, Penner R. 1993. Calcium release-activated calcium current in mast cells. *J. Physiol.* 465:359–86
144. Parekh AB, Terlau H, Stühmer W. 1993. Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. *Nature* 364:814–18
145. Hoth M, Fasolato C, Penner R. 1993. Ion channels and calcium signaling in mast cells. *Ann. NY Acad. Sci.* 707:198–209
146. Fasolato C, Innocenti B, Pozzan T. 1994. Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels? *Trends Pharmacol. Sci.* 15:77–83
147. Byerly L, Hagiwara S. 1988. Calcium channel diversity. In *Calcium and Ion Channel Modulation,* ed. AD Grinnell, D Armstrong, MB Jackson, pp. 3–18. New York: Plenum
148. Franzius D, Hoth M, Penner R. 1994. Non-specific effects of calcium entry antagonists in mast cells. *Pflügers Arch.* 428:433–38
149. Mason MJ, Mayer B, Hymel LJ. 1993. Inhibition of Ca^{2+} transport pathways in thymic lymphocytes by econazole, miconazole, and SKF 96365. *Am. J. Physiol.* 264:C654–62
150. Chung SC, McDonald TV, Gardner P. 1994. Inhibition by SK&F 96365 of Ca^{2+} current, IL-2 production and activation in T lymphocytes. *Br. J. Pharmacol.* 113:861
151. DeCoursey TE, Chandy KG, Gupta S, Cahalan MD. 1985. Voltage-dependent ion channels in T-lymphocytes. *J. Neuroimmunol.* 10:71–95
152. Wacholtz MC, Cragoe EJ Jr, Lipsky PE. 1993. Delineation of the role of a Na^+/Ca^{2+} exchanger in regulating intracellular Ca^{2+} in T cells. *Cell. Immunol.* 147:95–109
153. Donnadieu E, Trautmann A. 1993. Is there a Na^+/Ca^{2+} exchanger in macrophages and in lymphocytes? *Pflügers Arch.* 424:448–55
154. Ng J, Fredholm BB, Jondal M, Andersson T. 1988. Regulation of receptor-mediated calcium influx across the plasma membrane in a human leukemic T-cell line: evidence of its dependence on an initial calcium mobilization from intracellular stores. *Biochim. Biophys. Acta* 971:207–14
155. Ng J, Gustavsson J, Jondal M, Andersson T. 1990. Regulation of calcium influx across the plasma membrane of the human T-leukemic cell line, JURKAT: dependence on a rise in cytosolic free calcium can be dissociated from formation of inositol phosphates. *Biochim. Biophys. Acta* 1053:97–105
156. Haverstick DM, Gray LS. 1993. Increased intracellular Ca^{2+} induces Ca^{2+} influx in human T lymphocytes. *Mol. Biol. Cell* 4:173–84
157. Alvarez J, Montero M, García-Sancho J. 1991. Cytochrome P-450 may link intracellular Ca^{2+} stores with plasma membrane Ca^{2+} influx. *Biochem. J.* 274:193–97
158. Koch BD, Faurot GF, Kopanitsa MV, Swinney DC. 1994. Pharmacology of a Ca^{2+} influx pathway activated by emptying the intracellular Ca^{2+} stores in HL-60 cells: evidence that a cytochrome P-450 is not involved. *Biochem. J.* 302:187–90
159. Randriamampita C, Tsien RY. 1993. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that

stimulates Ca^{2+} influx. *Nature* 364:809–14

160. Bird GSJ, Putney JW Jr. 1993. Inhibition of thapsigargin-induced calcium entry by microinjected guanine nucleotide analogues. *J. Biol. Chem.* 268:21486–88
161. Fasolato C, Hoth M, Penner R. 1993. A GTP-dependent step in the activation mechanism of capacitative calcium influx. *J. Biol. Chem.* 268:20737–40
162. Vostal JG, Jackson WL, Shulman NR. 1991. Cytosolic and stored calcium antagonistically control tyrosine phosphorylation of specific platelet proteins. *J. Biol. Chem.* 266:16911–16

162a. Lee K-M, Toscas K, Villereal ML. 1993. Inhibition of bradykinin- and thapsigargin-induced Ca^{2+} entry by tyrosine kinase inhibitors. *J. Biol. Chem.* 268:9945–48

162b. Sargeant P, Farndale RW, Sage SO. 1994. Calcium store depletion in dimethyl BAPTA-loaded human platelets increases protein tyrosine phosphorylation in the absence of a rise in cytosolic calcium. *Exp. Physiol.* 79:269–72

163. Xu X, Star RA, Tortorici G, Muallem S. 1994. Depletion of intracellular Ca^{2+} stores activates nitric-oxide synthase to generate cGMP and regulate Ca^{2+} influx. *J. Biol. Chem.* 269:12645–53
164. Bahnson TD, Pandol SJ, Dionne VE. 1993. Cyclic GMP modulates depletion-activated Ca^{2+} entry in pancreatic acinar cells. *J. Biol. Chem.* 268:10808–12
165. Zweifach A, Lewis RS. 1995. Rapid inactivation of depletion-activated calcium current (I_{CRAC}) due to local calcium feedback. *J. Gen. Physiol.*, In press
166. Zweifach A, Lewis RS. 1994. Two mechanisms of Ca^{2+}-dependent inactivation of depletion-activated Ca^{2+} channels. *Biophys. J.* 66:A153
167. Choquet D, Partiseti M, Amigorena S, Bonnerot C, Fridman WH, Korn H. 1993. Cross-linking of IgG receptors inhibits membrane immunoglobulin-stimulated calcium influx in B lymphocytes. *J. Cell Biol.* 121:355–63
168. Diegel ML, Rankin BM, Bolen JB, Dubois PM, Kiener PA. 1994. Cross-linking of Fc gamma receptor to surface immunoglobulin on B cells provides an inhibitory signal that closes the plasma membrane calcium channel. *J. Biol. Chem.* 269:11409–16
169. Hess SD, Oortgiesen M, Cahalan MD. 1993. Calcium oscillations in human T and natural killer cells depend upon membrane potential and calcium influx. *J. Immunol.* 150:2620–33
170. Wilson HA, Greenblatt D, Poenie M, Finkelman FD, Tsien RY. 1987. Cross-linkage of B lymphocyte surface immunoglobulin by anti-Ig or antigen induces prolonged oscillations of intracellular ionized calcium. *J. Exp. Med.* 166:601–6
171. Berridge MJ. 1993. Inositol trisphosphate and calcium signalling. *Nature* 361:315–25
172. Fewtrell C. 1993. Ca^{2+} oscillations in non-excitable cells. *Annu. Rev. Physiol.* 55:427–54
173. Lewis RS, Grissmer S, Cahalan MD. 1991. Role of K^+ channels in regulating calcium signaling in leukemic T lymphocytes. *Biophys. J.* 59:13a

ANNUAL REVIEW OF IMMUNOLOGY
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CONTENTS

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