Ion channels in the immune system as targets for immunosuppression

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The discovery of a diverse and unique subset of ion channels in T lymphocytes has led to a rapidly growing body of knowledge about their functional roles in the immune system. Potent and specific blockers have provided molecular tools to probe channel structure–function relations and to elucidate the involvement of K^+, Ca^{2+}, and Cl^- channels in T-cell activation and cell volume regulation. Recent advances in analyzing Kv1.3 channel structure–function relationships have defined binding sites for channel blockers, which have now been shown to be effective in suppressing T-cell function *in vivo*. Ion channels may provide excellent pharmaceutical targets for modulating immune system function.

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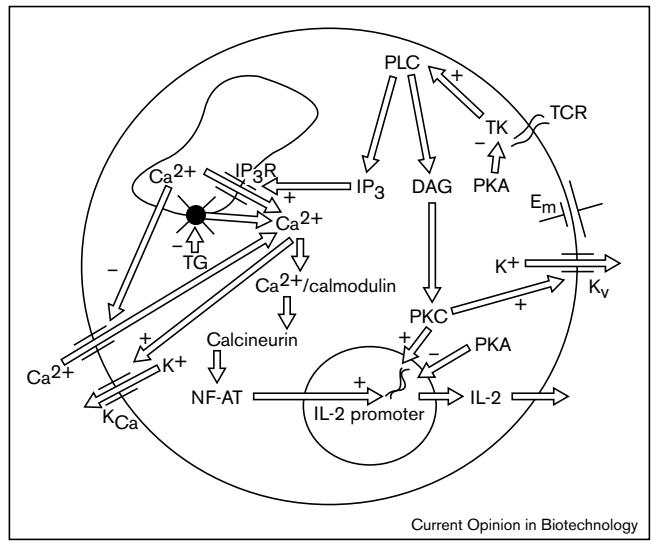
Abbreviations

$[Ca^{2+}]_i$	cytoplasmic Ca^{2+} concentration
CTX	charybdotoxin
IP_3	inositol 1,4,5-trisphosphate
K_V	voltage-gated K^+
K_{Ca}	calcium-activated K^+
PKC	protein kinase C

Introduction

The early stages of T-cell activation may be conceptually separated into pre-Ca^{2+} and post-Ca^{2+} events [1,2]. Initiated within 1–100 seconds of T-cell receptor engagement, pre-Ca^{2+} events include the activation of tyrosine kinases and the generation of inositol 1,4,5-trisphosphate (IP_3), leading to the release and influx of Ca^{2+} and the rise in cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$. Ranging from minutes to several hours, post-Ca^{2+} events involve changes in serine/threonine kinase and phosphatase activities, alterations in cytoskeleton and ion channel activity, and gene transcription. The rise in $[Ca^{2+}]_i$ activates the phosphatase calcineurin which then dephosphorylates a cytoplasmically localized transcription factor (nuclear factor of activated T cells) enabling it to accumulate in the nucleus and bind to a promoter element of the interleukin 2 gene. Along with other parallel events, involving the activation of protein kinase C (PKC) and ras, gene transcription leads to lymphokine secretion and to cell proliferation. A sustained or oscillatory Ca^{2+} signal results in dynamic changes in motility, morphology, and gene expression in T cells, with cytoskeletal elements and gene transcription exhibiting varying requirements for activation by $[Ca^{2+}]_i$; some genes requiring only a transient rise and others a long lasting or oscillatory Ca^{2+} signal [3,4,5•,6•]. Ca^{2+}-dependent immobilization of the T cell at the site of antigen presentation may help to cement the interaction between T cell and antigen-presenting cell and thereby facilitate the maintenance of local signaling between cells [5•]. Separate Ca^{2+}-dependent pathways control gene expression and motility; a pathway leading to interleukin 2 secretion is highlighted in Figure 1.

Figure 1



Signaling pathways in T cells. A signal transduction cascade leading from the T-cell receptor (TCR) to interleukin (IL)-2 secretion. DAG, diacylglycerol; E_m, plasma membrane potential; IP_3R, inositol 1,4,5-trisphosphate receptor; NF-AT, nuclear factor of activated T cells; PKA, protein kinase A; TG, thapsigargin; TK, tyrosine kinase. Plus signs indicate activation and minus signs indicate inhibition.

