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Physiological role, molecular structure and evolutionary relationships of voltage-gated potassium channels in T lymphocytes

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T lymphocytes display three distinct types of voltage-gated K^+ channels, types n, n' and l, that are expressed in an activation- and development-dependent fashion. These channels regulate the membrane potential of T cells and thereby control the calcium signaling response necessary for lymphocyte activation. Abnormal over-expression of type l K^+ channels is a marker for a subset of CD4/CD8-negative T cells associated with autoimmune disorders, and may reflect the ability of these cells to trigger the autoimmune process. The types n and l K^+ channels are encoded by genes belonging to two distinct subfamilies, showing only 51% sequence identity at the nucleotide level; the Shaker-related Kv1.3 gene on human chromosome 1 encodes the type n K^+ channel, whereas the type l K^+ channel is the product of the Shaw-subfamily gene Kv3.1 on human chromosome 11. Drugs which block the Kv1.3 channel inhibit T cell activation, and might therefore serve as immunosuppressants for the prevention of graft rejection and treatment of T cell-mediated autoimmune diseases. Agents which block type l K^+ channels, on the other hand, could be used to treat autoimmune disorders in a highly specific manner by targeting a disease-relevant subset of T lymphocytes.

Key words: K^+ channels / T lymphocytes / molecular structure / evolution / autoimmune disease

VOLTAGE-GATED potassium (K^+) channels are proteins which allow the rapid and passive passage of K^+ ions across lipid bilayers. They maintain the membrane potential, modulate electrical excitability in the nervous and cardiovascular systems, regulate hormone and neurotransmitter secretion, and control cell volume in many different tissues.[1] An extended family of genes (see K^+ channel gene nomenclature in this issue, pp 101-106, each of which encodes a K^+ channel with a unique complement of electrophysiological and pharmacological properties, is responsible for this diversity of functions.[2] Further diversity is achieved by modification of these channels by intracellular calcium and by neurotransmitter-mediated second messenger pathways.[1] Drugs which either increase or reduce ion flux through K^+ channels are currently used to treat cardiac arrhythmias, type-2 diabetes mellitus, baldness and hypertension,[3] and additional therapeutic applications are being explored. In this review we will describe the physiological role and the molecular structure of voltage-gated K^+ channels in T lymphocytes, and discuss their evolutionary relationships to the extended family of voltage-sensitive K^+ channels that have been characterized by electrophysiological and molecular means. It should be noted that other types of voltage-insensitive K^+ channels are present in T cells,[4-7] but these will not be discussed here.

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Potassium channels in lymphocytes

Electrophysiological studies have revealed the existence of three distinct voltage-gated K^+ channels in mouse T lymphocytes, types *n*, *n'* and *l*,[8-11] whose properties are outlined in Table 1. The expression of these K^+ channels varies in a development- and activation-dependent manner. Quiescent mouse T cells express ~20 type *n*, *l* or *n'* K^+ channels, depending on the cell surface phenotype.[9,10] Mouse helper T cells, identified by the presence of the CD4 cell surface molecule, express ~20 type *n* K^+ channels per cell.[9,10] Mouse cytotoxic T cells that display CD8 molecules on their cell membranes express types *l* or *n'* K^+ channels, also about 20 per cell.[9,10] Mitogen-activation with concanavalin A (Con A) or with allogeneic cells, induces a ~10-fold increase in type *n* channel expression in both T cell subsets,[12,13] and channel expression remains at a high level for at least

Table 1. Voltage-gated K^+ channels in T lymphocytes

	Type *n*	Type *l*	Type *n'*
Activation			
midpoint	- 35 mV	- 5 mV	- 10 mV
steepness	6	6	~9
τ_{tail} (at - 60 mV)	30 ms	2 ms	30 ms
Inactivation	cumulative		
τ_h (at 40 mV)	180 ms	300 ms	> 300 ms
Channel conductance	18 pS	27 pS	18 pS
Pharmacology			
TEA (mM)	10	0.1	100
CTX (nM)	3	> 100	~3
NTX (nM)	2	> 100	n.d.*

*n.d., not done.

