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Molecular Properties and Physiological Roles of Ion Channels in the Immune System

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The discovery of a diverse and unique set of ion channels in T lymphocytes has led to a rapidly growing body of knowledge about their functional roles in the immune system. Here we review the biophysical and molecular characterization of K^+, Ca^{2+}, and Cl^- channels in T lymphocytes. Potent and specific blockers, especially of K^+ channels, have provided molecular tools to elucidate the involvement of voltage- and calcium-activated potassium channels in T-cell activation and cell-volume regulation. Their unique and differential expression makes lymphocyte K^+ channels excellent pharmaceutical targets for modulating immune system function. This review surveys recent progress at the biophysical, molecular, and functional roles of the ion channels found in T lymphocytes.

KEY WORDS: T-cell activation; potassium channel; calcium channel; immunosuppression; toxins; (patch-clamp).

ELECTROPHYSIOLOGY: BIOPHYSICAL FINGERPRINTING

Development of the patch clamp technique by Neher and Sakman (Nobel prize winners in 1991) enabled the exploration of ion channels in a wide variety of cell types, including hematopoietic cells. By the mid 1980s, several groups were characterizing channels from red blood cells, T and B lymphocytes, macrophages, mast cells, and neutrophils. The patch clamp technique makes it possible to measure currents across a cell membrane with single channel resolution on a submillisecond time scale. A variety of recording configurations including whole-cell, on-cell (cell-attached), excised outside-out or inside-out patches, and perforated-patch recording allowed cells to be phenotyped for expression of an impressive variety of novel channel types. Ion channels are classified first according to their ion selectivity for permeant ions that pass through the channel. Thus, channels selective for K^+, Na^+, Ca^{2+}, Cl^-, and H^+ have been identified in various cells of the body. Distinguishing properties of an ion channel include the single-channel conductance (a measure of how many ions per second can pass through the channel—typically for ion channels this number is 10^6 or greater), how this conductance is affected by the concentration of permeant ions, the sequence of permeability or conductance for different ions, the size of the channel judged from the bulkiest permeant ion, and the "rectification" of the open channel (favoring inward or outward movement of ions). Then specific types of selective channels are characterized according to their gating mechanisms—how they can be opened and closed. For example, voltage-dependent channels are activated by depolarization of the membrane potential from its normal resting level (about -50 mV in T cells). Other channels are activated by cytosolic calcium or by other intracellular messengers. A third distinguishing characteristic of ion channels is their sensitivity to various channel blockers including metal ions, toxins, and organic compounds. Channel blockade can be accomplished by the agent directly plugging the channel pore or by altering the ability of the gates to move. Through its biophysical fingerprint (ion selectivity, kinetic features of gating, and sensitivity to pharmacological blockade), an ion channel can be unambiguously identified solely from electrophysiological experiments.

Voltage-Gated K^+ (K_V) Channels

From the initial patch clamp studies on T lymphocytes, the most commonly observed K_V channel was clearly related to delayed rectifier channels in excitable cells such as nerve and muscle (1, 2). But the lymphocyte K_V channel also exhibited some unusual properties

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including an unusually broad sensitivity to a variety of pharmacological agents and use-dependent or frequency-dependent inactivation (the more frequently the channels are used, the less likely they are to open). By recording from a variety of murine thymocyte and mature T cell subsets, it was possible to distinguish three distinct K_V channels called type *n* (normal) for the most commonly observed type, *n′* (nearly normal) for a channel that resembled type *n* but differed in the extent of use dependent inactivation and some pharmacological sensitivities, and *l* (large-conductance) for a larger conductance channel with a distinctly different voltage activation threshold (3, 4). The most important of these K_V channels is the *n*-type channel, which is encoded by the *Kv1.3* gene, as described below.

Calcium-Activated K^+ (K_{Ca}) Channels

Two different Ca^{2+}-activated K^+ channels were described in the early 1990s—a small-conductance channel found particularly in Jurkat T cells and an intermediate-conductance channel found in normal human and murine T cells, as well as in B lymphocytes (5–7). These channels are activated by a rise in cytosolic Ca^{2+} ($[Ca^{2+}]_i$).[3] The Ca^{2+} sensitivity is steep. At the resting $[Ca^{2+}]_i$ level of ~100 n*M*, all of the K_{Ca} channels are closed, but a 10-fold rise, to 1 μM, opens them all. The exact Ca^{2+} dependence has been measured by dialyzing buffered Ca^{2+}–EGTA mixtures into cells, by simultaneously monitoring $[Ca^{2+}]_i$ with the Ca^{2+} indicator fura-2 while measuring K_{Ca} currents and by exposing inside-out patches to varying Ca^{2+} levels. The IKCa1 gene encodes the intermediate conductance KCa channel in human lymphocytes, while the small-conductance K_{Ca} channel in Jurkat T cells is a product of the *SKCa2* gene.

[3] *Abbreviations used:* TCR, T-cell receptor; $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; IL-2, interleukin-2; K_V channel, voltage-gated K^+ channel; K_{Ca} channel, calcium-activated K^+; CRAC channel, calcium release-activated Ca^{2+} channel; SWAC channel, swelling-activated Ca^{2+}-permeable channel; ChTX, charybdotoxin; TEA, tetraethylammonium; ShK, *Stichodactyla helianthus* toxin; DAP, diaminopropionic acid; EAE, experimental autoimmune encephalomyelitis; AP1, heterodimer of C-Fos and C-Jun; CN, calcineurin; NFAT, nuclear factor of activated T cells; CsA, cyclosporin A; RVD, regulatory volume decrease; PKC, protein kinase C; PKA, protein kinase A; TK, tyrosine kinase; IP_3, 1,4,5-inositotriphosphate; PHA, phytohemagglutinin; TM, transmembrane; NMR, nuclear magnetic resonance; CaM, calmodulin; CCE, capacitive Ca^{2+} entry; SOC, store operated Ca^{2+}; CIF, calcium influx factory; BoTX, botulinum toxin; ER, endoplasmic reticulum; MBP, myelin basic protein; APC, antigen presenting cells; MHC, major histocompatibility complex; TG, thapsigargin; JNK, jun kinase; EST, expressed sequence tag.

Calcium Release-Activated Calcium (CRAC) Channels

Right from the start of patch clamp investigation of lymphocytes, researchers searched for calcium influx channels but were unable to detect conventional voltage-gated calcium channels of the type found in heart or in neurons. However, calcium-imaging studies convincingly demonstrated that calcium influx was evoked by mitogenic lectins such as phytohemagglutinin (PHA). A small, highly selective calcium conductance activated by PHA or by whole-cell dialysis with Ca^{2+} buffers was described by Lewis and Cahalan (8). A similar conductance was found in mast cells and named the CRAC channel (9). CRAC channels are activated indirectly by stimuli that result in depletion of Ca^{2+} from intracellular stores, including IP_3, ionomycin, and SERCA pump inhibitors such as thapsigargin, in addition to receptor activation or passive store depletion during dialysis by Ca^{2+} buffers (10, 11; reviewed in Ref. 12). The CRAC channel carries a tiny current of calcium ions into the cell that sustains the calcium signal and leads to lymphocyte activation. Their physiological importance for the immune response was reinforced by the discovery that CRAC channels are absent in some patients with severe immunodeficiency disorders (13, 14).

Swelling-Activated Cl^- (Cl_{swell}) Channels

By accident, in the late 1980s we discovered a novel-class of Cl^- channels activated by cell swelling that function as the trigger for RVD (15). A slight mismatch of the bath and pipette solution osmolarity, along with the presence of ATP in the pipette, permitted the activation of a large anion-selective conductance in response to cell swelling. This channel is perhaps the most abundant channel in T cells and it plays a critical role in homeostatic volume regulation.