Presently, a post-Ca^{2+} target, calcineurin, is the site of action for immunosuppression. Unfortunately the calcineurin inhibitors cyclosporin A and FK506 are toxic, with liver and renal disease limiting their use. Thus, the search for additional immunosuppressive agents for transplantation or inflammatory diseases occupies considerable attention in the pharmaceutical industry. There is an excellent track record of treating nervous and cardiovascular disorders with channel modulators — either blockers or openers. Channel blockers, as a general class, represent the major therapeutic agents for treatment of stroke, epilepsy, and arrhythmia. These considerations suggest that ion channels may represent attractive sites

for pharmaceutical immunomodulation, targeting the pre-Ca^{2+} stage of activation. In this review, we consider Ca^{2+}-dependent signal transduction pathways that depend upon the activity of ion channels and survey progress in identifying and characterizing a surprisingly diverse and functionally significant population of ion channels in T cells.

Signaling pathways in T-cell activation: the role of ion channels

Ion channels underlie the Ca^{2+} signal of T cells [2]. Initially, phospholipase C-mobilized IP_3 produces a transient $[Ca^{2+}]_i$ rise by activating the IP_3 receptor, a Ca^{2+}-permeable ion channel located in the endoplasmic reticulum. IP_3 receptors have also been reported to reside in the surface membrane of T cells [7,8], but this result remains controversial. Functionally, IP_3 receptors, even if present in the plasma membrane, appear not to participate in the Ca^{2+} influx mechanism [9–11]. Ca^{2+} ions move across the plasma membrane through a Ca^{2+}-selective channel that is activated through an unknown mechanism by the depletion of Ca^{2+} from the endoplasmic reticulum [4,9,10,12–19]. Termed a store-operated Ca^{2+} channel, or a calcium release-activated Ca^{2+} channel, the lymphocyte Ca^{2+} channel is not gated by voltage, in contrast to voltage-gated Ca^{2+} channels found in neurons, muscle, and the heart. Once the Ca^{2+} channel is opened by store depletion, Ca^{2+} influx depends upon the plasma membrane potential to provide the electrical driving force to pull Ca^{2+} ions inward. Compared to conventional voltage-gated Ca^{2+} channels, the inverse dependence of Ca^{2+} influx on the plasma membrane potential is especially pronounced because current through the open Ca^{2+} channel rectifies inwardly.

Two distinct types of K^+ channels, a voltage-gated K^+ (K_V) channel and a Ca^{2+}-activated K^+ (K_{Ca}) channel, indirectly determine the driving force for Ca^{2+} entry [2]. When K^+ channels are open, the resulting efflux of K^+ drives the membrane potential to a negative voltage. The resting membrane potential of –50 to –60 mV in T cells is uniquely set by the voltage-gated channel (type *n* encoded by Kv1.3), which resists depolarization through its ability to open when the membrane is depolarized [20,21]. K_{Ca} channels are opened in a steeply cooperative manner by a rise in $[Ca^{2+}]_i$ following T cell receptor engagement, and these channels serve to hyperpolarize the membrane even further to –80 mV [20,22–25]. The hyperpolarization may accentuate Ca^{2+} influx in a positive feedback manner to promote the upstroke of the Ca^{2+} signal [26,27,28•].

Diversity of ion channels in T lymphocytes

In the early 1980s, development of the patch clamp technique and its unique applicability to a wide variety of unexplored cell types led to the first electrophysiological studies in T cells and to the identification of a K_V channel in resting human T cells [29–31]. Patch clamping permits electrical recording to identify and characterize ion channels with resolution to the level of single channel molecules. Several additional ion channels have since been characterized in T cells, as summarized in Table I.