7 days.[13] Interleukin 2 (IL2) also stimulates a ~ fourfold increase in the number of channels in the mouse helper T cell line L2.[14] Interestingly, interleukin 3 and H-*ras* expression both appear to stimulate type *l* K^+ channel expression.[15,16]

The pattern of expression of K^+ channels in T cells varies in a species-dependent manner. Type *n* K^+ channels are present in peripheral blood human T cells,[8,17-19] while types *l* and *n'* K^+ channels have not been identified electrophysiologically in these cells. Type *l* K^+ channels are, however, expressed by the human Burkitt's lymphoma line, Louckes,[20,21] and a biophysically similar channel, G_{f2}, is present in myelinated nerves.[22] Another species-related difference is that quiescent human helper and cytotoxic cells possess ~200 type *n* channels,[8,17-19,23] in contrast to the 10-20 in resting mouse T cells, and respond with only a ~twofold increase in type *n* K^+ channels expression following activation with Con A, phorbol esters, anti-CD3, or allogeneic cells.[24-26] Surprisingly, phytohemagglutinin-activated human thymocytes and peripheral blood T cells have been reported to express substantially fewer channels than resting cells.[23,25] This phenomenon may, however, be due to a toxic effect of PHA, as activating human T cells with a lower concentration of PHA (1 $\mu g\,ml^{-1}$) results in a two- to fourfold *increase* in channel density (S. Grissmer, D. Hanson, personal communication).

Physiological role of lymphocyte voltage-gated K^+ channels

A pharmacological approach has been used to define the role of the type *n* K^+ channel in human and mouse T cells. Seven agents (tetraethylammonium, 4-aminopyridine, quinine, diltiazem, verapamil, ceteidil, nifedipine) that block type *n* K^+ channels were used to inhibit selected events during T cell activation.[8,11,14,25,27] These compounds, although differing in chemical structure, all block type *n* channels and, in a parallel potency sequence, inhibit mitogen-induced ^3H-thymidine and ^3H-leucine incorporation, production and secretion of IL2, induction of c-*myc* and c-*fos* mRNA, and transport of metabolites, but not the expression of the IL2 receptor.[8,27,28] Inhibition is not due to simple toxicity since washout of the blockers and subsequent challenge with fresh mitogen causes a normal activation response showing all of the above features.[8,27] In interpreting such experiments, it is important to keep in mind that these agents may not be specific for type *n* K^+ channels.[29] However, studies with more selective blockers of type *n* K^+ channels, charybdotoxin (CTX),[7,30-32] noxius toxin[7,30] and margatoxin,[7,33-35] have supported these findings. CTX, which blocks both the type *n* K^+ channel and the calcium-activated K^+ channel in T cells,[5-7] inhibits T cell activation at nanomolar concentrations.[31-35] Margatoxin and noxius toxin, which selectively block the type *n* K^+ channels without affecting the calcium-activated K^+ channel,[6,7] also inhibit T cell activation at concentrations which block the type *n* K^+ channel.[33-35] Interestingly, both these toxins suppress only calcium-dependent mitogen-activation, and have little effect on activation by treatments (e.g. anti-CD28 plus PMA) that do not cause an increase in intracellular calcium.

Several lines of evidence suggest that type *n* K^+ channels influence mitogenesis by regulating the membrane potential, which in turn modulates the influx of calcium through mitogen-responsive calcium channels. The resting membrane potential of T cells is estimated to range from - 50 to - 70 mV and represents primarily a K^+ diffusion potential.[36-39] The role of type *n* K^+ channels in setting the resting potential of human T cells is supported by experiments showing that blockade of these channels results in membrane depolarization.[7,39-41] Membrane depolarization, either by elevation of the external K^+ concentration[41] or by specific blockade of type *n* K^+ channels (i.e. with margatoxin or noxiustoxin), inhibits the calcium signal,[7,41] presumably by reducing the electrical driving force for calcium influx. Hess, Oortgiesen and Cahalan have more recently shown that the thapsigargin-induced rise in internal calcium in T

