Calcium channels in lymphocytes

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INTRODUCTION

On B and T lymphocytes, ligation of the antigen receptor (AgR) induces a biphasic Ca^{2+} response. In the initial phase there is a large elevation in the intracellular Ca^{2+} concentration as a consequence of Ca^{2+} release from intracellular stores. This is followed by a lower, but prolonged elevation that is dependent on extracellular Ca^{2+}.[1,2] This simple description belies the complexity of the response. The initial phase may involve as many as three different intracellular Ca^{2+} channels, while the second phase depends not only on plasma membrane Ca^{2+} channels, but also on at least two different intracellular channels. The complexity of the signal, and the many opportunities for regulation of individual components of the signalling mechanism, lead to a tremendous flexibility in outcome, ranging from single, brief elevated Ca^{2+} transients, through a range of oscillatory responses, each of which can be decoded by the cell into a differing outcome.[2] In this review we concentrate on the Ca^{2+} channels involved in the AgR-mediated Ca^{2+} signal, but we briefly discuss other Ca^{2+} channels present in lymphocytes. Figure 1 shows two possible schemes for the involvement of Ca^{2+} channels in TCR signalling, and Fig. 2 shows possible roles for Ca^{2+} channels in B cells.

INTRACELLULAR Ca^{2+} CHANNELS

A plethora of studies of AgR signalling have highlighted the role of inositol trisphosphate [Ins(1,4,5)P_3]-mediated release of Ca^{2+} from internal stores (reviewed in refs 1–3). However, it is becoming apparent that there is more to the regulated release of intracellular Ca^{2+} in lymphocytes than inositol trisphosphate receptors ($InsP_3Rs$). Recent studies are beginning to unravel roles for ryanodine receptors (RyRs) and the newly described and little understood NAADP receptor.

Inositol trisphosphate receptors

Three types of $InsP_3R$ are known, and they vary in their sensitivities to Ins(1,4,5)P_3 and in the properties of their activation by Ca^{2+}. $InsP_3Rs$ must bind Ins(1,4,5)P_3 for Ca^{2+} release to occur. The response of the $InsP_3R$ can be regulated by phosphorylation, by various accessory proteins and by ATP, but by far the most important regulator is Ca^{2+}. The exact mechanism is disputed[4–6] but it is apparent that the differing sensitivities of the $InsP_3R$ isoforms to regulation by Ca^{2+} allow cells to fine-tune the temporal and spatial aspects of the Ca^{2+} signal.[5]

Much recent work has been directed towards determining the roles of the various isoforms. B and T cells express all three types of $InsP_3R$ to varying degrees depending on their stage of differentiation.[7–11] It is not clear why lymphocytes simultaneously express all three isoforms, particularly since Sugawara *et al.*[8] clearly demonstrated the redundancy of $InsP_3R$ expression. In a series of knockouts in DT40 B lymphocytes the BCR-induced Ca^{2+} signal could not be ablated until all three $InsP_3R$ isoforms were simultaneously knocked out.[8]

$InsP_3R3$ was the first isoform to have a specific role ascribed to it. In both T and B cells, $InsP_3R3$ is up-regulated in cells undergoing apoptosis.[10] Inhibition of $InsP_3R3$ expression using antisense RNA prevents TCR-induced apoptosis.[10] However, Sugawara *et al.*[8] provided convincing evidence against a specific role for $InsP_3R3$ in apoptosis when they showed that knockout of any two isoforms in DT40 cells inhibited BCR-induced apoptosis.

$InsP_3R3$ can be expressed on the external surface of the plasma membrane of T and B cells and in T cells it can cocap with the TCR.[9,10] The validity of this observation has been questioned, but support was provided by Tanimura *et al.*[12] who demonstrated $InsP_3R3$ expression on the external surface of Jurkat T cells. They showed that this pattern of expression was not limited to $InsP_3R3$, and in fact the predominant isoforms in the plasma membrane were $InsP_3R1$ and $InsP_3R2$.[12] The role of plasma membrane $InsP_3Rs$ is unknown, but Putney[13] has suggested that, in some cell types, $InsP_3R3$ may be expressed as an integral plasma membrane protein and function as all or part of a store-operated Ca^{2+} channel. However, the properties of $InsP_3R$, in particular its Ca^{2+} selectivity profile, do not

Received 16 August 2001; revised 28 August 2001; accepted 28 August 2001.

