Regulating the Immune Response

The unexpected role of ion channels

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The immune response is a tightly regulated process, in which any breach of this strict regulation could lead to pathological phenomena. Lately, the key role of ion channels in immune function has become apparent. In this review we will discuss the latest data concerning the prominent involvement of the K⁺ channels Kv1.3 and KCa3.1 (IKCa1) and of the purinergic receptor P2X7 in the regulation of key elements of the immune response.

Although not readily apparent, there are more than a few similarities between the immune system and the central nervous system (CNS). Both systems can process complex and dynamic input signals and produce an appropriate and timely response. Both systems use exquisitely complex mechanisms that ensure that the response will match the input signal and thus prevent potential damage. Therefore, it is fitting that immunologists are now using the term “synapse” (or “immunological synapse”) to describe the close encounter and interaction between an antigen presenting cell (APC) and a T lymphocyte cell. However, until recently the cells of the immune system were categorized as “electrically non-excitable”, that is cells that do not have action potentials generated by voltage-gated ion channels, the hallmark of CNS cells. In fact it is now well established that immune system cells do express a remarkable array of voltage-gated and other types of ion channels. The function of these channels in the context of the immune system has only recently began to be understood. It appears that ion channels play a prominent role in the regulation of the immune response much as they do in the context of the CNS. In the present review, we will focus on two different examples of ion channel function in the immune system: the involvement of the K⁺ channels Kv1.3 and KCa3.1 in T cell activation, and of the purinergic receptor P2X7 in the modulation of the immune response.

Regulation of T cell activation: The Kv1.3 and KCa3.1 channels play a key role

Kv1.3 belongs to the Shaker family of voltage-dependent K⁺ channels. The channel is expressed in various populations of hematopoietic origin such as T and B lymphocytes, macrophages and natural killer (NK) cells. The functional channel in T lymphocytes is composed of four Kv1.3 subunits and is responsible for maintaining the membrane potential in the resting cell. The Kv1.3 channel regulates the immune response through the regulation of T cell activation. The activated T cell can now stimulate effector mechanisms such as plasma cell-secreted antibodies and T cytotoxic cells (Tc) that will help contain the invading pathogen. Finally, the activated Tc either dies by apoptosis or differentiates to long-lived memory cells.

Figure 1:
The Kv1.3 channel regulates the immune response through the regulation of T cell activation

Antigen presenting cells such as dendritic cells (DCs) and macrophages phagocyte and digest invading pathogens such as bacteria. Selected peptides of the pathogen (antigens) are “presented” to T helper (TH) cells. Only the TH with the appropriate T cell receptor can proliferate and become activated. The activated TH can now stimulate effector mechanisms such as plasma cell-secreted antibodies and T cytotoxic cells (Tc) that will help contain the invading pathogen. Finally, the activated Tc either dies by apoptosis or differentiates to long-lived memory cells.
K+ channels KV1.3 and KCa3.1 are believed to be involved. The current model for understanding the involvement of K+ channels in T cell activation implies that these channels indirectly control the intracellular Ca2+ signals that are indispensable to T cell proliferation. It is well known that elevated levels of intracellular Ca2+ must be maintained for a relatively long time (a few hours) in order to allow the activated T cell to proliferate. Extracellular Ca2+ enters the cell based on its electrical driving force and therefore depolarizes the cell membrane. This induces the activation of the K+ channel which, via K+ efflux, helps to maintain a negative membrane potential, thus allowing for continuous Ca2+ entry. As mentioned above, T cell activation enhances the expression of KCa3.1 channels, which open in response to the increased cytosolic Ca2+ and thus further hyperpolarize the membrane potential.6,7

In summary, the concerted action of both KV1.3 and KCa3.1 allowed the sustained rise in intracellular Ca2+ that permit the Ca2+-dependent activation of calcineurin and the transcription factor NF-AT. Consistent with this model, blockers of K+ channels such as Charybdotoxin and Margatoxin effectively inhibit antigen-dependent T cell activation and IL-2 secretion.6,8,10 Additionally, specific inhibitors of KCa3.1 were able to inhibit the activation of previously activated T cells, again, consistent with the model in which the increased expression of these channels after activation plays a significant role in a subsequent antigen challenge.11 Moreover, KV1.3 channel inhibitors were able to inhibit T cell-mediated immune responses in vivo such as delayed type hypersensitivity or antibody response to foreign antigens.12 These results prompted the notion that K+ channel blockers could be used as effective immunosuppressants. Indeed, in vivo treatment with the highly specific KV1.3 polypeptide inhibitor ShK was able to inhibit the onset of autoimmune encephalomyelitis (EAE), a widely used rodent model for multiple sclerosis.13