K_V channels

Soon after the electrophysiological characterization of the dominant K_V channel and the identification of an array of chemically distinct K^+ channel blockers, it was discovered that these same channel-blocking agents are able to inhibit T-cell activation, including secretion of lymphokines, cell proliferation, and killing of target cells [29,32]. Two developments have greatly increased the pace of discovery regarding structure and function of the type *n* K_V channel. First, increasingly potent and selective peptide toxins are providing important tools for investigating the channel's functional role, for protein purification, for mapping the topology of the channel vestibule, and for high-throughput screens that have identified a new generation of highly potent blockers currently being pursued in the pharmaceutical industry [33,34,35•,36–46]. In 1989, two groups reported that peptide scorpion toxins can block the type *n* K_V channel at nanomolar concentrations [33,34]. At the time, the pharmacology of K^+ channels lagged behind other channel types, but now toxins have become important research tools and are also being explored for their therapeutic possibilities. In addition, the cloning of a series of K^+ channel genes and identification of Kv1.3 as the gene encoding the type *n* channel have facilitated drug discovery and structure-function relations [47–51]. The type *n* channel is a 65×65 Å homotetramer of Kv1.3 subunits, which contains several important functional domains that regulate channel gating, ion selectivity, and binding of drug molecules (Figure 2) [52–55].

Recently, the use of a peptide toxin as an immunosuppressant was validated by an *in vivo* mini-pig model of delayed-type hypersensitivity and allogeneic responses [56••]. Margatoxin was found to be safe and significantly more potent than FK506 as an injectable immunosuppressant. Peptide toxins block the channel like a cork in a bottle, by binding to a 30 Å wide by 6 Å deep external vestibule, with several contact sites between the channel and the toxin molecule having been identified [36,37]. Mutagenesis of Kv1.3 has pointed to interactions between nonpeptidyl channel blockers, including dihydroquinolines and benzhydryl piperines for which considerable structure-activity information exists, and the inactivation gating mechanism which normally shuts the channel during prolonged depolarization [43]. A unique histidine in the outer mouth of the Kv1.3 channel, in addition to being involved in the inactivation gating mechanism, may confer selectivity upon the Kv1.3 channel for certain channel blockers [57–60]. Other classes of blockers, including verapamil, a classical Ca^{2+}-channel antagonist, may interact with residues near the inner channel vestibule [61,62]. The diversity of Kv1.3 channel blockers has recently been reviewed [44].

Table 1

Ion channels in lymphocytes.

Name	Gene	Conductance (pS)	Activation (midpoint)	Blockers (K_d range)	Expression (resting→proliferating)	Functional role
K_V						
n	Kv1.3	10–18	Voltage –40 mV	CTX nM MgTX nM TEA 10 mM	Human T cell (++→+++) Mouse T cell (+→+++) Mouse immature thymocytes +++ Jurkat ++, numerous cell lines	Sets resting membrane potential Indirectly modulates Ca^{2+} influx RVD
l	Kv3.1	27	Voltage 0 mV	TEA 100 μM	Mouse $CD8^+$ thymocyte ++ *lpr*, *gld* $CD4^-CD8^-$ T cell +++	?
Other	Kv1.1?	?	Voltage –20 mV	DTX nM TEA 1 mM	Human T cell + Mouse thymocytes +, B3Z +	Supports membrane potential if Kv1.3 is blocked
K_{Ca}						
SK	SKCa3?	2–8	$[Ca^{2+}]_i$ 400 nM	Apamin pM TEA 2 mM	Jurkat +++	Hyperpolarization during Ca^{2+} signal promotes Ca^{2+} influx Ca^{2+}-dependent RVD
IK	IKCa1?	11–35	$[Ca^{2+}]_i$ 300 nM	CTX nM TEA 40 mM	Human T cell (+→+++) Mouse thymocytes ++	Mediates Ca^{2+} influx, oscillations
Ca^{2+}						
CRAC	TRP?	0.01	Ca^{2+} stores depletion	La^{3+} nM< Gd^{3+} nM	Jurkat +++ Human T cell Mouse thymocytes	
SWAC	?	?	Swelling	Gd^{3+} μM< La^{3+} μM	Mouse immature thymocytes	Ca^{2+}-dependent RVD
P2X	P2X7?	?	μM ATP_o		Mouse thymocytes, T cells	Differentiation, activation?
Cl^-						
Mnini	?	2–3	Swelling ATP_i	DIDS μM< NPPB μM	Human T cell (+++→+++) Mouse T cell (+++→+++)	Trigger for RVD