Other Channels

In addition to the four main channels described above, a variety of other channel types has been reported, including cation channels activated by cell swelling, cation channels activated by extracellular ATP, and proton channels that are gated by voltage (16–18). Proton channels play an important role in neutrophils and macrophages during the respiratory burst (19–21), but their role in T-cell physiology is uncertain. In addition to the surface membrane channels, there are channels found in intracellular organelles, including, most importantly, the IP_3-receptor gated channel that provides for release

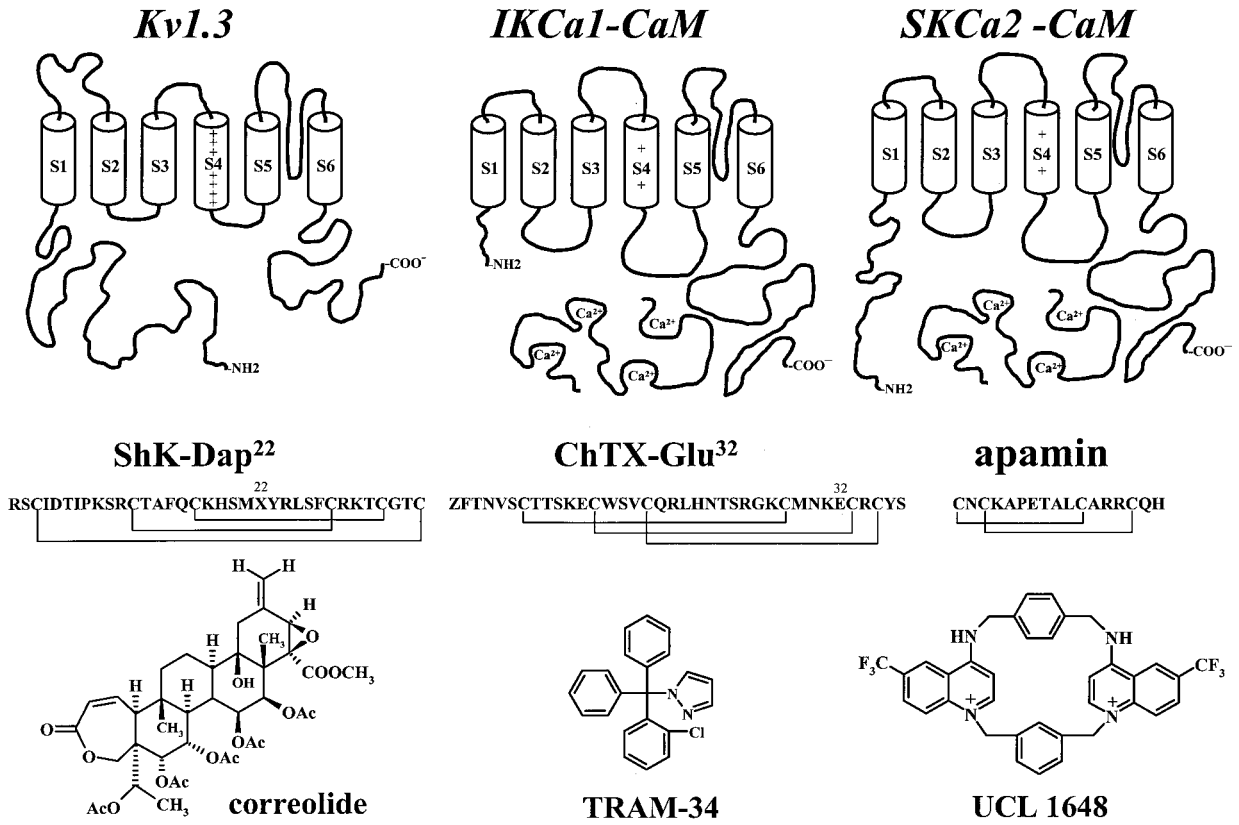

Fig. 1. Scheme of Kv1.3, IKCa1, and SKCa2, with their major functional domains. Also shown are the structures of their most potent polypeptide and small-molecule inhibitors. Kv1.3: ShK-Dap^{22} K_d = 52 p*M*, correolide K_d = 90 n*M*. IKCa1: ChTX-Glu^{32} K_d = 33 n*M*, TRAM-34 K_d = 20 n*M*. SKCa2: apamin K_d = 200 p*M*, UCL = 1684 K_d = 200 p*M*.

of calcium from the intracellular store that initiates the calcium signal.

MOLECULAR IDENTIFICATION AND STRUCTURE–FUNCTION ANALYSIS OF LYMPHOCYTE ION CHANNELS

Potassium Channels (in General)

Molecular cloning, initially in *Drosophila* and later by homology from mammalian cDNA and genomic libraries, led to the identification of a diverse family of K^+ channels in humans. The pace of discovery intensified with the advent of functional genomic approaches, especially the availability of EST and the human genomic DNA databases. Humans are currently thought to possess at least 80 different K^+ channel genes that encode proteins that can be grouped into four structural classes. The largest class is comprised of proteins containing six transmembrane (TM) segments (S1–S6) with the N- and C-termini on the cytoplasmic side of the membrane (Fig. 1). The S4 segment forms the voltage sensor of the K_V channels, while the P-loop ("pore" region, located between S5 and S6) and the S6 segment together form the ion conduction pathway. Residues in the outer vestibule between S5 and S6 also constitute the binding site for peptide toxins and for the classical blocker TEA (22–26). The functional channel is a tetramer. Based on phylogenetic analysis, the 46 proteins in this class have been clustered into 19 subfamilies (http://www.ucihs.uci.edu/pandb/channels). These proteins include the voltage-gated and calcium-activated K^+ channels, and mutations of these genes are known to underlie the long QT syndrome, benign familial convulsions, and other genetic disorders. A second structural class consists of proteins with two TMs (M1 and M2) and a P-loop, and the functional channel is a tetramer. These proteins include the inward rectifier K^+ channels that repolarize the membrane during the cardiac action potential as well as the ATP-modulated channels that are targets for antidiabetic and antihypertensive medications. The proteins that belong to this class have been organized into six

subfamilies based on phylogenetic criteria (27, 28). The third and fourth structural classes consist of proteins with two P-loops and the functional channels are thought to be dimers.

The biophysical properties of cloned K^+ channels have been determined by heterologously expressing the genes in *Xenopus* oocytes or in mammalian cells and then analyzing the proteins using electrophysiological methods. Each cloned channel has a unique "fingerprint" of biophysical and pharmacological properties and each gene has a unique tissue distribution. Thus, mammalian cells use a functionally diverse superfamily of K^+ channel proteins to fine-tune the regulation of membrane potential and signaling cascades. Additional functional diversity results from the heteromultimeric assembly of subunits encoded by different genes and, also, by the association of these α-subunits with accessory proteins.

Voltage-Gated K^+ Channels

Comparison of the biophysical "fingerprints" of the cloned K_V genes with native currents in T cells, coupled with molecular studies, led to the discovery of the *Kv1.3* gene that encodes the type *n* channel (29–32). Functional lymphocyte K_V channels are composed of four identical *Kv1.3* subunits (31, 33–37). The closely related *Kv1.1* gene is present in mouse thymocytes, while the distantly related *Kv3.1* gene is present in resting $CD8^+$ mouse T cells and is abnormally overexpressed in $CD4^-CD8^-TCR^+$ T cells from autoimmune mice. Despite extensive efforts, the human *Kv3.1* homologue has not been found in human T cells, although the human B-cell leukemia line Louckes expresses this channel in abundance (38).

Overall Topology of the Tetramer

Each subunit is comprised of a hydrophobic core consisting of six TM segments (S1–S6) and a P-loop between S5 and S6 and long hydrophilic N- and C-termini that extend into the cytoplasm. Low-resolution electron micrographs of purified *Kv1.3* tetramers reveal x–y dimensions of 64 Å.