Although a great deal of progress has been made in the last few years regarding the involvement of K+ channels in general, in the immune response, and in particular the involvement of KV1.3 and KCa3.1, it seems that there is a lot yet to be learned. For instance, a recent study suggested that the oligomeric composition of the main KV channel might vary in different functional pathways. of different functional pathways. For example, anergized T cells (live cells that are unable to proliferate following stimulation) may express KV1.2, KV1.1 or KV1.6 along with KV1.3, indicating that the substantial physiological and functional changes undergone by a responding T cell may be paralleled by changes in the subunit composition and/or function of the KV channel.14 Consistent with this idea, a recent report showed that exposure of T cells to hypoxia (low oxygen availability) downregulates the KV1.3 channel at the protein level, an effect that could account for the previously described inhibition of T cell proliferation under hypoxic conditions.15

Controlling inflammation:
The central role of P2X7

The P2X7 receptor is a member of a large family of ligand-gated ion channels that open in response to extracellular ATP. The family comprises 7 members (P2X1-7), which share between 40-50% identity in their amino acid sequence. Activation of all the P2X receptors leads to increased membrane permeability to small cations (Na+, K+ and Ca2+), while sustained ATP concentrations (in the range of millimolar) to become active. This prompted the speculation that there may be another endogenous ligand for the P2X7 receptor.

The P2X7 receptor is widely expressed throughout the hematopoietic lineage. The receptor has been found on T and B cells, dendritic cells (DCs), macrophages and mast cells and has been implicated in several physiological activities. Although inflammation is often alluded to in the

**Figure 2**

P2X7 controls inflammation by regulating the expression of inflammatory mediators

Bacteria activate macrophages through specific receptors in the cell membrane. The activated macrophage secretes a great number of inflammatory mediators including chemokines, cytokines and others that help recruit and activate other immune cells.
context of pathological conditions such as asthma and atherosclerosis, it is in fact an essential component of a complex mechanism designed to deal with tissue injury and infection. Inflammation often starts when tissue injury (caused for example by invading bacteria) initiates the secretion of chemical signals that recruit different cell populations such as neutrophils, mast cells, dendritic cells and macrophages. These cells constitute the first line of defense and will on one hand attack the invading bacteria by phagocytosis (macrophages and neutrophils) and on the other hand enlist the cells that are responsible for the permanent eradication of the pathogen (T cells).

The P2X7 receptor that is expressed on all the cells involved in the inflammatory process has been identified as a key player in the regulation of inflammation (see figure 2).

One of the most surprising roles of P2X7 in the immune system, is its key role in the secretion of the biologically active interleukin 1β (IL-1β). IL-1β is often regarded as the master proinflammatory cytokine based in its ability to initiate a wide variety of proinflammatory responses from different cell types, ranging from upregulation of metalloproteinases to secretion of IL-6. IL-1β is abundantly expressed by macrophages that have been activated, for example with bacterial LPS present in the sites of infection. However, activated macrophages produce only the inactive propropolypeptide form that must be cleaved by caspase-1 before it becomes active. Remarkably, activated macrophages need a second stimulus (independent of LPS) to activate caspase-1 and therefore produce mature IL-1β. One of the most powerful signals that operate in this context is extracellular ATP through the P2X7 receptor.18

The central role of P2X7 in ATP-mediated IL-1β maturation was demonstrated in macrophages isolated from P2X7 knockout mice. These cells failed to produce mature IL-1β despite the fact that they produced high quantities of proIL-1β in response to LPS.19 Other known functions of P2X7 in the immune response include shedding of L-selectin in leukocytes and lymphocytes, ATP-mediated proliferation and apoptosis, killing of intracellular bacteria and activation of intracellular inflammatory-related signal transduction pathways such as translocation of NF-κB to the nucleus or upregulation of cyclooxygenase-2 (COX-2) in monocytes and macrophages.20,21,22 Indeed, mice deficient in P2X7 (P2X7 knockout) showed a marked reduction of disease severity in a mouse model of inflammatory arthritis, prompting the suggestion that P2X7 can be an attractive target for anti-inflammatory drugs.23 Despite all this, it was difficult to reconcile these findings with the fact that very high concentrations of extracellular ATP are necessary to activate P2X7, since these ATP concentrations seemed unphysiological. A recent study might help to dispel the mystery. The authors showed that nicotinamide adenine dinucleotide (NAD) functions as a substrate for ADP-ribosyltransferase 2 (ART2), an ectoenzyme that catalyses the linkage of ADP-ribose to an acceptor protein, in this case P2X7.21 ART2-mediated ADP-ribosylation is able to activate P2X7 and all the known downstream signaling, such as Ca2+ mobilization and L-selectin shedding in the absence of ATP. For these effects to take place, only relatively small concentrations of extracellular NAD were required (in the micromolar range). Thus a putative model for the physiological activation of P2X7 is that, extracellular NAD (as extracellular ATP) that is released at the sites of inflammation by the lysis of dying cells, is catalysed by ART2 expressed on the cell membrane of inflammatory cells attracted to the site, and therefore activates the P2X7 channel on these cells.

