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COMMENTARY

MODULATION OF VOLTAGE-DEPENDENT POTASSIUM CHANNELS IN B LYMPHOCYTES

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The responses of the immune system to an antigen involve a complex network composed of different cell types. Among them, two main classes of lymphocytes can be distinguished: the B-cells which are the antibody producing cells, and different subsets of T-cells which carry out various effector functions such as secretion of lymphokines and killing of infected or antigen bearing cells. On B cells, ligation of an antigen to its receptors, which are the membrane immunoglobulins (mIg), leads, with the help of T-cell derived factors, to cell proliferation, differentiation and ultimately antibody production. Crosslinking of the mIg by antibodies mimicks the action of antigens and, in recent years, there have been many investigations of the early cellular events that occur with activation of B-cells by anti-IgM or mitogens [1, 2]. One of the earliest responses is a breakdown of phosphatidylinositid, presumably due to activation of the phospholipase C (PLC) [3, 4] which takes place within minutes. It is associated with an increase in intracellular Ca^{2+} [5–7] and a translocation of protein kinase C [8] from the cytosol to the membrane. Concomitantly, there is an efflux of K^+ and membrane potential variations [2, 9, 10]. This initial response is followed by changes in expression of different surface markers as the cell leaves the resting (G_0) state and enters the cell cycle. Of particular interest here is the hyperexpression of the surface Ia antigen, which is thought to improve the ability of the B cells to interact with helper T cells and which can be triggered by membrane depolarization alone [11]. It is reasonable to assume that ion channels play an important role in the early and late processes of B cell activation since, in addition to the observed K^+ and Ca^{2+} fluxes [2, 6, 12, 13], drugs known to be channel blockers inhibit at least some of these early responses [2, 13–15] as well as cellular proliferation [13, 16, 17]. But up to the recent present, no method was available to investigate directly channel activity, so that the type(s) and possible role of channels present in lymphocytes have been inferred indirectly on the basis of pharmacological experiments alone [13–16], which, as discussed below, can be misleading.

The patch-clamp technique [18] is a powerful electrophysiological method which now allows one to study directly the properties of membrane bound channels, and it has been applied in the last few years to cells of the immune system [19–21] in order to characterize their channels and their possible involvement in the activation process or other physiological functions. This review focuses on voltage-dependent K^+ channels in B cells, whose properties have been investigated only recently [21], and their modulation by intra- and extracellular signals. For the purpose of comparison, we will, however, often also refer to T cells, which have been documented extensively elsewhere [19, 20].

K^+ *Channels in B lymphocytes*

We have shown [21] that, in both immature and stimulated B cells, the main ion conductance gated by voltage is a K^+-dependent one analogous to the delayed rectifier of nerve and muscle. In the whole-cell clamp mode, the current carried through these K^+ channels is most easily revealed by holding the cell at a membrane potential around −80 millivolts (mV), at which they are closed, and then applying depolarizing jumps which activates them (Fig. 1). With this protocol, an outward current is generated for depolarizations of 40 mV or more, with the potential for half-maximum activation being around −30 mV. Upon prolonged depolarizing steps the current inactivates, or as otherwise stated, the channels enter into a non-conductive state. Both activation and inactivation kinetics are voltage dependent, developing faster as the cell is more depolarized. (Half-times for these two processes are on the order of 3 msec and 300 msec, respectively, for depolarizations from −80 mV to 0 mV.) While inactivation is complete at −40 mV, 20–30% of the channels remain functional at −50 mV [22]. It is worthwhile to note that the resting potential of lymphocytes is between −50 and −60 mV, as estimated with voltage-sensitive dyes [23–25]. Thus, in this range, a substantial number of channels can become activated. Since the input resistance of these cells is high (1–10 GΩ) [21], opening of a few K^+ channels would be sufficient to alter significantly