+ represents ~5–50, ++ 100–500, and +++ >500 channels per cell. CRAC, calcium release activated Ca^{2+} channel; DIDS, 4,4′-diisothiocyanatost -ilbene-2,2′-disulfonic acid; DTX, dendrotoxin; IK, intermediate conductance calcium-activated potassium channels; MgTx, margatoxin; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; RVD, regulatory volume decrease; SK, small conductance calcium-activated potassium channel; SWAC, swelling-activated Ca^{2+}-permeable channel; TEA, tetrathylammonium.

In general, the activity of ion channels can be modulated by direct channel block, phosphorylation or some other post-translational modification of the protein, or by altered levels of expression. The Kv1.3 channel is a substrate for protein kinase A, PKC, and tyrosine kinases in T cells; modulation of channel properties by phosphorylation may in turn impact signalling pathways involving the membrane potential [63–70]. Kv1.3 contains an adapter sequence at the carboxy terminus for the hDLG protein, which in turn associates with the tyrosine kinase p56*lck*, suggesting the existence of complexes of enzymes and channels at the membrane [71]. Functional effects of phosphorylation are still being clarified. Recently, attention has focused on tyrosine kinases, with papers documenting biochemical phosphorylation and decreased K^+ currents through Kv1.3 channels that could be relevant to fas-mediated apoptosis [66,67]. PKC stimulation was reported to decrease K^+ currents through Kv1.3 channels expressed in oocytes [63], but recently PKC stimulation has been reported to increase Kv1.3 currents in human T cells [64].

The tissue-specific and activation-dependent regulation of ion channel expression is poorly understood. The Kv1.3 gene contains an intronless coding region of 1.5 kilobases, yet mRNA species of 3.5–9.5 kilobases have been identified in lymphoid cells [47,51]. Regulatory elements controlling transcription have been described [72]. The levels of Kv1.3 expression are increased during T-cell activation, further suggesting a role in mitogenesis [2]. Kv1.3 is found associated with a β2 subunit which is also up-regulated during activation and may serve to stabilize the integrity of the channel complex [73].

Several groups have now identified additional K_V channels expressed at lower levels in lymphocytes, including charybdotoxin (CTX)-insensitive channels that may contribute to the maintenance of the membrane potential if Kv1.3 is blocked [25,74–76]. Inhibition of immune function by CTX and other toxins is generally not as complete as with less specific K^+ channel antagonists [2,27,34,39]. In murine T cells, another class of CTX-insensitive K_V channel, encoded by Kv3.1, is normally expressed in $CD8^+$ T cells, and aberrantly overexpressed in $CD4^-CD8^-$ T cells in several models of autoimmune disease, including lupus erythematosus, type 1 diabetes mellitus, collagen arthritis, and experimental allergic encephalomyelitis [2,77].

K_{Ca} channels

In addition to K_V channels, K_{Ca} channels are also attracting attention. Again, peptide toxins are providing useful tools for pharmacological separation of different channel components. The most common K_{Ca} channel in