Other functions, other channels

The above account of the involvement of K1,3, K3,1 and P2X7 in the modulation of the immune response by no means summarizes the contribution of ion channels in general to the immune response. Rather, there are many ion channels whose involvement has already been observed and whose function is no less central to the immune response.

The primary example is Ca2+ channels. As mentioned above, rise in intracellular Ca2+ concentration is crucial to the activation of lymphocytes (both T and B cells) and leukocytes (macrophages, neutrophils, mast cells). The release of Ca2+ from intracellular stores following stimulation is well characterized. However, the molecular identity of the channel that mediates the subsequent Ca2+ entry from the extracellular medium, which is actually responsible for the majority of the elevated Ca2+ levels, is still elusive. The uncertainty starts with the term used to describe the plasma membrane Ca2+ channel. The names Ca2+-release-activated Ca2+ channels (CRAC) and also store-operated Ca2+ channel (SOCC) are used interchangeably, although there may be differences in the biophysical properties of both channels. The leading candidates for the molecular identity of the CRAC channels are members of the TRP gene superfamily. TRPC3 and TRPC6 have been studied in T cells, although it appears that the current favorite is TRPV6 (CaT1).24,25

Cl channels have also been observed in lymphocytes where they function to decrease the volume of the cell following osmotic stress.1 In addition, blockers of Cl channels have been shown to inhibit lymphocyte activation and proliferation.26 As in the case of the CRAC channels, the molecular identity of the Cl channels is not clear, although mRNA from the CLC3 channel has been detected in several hematopoietic cell lines.27

Finally, there are several ion channels that have been identified in cells of the immune system but whose function has not yet been thoroughly investigated. This group includes epithelial Na+ channels (ENaC) in lymphocytes,28 the water channel aquaporin 9 in neutrophils,29 and the nicotinic acetylcholine receptor in B lymphocytes.30

Undoubtedly, in the next few years further studies will reveal new and perhaps not so unexpected roles of ion channels in the immune response.

References:

Molecular Tools for the Study of Living Cells

As part of our on-going effort to provide the research community with novel molecular tools, Alomone Labs is pleased to announce the first FITC labeled antibodies to K1,3 and P2X7 which are part of our growing line of ion channel markers which can be used in the study of living cells. We welcome ideas for future products.
## Related Products

<table>
<thead>
<tr>
<th>Antibodies to Ion Channels</th>
<th>Product #</th>
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<tbody>
<tr>
<td>Anti-P2X7</td>
<td>APR-004</td>
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<tr>
<td>Anti-P2X7 extracellular</td>
<td>APR-008</td>
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<tr>
<td>Anti-P2X7 extracellular-FITC</td>
<td>APR-008-F</td>
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<tr>
<td>Anti-Kv1.3 extracellular-FITC</td>
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<tr>
<td>Anti-KvCa3.1</td>
<td>APC-064</td>
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**Ion Channel Blockers**
- rAgitoxin-1: RTA-150
- rAgitoxin-2: RTA-420
- rAgitoxin-3: RTA-390
- rCharybdotoxin: RTC-325
- rHongotxin-1: RTH-400
- rMargatoxin: RTM-325

### Anti-Kv1.3

**Product #: APC-002**

**Host:** Rabbit.
**Epitope:** GST fusion protein TLSKSEYMWIEEGGM NHSAF PQTP PKTNSTACTCTNNPNSCNVIKIF DDD corresponding to residues 471-523 of human Kv1.3.
**Epitope location:** Intracellular, C-terminus.

**Homology with other species:** Rat, rabbit, mouse – identical.
**Reactivity Confirmed:** Rat, mouse, human.

**Applications:**
- Immunohistochemistry: Rat brain sections.
- Western Blotting:

### Anti-Kv1.3 (extracellular)

**Product #: APC-010**

**Host:** Rabbit.
**Epitope:** Peptide KDYPASQDSFEA(C) corresponding to residues 211-224 of human Kv1.3.
**Epitope location:** Extracellular, between S1 and S2 domains.