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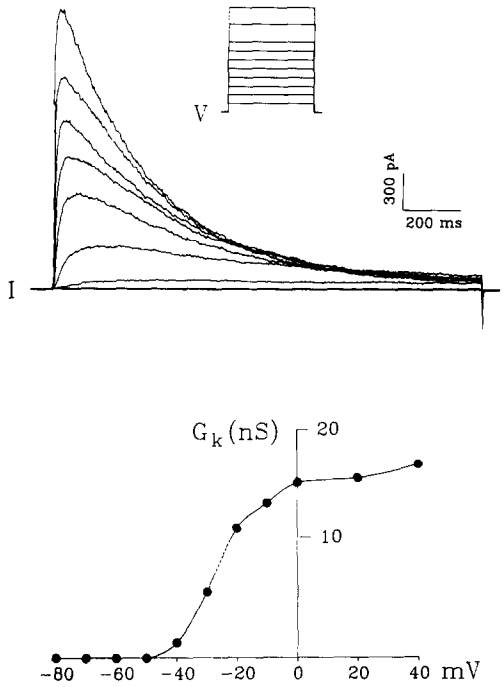

Fig. 1. Activation of voltage-dependent K^+ currents in a pre-B lymphocyte. Upper part: superposed traces of currents (I) recorded in the whole-cell clamp mode, elicited from a holding potential of −80 mV by successive depolarizing pulses (V) separated by 10 mV increments. Note that the outward current grows bigger as the cell is more depolarized, and inactivates upon sustained depolarization. Lower part: Corresponding plot of the K^+ conductance (G_k) versus voltage.

the resting potential (along this line, we have not detected Na^+ currents, or K^+ inward rectifiers, in pre-B and stimulated B cells, although there must be another ion, for instance Cl^-, involved in keeping the cell potential away from the K^+ reversal potential). A similar K^+ channel has been described in a variety of T cell types [20] and is referred to as type n (normal) [26, 27], because in these cells other K^+ channels with somewhat different properties have also been detected.

This K^+ current is present throughout pre-B cell differentiation since it was detected in a variety of cell lines representative of the various stages of development [21] and, furthermore, stimulated cells also possess high numbers of channels (200–500 per cell) [21], whereas resting B cells express a much lower number of them (10–15 per cell) [17]. These figures were obtained by dividing the total conductance of the cell (as measured in the whole-cell recording mode) by the single channel conductance. They can further be related to cell size (as estimated by membrane capacitance), and they indicate an increase in the number of channels per unit surface area after mitogenic stimulation [17]. The fact that stimulated lymphocytes express a higher number of K^+ channels than do their resting counterparts has also been observed in a variety of T cells such as those from helper T clones stimulated with Il2 [28], human peripheral blood [29] or mice [30], activated, respectively, with phorbol esters or the plant lectin ConA. Thus, the level of expression of this channel is highly regulated within the cell cycle. This general finding, coupled with the fact that several drugs, such as tetraethylammonium (TEA), 4-aminopyridine (4-AP), quinine, and verapamil, inhibit mitogen-induced proliferation of lymphocytes with potencies similar to those at which they block the K^+ current [16, 17, 31], already suggests a link between these two cellular processes.

Modulation by intracellular calcium

Since certain types of K^+ channels can be activated by Ca^{2+} [32], it was natural to investigate whether such is the case in B cells. It was found, however, that this cation does influence K^+ conductances, but in a different and more complex manner. A property of the type n K^+ channel is its inhibition by substances reputed to be Ca^{2+} channel blockers, such as certain divalent cations (Cd^{2+}, Co^{2+}, Ni^{2+}), or organic compounds (nifedipine, verapamil), as well as by substances known to block classical Ca^{2+}-activated K^+ channels such as quinine and charybdotoxin [17, 20, 21, 27]. However, a number of arguments indicate that in B and T cells this K^+ conductance is not activated by an influx of Ca^{2+}: (i) the current is little affected when recorded in a medium nominally free of Ca^{2+}, or with a pipette solution highly buffered at low Ca^{2+} concentration [21], and (ii) raising extracellular calcium shifts the voltage dependence of the K^+ channel to more positive potentials, an effect opposite to that expected for a Ca^{2+}-activated process and that probably can be explained by modifications of membrane surface charges. Moreover, although voltage-dependent Ca^{2+} channels are found in myelomas and hybridomas [33], they were not detected in normal B or T cells with the patch-clamp technique. Finally, depolarization of B cells by raising extracellular K^+, which would open such channels, induces no changes in internal Ca^{2+} levels, as measured by fluorescent Ca^{2+} indicators [7, 34].