Abbreviations: AgR, antigen receptor; BCR, B-cell antigen receptor; cADPR, cyclic ADP ribose; I_{CRAC}, Ca^{2+}-release-activated Ca^{2+} current; I_{CRANC}, Ca^{2+}-release-activated non-selective cation current; $InsP_3R$, inositol trisphosphate receptor; K_{Ca}, Ca^{2+}-activated K^+ channel; NAADP, nicotinic acid adenine dinucleotide phosphate; RyR, ryanodine receptor; TCR, T-cell antigen receptor.

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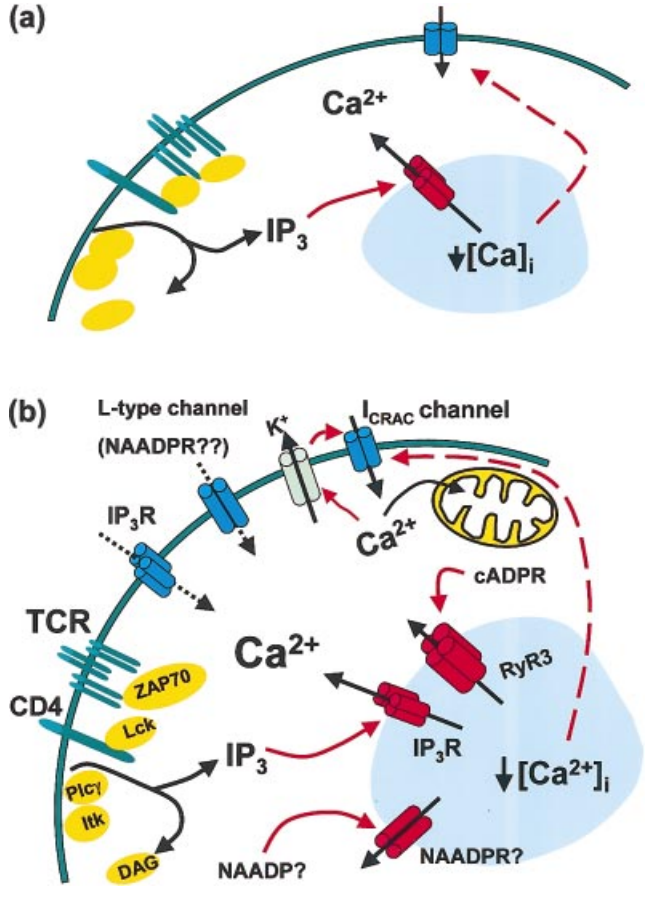

Figure 1. A possible scheme for the involvement of Ca^{2+} channels in TCR signalling (a) depicts the simplest possible scheme for the role of Ca^{2+} channels in TCR-induced Ca^{2+} signalling. TCR-induced Ins(1,4,5)P_3 production causes Ca^{2+} release from intracellular stores, which in turn relays a signal to the plasma membrane store-operated Ca^{2+} channel (I_{CRAC} channel), causing it to open. In an alternative scheme (b) intracellular Ca^{2+} flux results from the TCR-induced production of Ins(1,4,5)P_3, cADPR and possibly NAADP, in concert with the activation of the I_{CRAC} channel in the plasma membrane. The Ca^{2+} signal is sustained by the activity of mitochondria (shown in yellow), K_{Ca} channels, and cADPR. Note that the localization of the NAADP receptor is unknown, and that RyR3 and $InsP_3R$ may not be present on the same intracellular stores. The roles of plasma membrane $InsP_3Rs$ and the L-type Ca^{2+} channel are unknown – the possibility that they may mediate Ca^{2+} influx is indicated by dotted lines. The identification of the L-type Ca^{2+} channel as an NAADP receptor is speculative. Intracellular stores are depicted in blue, and activation steps are shown by red arrows.

accord with those of known store-operated Ca^{2+} channels and the prevailing evidence strongly indicates that these channels are formed by distinct molecules from $InsP_3Rs$ (discussed below).

