B lymphocytes taken to task: a role for a background conductance \( K_{2p} \) \( K^+ \) channel in B cells. Focus on “Expression of TASK-2 and its upregulation by B cell receptor stimulation in WEHI-231 mouse immature B cells”

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The pivotal role played by \( K^+ \) channels in lymphocyte biology has long been recognized (4). Two \( K^+ \) channels have been reported in T and B lymphocytes, a voltage-gated Kv1.3 (or KCNA3) channel and the \( Ca^{2+} \)-dependent IK1 (KCNN4) channel. These \( K^+ \) channels are involved in the intracellular \( Ca^{2+} \) signaling that triggers lymphocyte activation via the control they exert on the lymphocyte membrane potential. Activation of lymphocytes takes place by inositol 1,4,5-trisphosphate-stimulated \( Ca^{2+} \) release from intracellular stores. As the stores are depleted, calcium-release-activated calcium (CRAC) channels come into action to sustain a continued entry of \( Ca^{2+} \) and an increase in intracellular \( Ca^{2+} \) activity. The role of Kv1.3 and IK1 is to maintain a polarized membrane potential to drive a continuous \( Ca^{2+} \) influx. They do so through their respective depolarization and intracellular \( Ca^{2+} \) dependence. In addition to these dynamic functions, Kv1.3 is thought to be involved in the control of the membrane potential under resting conditions. Although Kv1.3 is open at only a very small fraction of its full depolarized activity at around −50 mV, the membrane potential of peripheral human blood T lymphocytes, its contribution to total conductance is believed to suffice to bring the membrane potential of the lymphocyte toward \( E_K \) (4).

The class of \( K^+ \) channels responsible for the leak (also known as resting or background) conductance that occurs in the majority of cells, including excitable cells, is conspicuously absent from early studies of the lymphocyte ion channel repertory. This type of conductance has been known for over 60 years but it is only recently that their molecular counterparts have been identified. Leak conductances correspond to \( K^+ \) channels of the two-pore domain (\( K_{2p} \) or KCNK) superfamily (6, 9). These channels are remarkable in that they possess two P-domains and four \( \alpha \)-helices in each subunit (see Fig. 1A), form dimers in which each monomer contributes two P-domains to the permeation pore with a pseudo-fourfold symmetry, and are mostly open at resting potential. This unique type of organization contrasts with that of all other \( K^+ \) channels, which are tetramers where each subunit contributes one P-domain to the selectivity filter with fourfold symmetry. \( K_{2p} \) leaks are fundamental to the function of various cells including neurons, muscle, and epithelial cells. Their major contribution is in setting the membrane potential, but far from being passive leaks they are exquisitely regulated. There are fifteen human members to the \( K_{2p} \) family (Fig. 1C), and their gating is regulated by free fatty acids, membrane tension, \( G \) protein-generated signaling, fatty acids, and changes in intra- and extracellular pH.

A first inkling of the presence of \( K_{2p} \)-type leaks in lymphocytes came from work describing two background type \( K^+ \) channels of large and intermediate conductance (\( LK_{bg} \) and \( MK_{bg} \), respectively) in WEHI-231 cells, a murine lymphoma generally considered to preserve the characteristics of immature B lymphocytes (14). Further to this publication, the \( LK_{bg} \) background \( K^+ \) channel of WEHI-231 cells was identified as TREK-2 but a role in B cell function has not been advanced (23). Work with human T cells purified from peripheral blood has shown the presence of TWIK-related acid-sensitive \( K^+ \) channel 1 (TASK-1) and TASK-3, acid-sensitive and \( G_q \) protein-regulated \( K_{2p} \) channels. Their involvement in T cell receptor-mediated effector functions has been demonstrated in vitro and in experimental autoimmune encephalomyelitis model (12). Examples of more recent work supporting the concept of a role for \( K_{2p} \) channels in lymphocytes include experiments demonstrating the importance of TASK-1 in autoimmune inflammation of the central nervous system using a TASK-1 knockout mouse (3) and work with human T cells that express TASK-2, which becomes upregulated after T-cell activation and in CD4 and CD8 T cells in relapsing/remitting multiple sclerosis patients (2).

In a report in the present issue of American Journal of Physiology-Cell Physiology, Nam et al. (13) extend their work on WEHI-231 immature B cells by identifying the \( MK_{bg} \) background \( K^+ \) channel as TASK-2, demonstrating its activation by stimulation of B cells receptors (BCR ligation) and its participation in BCR-ligation-dependent apoptosis.