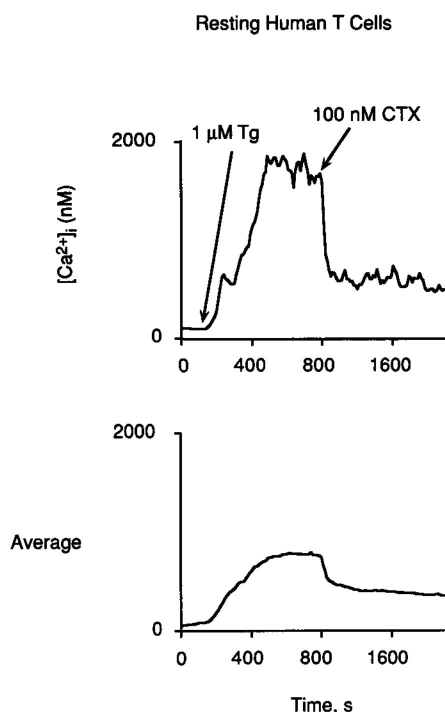

Figure 1. Effect of 100 nM charybdotoxin (CTX) on thapsigargin (Tg) induced rise in $[Ca^{2+}]_i$ in resting human T cells. Cells were loaded with fura-2, plated on a microscope stage and $[Ca^{2+}]_i$ was monitored in single cells during the application of Tg and Tg + CTX using an image processor. $[Ca^{2+}]_i$ is shown for one individual cell (top) and for the average of all the cells in the field of view (bottom). Data courtesy of S. Hess, M. Oortgiesen and M.D. Cahalan.

cells can be blocked by CTX, as seen in Figure 1. Membrane depolarization also suppresses T cell activation as assessed by IL2 mRNA production, IL2 secretion and ^{3}H-thymidine incorporation.[32,35] This inhibition does not appear to be due to a non-specific toxic effect on generalized metabolism, as had been previously suggested.[29] *In situ* NMR studies of human T lymphocytes show that membrane depolarization up to -38 mV does not suppress glycolysis or lactate production, nor does it affect the levels of phosphorus metabolites.[42] Thus, the initial mitogen-induced entry of calcium may be enhanced by the hyperpolarization associated with type *n* K^+ channel opening. Further increases in internal calcium will open calcium-activated K^+ channels contributing to membrane hyperpolarization. Higher cytosolic calcium concentrations enhance the inactivation of type *n* K^+ channels and inhibit further calcium entry. These data collectively suggest that type *n* K^+ channels are required for activation of T cells via calcium-dependent pathways. Type *n* K^+ channel blockers may therefore be immunosuppressive, and, if found to selectively affect lymphocyte function, could be used therapeutically for the prevention of graft rejection or T cell-mediated autoimmune diseases.

Voltage-gated K^+ channels have also been implicated in volume regulation in T cells.[40,43,44] Following hypoosmotic challenge T cells swell due to the influx of water, but reassume their original volume by losing Cl^-, K^+, and water. A working hypothesis for this regulatory volume decrease was originally suggested by Cahalan and Lewis.[43] They characterized 'stretch-activated' mini-Cl^- channels in T cells that were activated by a transmembrane osmotic gradient in the presence of intracellular ATP. Activation of these Cl^- channels would depolarize the membrane which in turn would open voltage-gated K^+ channels; under these conditions both Cl^- and K^+ would leave the cell and water efflux would follow. As predicted by this hypothesis, type *n* K^+ channel blockers inhibit volume regulation in T cells.[40,43,44]

Over-expression of type *l* K^+ channels in T cells associated with autoimmune diseases

Lymphocytes which recognize and destroy tissues within the body are termed auto-reactive or self-reactive cells, and are normally eliminated during fetal development. Cells which escape this fate may reside in peripheral lymphoid tissues (spleen and lymph nodes) in a quiescent state until triggered by exogenous agents such as viruses or toxins. These activated self-reactive lymphocytes then damage tissues within the body causing a group of disorders collectively called autoimmune diseases. Examples of such autoimmune disorders include systemic lupus erythmatosus (SLE), rheumatoid arthritis, type-1 diabetes mellitus, multiple sclerosis, myasthenia gravis and Graves' disease. Studies of mouse and rat models have provided important insights into the mechanisms underlying autoimmunity, and have focused attention on a minor subset of T cells which lack both CD4 and CD8 cell surface markers ('double negative' T cells). These double negative T cells have been reported to induce autoantibody production in mice and humans with SLE,[45,46] possibly by secreting lymphokines such as IL6, tumor necrosis factor and interferon-γ.[47-49] Double negative T cells which are activated by heat-shock proteins