Ion Conduction Pathway

The channel's ion conduction pathway lies at the center of the tetramer. At the outer entrance to the channel pore forms a shallow vestibule to which bind several peptide toxin inhibitors derived from scorpion venoms and marine extracts. The NMR structures of these toxins have been determined. Using a complementary mutagenesis approach, several contact points were identified between these structurally defined toxins and the Kv1.3 channel. Knowing the distance between key residues in these toxins, the dimensions of the external vestibule of Kv1.3 have been deduced to be 28 Å wide and 6–8 Å deep. The outer entrance to the Kv1.3 pore is estimated to be ~9–14 Å wide, tapering to a width of 4–5 Å at a depth of 5–7 Å from the vestibule. These predicted dimensions proved to be remarkably accurate when compared with the crystal structure of the phylogenetically related, proton-activated, bacterial K^+ channel, KcsA, published 3 years later (39, 40). Molecular models of the Kv1.3 pore have been generated based on its architectural similarity to the KcsA structure (41, 42). The outer third of the KcsA pore is lined by the four P-loops and forms a narrow, ~3-Å-wide, K^+-selective filter (39). The inner two-thirds of the pore is lined by the four cytoplasmic halves of S6. The S6 segments are arranged like an inverted teepee and cross each other at the cytoplasmic end of the pore. This "bundle crossing" is thought to constitute the channel gate (43), which has to open for K^+ ions to transverse the pore.

Voltage Sensor

A voltage-dependent process is responsible for opening of the gate in Kv1.3. Mutagenesis studies suggest that the S4 segment containing seven repeats of the motif Arg–X–X–Arg, along with residues in S2 and S3, constitute the voltage sensor of the channel (44–46). Changes in membrane potential induce a conformational change in the Kv1.3 protein involving the movement of the charged residues in S4, which in turns leads to the opening of the channel gate. The recent structure of the voltage-gated sodium channel from the electric eel suggests that the S4 segments reside in a "tunnel" that runs through the protein (47).

Inactivation

Kv1.3 channels inactivate during prolonged or repetitive episodes of depolarization. The type of inactivation is distinct from N-terminal ball-and-chain inactivation in *Shaker* and several other mammalian K^+ channels, because it can be abolished by extracellular TEA and involves residues near the external vestibule of the channel (48, 49). The dihydoquinolone and piperidine compounds CP-339818 and UK-78282 (see below) preferentially interact with the C-type inactivated state of the channel, and point mutations in the external vestibule that abolish inactivation also render the channel resistant to these compounds (50, 51).

Tetramerization Domain and Accessory Proteins

An N-terminal segment in the Kv1.3 channel called the T1 domain contributes to the formation of the

channel tetramer along with residues in the hydrophobic core. This segment forms stable tetramers when purified on its own, and its structure has been determined (52, 53). The T1 tetramer is physically associated with a tetramer of accessory proteins composed of $K_v\beta$ subunits and a structure of the *Shaker* T1 domain complexed to the $K_v\beta_1$ tetramer has been determined (54). The Kv1.3 channel is associated with the related $K_v\beta_2$ protein (55–58). A low-resolution image of $K_v\beta_2$ tetramers has been obtained from studies with atomic force and electron microscopy techniques. The square-shaped tetrameric complex has x–y dimensions of 100 × 100 Å and a height of 51 Å (59). $K_v\beta_2$ proteins are known to stabilize K^+ channel tetramers (60) and physically couple the channel to signaling molecules including PKC and the p56^{lck} tyrosine kinase via the adaptor proteins ZIP1 and ZIP2 (61). During T-cell mitogenesis, $K_v\beta_2$ mRNA expression increases substantially, and this increase is reflected in an enhancement of Kv1.3 channel expression. The three C-terminal residues of *Kv1.3* (Thr/Asp/Val) are associated with the PDZ-domain proteins hDlg and PSD-95 (62, 63); the structure of a PDZ domain complexed to a peptide containing the Thr–X–Val motif has been determined (64). The hDlg protein has also been reported to link Kv1.3 to the p*56*^{lck} tyrosine kinase (62). Thus, the Kv1.3 channel could be physically linked to p*56*^{lck} via its N and C termini. Functional coupling between p*56*^{lck} and the *Kv1.3* channel has also been demonstrated (65, 66). Since p*56*^{lck} is known to associate with the CD4 accessory protein, one can envision a large complex consisting of the Kv1.3 channel, critical signaling molecules, and the CD4 protein (depicted in Fig. 2). Kv1.3 channels have also been reported to be associated with β-integrins (67).

Calcium-Activated K^+ Channels

An EST-based approach led to the discovery of a subfamily of four K_{Ca} genes called *SKCa1-3* and *IKCa1*. The *IKCa1* gene on human chromosome 19q13.2 is expressed in human T cells and stem cells (accession number AA558247) and encodes a channel with biophysical properties identical to those of the intermediate conductance K_{Ca} channel in these cells (68–72). The *SKCa2* gene on human chromosome 5q23.1–q23.2 is expressed in the human Jurkat T cell line and encodes the small-conductance K_{Ca} channel in these cells (73–75). The *SKCa3* gene on human chromosome 1q21 is expressed in CD20$^+$ germinal-center B lymphocytes (accession numbers AA767647, AA741463, AA731772, AA490752, AA256031) and gene array analysis suggests that it is overexpressed in follicular B-cell lymphomas (76) (http://genome-www4.stanford.edu/MicroArray/SMD). A *SKCa3* EST has also been isolated from mouse thymocytes (accession number AI121990).

Overall Topology

Each IKCa1, SKCa2, and SKCa3 subunit is organized in a fashion analogous to that of *Kv1.3*, encompassing the six TMs and a P-loop (Fig. 1). The topology of the external vestibule of IKCa1 inferred from mapping studies with the peptide toxins, charybdotoxin and ShK toxin, indicate that the IKCa1, K_V, and KcsA channels are architecturally conserved in this region (42). The S4 segments of IKCa1, SKCa2, and SKCa3 contain fewer charged residues, consistent with their lack of voltage dependence. Instead, these channels are opened in response to a rise in intracellular Ca^{2+}.

Calmodulin, the Calcium Sensor

Each IKCa1, SKCa2, and SKCa3 subunit is tightly bound via its C-terminus to the Ca^{2+} binding protein calmodulin (CaM) (77, 78). Unlike typical CaM–protein associations, K_{Ca} channels bind CaM even in the absence of calcium. The site where CaM binds these channels lies 6–60 residues distal to the channel gate formed by the "bundle crossing" in S6. This C-terminal region is thought to be α-helical and any Ca^{2+}–CaM-induced conformational change could conceivably be transmitted along the helical rod, resulting in channel opening. When cytoplasmic Ca^{2+} rises from its resting level of ~100 n*M*, the K_{Ca} channels are activated via a mechanism involving Ca^{2+} binding to the EF hands of CaM (~K_d for Ca^{2+} binding, 300 n*M*), which possibly causes a conformational change leading to the opening of the channel pore (77, 78).

Tetramerization Domain

The site of tetramerization of K_{Ca} channels has not been determined. In K_V channels, overexpression of an N-terminal fragment containing the T1 tetramerization domain results in dominant-negative suppression due to the coassembly of truncated fragments with full-length subunits in nonfunctional tetramers. In a recent study, we were able to demonstrate similar dominant-negative suppression of SKCa2 currents in Jurkat T cells by overexpression of truncated N-terminal fragments of SKCa2 or SKCa3, indicating that SK_{Ca} channels, like K_V channels, are likely to contain an N-terminal tetramerization domain (75). The IKCa1 channel may lack an N-terminal T1 domain since it has a short, 25-residue N-terminus, unlike the long N-termini in SK_{Ca} and K_V channels.