Although not a classical Ca^{2+}-activated K^+ channel, type n channel displays a special sensitivity to this cation. We have shown that, in B cells, raising intracellular Ca^{2+} speeds up inactivation (Fig. 2) [21], and shifts the voltage dependence of activation* to more negative potentials, with little effect on the maximum conductance. These results are somewhat different from those observed on T cells, where increased intracellular Ca^{2+} primarily decreases K^+ current amplitude [35].

The origin of the mitogen-induced rise in internal Ca^{2+} in T or B lymphocytes, and its relation to both membrane potential and K^+ fluxes, are still subject to controversy, hampered by the different approaches used. In B cells, crosslinking of mIg by antibodies causes a rapid (less than 1 min) increase of cytoplasmic Ca^{2+} [4–7]. This Ca^{2+} rise is likely to be due to both a release from internal stores [5, 6, 12, 34, 36] and an influx through the plasma membrane [6, 12, 13], since (i) it is diminished, but not abolished, when cells are incubated in a medium without Ca^{2+}, and (ii) there is an increase in extracellular $^{45}Ca^{2+}$ uptake when cells are challenged with

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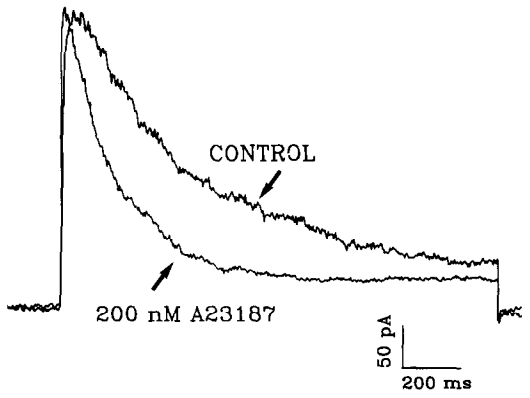

Fig. 2. Inactivating effect of internal Ca^{2+}. Currents were produced by a depolarizing step from −80 mV to 0 mV, recorded before and 2 min after perfusion with the Ca^{2+} ionophore A23187. The pipette solution contained no Ca^{2+} and 0.2 mM ethyleneglycolbis(amino-ethylether)tetra-acetate (EGTA) in order to allow internal Ca^{2+} changes. The increase in internal Ca^{2+} induces the K^+ channel to close earlier than in the control, as indicated by the faster decay time of the current.

anti-Igs. The release of Ca^{2+} is most likely mediated by the action on endoplasmic reticulum of inositol phosphate (IP_3) [36] that is generated as a product of the breakdown of phosphoinositides following receptor-induced activation of PLC. The mechanism underlying the increase in influx is still unknown. It appears to consist of an increase in plasma membrane permeability rather than a carrier-mediated process, because it does not depend on extracellular Na^+ [10, 34], and it is inhibited by depolarization and channel blockers. But although inhibited by Ca^{2+} channel antagonists [2, 10, 15], it is probably not mediated through voltage-dependent Ca^{2+} channels. Indeed, both B and T cells appear to lack such channels. The influx seems to be blocked by the specific K^+ blockers TEA and 4-AP in B cells [2], and in some [20], but not all [37], stimulated T cells. These observations led to the suggestion that Ca^{2+} permeation may be mediated through the K^+ channels [38]. The objection was raised that depolarizing the cells by raising extracellular K^+ (which would increase the K^+ conductance) does not increase intracellular Ca^{2+} [7, 34, 37], but in this case Ca^{2+} movement through the K^+ channel would probably be blocked by either (i) a large K^+ efflux, or (ii) inactivation of the channel, as a result of prolonged depolarization. In fact, in T cells, the phytohemagglutinin (PHA)-triggered Ca^{2+} influx is inhibited by K^+-induced depolarizations [39]. Previous reports on B cells have failed to find such an effect [7, 12], but in those cases the external K^+ concentrations used were much smaller than in the T cell experiments, and thus the extent of the potential shift may have been insufficient. In fact, sensitivity of anti-Ig triggered Ca^{2+} influx to depolarization has been alluded to recently [2].