In an attempt to explore the role of $InsP_3R1$, Jayaraman *et al.*[11] used antisense RNA to show that TCR-induced Ca^{2+} signals are exclusively transduced through this subtype. However, this is a controversial finding since the construct they used could cross-react with the type 2 and type 3 receptors. Hirota *et al.*[14] subsequently demonstrated that T lymphocytes could develop normally in $InsP_3R1$ knockout mice and showed normal Ca^{2+} fluxes in response to anti-CD3 stimulation, contradicting the claim that $InsP_3R1$ was absolutely required for TCR signalling. In the most convincing study to ascribe roles for the various isotypes, Miyakawa *et al.*[15] showed that $InsP_3R3$ was involved in the generation of monophasic single Ca^{2+} transients following BCR ligation, whereas $InsP_3R1$ and $InsP_3R2$ were involved in the generation of Ca^{2+} oscillations with differing frequencies.

The knockouts studied by Sugawara *et al.*[8] suggest that inhibition of downstream events may be achieved simply by reducing the overall levels of $InsP_3Rs$, rather than the specific levels of one particular isotype. This raises the possibility that cells do not express homotetramers of $InsP_3Rs$; rather they may express heterotetramers of varying amounts of each isoform, allowing the cell to express a graded array of hybrid receptors with a wide variety of subtly different properties made up of combinations of the properties of the 'pure' homotetramers. Clearly much remains to be done to unravel the role of $InsP_3Rs$ in AgR signalling.

Ryanodine receptors

RyRs are large (~560 kDa) homotetrameric receptors that mediate Ca^{2+} release from the endoplasmic reticulum stores. Three isoforms, encoded by separate genes, have been described. RyRs can be gated by allosteric coupling to plasma membrane voltage-gated Ca^{2+} channels (in the case of RyR1) and by Ca^{2+} (all isoforms). All three isoforms can be activated by cyclic ADP ribose (cADPR). cADPR can initiate Ca^{2+} release and sensitise the RyR to further activation by Ca^{2+}. This feed-forward mechanism, known as Ca^{2+}-induced Ca^{2+} release, can lead to the prolonged propagation of Ca^{2+} signals.[16,17] In contrast to $InsP_3Rs$, only one isotype of RyR is expressed in lymphocytes, RyR1 in B lymphocytes and RyR3 in T lymphocytes, therefore there is no question of heterotetramer formation.[18–22]

The role of RyRs in AgR signalling is beginning to be addressed and appears to differ between B and T cells. In T cells the initial peak of Ca^{2+} release following TCR engagement is due to Ins(1,4,5)P_3-mediated Ca^{2+} release from intracellular stores. This peak is followed by a sustained Ca^{2+} flux that requires both the influx of extracellular Ca^{2+} and the production of cADPR. Guse *et al.*[22] showed that cADPR is produced following TCR ligation and that antagonists of cADPR inhibited TCR-induced proliferation and the expression of early and late activation markers. They showed that the long-lasting Ca^{2+} influx, rather than the initial Ca^{2+} peak, depended on cADPR production. In contrast, in B cells depletion of RyR1-gated stores significantly inhibits the BCR-induced Ca^{2+} peak, suggesting that in B cells the RyR is activated at the same time as Ins(1,4,5)P_3-induced Ca^{2+} release.[18]

The mechanism that activates RyRs in B cells is unknown, but in T cells Guse *et al.*[22] showed that it is TCR-induced cADPR production. The source of TCR-induced cADPR is controversial. cADPR is produced from β-NAD by the action of ADP-ribosyl cyclase. This enzyme was first isolated from *Aplysia*, and its mammalian homologues include CD38. CD38 is an ecto-enzyme having both ADP-ribosyl cyclase and cADPR-hydrolase activities. The extracellular location of CD38 has proved puzzling since the site of action of its