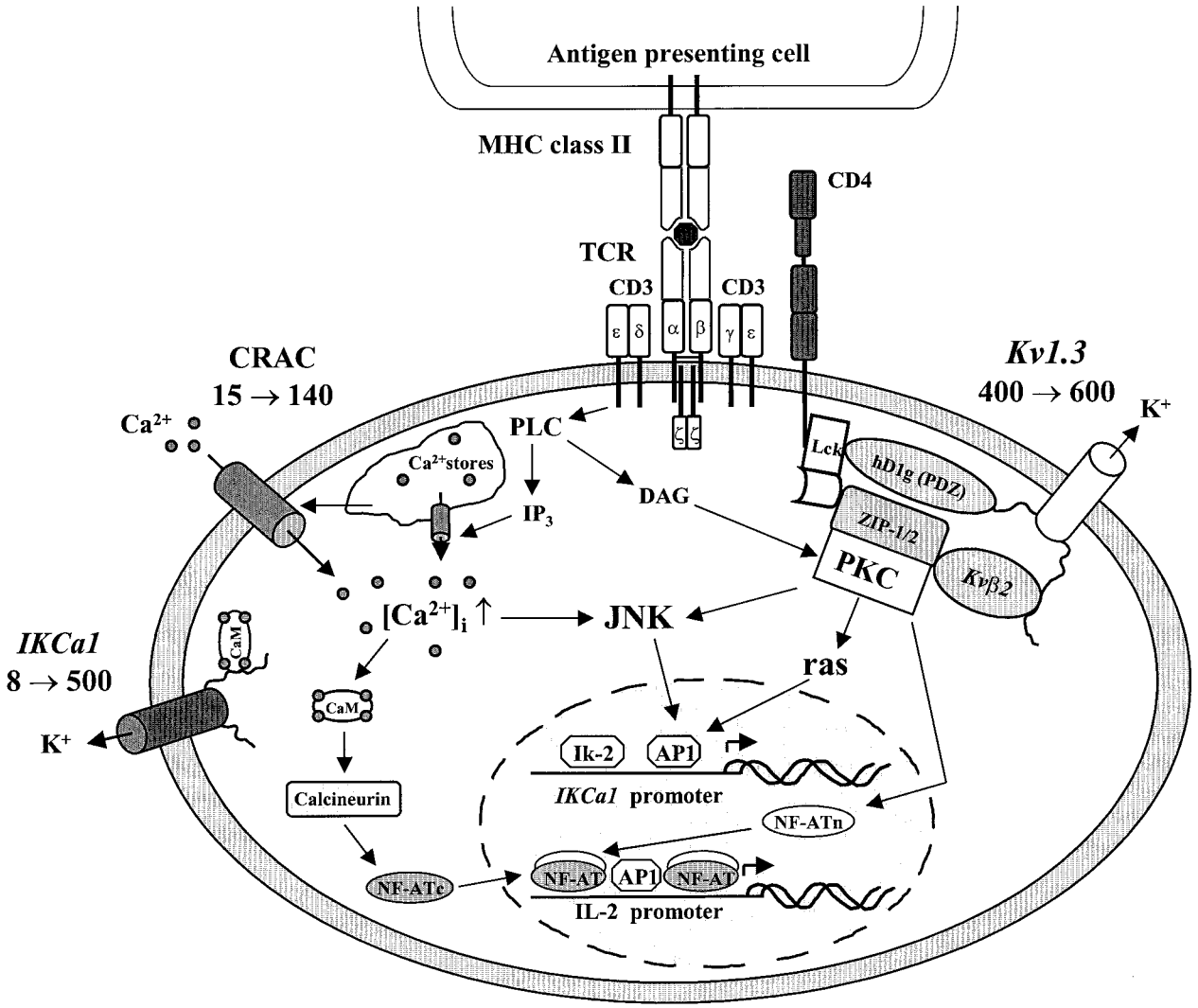

Fig. 2. Scheme showing the involvement of Kv1.3, IKCa1, and CRAC in the T-cell signaling cascade following antigen presentation. The number of channels expressed in resting and activated T cells is indicated next to each channel type. CaM, calmodulin; hDlg, human homologue of the *Drosophila* disc-large tumor suppressor protein (a PDZ domain protein); Ik-2, Ikaros-2; JNK, c-Jun N-terminal kinase; LcK, p56lck tyrosine kinase; ZIP1/2, PKC-ζ interacting protein.

CRAC Channels

The molecular identity of the CRAC channel remains a mystery, although several candidate genes have been proposed, including several TRP-gene family members (79). ESTs encoding a TRPC- and melastatin-related gene named *ChaK* or *TRP-PLIK* or *LTRPC7* (accession numbers NM_021450, AF149013, AK000124) have been isolated from mouse T cells (accession numbers AA596604, AI050262, AI153682, AA200403) and human B cells (accession number AW503077). Recent work indicates that the channel expressed in mammalian cells functions as a cation channel that can carry calcium ions, but the mechanism of activation is controversial (80, 81). A gene encoding a calcium transport protein that has been referred to as CAT-1 (accession numbers AF304463, AF160798, AB037373, AJ277909, AF209196), TRP8b (accession numbers AJ243501, AJ243500), ECAC1 (accession numbers NM_019841, AJ271207, XM_005003, AB032019), and OTRPC3, (accession number NM_022413) has been found in mouse thymocytes (accession number AI007094) and human lymphocytes (accession numbers U82613, X83877, NM_014274). When heterologously expressed in COS-7 (82) cells, this clone produces a channel with permeation and pharmacological properties similar to the CRAC channel. At low expression levels, the channel exhibits

store dependence, in accord with expectations for the CRAC channel.

CRAC Channels Are Permeable to Monovalent Ions in the Absence of Divalent Ions

When deprived of extracellular divalent ions, CRAC channels allow monovalent cations to permeate, a property shared with voltage-gated Ca^{2+} channels (9, 83, 84). Initially, we mapped the size of the pore using various organic and inorganic permeant monovalent cations of varying sizes, determined the role of intracellular Mg^{2+} in the inactivation and rectification of the channel, and measured its sensitivity to block by divalent ions. We discovered that even fairly large monovalent cations (up to 6 Å in diameter) can permeate through the CRAC channel when external divalent ions are removed (85). We reasoned that CRAC channels and voltage-gated Ca^{2+} channels share many features of ion permeation. The CRAC channel, like voltage-gated Ca^{2+} channels, achieves selectivity for Ca^{2+} by selective binding in a large pore with *I–V* characteristics shaped by internal Mg^{2+}. Taking advantage of the fact that CRAC channels are highly permeable to Na^+ ions when external divalents are reduced, we were able to resolve single-CRAC channel currents during whole-cell recording (86). Our results provide a detailed biophysical description of ion selectivity and gating of native CRAC channels that will be useful for comparison with candidate genes. In addition, this method enables us to count the number of channels per cell with single-channel resolution, the ultimate in phenotypic analysis. As a result, we now know that the number of CRAC channels that regulate Ca^{2+} influx is surprisingly low (10–15 channels per cell) in resting T cells and is up-regulated (~150 per cell) in activated T cells (87).