Thus, the substrate for Ca^{2+} fluxes remains to be determined. Along this line, in T cells the mitogen PHA and intracellular IP_3 [40, 41] have been shown to trigger the opening of a voltage-independent conductance that is permeable to Ca^{2+} and may account for the PHA-induced increase in Ca^{2+} permeability observed with fluorescent dyes or $^{45}Ca^{2+}$ uptake. It would be worthwhile to know if such a channel is activated by IgM crosslinking in B cells.

Changes in membrane potential following stimulation: An hypothesis

There are two reports concerning changes in membrane potential occurring upon mitogenic stimulation of lymphocytes. First, a somewhat delayed (3–5 min) and sustained (2 hr) depolarization following activation of B lymphocytes with anti-IgM, or lipopolysaccharide (LPS) [9] was observed using cyanine dyes and flow cytometry. Second, a recent study demonstrates an early hyperpolarization upon crosslinking of IgM [10] evidenced with spectrometry and the anionic probe bis-oxonol. Although the reliability of cyanine dyes in measuring transmembrane potentials has been questioned, these results may be complementary since flow cytometry does not allow detection of early changes, whereas spectrometry only analyzes short-term effects. Thus, it could be that cells undergo an initial hyperpolarization followed by a sustained depolarization. It was proposed [10] that the hyperpolarization is due to an efflux of K^+ triggered by an increase in internal calcium following IgM crosslinking, through activation of Ca^{2+}-dependent-K^+ channels. Indeed, this potential shift is mimicked by addition of Ca^{2+} ionophores, is inhibited in conditions that minimize Ca^{2+} influx, and is sensitive to the K^+ gradient. Similar results have been shown in T cells stimulated with PHA or ConA [42, 43]. In that case, the hyperpolarization is further inhibited by the Ca^{2+}-activated K^+ channel blockers quinine and charybdotoxin. An alternative interpretation to the presence of such channels in B and T cells could be that raising internal Ca^{2+} induces K^+ efflux, and hyperpolarization, by shifting the voltage dependency of activation of the K^+ channel (which is the only one characterized so far in B cells) to more negative potentials. Furthermore, the sustained increase in intracellular Ca^{2+} would then inactivate the K^+ channel, with subsequent cell depolarization. Such a sequence may provide a basis for the cell to regulate Ca^{2+} entry. If so, the early opening of K^+ channels would maintain an electrical gradient necessary for enough Ca^{2+} to flow in, whereas their secondary inactivation and, thus, cell depolarization, would prevent an overload of Ca^{2+} known to be toxic. It is worthwhile to note that this scheme would pertain regardless of whether Ca^{2+} influx were to occur through an independent channel or through the K^+ one. The arguments developed above favor such a model, although it remains to be determined whether the various drugs that block K^+ channels inhibit Ca^{2+} influx by directly blocking a putative Ca^{2+} channel or by preventing K^+ channels from playing their permissive role.

Modulation by cyclic nucleotides

In pre-B and stimulated B lymphocytes, elevation of intracellular cyclic AMP induces a marked

decrease (50–100%) in the maximum K^+ conductance, associated with a less prominent increase (10–30%) in the rate at which it inactivates. This was shown by stimulating the adenylate cyclase with forskolin, by including cAMP directly into the recording pipette [21], and by incubating the cells with cholera toxin (unpublished observation) which irreversibly activates the cyclase by ADP-ribosilating the associated stimulatory G protein. The results with the last two approaches suggest that forskolin does not only act on K^+ channels in B cells through a cAMP-independent mechanism, as it does in human T cells [44].

Correlation with cell function is not obvious. Although transient increases in cAMP levels (which reduce K^+ conductance) can be measured early after mitogenic stimulation [45, 46], agents that elevate intracellular cyclic AMP such as forskolin, cholera toxin or dB-cAMP are inhibitory to lymphocyte proliferation [47, 48], but at later stages they stimulate differentiation. To our knowledge, no studies have been carried out to determine the effect of cAMP on membrane potential or K^+ fluxes. However, a cAMP-mediated reduction in K^+ conductance would probably not depolarize the cell by itself, unless an inward current were generated. Along this line, it has been shown that cAMP inhibits anti-Ig induced depolarization of small B cells, most likely by inhibiting phosphatidylinositid hydrolysis and subsequent Ca^{2+} influx [49, 50].