have also been implicated in rheumatoid arthritis.[50] We have reported that double negative T cells from the lymph nodes and spleens of mice with SLE, type 1 diabetes mellitus, multiple sclerosis or type II collagen arthritis, all exhibit abnormally large numbers of type *l* K^+ channels (~200/cell), a pattern not seen in normal mice.[51-54] The increased expression of type *l* K^+ channels in double negative T cells present at sites of inflammation may reflect their activated status and their ability to secrete specific lymphokines. Thus, blockers of type *l* K^+ channels, if found to selectively suppress 'diseased' double negative T cell function, could be clinically useful for treatment of autoimmune diseases.

Molecular structure of T lymphocyte K^+ channels

What are the mechanisms which underlie expression of these channels in normal and diseased T cells? Can drugs which selectively modulate these channels be designed for use as immunosuppressives? It was to address these issues that we began studying the genes encoding the T cell K^+ channels.

K^+ channels are thought to consist of homotetramers, each subunit of which contains six membrane-spanning helices (termed S1 through S6), and intracellular amino- and carboxy-terminal domains of varying lengths.[55] A portion of the extracellular loop connecting S5 and S6 is thought to re-enter the cell membrane and participate in the formation of the channel pore[55] (for a discussion of K^+ channel structure see paper by O. Pongs, this issue, pp 93-100). Each of the four *Drosophila* K^+ channel genes, *Shaker*, *Shab*, *Shal*, and *Shaw*, has one or more known vertebrate homologues[2] (also see K^+ channel gene nomenclature table in this issue, pp 101-106) which belong to a large supergene family of ion channels.[56] These genes form three evolutionarily related monophyletic groups, one consisting of the voltage-sensitive Na^+ and Ca^{2+} channels, another comprising the cyclic nucleotide-modulated channels, and a third including the voltage-sensitive K^+ channels.[56] The *Shaker* and *Shal* subfamily of K^+ channel genes appear to have arisen from one common precursor, while the *Shaw* and *Shab* subfamily genes arose from a separate but related precursor (Figure 2). These genes do not cluster together in the human genome.[21,57-64] Interestingly, the type *n* and *l* channels in T cells are encoded by two genes which share only about 50% sequence identity, the *Shaker*-related Kv1.3 gene (*n* channel) and the *Shaw*-related Kv3.1 gene (*l* channel).

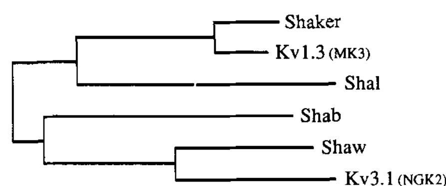

Figure 2. Phylogenetic tree based on parsimony analysis (using the program PAUP[98]) of aligned amino acid sequences of four *Drosophila* K^+ channel proteins (*Shaker*, *Shal*, *Shab* and *Shaw*), and two mouse proteins (Kv1.3[MK3] and Kv3.1[NGK2]) which correspond to the type *n* and *l* lymphocyte K^+ channels (see text, and ref 56).

The Kv1.3 gene encodes the type *n* K^+ channel in T cells

The functional properties of the mouse Kv1.3[65] gene product, expressed in *Xenopus* oocytes, were compared in detail with channel 'fingerprints' of the native type *n* K^+ channel in lymphocytes.[57] As an additional biophysical test, we expressed mouse Kv1.3 in rat basophilic leukemic cells using the vaccinia viral vector,[66] and examined the electrophysiological properties of the cloned channel. The mouse Kv1.3 channel, when expressed in *Xenopus* oocytes or in mammalian cells, has gating properties and pharmacological sensitivities (Figures 3 and 4) that are so remarkably similar to those of type *n* K^+ channels that it is likely the native channel is a homomultimer of the Kv1.3 protein. Kv1.3 transcripts have been identified in mouse T cells,[57,67] and vary in size from 9.5 kb to 3.5 kb.[61,64,67-69] Since Kv1.3 has an intronless coding region[64,65,68] the multiple transcripts are probably the result of alternative splicing within the 5′ or 3′ non-coding regions. Using similar approaches, the rat and human Kv1.3 genes have also been reported to encode the type *n* K^+ channels in T cells in these species.[61,64,68] Recently, Honore and colleagues[70] have reported that injection of large amounts of hKv1.3 cRNA (>35 ng/oocyte, equivalent to a total current of ~8 μA) generates typical type *n* K^+ currents along with a second, non-inactivating, CTX-insensitive K^+ current. We have never observed this phenomenon, even when our oocytes express mKv1.3