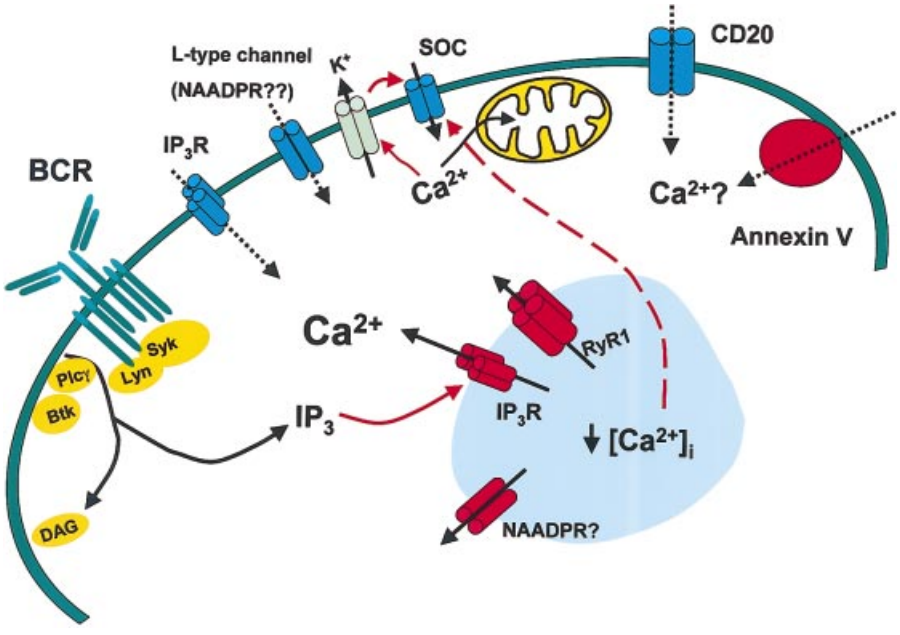

Figure 2. Possible roles for Ca^{2+} channels in B cells. The BCR-induced Ca^{2+} signal involves the production of Ins(1,4,5)P_3 and the release of Ca^{2+} from intracellular stores gated by InsP_3Rs and RyR1. This is followed by an influx of Ca^{2+} through an unidentified store-operated channel (SOC). The mechanism of activation of RyR1 is unknown. Note that RyR1 and InsP_3R are unlikely to be present on the same intracellular stores. The possible involvement of NAADP receptors in BCR signalling is highly speculative. The roles of plasma membrane InsP_3Rs and the L-type Ca^{2+} channel are unknown – the possibility that they may mediate Ca^{2+} influx is indicated by dotted lines. The identification of the L-type Ca^{2+} channel as an NAADP receptor is speculative. CD20 and annexin V are shown as possible Ca^{2+} channels. Intracellular stores are depicted in blue, and activation steps are shown by red arrows.

product (cADPR) is intracellular. One study suggests that cADPR produced by CD38 on human haemopoietic precursors can raise intracellular Ca^{2+} levels via a specific plasma membrane cADPR transporter.[23] However, da Silva *et al.*[24] have shown that the TCR-induced production of cADPR is intracellular and independent of extracellular production by CD38. Supporting the intracellular origin of TCR-induced cADPR, Guse *et al.*[22] demonstrated the presence of an ADP ribosyl cyclase activity in the cytosol of T cells.

The presence of RyR in lymphocytes is an interesting recent discovery. The Ca^{2+}-induced Ca^{2+} release properties of these receptors suggest a role in maintaining a long-term Ca^{2+} signal. In turn, this may suggest that InsP_3Rs are more concerned with triggering of the Ca^{2+} signal than with its long-term propagation.