Figure 2

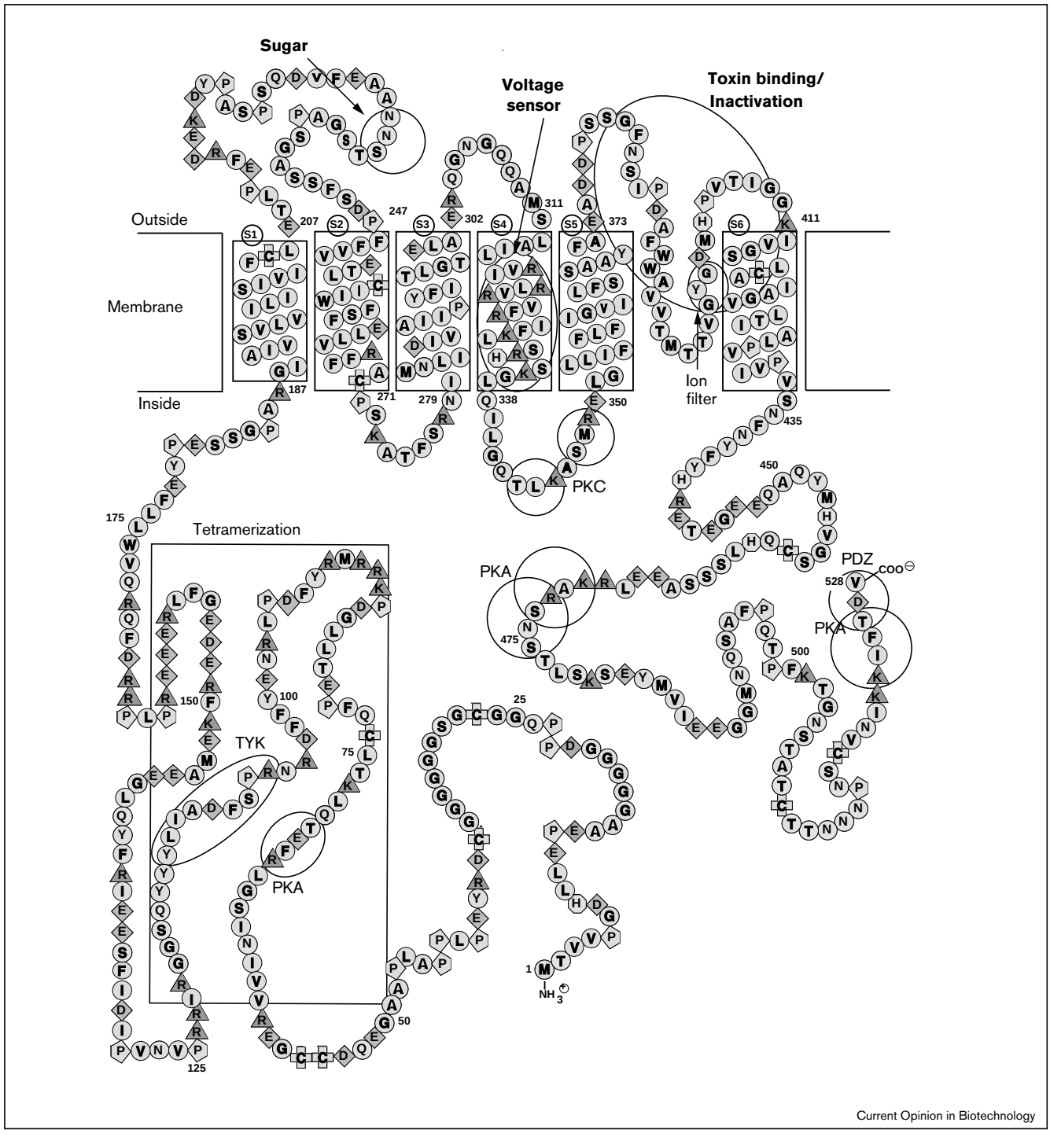


Diagram of Kv1.3. Major functional domains within the amino acid sequence (amino acid single letter code) of Kv1.3 are indicated. The channel is composed of four identical Kv1.3 subunits; the rectangle with 'tetramerization' shows the site of monomer–monomer interaction. The circles show sites that are functionally associated with various properties. S1, S2 etc., denote transmembrane segments. PKA, protein kinase A; TYK, tyrosine kinase.

human T cells is activated by a rise in $[Ca^{2+}]_i$ to 200 nM or more, has an intermediate single-channel conductance of 11–35 pS, and is blocked by nanomolar concentrations of CTX but not by margotoxin, kaliotoxin, or noxiustoxin, other toxins that block K_V channels at nanomolar levels or below [23]. Just to complicate matters, a commonly used T-cell lymphoma line (Jurkat) expresses a different, smaller conductance K_{Ca} channel that is blocked by