**Homology with other species:** Rat, mouse – 12 out of 14 residues identical.
**Reactivity Confirmed:** Rat, Human.

**Applications:**
- Immunohistochemistry: Rat brain sections.
- Western Blotting:

### Anti-Kv1.3 (extracellular)-FITC

**Product #: APC-010-F**

**Host:** Rabbit.
**Epitope:** Peptide KDYPASQDSFEA(C) corresponding to residues 211-224 of human Kv1.3.
**Epitope location:** Extracellular, between S1 and S2 domains.

**Label:** Fluorescein isothiocyanate (FITC).
**Homology with other species:** Rat, mouse – 12 out of 14 residues identical.
**Reactivity Confirmed:** Rat, Human.

**Applications:**
- Western Blotting:

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**Anti-KvCa3.1 (SK4, IKCa1)**

**Product #: APC-064**

**Host:** Rabbit.
**Epitope:** Peptide RQVRLHKLREKVL(C), corresponding to amino acid residues 350-363 of rat KvCa3.1.
**Epitope location:** Intracellular, C-terminal part.
**Homology with other species:** Mouse, human, pig – identical.
**Reactivity Confirmed:** Rat, human.

**Applications:**
- Western Blotting:

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**Anti-KvCa3.1 (extracellular) FITC**

**Product #: APC-010-F**

**Host:** Rabbit.
**Epitope:** Peptide KDYPASQDSFEA(C) corresponding to residues 211-224 of human Kv1.3.
**Epitope location:** Extracellular, between S1 and S2 domains.

**Label:** Fluorescein isothiocyanate (FITC).
**Homology with other species:** Rat, mouse – 12 out of 14 residues identical.
**Reactivity Confirmed:** Rat, Human.

**Applications:**
- Western Blotting:

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**Unstained cells**

**Anti-Kv1.3 extracellular-FITC (0.5µl per 1x10^6 cells)**

**Western blotting of rat brain membranes:**
1. Anti-Kv1.3 antibody (APC-002) (1:200).
2. Anti-Kv1.3 antibody, preincubated with the control antigen.

**Western blotting of human Jurkat T cells:**
1. Anti-Kv1.3 (extracellular) antibody (APC-101) (1:500).
2. Anti-Kv1.3 (extracellular) antibody, preincubated with the control peptide antigen.
**Anti-P2X7 (extracellular)-FITC**

**(P2Z)**

**Product #: APR-008-F**

Host: Rabbit.
Epitope: Peptide KKGWMDPQSKGIQTGRC, corresponding to residues 136-152 of mouse P2X7 receptor.
Putative epitope location: Extracellular.
Label: Fluorescein isothiocyanate (FITC).
Homology with other species:
Human, rat - identical;
bovine - 14/17 residues identical.
Reactivity Confirmed: Human.

Applications:
Immunocytochemistry:

Flow Cytometry:
Flow cytometry analysis of Jurkat T-cells.

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**Anti-P2X7 (extracellular)**

**(P2Z)**

**Product #: APR-008**

Host: Rabbit.
Epitope: Peptide KKGWMDPQSKGIQTGRC, corresponding to residues 136-152 of mouse P2X7 receptor.
Putative epitope location: Extracellular.
Homology with other species:
Human, rat - identical;
bovine - 14/17 residues identical.
Reactivity Confirmed: Human.

Applications:
Indirect flow cytometry.
Western Blotting:

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**Anti-P2X7**

**(P2Z)**

**Product #: APR-004**

Host: Rabbit.
Epitope: Peptide (C) KIRKEFPKTQGQYS GFKYPY, corresponding to residues 576-595 of mouse P2X7 receptor.
Putative epitope location: Intracellular, C-Terminus.
Homology with other species:
Mouse - 18/20 residues identical;
Human - 16/20 residues identical.
Reactivity Confirmed: Rat, mouse.

Applications:
Immunohistochemistry:

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Contributed by Amadio, S., Cavaliere, F. and Volonte, C. CNR Fondazione Santa Lucia, Italy

Immunofluorescence staining of P2X7 using Anti-P2X7 antibody (APR-008) in rat organotypic hippocampal cultures, visualized by confocal microscopy P2X7 (green) and PI (red).

Western Blotting:

Western blotting of rat brain membrane (lanes 1,5) and human cell lines:
K562 (lanes 2,6), WEHI-231 (lanes 3,7) and HL-60 (lanes 4,8).

Lanes 1-4. Anti-P2X7 (extracellular) antibody (APR-008) (1:200).
Lanes 5-8. Anti-P2X7 (extracellular) antibody, preincubated with the control peptide antigen.