NAADP receptors

Nicotinic acid adenine dinucleotide phosphate (NAADP) is a recently described intracellular Ca^{2+} mobilizing agent. NAADP is synthesized from NADP by the actions of ADP-ribosyl cyclase. Its actions are poorly understood, but it is increasingly evident that it mobilizes Ca^{2+} via a specific receptor, distinct from InsP_3Rs and RyRs, and which is a channel in its own right.[25] The actions of NAADP appear to vary depending on cell type (reviewed in refs 25 and 26). In Jurkat T lymphocytes, recent evidence suggests that mobilization of NAADP is an absolute requirement for signalling through the TCR.[27] NAADP appears to act before Ins(1,4,5)P_3 and cADPR to provide sufficient Ca^{2+} to sensitize the InsP_3R and RyR to Ins(1,4,5)P_3 and cADPR, respectively.

The nature of the NAADP receptor is unknown, but recent data suggest that it is an intracellular Ca^{2+} channel. Its pharmacology is beginning to be explored. It is known to be inhibited by high concentrations of L-type voltage-gated Ca^{2+} channel inhibitors, and curiously also by activators of these channels. It is also sensitive to inhibitors of some classes of voltage-gated K^+ channels.[28] There are currently no reports of NAADP involvement in B-cell signalling, but the persistent reports of the effects of inhibitors of L-type Ca^{2+} channels on BCR signalling (discussed below) suggest the possibility of this system also being involved in the BCR-induced Ca^{2+} flux.

Perhaps the most interesting feature of the NAADP receptor is its inactivation properties. Prior exposure to high concentrations of NAADP, such as might be expected if the TCR had previously been cross-linked, render the receptor inactive and unable to respond to further stimulation. In effect it allows the cell to retain a memory of a previous Ca^{2+} signal. This property led Berg *et al.*[27] to speculate that the NAADP/Ca^{2+} system may provide a mechanism underlying anergy in T cells. Together, InsP_3Rs, RyRs and the NAADP receptor form a complex web for priming, initiating and maintaining a signal, and then 'remembering' that is has been transduced.

PLASMA MEMBRANE CALCIUM CHANNELS

Much more is known about the AgR-activated Ca^{2+} channel in T cells than in B cells. In T cells there is strong evidence

that the TCR-activated Ca^{2+} channel is identical to the store-operated I_{CRAC} channel (reviewed in detail in ref 2). For example, both store depletion and TCR ligation activate a channel with identical properties[29] and in a human primary immunodeficiency caused by an absence of I_{CRAC}, there is no TCR-activated Ca^{2+} influx.[30] Ca^{2+} influx occurs following BCR ligation or pharmacological depletion of intracellular stores. However, to date, there have been no electrophysiological studies demonstrating the presence of the I_{CRAC} channel in B lymphocytes and it is not known whether B cells express this channel.

While the majority of studies on transmembrane Ca^{2+} flux have been aimed at identifying the AgR-activated Ca^{2+} channel, it is unlikely to be the only plasma membrane Ca^{2+} channel present in lymphocytes. A number of other potential Ca^{2+} channels have been identified but their unambiguous identification as *bona fide* Ca^{2+} channels is still lacking.

Store-operated calcium channels

Properties

AgR ligation leads to a release of Ca^{2+} from intracellular stores. The decrease in store Ca^{2+} concentration causes the activation of Ca^{2+} channels (store-operated Ca^{2+} channel: SOC) in the plasma membrane by an as yet unresolved mechanism.[31–33] Store-operated Ca^{2+} channels have been the subject of intensive research, but are still best described in terms of the current passing through the channel. The best characterized store-operated Ca^{2+} current in haemopoietic cells is the Ca^{2+}-release-activated Ca^{2+} current (I_{CRAC}), which was first identified in mast cells[34] and subsequently in Jurkat T lymphocytes.[29] The I_{CRAC} channel appears to be widely distributed, but other types of store-operated current have been described, including the Ca^{2+}-release activated non-selective cation current (I_{CRANC}) in Jurkat T cells.[35] There are several defining features of the I_{CRAC} Ca^{2+} influx pathway. These include a high selectivity for Ca^{2+},[36] a very small single channel conductance and blockade by divalent cations with a characteristic selectivity profile.[29,37,38] The other notable feature of the I_{CRAC} channel is Ca^{2+}-dependent modulation. Ca^{2+} influx can be enhanced through the binding of Ca^{2+} to an extracellular site on the channel[39] and the channel can be rapidly inactivated due to accumulation of Ca^{2+} close to its intracellular mouth.[40]

The properties of the I_{CRAC} channel have been determined by electrophysiological means and obviously must be met by any candidate protein. In this context, it is important to note that, although these properties describe one current, they may represent the averaged properties of a number of individual different subunits. This in turn may explain why the molecular identity of the I_{CRAC} channel has proved to be so hard to determine (discussed in more detail below).

