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+, Ca2+, 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
[Ca2+]i: cytoplasmic Ca2+ concentration
CTX: charybdotoxin
IP3: inositol 1,4,5-trisphosphate
Kv: voltage-gated K+
KCa: calcium-activated K+
PKC: protein kinase C

Introduction
The early stages of T-cell activation may be conceptually separated into pre-Ca2+ and post-Ca2+ events [1,2]. Initiated within 1–100 seconds of T-cell receptor engagement, pre-Ca2+ events include the activation of tyrosine kinases and the generation of inositol 1,4,5-trisphosphate (IP3), leading to the release and influx of Ca2+ and the rise in cytoplasmic Ca2+ concentration [Ca2+]i. Ranging from minutes to several hours, post-Ca2+ events involve changes in serine/threonine kinase and phosphatase activities, alterations in cytoskeleton and ion channel activity, and gene transcription. The rise in [Ca2+]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 Ca2+ 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 [Ca2+]i; some genes requiring only a transient rise and others a long lasting or oscillatory Ca2+ signal [3,4,5•,6•]. Ca2+-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 Ca2+-dependent pathways control gene expression and motility; a pathway leading to interleukin 2 secretion is highlighted in Figure 1.

Presently, a post-Ca2+ 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-
Ca²⁺ stage of activation. In this review, we consider
Ca²⁺-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 Ca²⁺ signal of T cells [2]. Ini-
nally, phospholipase C-mobilized IP₃ produces a transient
[Ca²⁺]ᵢ rise by activating the IP₃ receptor, a Ca²⁺-perme-
able ion channel located in the endoplasmic reticulum.
IP₃ receptors have also been reported to reside in the
surface membrane of T cells [7,8], but this result remains
controversial. Functionally, IP₃ receptors, even if present
in the plasma membrane, appear not to participate in
the Ca²⁺ influx mechanism [9–11]. Ca²⁺ ions move across
the plasma membrane through a Ca²⁺-selective channel
that is activated through an unknown mechanism by
the depletion of Ca²⁺ from the endoplasmic reticulum
[4,9,10,12–19]. T ermed a store-operated Ca²⁺ channel, or
Ca²⁺-activated K⁺ (KCa) channel, the lymphocyte
Ca²⁺ channel is not gated by voltage, in contrast to
voltage-gated Ca²⁺ channels found in neurons, muscle,
and the heart. Once the Ca²⁺ channel is opened by
store depletion, Ca²⁺ influx depends upon the plasma
membrane potential to provide the electrical driving force
to pull Ca²⁺ ions inward. Compared to conventional
voltage-gated Ca²⁺ channels, the inverse dependence of
Ca²⁺ influx on the plasma membrane potential is
especially pronounced because current through the open
Ca²⁺ channel rectifies inwardly.

Two distinct types of K⁺ channels, a voltage-gated K⁺
(Kᵥ) channel and a Ca²⁺-activated K⁺ (KᵥCa) channel,
indirectly determine the driving force for Ca²⁺ 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]. KᵥCa
channels are opened in a steeply cooperative manner by a
rise in [Ca²⁺]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 Ca²⁺ influx in a positive feedback manner to
promote the upstroke of the Ca²⁺ 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 Kᵥ
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.