TASK-2 belongs to the TALK group of \( K_{2p} \) channels comprising TASK-2, TALK-1, and TALK-2, which share the characteristic of being activated by extracellular alkalinization (17, 19). Figure 1B shows the extracellular pH dependence of TASK-2 activity. TASK-2 has been shown to be important in the regulation of cell volume (1, 15), and physiological roles have been suggested by work using a TASK-2 knockout mouse. These animals present metabolic acidosis and hypotension secondary to renal loss of \( HCO_3^- \) (22). It is thought that transport of \( HCO_3^- \) in the proximal tubule is coupled to TASK-2 activity through extracellular alkalinization. The knockout mouse has also served to show that TASK-2 channels are expressed in a very restricted region in the brain in retrotrapezoid nucleus neurons that are important actors in central \( CO_2 \) and \( O_2 \) chemosensitivity (5). Genetic inactivation of TASK-2 impairs the central response to \( CO_2 \) and \( O_2 \). The ventilatory drive at high partial pressure of \( CO_2 \) could be secondary to a decrease in TASK-2 channel activity, and
The process of programmed cell death known as apoptosis is an essential feature in the function of the immune system (11). Apoptosis is central to the prevention of autoimmune reaction and in the maintenance of lymphocyte populations. The process of cell death of lymphocytes is closely controlled, and derangements of its regulation have dramatic pathological consequences. An abnormal enhancement of apoptosis can lead to immunodeficiency. Prevention of apoptosis, on the other hand, can lead to the development of autoimmune disease or be the cause of malignant growth leading to lymphoma. An early process associated with cell death by apoptosis is a decrease in cell volume. By analogy with the process of regulatory volume decrease (RVD), which occurs when cells are swollen by exposure to hypotonic solutions, the apoptosis-associated shrinkage has been termed AVD, apoptotic volume decrease (10). AVD is a well-established phenomenon that occurs shortly after death stimulus and precedes caspase activation and DNA and cell fragmentation. AVD is the consequence of KCl efflux through K⁺ and Cl⁻ channels, and their pharmacological inhibition prevents the progress of the apoptotic program. The Cl⁻ channel implicated in AVD is a ubiquitous volume-sensitive, outwardly rectifying channel that appears to be the same as that involved in RVD and has been shown to be prominent in T lymphocytes (4). This anion channel has yet to be identified molecularly. Various K⁺ channels have been proposed to mediate the conductance leaving K⁺ in the early phases of apoptosis in the Jurkat leukemic cell line of T lymphocytic origin (21).

Nam et al.'s demonstration that TASK-2 occurs in immature B lymphocytes rests first on the use of RT-PCR and Western blot analysis to show the presence of transcript and protein in WEHI-231 cells. The second line of evidence comes from electrophysiological recordings comparing macroscopic and single-channel currents of WEHI-231 cells with those arising from heterologous expression of TASK-2. Finally, decreased expression of TASK-2 by small inhibitory RNA (TASK-2 siRNA) also supports the presence TASK-2 in WEHI-231 cells. Importantly, similar pH-sensitive macroscopic and single-channel currents can be recorded from freshly isolated mouse splenic B cells, where the TASK-2 transcript is prominent in RT-PCR assays. The findings by Nam et al. extend our understanding of a function of great importance in lymphocyte biology, namely, the mechanism of apoptosis of immature B lymphocytes. BCR ligation, which induces apoptosis in B lymphocytes rests first on the use of RT-PCR and Western blot analysis to show the presence of transcript and protein in WEHI-231 cells. The second line of evidence comes from electrophysiological recordings comparing macroscopic and single-channel currents of WEHI-231 cells with those arising from heterologous expression of TASK-2. Finally, decreased expression of TASK-2 by small inhibitory RNA (TASK-2 siRNA) also supports the presence TASK-2 in WEHI-231 cells. Importantly, similar pH-sensitive macroscopic and single-channel currents can be recorded from freshly isolated mouse splenic B cells, where the TASK-2 transcript is prominent in RT-PCR assays. The findings by Nam et al. extend our understanding of a function of great importance in lymphocyte biology, namely, the mechanism of apoptosis of immature B lymphocytes. BCR ligation, which induces apoptosis in B cells, increases TASK-2 activity, and both this activity increase and the apoptosis process are inhibited by TASK-2 siRNA treatment. This suggests a central role for TASK-2 in B cell apoptosis, a crucial mechanism for the prevention of autoimmune disorders.

These results raise a number of very interesting and important questions that will be worth tackling in the future. For example, is the presence of TASK-2 in murine B cells replicated in their human counterparts? This is particularly important given the fact that inhibition of Kv1.3 and IK1 in human T lymphocytes appears to abolish all K⁺ conductance leaving little room for K₂P-mediated currents (4). It will also be important to take advantage of the TASK-2 knockout murine model to substantiate the observations reported here. The TASK-2 null animals should provide an ideal model to investigate potential immunological disorders caused by the channel deficiency. The future development of specific inhibitors for...
K_{2P} channels, which are remarkably insensitive to conventional K\(^+\) channel blockers, might result in useful pharmacological tools in the field of lymphocyte pathology.

Although further work with primary lymphocytes appears necessary to ascertain the presence and the importance of K_{2P} channels in cells from the immune system, the work of Nam et al. strongly supports the presence of TASK-2 and its possible biological role in B lymphocytes. This work, and the weight of evidence from previous reports that have implicated TASK-1, TASK-3, and TREK-2 in mouse and human lymphocytes, seems to announce that K_{2P} channels have burst into immunology to stay.

REFERENCES