Regulation

The importance of Ca^{2+} entry in normal lymphocyte function was highlighted when defects in this pathway were discovered. In 1994, Partesi *et al.*[30] described a patient with an immunodeficiency associated with defective T lymphocyte proliferation, where the defect was shown to be in the Ca^{2+} entry pathway. This defect was sufficient to alter T lymphocyte expansion and function. More recently, two severe-combined immunodeficiency patients have been shown to suffer from defective Ca^{2+} influx in both T and B lymphocytes resulting in impaired lymphocyte activation.[41]

It is not surprising, then, that Ca^{2+} entry turns out to be a regulated event in lymphocyte activation. The entry of Ca^{2+} through I_{CRAC} channels is ultimately driven by the membrane potential. Although lymphocytes are non-excitable cells, both B and T lymphocytes express voltage-gated K^+ channels and Ca^{2+}-activated K^+ channels (K_{Ca}) whose function is to maintain the resting membrane potential.[42] Following the AgR-induced rise in intracellular Ca^{2+} concentration, K_{Ca} are activated and hyperpolarize the membrane. This makes the membrane potential more negative and enhances Ca^{2+} influx by increasing the inward driving force for Ca^{2+} entry. Ca^{2+} entry can be sustained by the action of mitochondria strategically placed close to the mouth of the I_{CRAC} channel. They sequester Ca^{2+} following rapid Ca^{2+} entry preventing the Ca^{2+}-dependent inactivation of the channel.[43]

Co-receptors on lymphocytes can also regulate Ca^{2+} entry. Activation of acidic sphingomyelinase following Fas (CD95) ligation on T lymphocytes results in the release of ceramide which is further metabolized to sphingosine. Both ceramide and sphingosine can inhibit Ca^{2+} currents through I_{CRAC} channels and this may be the mechanism behind Fas-dependent immune suppression and impairment of lymphocyte function.[44] It has been suggested that blockade of I_{CRAC} channels could be responsible for anergy in lymphocytes.[2] In B lymphocytes, engagement of FcγRIIb is important in the negative regulation of Ca^{2+} influx. SHIP, a lipid phosphatase, is recruited to FcγRIIb following coligation with the BCR, and plays a key role in down-regulating the BCR-induced Ca^{2+} influx.[45]

Ca^{2+} influx can be regulated by protein kinases activated downstream of AgRs. B lymphocytes lacking Btk activity are deficient in Ca^{2+} influx following BCR ligation.[46] Similarly, in Itk-deficient T cells there is defective TCR-induced Ca^{2+} influx.[47] These effects are due to a defect in the sustained production of $Ins(1,4,5)P_3$, rather than a direct effect of the kinases on the Ca^{2+} channel. Using Lyn-knockout DT40 B cells, Hasimoto *et al.*[48] showed that Lyn negatively regulates BCR-induced Ca^{2+} influx, possibly through phosphorylation of the channel. PKCβ1 is reported to have a role in the down-regulation of Ca^{2+} entry in Jurkat T lymphocytes, although the exact mechanism is unclear.[49]

There are several estimates for the number of I_{CRAC} channels expressed on T lymphocytes. Kerschbaum and Cahalan[50] estimated that there are 100–400 channels per T lymphocyte, whereas Fomina *et al.*[38] estimated that resting T cells have 15 channels, rising to 140 channels in activated T cells. Fomina *et al.*[38] suggested that this up-regulation in the number of channels during T cell activation is necessary for sustaining proliferation and for enhancing Ca^{2+} signalling during secondary T lymphocyte activation.