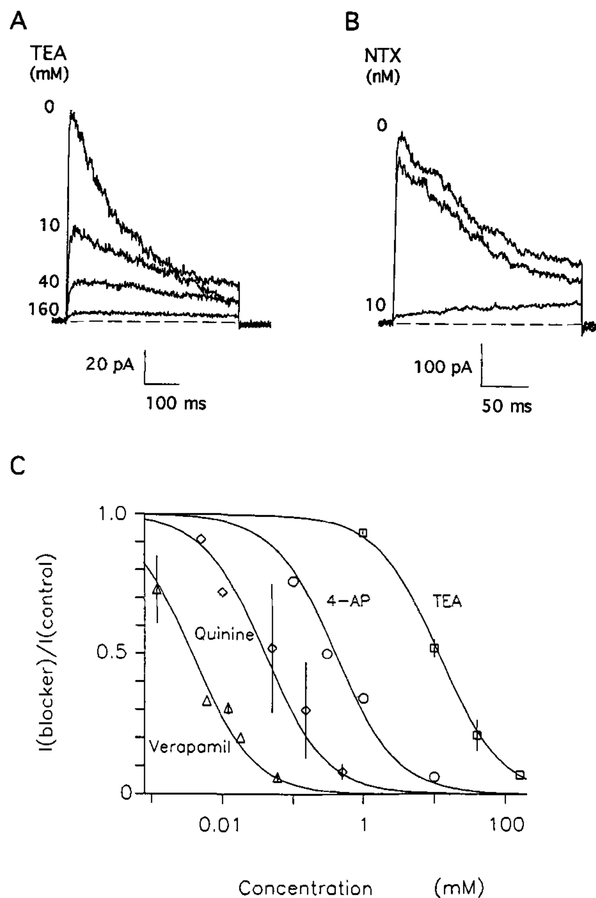

Figure 3. Pharmacology of K^+ currents in an outside-out patch from an oocyte injected with Kv1.3 cRNA. (A) K^+ currents were activated with 400 ms depolarizing pulses from − 80 mV to + 40 mV every 30 s before and during application of 10, 40, and 160 mM tetraethylammonium (TEA). (B) K^+ currents were activated with 200 ms depolarizing pulses from − 80 mV to + 40 mV every 30 s before, during, and after application of 10 nM noxiustoxin (NTX). (C) Dose-response relationship for verapamil, quinine, 4-amino-pyridine (4-AP), and TEA to reduce K^+ currents in outside-outpatches from oocytes injected with Kv1.3 cRNA. The fits of the dose-response curves for the reduction of current yielded apparent dissociation constants of 4 μM, 40 μM, 0.4 mM and 11 mM, respectively. Internal pipette solution was KF.[18,21,57]

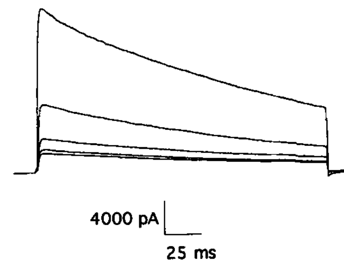

Figure 4. Voltage-gated K^+ currents in rat basophilic leukemia (RBL) cells infected with vaccinia virus and Kv1.3. K^+ currents were activated with 200 ms depolarizing pulses from − 80 mV to + 40 mV every second. This pulse interval is too short to allow complete recovery from inactivation and the current amplitude decreases with each depolarizing pulse. Uninfected RBL cells do not express voltage-gated K^+ channels of similar magnitude (< 200 pA). Internal pipette solution was KF.[18]

currents > 50 μA, and the reasons underlying these differences in results remain unclear.