In lymphocytes, cGMP and cAMP have opposite effects. Elevation of cyclic GMP has a stimulatory effect on cell proliferation [45, 47], and preliminary experiments indicate that this cyclic nucleotide induces an increase in peak amplitude of the K^+ current in pre-B cells [22]. It is worthwhile to note that, since functional K^+ channels [16, 17, 31] are required for the mitogenic response, cAMP and cGMP could exert part of their regulatory role through their opposite effects on the K^+ conductance.

Hormone regulation of potassium channels

Lymphocytes bear receptors for a variety of transmitters [48] that influence cellular function positively and negatively. In view of the involvement of K^+ channels in the response of immune cells to mitogens, it is of great interest to determine whether immunomodulatory substances affect the behavior of these channels. We have shown recently that the amine serotonin (5-HT) at doses between 1 and 20 μM has a powerful effect on the voltage-dependent K^+ conductance of a pre-B cell line (Fig. 3) [22]. Surprisingly, 5-HT both increases the amplitude of the current (30–50%) through activation of 5-HT_1 receptors, and speeds up the inactivation process (20–40%) through 5-HT_3 receptors. This was shown by using classic and recently described [51] agonists and antagonists specific for each receptor subtype. There are several noticeable features in these results; they represent a clear example of the regulation of an ion channel by two different sets of receptors to a single molecule, and furthermore, to our knowledge, 5-HT_3 receptors have not been implicated yet in the control of channel inactivation. The long-term effect of 5-HT is to decrease the number of channels available for activation, and this could be related to the ability of this amine to inhibit the proliferative response of murine lymphocytes to PHA [52].

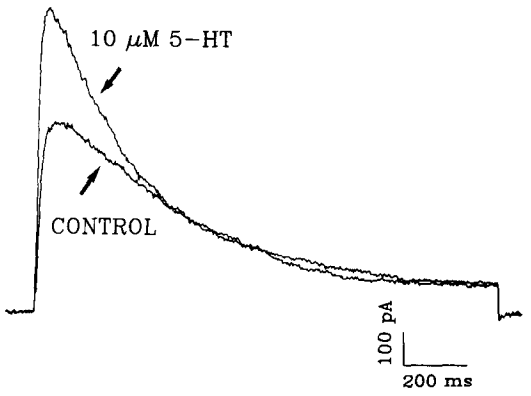

Fig. 3. Evidence that 5-HT enhances K^+ currents in pre-B lymphocytes and speeds up their inactivation. Records were taken before, and 3 min after, perfusion with the hormone.

Reports from other groups confirm that transmitters also modulate the analogous channel in T cells: activation of the β-adrenergic receptor with isoproterenol induces a cAMP-like effect [53], and β-endorphin reduces the frequency of opening of K^+ channels in murine T cells [54].

Conclusions

K^+ channels are present in the membrane of B cells where they have been identified only recently, and in other cells of the immune system. They are subject to regulatory mechanisms at the level of their expression and of their biophysical properties as well. For example, several second messengers such as Ca^{2+}, cAMP and cGMP, may speed channel inactivation, and decrease or increase K^+ current amplitude.

These channels are required in mitogenesis since K^+ channel blockers may inhibit proliferation, their expression is highly regulated during the cell cycle, and K^+ fluxes occur during stimulation. However, caution is needed in interpreting this notion, because a distinction has to be made regarding their involvement in early and later events during activation: different mitogens do not use the same pathways to activate the cells (for example LPS, as compared to anti-Igs), and K^+ channels may be implicated in some but not all of these routes. The data reviewed here suggest that these channels act primarily at stages where Ca^{2+} influx is the determinant, although the manner in which these ionic fluxes are linked is still unclear. Thus, their function at early stages most probably depends upon the kind of stimuli to which B cells are exposed. In contrast, blocking the channels inhibits cell cycling regardless of the triggering signal, and their role in this process remains to be determined.

In the course of proliferation and differentiation, B cells encounter a variety of growth factors and stimuli. Among them, the actions of hormones are

of particular interest since they provide a link between the immune and nervous systems. In the latter, neurotransmitters primarily act by modifying ionic conductances directly, or by way of second messengers. In lymphocytes, it was found that 5-HT can affect K^+ conductances, as it does in a number of neuronal cells [55]. This finding may become relevant when the function(s) of ion channels in the immune system is clarified.

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