It is clear that I_{CRAC} can be regulated by a number of mechanisms. Regulation by intracellular Ca^{2+} and by lymphocyte co-receptors allows lymphocytes the opportunity to fine-tune the Ca^{2+} signal in the short term. In the longer term, activation can be sustained by up-regulation of the number of channels expressed on the cell surface.

Identity

The molecular identity of the I_{CRAC} channel is unclear but the current view is that it is formed by one or more members of the Trp family. Trp proteins were originally discovered in *Drosophila* photoreceptor cells as ion channels required for sustained Ca^{2+} entry. Mammalian homologues of Trp fall into three subfamilies: TrpC, TrpV and TrpM.[51] TrpC1, TrpC3, TrpC5 and TrpC6 have all been reported to be expressed in Jurkat T lymphocytes.[52] Su *et al.*[35] described a store-operated non-selective cation channel (I_{CRANC}) in Jurkat. They suggest that TrpC3 and TrpC6 are likely to be subunits of this channel since, when TrpC3 and TrpC6 are expressed together, they form a channel with very similar properties to I_{CRANC}.

It is possible that Trp proteins are regulators of Ca^{2+} currents, are accessory subunits in a channel complex, or form the channel themselves. It may be necessary for a number of different Trp proteins to form heteromeric complexes in order to produce functional ion channels. Many of the studies that have sought to assign roles to ion channels formed by Trp proteins have relied on the ectopic overexpression of these proteins. This is likely to produce a channel that does not represent the native stoichiometry. In turn, this may explain some of the conflicting data on the functions of Trp family members. For just about every Trp family member, it is possible to find studies claiming and refuting its role as a store-operated Ca^{2+} channel.[53,54]

Currently the best candidate for the I_{CRAC} channel is the TrpV family member CaT1, which was originally cloned from small intestine[55] and subsequently recloned from Jurkat T cells.[56] Yue *et al.*[56] showed that transient expression of CaT1 in CHO-K1 cells results in a Ca^{2+} current with properties similar (but not identical) to those of I_{CRAC}. These similarities include activation by store depletion, cation selectivity, and estimates of single channel conductance. By determining some of the properties in CHO-K1 cells expressing submaximal levels of CaT1, Yue *et al.* address the issue of stoichiometry and association with other regulatory proteins. Their data provide compelling evidence that CaT1 may form all or a component of the I_{CRAC} channel. As yet the expression of CaT1 in B lymphocytes has not been addressed.

Other Ca^{2+} channels

Store-operated Ca^{2+} channels are unlikely to be the sole Ca^{2+} channels present in lymphocytes. There are numerous reports suggesting the presence of other types of Ca^{2+} channel such as the I_{CRANC} channel (discussed above), annexin V, CD20 and channels related to voltage-gated Ca^{2+} channels.

Annexins potentially form Ca^{2+} channels in lymphocytes. The annexins are a large diverse family of Ca^{2+}-dependent phospholipid binding proteins that can form voltage-gated Ca^{2+} channels in cell-free systems.[57,58] Annexin V (also known as endonexin II) is expressed intracellularly in B and T lymphocytes where its physiological function is unknown.[58]

Two recent studies have addressed the involvement of annexin V in Ca^{2+} influx in lymphocytes. In CEM T cells, the pharmacological extracellular application of annexin V was associated with an increased intracellular Ca^{2+} concentration, which in turn was associated with an inhibition of etoposide-induced apoptosis.[59] However, the extracellular application of large amounts of a protein that normally has an intracellular localization does not represent a physiologically realistic situation. Nor was the mechanism of formation and activation of the supposed annexin V Ca^{2+} channel explored. In a more convincing study, Kubista *et al.*[57] demonstrated annexin V-mediated H_2O_2-induced Ca^{2+} influx in B lymphocytes. Using DT40 B lymphocytes with targeted gene disruptions in annexin V, they showed that thapsigargin- and anti-IgM-induced store-operated Ca^{2+} entry was normal in these cells but that Ca^{2+} elevations induced by 2 mM H_2O_2 were reduced. However, this study could not rule out a role of annexin V as an activator of a Ca^{2+} channel rather than an authentic Ca^{2+} channel in its own right.