We had previously mapped the Kv1.3 gene to human chromosome 13.[57] More recently, Attali and colleagues[61] reported that Kv1.3 was located on human chromosome 1p13.3, and Tempel's group have mapped this gene to mouse chromosome 3 in a region homologous to human 1p21 (B. Tempel, personal communication). We are currently re-examining our chromosomal localization data to clarify this issue.

The Kv1.3 protein has a predicted molecular mass of ~ 59 kDa, based on its deduced amino acid sequence.[57,61,64,65,68] A consensus sequence for N-glycosylation[71] in the external loop linking the S1-S2 segments (position 232)[65] is conserved in all the *Shaker*- and *Shaw*-subfamily channel proteins.[56] Other potential N-glycosylation sites in Kv1.3[65] are located on the cytoplasmic surface of the protein and are therefore unlikely to be used. Potential sites for phosphorylation[72] by tyrosine kinase (position 114), cyclic-AMP-dependent protein kinase (positions 70, 472, 474, 526) and protein kinase C (position 343, 347) are also present in Kv1.3.[65] We have generated a polyclonal antibody against a synthetic peptide (position 369-388, corresponding to the external loop linking the S5 segment and the pore of mKv1.3[69]), which recognizes a ~ 60-70 kDa membrane protein in mouse and human T cells. A second polyclonal antibody produced against the extracellular S1-S2 loop of the rat Kv1.3 protein has been used in immunoprecipitation experiments to demonstrate that Kv1.3 is phosphorylated *in vivo* at serine residues (Y.C. Cai, J. Douglass, personal communication). In *in vitro* experiments, PKA and PKC have been shown to phosphorylate Kv1.3 (Y.C. Cai, J. Douglass, personal communication), possibly at serine 347, 472 or 474. Both Kv1.3-specific antibodies will be valuable tools for the biochemical characterization of the Kv1.3 protein, and for the identification of post-translational modifications.

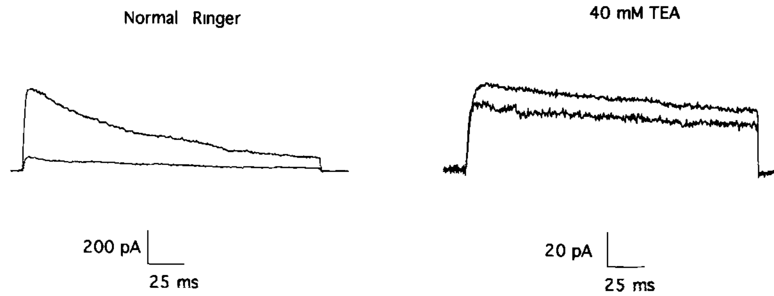

Figure 5. Effect of 9.2 nM charybdotoxin (CTX) on T cell K^+ channels in the absence (left) and presence of 40 mM TEA (right). K^+ currents were activated with 200 ms depolarizing pulses from −80 mV to +40 mV every 30 s. Internal pipette solution was KF.[18]

External TEA[73] interacts with a histidine at the mouth of the Kv1.3 channel (GYGDM*H*PVTIG),[65] thereby preventing slow C-type inactivation[74] while blocking opened Kv1.3 channels.[75] Extracellular acidification protonates this histidine, consequently slowing C-type inactivation[74] and reducing the sensitivity of the channel to block by TEA.[73] Substitution of a glycine (SS*G*FNSIPD), in the loop linking S5 with the putative pore (P-region), with a phenylalanine appears to interfere with the high affinity interaction between CTX and Kv1.3, probably by sterically hindering CTX binding (R. Swanson, personal communication). Conversely, replacing phenylalanine at the homologous position in the CTX-insensitive *Shaker*B channel with a glycine facilitates a high affinity interaction between CTX and the channel.[76] Quinine and TEA applied externally interfere with CTX block of Kv1.3 channel, as seen in Figure 5, and quinine has been previously shown to inhibit CTX binding to Kv1.3.[26] These three drugs therefore appear to occupy identical or overlapping sites in the mouth of the channel.