Mechanism of CRAC Channel Activation

Capacitive Ca^{2+} entry (CCE) through store-operated Ca^{2+} (SOC) channels was described several years ago by Putney (88–90). Despite the importance of SOC channels that mediate CCE, not only in lymphocytes but in a wide variety of cells, our level of understanding is cartoonish at best. Proposed signaling mechanisms have included direct actions of IP_3, cGMP, G proteins, tyrosine kinases, and an extractable calcium influx factor (CIF), channel delivery by vesicle fusion, conformational coupling between IP_3 receptors and surface membrane Ca^{2+} channels, *S*-nitrosylation by nitric oxide donors, and a role of lipid intermediaries. A low molecular weight CIF was isolated from store-depleted Jurkat T cells and shown to evoke Ca^{2+} signals when applied to cells (91). However, this material was later shown to activate nonspecific currents rather than I_{CRAC} (92), and this hypothesis has fallen into some disfavor, although it applies to some cell types including smooth muscle (93–95). The slow onset of CRAC channel activation suggested the possibility of exocytotic insertion of the Ca^{2+} channel upon store depletion. This hypothesis received some support from the finding that capacitative Ca^{2+} influx can be inhibited by GTPγS (96), an agent that disrupts membrane trafficking and vesicle fusion. More recently, experiments on a native SOC channel in *Xenopus* oocytes showed that channel activation was inhibited by cell-attached patch formation (physical separation of membrane from store?) and was blocked by pretreatment with botulinum toxin (BoTX), which inhibits synaptic transmission and membrane traffic (97, 98). Moreover, dominant-negative SNAP-25 constructs inhibited SOC channel activation, suggesting but not proving the existence of a vesicular delivery mechanism (98). In our single-channel recordings with Na^+ as the current carrier, CRAC channels appear one at a time during whole-cell recording, implying that the putative vesicles would contain no more than a single channel. Thus, the idea of CRAC channel delivery via vesicle fusion remains viable but with certain restrictions. A conformational coupling hypothesis, attributed to Irvine (99) and Berridge (100), proposes a direct physical link between the IP_3-sensitive Ca^{2+} store and the surface membrane. In its most specific form, the IP_3 receptor senses ER Ca^{2+} depletion and tells the surface membrane Ca^{2+} channel to open (like E–C coupling in muscle, but with information flow in the opposite direction). Experiments to test the conformational coupling hypothesis have been done mostly on *TRP*-transfected cells, using indirect measures of CCE. Several studies suggest that the IP_3 receptor is an essential component for mediating and maintaining coupling between store and CRAC channels. Besides its indirect role in emptying Ca^{2+} stores, a direct role for the IP_3 receptor in the regulation of h*TRP*3 channels was recently suggested (101). Boulay *et al* (102) recently presented direct evidence for IP_3R–TRP3 protein complexes by coimmunoprecipitating IP_3R with an antibody raised against TRP3 portions. Apparently, the TRP region interacting with the IP_3R is the C terminus. There is also evidence for involvement of the IP_3 receptor in regulating CCE (103).

Other Channels

The chloride-channel gene C1C3 is reported to behave as a volume-sensitive channel (104) and has been isolated from rat spleen (EST AA894109) and mouse

germinal-center B cells (EST BG089064), but it is not clear whether this gene encodes the SWAC channel in lymphocytes.

PHARMACOLOGY

Potassium Channels

The existence of K_V channels in electrically inexcitable T lymphocytes prompted the use of channel blockers to probe function. Initially, classical neuronal K^+ channel blockers such as 4-aminopyridine, tetraethylammonium (TEA), and quinine blocked lymphocyte K_V channels at micromolar to millimolar concentrations and also suppressed mitogen-induced ^{3}H-thymidine incorporation (1). Several additional low-potency inhibitors of the channel with micromolar affinities were discovered, including classical calcium channel blockers (verapamil, diltiazem, and nifedipine), CaM antagonists, and polyvalent cations (105, 106). Even the hormone progesterone directly blocks K_V channels (107). Furthermore, all of these compounds, despite having widely differing structures, inhibited cytokine production, gene expression, and T-cell proliferation with a potency parallel to channel block. Since these agents were not toxic, acted reversibly, inhibited only if administered within the first 24 hr, and did not suppress expression of IL-2 receptors or proliferation by exogenous IL-2, the concept was developed that K^+ channels are required for an early phase of T-cell activation (105). Later experiments measuring Ca^{2+}-dependent gene expression driven by the transcription factor NF-AT confirmed that at least this well-known signal transduction pathway is involved in the mechanism of inhibition (107, 108). Because these compounds blocked K_V channels with micromolar to millimolar potency and also affected other channel types, a clear need for specific blockers of the T-cell channels was perceived in the early nineties.

Polypeptide Toxins and the Development of Highly Selective Inhibitors

By the mid-1980s progress on the biochemical characterization of K^+ channels lagged far behind that of Na^+ channels and acetylcholine receptors, primarily because potent (submicromolar) K^+ channel blockers had not yet been identified. While teaching at a patch clamp course in the summer of 1986, one of us (M.C.), together with Dr. William Gilly, milked a scorpion (*Centruroides sculpturatus*) using electrical stimulation to obtain a small droplet of venom. A highly diluted sample blocked K_V channels in mouse thymocytes, initiating a search for the active constituent that identified charybdotoxin (ChTX isolated from the scorpion *Leiurus quinquestriatus*) as the first polypeptide shown to block any K_V channel; for Kv1.3 this was achieved with a nanomolar affinity (109–112). ChTX, however, also blocks large-conductance BK_{Ca} channels (113–115), IKCa1 channels (6, 116, 117), and other K_V channels (118, 119), necessitating a search for more selective and potent inhibitors of each T-cell K^+ channel. Other polypeptide inhibitors such as noxiustoxin, kaliotoxin, margatoxin, agitoxin-2, hongotoxin, HsTX1, Pi1, Pi2, and Pi3 with low-nanomolar and picomolar affinity for the Kv1.3 channel were discovered in other scorpion venoms (25, 26, 111, 119–131).

The lack of highly specific and potent nonpolypeptide inhibitors of Kv1.3 and IKCa1 initially slowed efforts to evaluate the functional roles of these channels acting separately and together in T cells and to determine whether *in vivo* channel blockade might have therapeutic value for immunosuppression. We developed highly selective and potent polypeptide inhibitors of Kv1.3 and IKCa1 on the basis of their known interactions with these channels (Fig. 1). A sea anemone polypeptide ShK, which blocked Kv1.3 and the neuronal channels Kv1.1, Kv1.4, and Kv1.6 with low-picomolar affinity, was engineered to be selective for Kv1.3 by replacing a critical lysine (Lys^{22}) with the shorter non-natural residue diaminopropionic acid (41, 42). ShK-Dap^{22} blocks Kv1.3 with a high degree of specificity and picomolar potency (41). A selective and potent polypeptide inhibitor of the IKCa1 channel, ChTX-Glu^{32}, was also developed by structure-guided transformation of charybdotoxin, a scorpion toxin that blocks several distinct K^+ channels (117).

Nonpolypeptide Small-Molecule Antagonists of T-Cell Channels

Using high-throughput toxin displacement, ^{86}Rb-efflux screening, or membrane potential assays, pharmaceutical companies and their academic collaborators discovered several submicromolar nonpolypeptide blockers of Kv1.3. The first was the dihydroquinoline CP339818 (50, 132), followed by the piperidine UK78282 (51), and the nor-triterpenoid correolide (133–136). Two recent patent applications describe novel classes of inhibitors including the phenyloxoazapropylcycloalkanes (137) and the sulfimidebenzamidoindanes (138). Other, less potent small-molecule inhibitors of Kv1.3 include 5,8-diethoxypsoralen (139, 140), the hormone progesterone (107), and cicutoxin, the poisonous principal of hemlock (141). Parallel efforts in our group led to the development of a highly selective and potent

inhibitor of the IKCa1 channel. The antimycotic clotrimazole potently blocks the IKCa1 channel (142) and also inhibits the activity of cytochrome P450 enzymes at picomolar to low nanomolar concentrations. Due to its channel blocking activity, clotrimazole is in human clinical trials for the treatment of sickle-cell anemia and secretory diarrhea, and early studies also reported therapeutic benefit for patients with rheumatoid arthritis (143). However, clotrimazole's clinical usefulness is impaired by its toxic side effects via inhibition of cytochrome P450 enzymes. We recently synthesized a novel clotrimazole analogue, TRAM-34 (Fig. 1), that blocks IKCa1 with a K_d of 20 n*M* but does not inhibit cytochrome P450 (144).

CRAC and Other Channels

CRAC channel pharmacology remains underdeveloped. SK 96365, 2-APB, and polyvalent cations such as La^{3+} and Gd^{3+} block CRAC channels in the micromolar range but also affect several other channel types. Similarly, a variety of compounds is reported to inhibit Cl^- channels, but not very potently (145). One compound, NPPB, blocks both CRAC and Cl^- channels (146).