apamin but not by CTX, suggesting that transformation or lymphoid differentiation may alter their pattern of K_{Ca} channel expression [22,24]. In normal human T cells, expression of the intermediate conductance CTX-sensitive K_{Ca} channel is dramatically increased as T cells become activated to proliferate [23]. In parallel, activated T cells gain the ability to exhibit stronger and more oscillatory Ca^{2+} signals, raising the possibility that K_{Ca} channels participate indirectly by hyperpolarizing the membrane and thereby promoting Ca^{2+} entry [20,26,27,28•]. Targeting K_{Ca} channels may be particularly attractive in modulating autoimmune responses by previously activated T cells. Recently, a family of voltage-insensitive K_{Ca} channel genes has been discovered, encoding at least two small-conductance apamin-sensitive K_{Ca} channels and an intermediate conductance K_{Ca} channel [78•,79]. One of these, hIKCa1 or hSKCA4, is found in thymocytes and has properties similar to the intermediate conductance channel found in T cells [79,80]. The channel has been proposed to underlie the earliest known K_{Ca} channel — the Gardos channel in red blood cells, a therapeutic target for sickle cell anemia.

Ca^{2+} channels

Conceptually, the most direct target for toning down the $[Ca^{2+}]_i$ signal would be the Ca^{2+} channel [4,9,10–15]. With Ca^{2+} channels blocked, T cells exhibit only a meager and transient rise in $[Ca^{2+}]_i$; the Ca^{2+} channel is absolutely required for long-lasting signals that are capable of stimulating transcription. Furthermore, a novel primary T-cell immunodeficiency is associated with defective Ca^{2+} entry via Ca^{2+} channels [81]. Unfortunately, progress in identifying channel blockers for the Ca^{2+} channel has been slow, no peptide toxins or sub-micromolar blockers exist except for nonspecific, but surprisingly potent, La^{3+} ions [82,83]. Furthermore, the activation mechanism linking store depletion to channel opening, as well as the channels molecular identity, are still enigmatic. Similarities in ion permeation (but not gating) between voltage-gated and stores-operated Ca^{2+} channels suggest commonalities in pore-lining sequences and enable much larger monovalent currents to be studied [84].

Other channels, other functions

Several other channel types have been reported in patch-clamp and Ca^{2+} imaging experiments. Chloride channels activated by cell swelling were initially discovered in T cells but are also present in a variety of other cell types [85–91]. In lymphocytes, Cl^- channels provide the trigger for a homeostatic volume regulatory mechanism which restores the cell to its normal volume following exposure to a dilute environment [85,90]. Cell swelling in hypotonic solution activates the chloride channels, resulting in the loss of Cl^- and other permeable osmolytes, depolarization, and consequent opening of Kv1.3 channels. The loss of Cl^- and K^+ through their respective channels, along with osmotically obligated water, reduces cell volume. Cl^- channels may also play a role in mitogenesis by helping to maintain membrane potential [76,91]. In thymocytes, a calcium-permeable cation channel activated by cell swelling may complement this normally Ca^{2+}-independent mechanism for regulatory volume decrease, by providing a Ca^{2+}-dependent pathway and bringing K_{Ca} channels into play as a second K^+ efflux pathway [83].

Certain thymocyte subsets and mature T cells express P2Z receptors activated by extracellular ATP [92–98]. In the thymic microenvironment, ATP levels are probably high enough to activate these receptors, leading to the opening of a large Ca^{2+}- and cation-permeable channel. $[Ca^{2+}]_i$ signaling evoked by P2Z receptors has been implicated in triggering or enhancing proliferation, differentiation, and apoptosis. The role that these and other channels may play in lymphocyte function merits further investigation.

Conclusions

During the past few years, considerable attention has been focused upon T cell ion channels as targets for immunomodulation. A voltage-gated K^+ channel encoded by Kv1.3 has been subjected to the most intense scrutiny, with the effectiveness of highly selective peptide toxins in an *in vivo* model proving the concept that channel blockade can work to suppress the immune response. We can anticipate that channel blockers, by inhibiting pre-Ca^{2+} steps in the activation cascade may synergize with post-Ca^{2+} agents presently used, enabling doses of cyclosporin or FK506 to be substantially lowered, thereby reducing toxicity. The mix of ion channels expressed in a subset- and activation-dependent manner offers opportunities for continued development of channel blockers as immunomodulatory agents.

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