**Kᵥ channels**

Soon after the electrophysiological characterization of
the dominant Kᵥ 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 Kᵥ 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 Kᵥ channel
at nanomolar concentrations [33,34]. At the time, the
pharmacology of Kᵥ 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
Kᵥ 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 immunosuppres-
sant 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 ben-
zydryl 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 Ca²⁺-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.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>Conductance (pS)</th>
<th>Activation (midpoint)</th>
<th>Blockers (K&lt;sub&gt;d&lt;/sub&gt; range)</th>
<th>Expression (resting→proliferating)</th>
<th>Functional role</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K&lt;sub&gt;V&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>Kv1.3</td>
<td>10–18</td>
<td>Voltage −40 mV</td>
<td>CTX nM MgTX nM TEA 10 nM</td>
<td>Human T cell (++→++++) Mouse T cell (++→++++) Mouse immature thymocytes +++</td>
<td>Sets resting membrane potential Indirectly modulates Ca&lt;sup&gt;2+&lt;/sup&gt; influx RVD</td>
</tr>
<tr>
<td>/</td>
<td>Kv3.1</td>
<td>27</td>
<td>Voltage 0 mV</td>
<td>TEA 100 µM</td>
<td>Mouse CD8&lt;sup&gt;+&lt;/sup&gt; thymocyte +++</td>
<td>Jurkat ++, numerous cell lines ?</td>
</tr>
<tr>
<td>Other</td>
<td>Kv1.1?</td>
<td>?</td>
<td>Voltage −20 mV</td>
<td>DTX nM TEA 1 mM</td>
<td>Human T cell + Mouse thymocytes +, B3Z +</td>
<td>Supports membrane potential if Kv1.3 is blocked</td>
</tr>
<tr>
<td><strong>K&lt;sub&gt;Ca&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK</td>
<td>SKCa3?</td>
<td>2–8</td>
<td>[Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;E&lt;/sub&gt; 400 mM</td>
<td>Apamin pM TEA 2 nM</td>
<td>Jurkat +++</td>
<td>Hyperpolarization during Ca&lt;sup&gt;2+&lt;/sup&gt; signal mediates Ca&lt;sup&gt;2+&lt;/sup&gt; influx Ca&lt;sup&gt;2+&lt;/sup&gt;-dependent RVD</td>
</tr>
<tr>
<td>IK</td>
<td>IKCa1</td>
<td>11–35</td>
<td>[Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;E&lt;/sub&gt; 300 nM</td>
<td>CTX nM TEA 40 mM</td>
<td>Human T cell (→++++) Mouse thymocytes ++</td>
<td></td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>CRAC</td>
<td>TRP?</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; stores depletion</td>
<td>La&lt;sup&gt;3+&lt;/sup&gt;nM&lt;sup&gt;c&lt;/sup&gt; Gd&lt;sup&gt;3+&lt;/sup&gt;nM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Jurkat +++ Human T cell Mouse thymocytes</td>
<td></td>
</tr>
<tr>
<td>SWAC</td>
<td>?</td>
<td>?</td>
<td>Swelling</td>
<td>Gd&lt;sup&gt;3+&lt;/sup&gt; µM&lt;sup&gt;c&lt;/sup&gt; La&lt;sup&gt;3+&lt;/sup&gt; µM</td>
<td>Mouse immature thymocytes</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;-dependent RVD</td>
</tr>
<tr>
<td>P2X</td>
<td>P2X7?</td>
<td>?</td>
<td>µM ATPo</td>
<td></td>
<td>Mouse thymocytes, T cells</td>
<td>Differentiation, activation?</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;−&lt;/sup&gt;</td>
<td>Mnini</td>
<td>?</td>
<td>2–3</td>
<td>DIDS µM&lt;sup&gt;c&lt;/sup&gt; NPPB µM</td>
<td>Human T cell (+++++++)</td>
<td>Trigger for RVD</td>
</tr>
</tbody>
</table>

+ represents ~5–50, ++ 100–500, and +++>500 channels per cell. CRAC, calcium release activated Ca<sup>2+</sup> channel; DIDS, 4,4′-disothiocyanatostilbene-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<sup>2+</sup>-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<sub>lk</sub>, 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<sup>+</sup> currents through Kv1.3 channels that could be relevant to fas-mediated apoptosis [66,67]. PKC stimulation was reported to decrease K<sup>+</sup> 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<sub>V</sub> 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<sup>+</sup> channel antagonists [2,27,34,39]. In murine T cells, another class of CTX-insensitive K<sub>V</sub> channel, encoded by Kv3.1, is normally expressed in CD8<sup>+</sup> T cells, and aberrantly overexpressed in CD4<sup>+</sup>CD8<sup>−</sup> T cells in several models of autoimmune disease, including lupus erythematosus, type 1 diabetes mellitus, collagen arthritis, and experimental allergic encephalomyelitis [2,77].