CHANNEL PHENOTYPE: CHANGES IN EXPRESSION PATTERNS DURING ACTIVATION AND DIFFERENTIATION

Human T Cells

Expression levels of ion channels vary dramatically during thymic development, activation, and differentiation to effector cells. Figure 2 shows a scheme of the signal transduction pathways initiated by antigen and illustrates changes in expression patterns of ion channels in human T cells. Human CD3 thymocytes and mature cells express roughly 300–400 Kv1.3 channels (1, 147, 148). In contrast, quiescent mature human T cells express small numbers of IKCa1 channels (~10/cell) and this number increases to ~500 channels per cell as cells move from resting to proliferating blast cells. This is due primarily to activation of PKC-dependent pathways and requires the participation of the transcription factors Ikaros and AP-1, which bind to two overlapping elements in the IKCa1 promoter. At the same time, the number of CRAC channels per cell also increases ~10-fold. A small increase in Kv1.3 channels is also noted during activation. Jurkat T cells express Kv1.3 as well as SKCa2 channels in place of IKCa1, but the functional roles of these K_{Ca} channels appear to be interchangeable (5, 73–75). Mitogen activation of Jurkat cells results in a significant reduction in the level of SKCa2 mRNA and functional channels, whereas overexpression of the Src family tyrosine kinase $p56^{lck}$ up-regulates SKCa2 expression (73).

Rodent T Cells

In murine T cells, both K_V and K_{Ca} channels vary dramatically in various subsets of T cells. Immature $CD4^+CD8^+$ mouse thymocytes express ~200–300 Kv1.3 channels, but this number decreases dramatically during maturation (4). Mature $CD4^+$ T cells down-regulate Kv1.3 expression to ~20 Kv1.3 channels/cell, while $CD8^+$ cells turn off Kv1.3 and turn on a different channel, called Kv3.1. Mitogen activation of either of these subsets results in a dramatic up-regulation of Kv1.3 and IKCa1 channels. Among mouse T helper cells, Th1 mouse T cells express higher levels of IKCa1 channels compared to Th2 cells and the increased number is partly responsible for the higher calcium rise in Th1 cells following thapsigargin stimulation (149). Interestingly, $CD3^+CD4^-CD8^-$ T cells in mice with autoimmune disorders [systemic lupus erythematosus, type-1 diabetes mellitus, experimental autoimmune encephalomyelitis (EAE), and type-II collagen arthritis] express large numbers of Kv3.1 channels. This phenotype is not seen in this subset or any other in normal mice, suggesting that the Kv3.1 overexpression might be associated with the development of these autoimmune disorders (150–153). We have not found Kv3.1 channels in human or rat T cells despite intense investigation.

Like mature mouse T cells, rat splenic and lymph node T cells express hardly any potassium channels, while mitogen activation up-regulates both Kv1.3 and IKCa1 channels (unpublished results). Activation of myelin basic protein (MBP)-specific encephalitogenic $CD4^+$ Lewis rat T cells with the antigen results in a dramatic increase in Kv1.3 expression peaking on days 2 and 3, temporally coinciding with the encephalitogenicity of these cells (154, 155).

FUNCTIONAL ROLES OF LYMPHOCYTE ION CHANNELS

To mount an effective immune response, T and B lymphocytes must migrate into tissue, encounter and recognize specific antigens presented by appropriate MHC/peptide-bearing antigen presenting cells (APC), secrete biologically active proteins (including lymphokines, chemotactic agents, perforins, antibodies depending upon the lymphoid subset and the stimulus), prolif-

erate (enabling clonal expansion in response to a specific antigen), and differentiate (to memory, anergic, apoptotic, or terminally differentiated effector subsets). Many of these processes involve Ca^{2+} signaling. Lymphocytes must also be able to regulate their volume and survive up to decades while "remembering" past encounters with antigen.

Membrane Potential

K_V channels normally set the resting potential of T lymphocytes at -50 to -55 mV (reviewed in Ref. 12). Because T cells are so small and electrically tight, only a few K_V channels need be open near the foot of the activation curve to maintain the resting potential. K_V channels, by their voltage dependence, serve to protect the cell against depolarization. K_{Ca} channels, by their $[Ca^{2+}]_i$ dependence, open as soon as the Ca^{2+} signal is initiated by release of Ca^{2+} from IP_3-sensitive stores. As a result, the membrane potential hyperpolarizes toward -80 mV. CRAC channels, activated by Ca^{2+} store depletion, exhibit an "upside-down" voltage dependence compared to voltage-gated Ca^{2+} channels in electrically excitable cells such as the heart or central neurons. Thus, the opening of K_{Ca} channels provides positive feedback to enhance Ca^{2+} entry through CRAC channels. Depolarization is inhibitory for Ca^{2+} influx, signaling, and lymphocyte activation because of the electrical driving force.

Motility, Chemotaxis, and Adhesion

Lymphocyte adhesion and migration in response to the chemokine MIP-1β is inhibited by blocking the Kv1.3 channel with various peptide toxins (margatoxin, kaliotoxin, noxiustoxin, ChTX) or by substance P (67). Moreover, β1-integrin and Kv1.3 can be coimmunoprecipitated, suggesting that they are not only functionally coupled but physically associated. High extracellular K^+ and ChTX were also observed to inhibit motility of human fibroblast and melanoma cells (156). Specifically, the IKCa1 channel has been shown to play an important part in polarization and migration of MDCK cells by regulating cell volume and by promoting the remodeling of the actin cytoskeleton (157–160). Thus, in several cell systems, there is evidence that K^+ channels play a role in motility and chemotaxis.

Calcium Signaling: NFAT

The immune response is initiated by contact between an APC and a T lymphocyte expressing a T-cell receptor (TCR) specific to a peptide antigen bound to MHC proteins on the presenting cell (Fig. 2). TCR aggregation within the contact zone, termed the "immunological synapse," leads to activation of tyrosine kinases resulting in phosphorylation and activation of phospholipase C (PLCγ). Using an optical trap to position anti-TCR-coated beads into contact with a T cell, we recently found that at least 300 TCR molecules must be engaged preferentially at the leading edge of the T cell to initiate $[Ca^{2+}]_i$ signaling (161). Both IP_3 and diacylglycerol are generated, resulting in the release of Ca^{2+} from internal stores and activation of PKC. Ca^{2+} release and influx through CRAC channels both contribute to the rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), but influx dominates quantitatively and is required for subsequent gene expression. T-cell $[Ca^{2+}]_i$ rises within seconds of receptor engagement, causing immediate changes in shape and motility and subsequent activation of new genes resulting in lymphokine secretion and cell proliferation. Thapsigargin (TG), a specific inhibitor of the Ca^{2+}-uptake pump in the ER, can be used to bypass the proximal signaling events by depleting Ca^{2+} stores without generating IP_3. The same population of CRAC channels is indirectly activated by TCR engagement, dialysis of IP_3, addition of TG or ionomycin, or strong intracellular Ca^{2+} buffering, all stimuli that deplete the Ca^{2+} store (8, 11). In addition to coactivating PKC, jun kinase (JNK), and calcium/calmodulin-dependent protein kinase (CaMK), the rise in $[Ca^{2+}]_i$ activates the phosphatase calcineurin (CN) resulting in dephosphorylation of NFAT (nuclear factor of activated T cells), a cytoplasmic transcription factor that then migrates to the nucleus and, in concert with additional transcription factors, initiates IL-2 gene transcription. IL-2 in turn stimulates newly expressed IL-2 receptors to drive proliferation. Blocking Ca^{2+} influx prevents activation of T cells, and CN is the site of action for the immunosuppressive drug cyclosporin A (CsA), underscoring the requirement for this pathway in the signaling cascade.