Modulation of the Kv1.3 channel

Kv1.3 expression could be regulated during transcription and/or translation, or post-translationally. The mitogen-stimulated ~twofold increase in Kv1.3 channel expression in human T cells[24-26] could be due to an increase in either transcription or translation, a decreased rate of degradation of either mRNA or protein, or it might be the result of posttranslational activation of previously non-functional cryptic molecules. Radiolabeled CTX binding to human T cells, a measure of the number of Kv1.3 molecules on the cell surface, increases two- to threefold following the activation of human T cells by anti-CD3 and IL2,[26] possibly reflecting the synthesis of new Kv1.3 proteins. However, Kv1.3 mRNA levels fall dramatically 16-48 h after activation of human peripheral blood T cells with Con A.[68] Thus, either increased translation of pre-existing Kv1.3 transcripts, or posttranslational activation of intracellular pools of inactive channel proteins, may underlie the mitogen-induced increase in Kv1.3 channels in human T cells. While Con A-stimulated mouse T cells show a similar time-dependent decrease in Kv1.3 mRNA,[68] the IL2-triggered ~fivefold increase in type *n* K^+ channel expression in the mouse T cell line L2, by contrast, appears to be associated with an equivalent increase in Kv1.3 mRNA expression.[67]

A number of second messengers can lead to changes in the electrophysiological properties of ion channels, thereby contributing to an additional level of K^+ channel diversity. Intracellular calcium at micromolar concentrations, levels reached during a normal activation response, increase type *n* K^+ channel inactivation.[77,78] Raising either external or internal Ca^{2+} accelerates inactivation in a similar fashion, suggesting that the binding site for this ion lies within the pore of the channel.[11,78] K^+ channel behaviour, it should be noted, may also be sensitive to changes in pH and temperature. Intracellular alkalinization enhances type *n* K^+ currents, whereas acidification inhibits these channels;[79] similarly, an increase in temperature from 22°C to 37°C enhances type *n* channel activity.[80,81]

Modulators of adenyl cyclase (PGE2 and cholera toxin) as well as a cyclic AMP analogue (8-bromo-cAMP), at concentrations which block phytohemagglutinin-stimulated IL2 production, have been reported to inhibit the activity of type *n* K^+ channels in the human Jurkat T cell line.[82] However,

these data have not been confirmed by Deutsch's group, who were unable to detect any effects of cyclic-AMP or cAMP-modulating agents on type *n* K^+ currents channels in resting human T cells.[80,83] Protein kinase C also has been reported to suppress type *n* K^+ currents in Jurkat T cells,[82] and we have shown that cloned mKv1.3 channels expressed in oocytes are similarly modulated.[84] Neurotransmitters have also been reported to affect type *n* K^+ channel currents. For example, β-adrenergic agonists and substance P interact with receptors on T cells and cause inhibition of the type *n* K^+ channel.[85,86] We have recently shown that 10 nM serotonin working via the $5HT_{1c}$ receptor completely inhibits Kv1.3 currents expressed in *Xenopus* oocytes within ~15 min;[84] inhibition is dependent on the presence of calcium, since EGTA (a calcium chelator) can abrogate the effect. Serotonin (1 μM) acting via the $5HT_2$ receptor, modulates rat and human Kv1.3 in a similar manner.[87,88] Thus, modification of K^+ channel activity in T cells may be a major factor in controlling the lymphocyte activation response.

The *Shaw*-related Kv3.1 protein encodes the type *l* K^+ channels in T cells

The *Shaw*-related mouse gene Kv3.1, when expressed in *Xenopus* oocytes, produces a K^+ channel with biophysical and pharmacological properties[21] identical to those of the type *l* K^+ channel in mouse T cells[9-11,51-54] and in the human B cell line, Louckes,[20,21] indicating that the native channel is composed of a homomultimer of the Kv3.1 gene product. Using Kv3.1-specific PCR primers we have detected Kv3.1 mRNAs in mouse T cells and in human Louckes cells,[21] and occasionally in human peripheral blood mononuclear cells. Such studies are facilitated by the fact that the amino acid sequences of the mouse, rat and human Kv3.1 gene products are very highly conserved.[21,63,89,90]