### K<sub>Ca</sub> channels

In addition to K<sub>V</sub> channels, K<sub>Ca</sub> channels are also attracting attention. Again, peptide toxins are providing useful tools for pharmacological separation of different channel components. The most common K<sub>Ca</sub> channel in
human T cells is activated by a rise in $\left[\text{Ca}^{2+}\right]_i$ 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 Kv channels at nanomolar levels or below [23]. Just to complicate matters, a commonly used T-cell lymphoma line (Jurkat) expresses a different, smaller conductance $\text{KCa}$ channel that is blocked by
apamin but not by CTX, suggesting that transformation or lymphoid differentiation may alter their pattern of KCa channel expression [22,24]. In normal human T cells, expression of the intermediate conductance CTX-sensitive KCa 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 Ca2+ signals, raising the possibility that KCa channels participate indirectly by hyperpolarizing the membrane and thereby promoting Ca2+ entry [20,26,27,28*]. Targeting KCa channels may be particularly attractive in modulating autoimmune responses by previously activated T cells. Recently, a family of voltage-insensitive KCa channel genes has been discovered, encoding at least two small-conductance apamin-sensitive KCa channels and an intermediate conductance KCa 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 KCa channel—the Gardos channel in red blood cells, a therapeutic target for sickle cell anemia.

**Ca2+ channels**

Conceptually, the most direct target for toning down the [Ca2+]i signal would be the Ca2+ channel [4,9,10–15]. With Ca2+ channels blocked, T cells exhibit only a meager and transient rise in [Ca2+]i; the Ca2+ 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 Ca2+ entry via Ca2+ channels [81]. Unfortunately, progress in identifying channel blockers for the Ca2+ channel has been slow, no peptide toxins or sub-micromolar blockers exist except for nonspecific, but surprisingly potent, La3+ 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 Ca2+ channels suggest commonalities in pore-linking 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 Ca2+ 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 osmoles, 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 Ca2+-independent mechanism for regulatory volume decrease, by providing a Ca2+-dependent pathway and bringing KCa 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 Ca2+- and cation-permeable channel. [Ca2+]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 vitro model proving the concept that channel blockade can work to suppress the immune response. We can anticipate that channel blockers, by inhibiting pre-Ca2+ steps in the activation cascade may synergize with post-Ca2+ 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.

**Acknowledgements**

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

With videos accompanying several figure legends, this paper shows the dynamic nature of the T cell’s response to antigen presentation. Overlays of Nomarski optics show membrane extensions and engulfment of the antigen-presenting cell, while the Ca\(^{2+}\) signal is illustrated by changes in color. The paper reveals the polarity of responsiveness to antigen using an optical trap to determine the point of cell–cell contact. Furthermore, the Ca\(^{2+}\) dependence of motility and shape is determined quantitatively and shown to differ from Ca\(^{2+}\) dependence of gene expression.


This paper documents the differential responsiveness of genes to varying Ca\(^{2+}\) signals. Nuclear factor KB, c-Jun amino-terminal kinase is shown to be selectively activated by a large transient rise in the intracellular Ca\(^{2+}\) concentration, whereas the nuclear factor of activated T cells is activated by a low, sustained Ca\(^{2+}\) plateau. Decoding the differential responsiveness of genes to a common second messenger reveals how signaling specificity can be achieved.


This paper describes a family of three genes, SKCa1–3, encoding small-conductance Ca²⁺-activated K⁺ channels. SKCa2 and 3 are apamin-sensitive, while SKCa1 is not. Several ESTs for SKCa3, isolated from lymphoid cells, exist in GenBank (GenBank:www.ncbi.nlm.nih.gov).