What is the relationship between the $[Ca^{2+}]_i$ signal and downstream gene expression? Using a reporter T-cell line expressing the *lacZ* gene driven by the NFAT element of the IL-2 promoter, we quantitatively related the pattern of $[Ca^{2+}]_i$ signaling to gene expression in individual T cells (108). The NFAT-*lacZ* signal appeared as an all-or-none increase in fluorescence. The frequency of $[Ca^{2+}]_i$ oscillations elicited by TCR engagement correlated with gene expression. We determined the $[Ca^{2+}]_i$ dependence of NFAT-regulated gene expression by elevating $[Ca^{2+}]_i$ using either TG or ionomycin and then "clamping" $[Ca^{2+}]_i$ to various, stable levels by altering either $[Ca^{2+}]_o$ or $[K^+]_o$. A rise in $[Ca^{2+}]_i$ from

resting levels of 70 n*M* to between 200 n*M* and 1.6 μM increased the fraction of activated cells, with a K_{eff} value of ~1 μM. Costimulation with PKC activators greatly enhanced the $[Ca^{2+}]_i$ sensitivity of gene expression [K_{eff} = 210 n*M*; similar to the steep $[Ca^{2+}]_i$ dependence found by Fanger *et al* (162)], whereas stimulation of PKA inhibited $[Ca^{2+}]_i$-dependent gene expression. These experiments provided the first single-cell measurements linking a second messenger to gene expression in individual cells.

We are just beginning to decode the frequency dependence of Ca^{2+} signals and to appreciate how varying outcomes of activation, apoptosis, or anergy may depend upon the pattern of Ca^{2+} signaling as well as the types of costimulatory input integrated by the cell. Specific pathways of transcriptional activation can be differentially "tuned" to particular frequencies or intensities of Ca^{2+} signaling within the cell (163). By using the intact cell as a reporter we can begin to ask questions about the interplay between the various elements that control the genetic machinery. The Ca^{2+} dependence of gene expression, along with knowing the channel "phenotype" of human T cells, provides a good explanation for inhibitory effects of K^+ channel blockers on IL-2 secretion and lymphocyte proliferation; the Ca^{2+} signal is attenuated by reduced Ca^{2+} influx through CRAC channels indirectly when the membrane potential is depolarized. The Ca^{2+} signal depends primarily upon influx through CRAC channels driven by a negative membrane potential. K_V channels provide the initial electrical driving force favoring Ca^{2+} entry by maintaining the resting membrane potential. K_{Ca} channels contribute positive feedback by hyperpolarizing the membrane potential and augmenting Ca^{2+} entry. Both K^+ channels oppose depolarization that would otherwise occur during Ca^{2+} entry through CRAC channels. The initial Ca^{2+} influx through CRAC channels is driven by the electrochemical gradient, but membrane depolarization resulting from movement of divalent Ca^{2+} ions across the membrane limits further entry. The opening of *Kv1.3* channels in response to membrane depolarization and the opening of *IKCa1* channels by the initial rise in cytosolic Ca^{2+} provide mechanisms to enhance efflux of K^+ ions and maintain membrane potential via positive feedback, promoting sustained Ca^{2+} influx in the time frame required for new gene transcription.

The relative contributions of K_V and K_{Ca} channels may vary in different T-cell subsets, since their levels of expression can change dramatically depending upon the state of activation. Helper T cells can be subdivided on the basis of cytokine production into two subsets. In Th1 and Th2 lymphocytes, activation begins with identical stimuli but results in the production of different cytokines: IL-2 and γ-interferon in Th1 cells and IL-4, IL-5, and IL-10 in Th2 cells. We noticed that the Ca^{2+} rise elicited following store depletion with TG was significantly lower in Th2 cells than in Th1 cells (149). Maximal Ca^{2+} influx rates and whole-cell Ca^{2+} currents showed that both Th1 and Th2 cells express CRAC channels. Whole-cell recording demonstrated that there is no distinction in the amplitudes of K_V currents in the two cell types, but K_{Ca} currents were significantly smaller in Th2 cells than in Th1 cells. Pharmacological equalization of K_{Ca} currents in the two cell types reduced but did not completely eliminate the difference between Th1 and Th2 Ca^{2+} responses; in addition, Th2 cells extrude Ca^{2+} more quickly than Th1 cells. The combination of a faster Ca^{2+} clearance mechanism and smaller K_{Ca} currents in Th2 cells accounts for the lower Ca^{2+} response of Th2 cells.

Ca^{2+} influx in T cells depends on both activation of CRAC channels and a membrane potential sufficiently large to drive Ca^{2+} influx through CRAC channels. In a recent series of experiments, we used specific channel blockers and manipulated channel expression levels to evaluate the functional role of K_{Ca} channels in Jurkat cells and in preactivated human T cells with up-regulated IKCa1 channels (75). Inhibition of endogenous *SKCa2* but not K_V or IKCa1 channels in Jurkat T cells significantly reduced the plateau Ca^{2+} following TG stimulation. In human T cells, inhibition of IKCa1 but not K_V or SKCa2 channels also reduced Ca^{2+} signaling. Genetic manipulation of K_{Ca} channel expression profoundly affected Ca^{2+} signaling. A dominant-negative construct of *SKCa2* blocked functional channel expression and inhibits Ca^{2+} signaling. Transfected *IKCa1*, tagged for identification with N-terminal GFP, rescued the Ca^{2+} signal. These results again point to tight coupling between Ca^{2+} signaling and functional K_{Ca} channels in T cells with up-regulated K_{Ca} channels.

Mitogenesis

From the use of pharmacological agents in functional assays, a better understanding has emerged of the roles of Kv1.3 and IKCa1 in resting and activated human T cells. Kv1.3 channels are essential for initiating the human immune response, and IKCa1 channels are required to sustain the activation process.

Resting T Cells: Dependence on Kv1.3

Kv1.3 is the dominant conductance in resting T cells, and our results using more potent and selective blockers have confirmed our previous conclusion that Kv1.3 is a

potential target for preventing an immune response. ShK-Dap[22], the selective K_V channel blocker, suppressed anti-CD3-induced ^{3}H-thymidine incorporation in human T lymphocytes at subnanomolar concentrations. Due to their greater abundance, *Kv1.3* channels are more important than IKCa1 channels in regulating the membrane potential of resting T cells. Selective blockade of Kv1.3 channels (but not IKCa1) chronically depolarizes the membrane of resting cells, attenuates Ca^{2+} entry, and suppresses the signaling cascade leading to cytokine production and cell proliferation (41, 121, 164, 165).

Activated T Cells: Dependence on IKCa1

Activation of human T cells results in a dramatic transcriptional up-regulation of IKCa1 channel expression (6, 70, 166) involving the transcription factors Ikaros-2 and AP1 (165). Consequently, activated human T cells express about 500 IKCa1 channels along with a roughly equivalent number of Kv1.3 channels. In parallel, with the enhancement in IKCa1 expression, the number of CRAC Ca^{2+} channels also increases approximately 10-fold (100–300 channels/cell) (87). Mitogen or antigen stimulation of these preactivated cells results in enhanced Ca^{2+} entry through the increased numbers of CRAC channels. The resulting membrane depolarization might overwhelm the ability of Kv1.3 channels to maintain the membrane potential, and the electrochemical gradient required for Ca^{2+} entry would dissipate. The compensatory increase in the number of IKCa1 channels might be sufficient to provide the counterbalancing cation efflux to compensate for enhanced Ca^{2+} entry. As the cytoplasmic Ca^{2+} concentration rises, IKCa1 channels would open via a CaM-dependent mechanism, the membrane would hyperpolarize, and the electrochemical gradient required for Ca^{2+} entry would be restored. Consistent with this hypothesis, Kv1.3 blockers do not suppress the proliferation of preactivated T cells, whereas blockade of K_{Ca} channels suppresses mitogen-induced proliferation of these cells (144, 165, 166).