Unlike the vertebrate *Shaker*-subfamily genes that have intronless coding regions, the mammalian *Shaw*-related genes can exhibit alternate splicing within the coding region.[89-92] The Kv3.1 gene yields at least two transcripts (Kv3.1a/NGK2; Kv3.1b/Kv4) which encode functionally indistinguishable K^+ channels.[89,90] These mRNAs are identical up to amino acid 501 in the C-terminal region and then diverge completely, the last 10 amino acids of Kv3.1a being replaced by a distinct sequence of 84 amino acids in Kv3.1b. The Kv3.1a transcripts vary in size from 7-11 kb,[89,90] whereas the Kv3.1b mRNA is about 4.5-5 kb.[89] Mouse thymocytes and splenic T cells express Kv3.1a mRNAs, whereas Louckes cells contain a ~5 kb transcript suggestive of Kv3.1b.[93] Using specific probes, Kv3.1b mRNAs have been shown to be confined mainly to the rat cerebellum, and are not found in heart, kidney, liver, lung and skeletal muscle.[94]

The predicted size of the Kv3.1a protein is ~58 kDa, based on its deduced amino acid sequence,[90] while the Kv3.1b protein is ~67 kDa.[89] Two putative glycosylation sites are present in the external S1-S2 loop (positions 220 and 229), the second of these sites being conserved in all *Shaw*- and *Shaker*-subfamily proteins.[90] Like the Kv1.3 protein, the Kv3.1 channel has a potential PKC phosphorylation site in the internal S4-S5 loop (position 337).[90] The exquisite sensitivity of the Kv3.1 channel to external TEA is probably due to the presence of a tyrosine (GDM*Y*PKQ) at the C-terminal end of the putative pore region.[73] Mutagenesis studies have also shown that a leucine (MTT*L*GYGD) in the pore region is one of two residues that determine the single-channel conductance and K^+/Rb^+ selectivity of Kv3.1.[95,96] The amino acid sequence of the central portion of this region (the 'deep pore') of Kv3.1 differs from Kv1.3 only at this position, with a valine replacing the leucine of Kv1.3. It will be interesting to determine whether this residue contributes in any way to the difference in single channel conductance of the two T cell K^+ channels.

We have localized Kv3.1 to human chromosome 11[21] and, more recently, Rudy's group has sub-localized the gene to 11p15.[63] We have also mapped the Kv3.1 gene to mouse chromosome 7 in a location homologous to human chromosome 11p15, and in close proximity to the H-*ras* protooncogene (R. Wymore, N.A. Jenkins, K.G. Chandy, unpublished data). The defective gene which is responsible for the long QT syndrome, a cardiac dysrhythmia due to improper repolarization during the action potential, has been localized to a region within 10 Mbp of the H-*ras* gene,[97] suggesting a possible connection with the Kv3.1 gene. Genes in the 11p15.4-15.5 region have also been implicated in acute T cell leukemias and type-1 diabetes mellitus (see ref 63). It may be fruitful, therefore, to consider the possibility that alterations in the Kv3.1 gene, either in its coding region or in its regulatory sequences, may alter its expression in T cells in patients with leukemia or type-1 diabetes.

T cell K^+ channels as potential targets for the development of anti-inflammatory drugs

The isolation of a vast family of K^+ channel genes expressed in many cell types, and the identification of sites within K^+ channel proteins which interact with channel modulating drugs, has, for the first time provided an opportunity to rationally design drugs which selectively bind to a specific K^+ channel in a particular tissue. Since type *n* K^+ channel blockers inhibit T cell activation, they could likely serve to treat a variety of T cell-mediated disorders, and may have fewer side effects than currently used immunosuppressive agents such as cyclosporine and FK-506. In fact, quinidine, a K^+ channel blocker, has been reported to ameliorate experimental allergic neuritis.[99] By extension, type *l* K^+ channel blockers would be expected to suppress the function of double negative T cells that abnormally overexpress the type *l* K^+ channel, and might therefore be used to selectively retard the progress of autoimmune disorders.

Conclusion

A combination of molecular biological, electrophysiological and cell biological techniques have been applied to investigate the role of K^+ channels in normal and diseased T cells. These approaches have enabled us to define the role of K^+ channels as important modulators of lymphocyte function and proliferation, and have facilitated the identification of an abnormality in K^+ channel expression in T cells associated with autoimmune diseases. Future studies will clearly be directed toward the identification of selective therapeutic agents which modulate the functions of these proteins and thereby ameliorate inflammatory and autoimmune disorders.

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