Possibly the best known candidate for a non-store-operated Ca^{2+} channel is CD20. CD20 is a marker of B lymphocytes that is expressed on resting and activated B cells[60] and on some T cell malignancies.[61–63] Transfection of CD20 into non-lymphoid cells induces the expression of a Ca^{2+} conductance identical to that seen when CD20 is overexpressed in T and B cells. This Ca^{2+} conductance is enhanced following the binding of anti-CD20 monoclonal antibodies to $CD20^+$ lymphoblastoid cells.[64] However, the anti-CD20 induction of Ca^{2+} influx is not a universal observation.[65] In some cases where anti-CD20 Ca^{2+} influx has been demonstrated there is evidence suggesting that it is a downstream consequence of phospholipase C activation.[66,67] Even in those studies that suggest that CD20 is a Ca^{2+}-permeable cation channel[68–70] the possibility that CD20 could be a regulatory subunit of a Ca^{2+} channel complex, rather than a Ca^{2+} channel in its own right, cannot be excluded. The role of CD20 in Ca^{2+} influx therefore remains unresolved.

An intriguing and persistent observation is that lymphocytes express non-voltage-gated Ca^{2+} channels that are related to classical voltage-gated Ca^{2+} channels. Early work relied heavily on pharmacological agents, typically the classical L-type Ca^{2+} channel antagonists such as diltiazem and verapamil. These were used at higher concentrations than required to block voltage-gated Ca^{2+} channels and at concentrations far higher than estimated therapeutic levels. Using L-type channel antagonists a number of studies showed profound effects on T- and B-cell activation and AgR-induced Ca^{2+} influx.[71–77] Whilst these pharmacological studies provide some evidence for the expression of Ca^{2+} channels related to voltage-gated Ca^{2+} channels, they should at the same time be treated with some caution due to the high concentrations of drugs used.

Akha *et al.*[78] demonstrated that anti-Ig induced Ca^{2+} influx in rat B lymphocytes occurred through a dihydropyridine-sensitive channel with similarities to the α_{1D} ($Ca_V1.3$) subtype of L-type Ca^{2+} channels. L-type channel antagonists and an anti-α_{1D} antibody blocked the anti-Ig induced Ca^{2+} response. The presence of α_{1C} ($Ca_V1.2$) and α_{1S} ($Ca_V1.1$) L-type Ca^{2+} channel transcripts in Jurkat T lymphocytes has been demonstrated by RT-PCR.[79] Recent studies show that a dihydropyridine-sensitive Ca^{2+} channel is involved in $HgCl_2$ and TCR-induced IL-4 synthesis in mouse T lymphocytes.[80,81] Savignac *et al.*[81] showed surface staining of mouse T lymphocytes with an anti-α_{1D} specific antibody, and the expression of an L-type Ca^{2+} channel transcript in these cells. These studies demonstrate the presence of an L-type channel transcript and protein in lymphocytes, but there appears to

be some confusion over which pore-forming subunit is expressed.

These reports represent an intriguing finding in the light of the reported pharmacology of the NAADP receptor. Could this elusive channel be in some way related to the non-voltage-gated L-type channels so persistently reported in lymphocytes? This question turns us full circle: it is clear that Ca^{2+} signals in lymphocytes exhibit a complex temporal and spatial pattern. The remarkable sophistication of something seemingly as simple as a Ca^{2+} flux enables the lymphocyte to translate an extracellular signal into an outcome finely tuned to the environment and needs of the cell. This is achieved via a web of intracellular and transmembrane Ca^{2+} channels interacting in a complexity of ways that we are only beginning to understand.

ACKNOWLEDGMENTS

GG is supported by the Medical Research Council; LT is supported by the University of Birmingham, School of Medicine. We are grateful to Dave Sansom for critical reading of this review.

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