K^+ Channel Block and Immunosuppression by Progesterone: Fetal–Maternal Protection

Progesterone is the obligatory hormone for the maintenance of pregnancy. Although the concentration of progesterone in peripheral blood is 0.1 μg/ml, it is much higher in the placenta, where trophoblast cells are devoted to its production. Thus, progesterone in the vicinity of the placenta may be the single most important immunoregulatory substance responsible for maintenance of the fetal allograft. The mechanism by which progesterone causes localized suppression of the immune response during pregnancy has remained elusive. Our results demonstrated that progesterone, at concentrations found in the placenta, rapidly and reversibly blocked K_V and K_{Ca} channels, resulting in depolarization of the membrane potential. As a result, Ca^{2+} signaling and NFAT-driven gene expression were inhibited. Progesterone acts distally to the initial steps of TCR-mediated signal transduction, since it blocked sustained Ca^{2+} signals after thapsigargin stimulation, as well as oscillatory Ca^{2+} signals, but not the IP_3-driven Ca^{2+} transient following TCR stimulation. K^+ channel blockade by progesterone is specific; other steroid hormones had little or no effect, although the progesterone antagonist RU 486 also blocked K_V and K_{Ca} channels. Progesterone effectively blocked a broad spectrum of K^+ channels with an affinity in the micromolar range, reducing both Kv1.3- and ChTX-resistant components of K_V current and K_{Ca} current in T cells, as well as blocking several cloned K_V channels expressed in cell lines, but had little or no effect on Na^+ channels, inward rectifier K^+ channels, or lymphocyte CRAC or Cl^- channels. Away from the placenta, progesterone would rapidly unbind from K^+ channels and allow normal signal transduction and effector responses in the maternal T cells. Thus, low-affinity K^+ channel block by progesterone may serve as a mechanism for localized immunosuppression of maternal T cells, providing protection of the developing fetus without suppressing the maternal immune response.

Volume Regulation

During a hypotonic osmotic challenge lymphocytes initially swell, but then exhibit a homeostatic behavior called RVD that restores the cell volume back to normal despite the maintained presence of the hypotonic challenge (145). This property makes it possible for lymphocytes to regulate cell volume during passage through the renal microcirculation and interstitial spaces that regularly experience large changes in osmolarity. RVD involves the efflux of chloride and potassium through separate conductive pathways along with water (Fig. 3). In mature T cells, the opening of swelling-activated Cl^- channels allows Cl^- ions to exit the cell but also depolarizes the membrane potential, indirectly opening K_V channels that would mediate K^+ efflux. This Ca^{2+}-independent mechanism of RVD may apply to a wide variety of cell types. In mouse thymocytes, osmotic swelling activates Ca^{2+}-permeable nonselective cation (SWAC) channels, producing a Ca^{2+} signal that activates K_{Ca} channels. When this occurs, volume regulation is enhanced and K_{Ca} channels play a more significant role than K_V channels, with Cl^- channels providing increased anion efflux because the hyperpolarized mem-

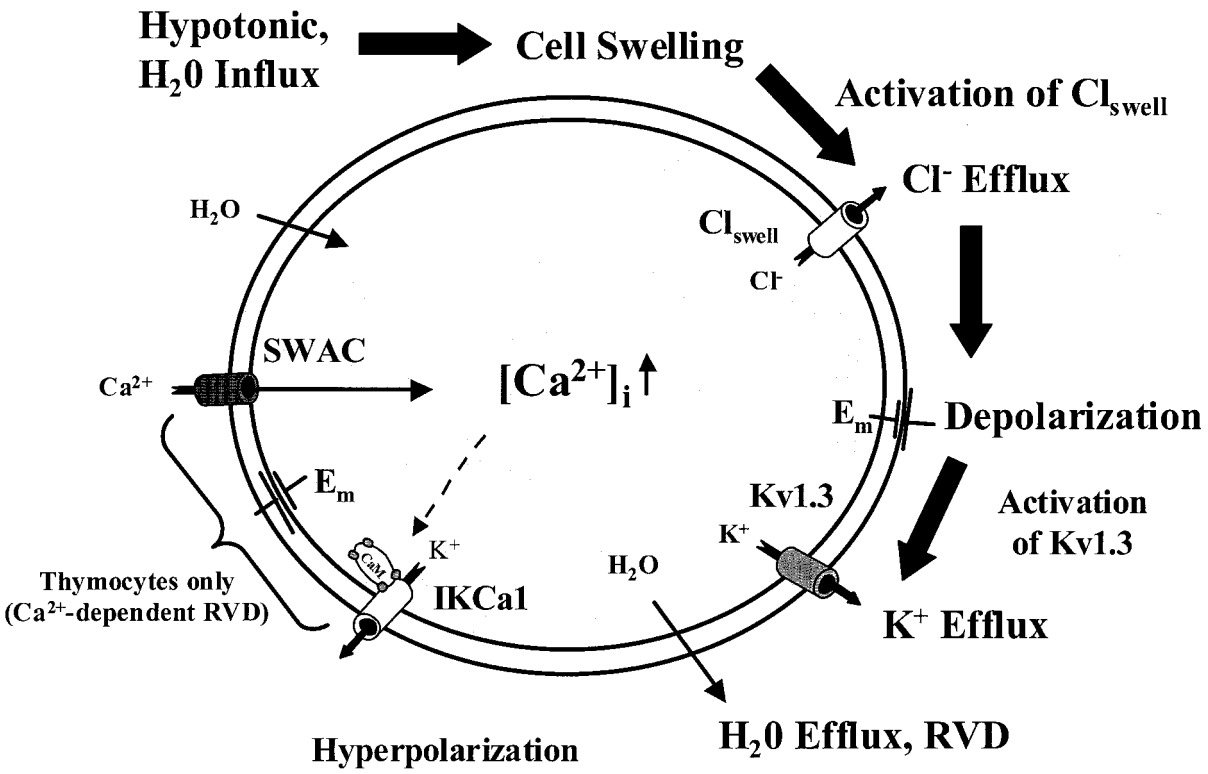

Fig. 3. Scheme of volume regulation and Ca^{2+} signaling.

brane potential provides an increased driving force for loss of Cl^- ions (16).

CHANNELS AS THERAPEUTIC TARGETS

Immunosuppressants such as CsA are effective in preventing graft rejection following organ transplants, but tissue damage in the liver and kidney limits its use. Immunosuppressants also have a place in the management of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and type-1 diabetes mellitus. The two T-cell K^+ channels are attractive targets for immunomodulation because of their functionally restricted tissue distribution and their critical role in T-cell activation. Gloria Koo's group at Merck provided the first proof of this concept. Using the polypeptide margatoxin, which potently blocks the Kv1.3 channel, Koo and her colleagues suppressed delayed type hypersensitivity responses *in vivo* in minipigs (164). Subsequently, the same group used nor-triterpenoid compounds related to correolide to suppress the delayed-type hypersensitivity response in minipigs. More recently, Evelyne Beraud's group at the University of Marseille tested whether *in vivo* blockade of K^+ channels by the polypeptide inhibitor kaliotoxin would ameliorate the clinical symptoms of EAE (167), a model for human multiple sclerosis. In this model, a $CD4^+$ T-cell line expressing Kv1.3 channels was injected into the abdomen of Lewis rats ("adoptive transfer") following *in vitro* activation with MBP, a component of the myelin sheath that covers neurons in the brain, providing insulation that enhances the velocity of action potential conduction. Within 5–6 days after adoptive transfer, the disease-inducing T cells invaded the central nervous system and caused severe demyelination, paralysis, and death. Kaliotoxin injected twice a day significantly reduced the severity of the clinical symptoms in these animals compared to saline-injected controls (167). Since kaliotoxin blocks both Kv1.3 (present in T cells) and Kv1.1 (present in neurons), these investigators were unable to determine whether the therapeutic effect was due to immunosuppression (via Kv1.3 blockade) or to enhanced neurotransmission (via Kv1.1 blockade). With the advent of many potent nonpeptide and polypeptide inhibitors of IKCa1 and Kv1.3, it should be possible to test more definitively whether *in vivo* blockade of the T-cell K^+ channels will help in the treatment of autoimmune diseases and in the management of graft rejection.

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