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Transient Receptor Potential (TRP) channels in T cells

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Abstract

The Transient Receptor Potential (TRP) family of ion channels is widely expressed in many cell types and plays various physiological roles. Growing evidence suggests that certain TRP channels are functionally expressed in the immune system. Indeed, an increasing number of reports have demonstrated the functional expression of several TRP channels in innate and adaptive immune cells and have highlighted their critical role in the activation and function of these cells. However, very few reviews have been entirely dedicated to this subject. Here, we will summarize the recent findings with regards to TRP channel expression in T cells and discuss their emerging role as regulators of T cell activation and functions. Moreover, these studies suggest that beyond their pharmaceutical interest in pain management, certain TRP channels may represent potential novel therapeutic targets for various immune-related diseases.

Keywords

T cells; T cell receptor; TRP channels; Ca²⁺ signaling

Introduction

This special issue of Seminars in Immunopathology focuses on “*The role of TRP Ion Channels in Physiology and Pathology*”. TRP channels have been studied for many years by neuroscientists, mostly as pain receptors [1]. Although the physiological roles of TRP channels are well characterized in the nervous system, growing evidence suggests that TRP channels are expressed and functional in a wide array of non-neuronal cells. Indeed, these channels are expressed in many organs and tissues, including brain, heart, liver, lung, small and large intestine, skeletal muscle, skin, pancreas and the immune system [2-4]. However, the role of TRP channels in immune cells including T cells is by far less understood.

The mammalian TRP family consists of 28 members divided into six subfamilies: 1 TRPA (Ankyrin), 7 TRPC (Canonical), 6 TRPV (Vanilloid), 8 TRPM (Melastatin), 3 TRPP (Polycystin) and 2 TRPML (Mucolipin) [5]. All TRP proteins have six putative transmembrane domains (S1-S6) with a cation-permeable pore region between S5 and S6

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and functional TRP channels are formed by homo- or hetero-tetramers with N- and C-termini being located intracellularly [3, 5, 6]. Most TRP channels are non-selective cation channels with a permeability for Ca^{2+} over Na^{+} (ratio $P_{\text{Ca}}/P_{\text{Na}}$) ranging from 0.1 to >100 [3, 6].

Ca^{2+} channels are mandatory molecules for T cell activation as they provide the Ca^{2+} influx necessary for the many signaling processes that direct cellular responses such as cytokine secretion, T cell proliferation and T cell differentiation in T helper (Th) effector cells [7-9]. A major target downstream of T cell receptor (TCR) activation is the phosphorylation of phospholipase $\text{C}\gamma 1$ ($\text{PLC}\gamma 1$), which catalyzes the hydrolysis of phosphatidylinositol (4,5) bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol (1,4,5) trisphosphate (IP_3). DAG and IP_3 promote protein kinase C (PKC) activation and a release of Ca^{2+} from intracellular stores, respectively, followed by an influx of Ca^{2+} from the extracellular space, a process called store-operated Ca^{2+} entry (SOCE) [10]. Significant advances in our understanding of Ca^{2+} signaling in T cells and of the molecular machinery (i.e., ion channels) involved in this complex process have occurred over the last decade. One particular ion channel, the Ca^{2+} release-activated Ca^{2+} (CRAC) channel has been described as the major player in mediating SOCE in T cells [8, 11]. However, several additional families of ion channels are expressed at the plasma membrane of T cells and may therefore contribute to the regulation of Ca^{2+} entry in T cells [12-14]. Thus, before the discovery of ORAI1 as the pore-forming plasma membrane subunit of the CRAC channel [8, 11], several members of the TRP channel family (e.g., TRPC1/3, TRPV6) were considered as candidates to form the CRAC channel. However, the pore properties of most TRP channels studied appeared not to match those of the CRAC channel [3, 6]. Nevertheless, an increasing amount of literature has reported the contribution of several TRP channels to Ca^{2+} entry in T cells via different mechanisms: either directly via SOCE or non-SOCE pathways, or indirectly through the regulation of the driving force for Ca^{2+} via other Ca^{2+} -permeable channels [12-14]. Although the evidences about TRP channel expression in T cells have been accumulating over the last decade, to the best of our knowledge only one review has been entirely dedicated to the expression and function of TRP channels in T cells so far [15].

In this review, we will attempt to summarize the current knowledge on the TRP channels that have been identified as functionally expressed in rodent or human T lymphocytes and discuss the mechanisms by which they regulate T cell activation and functions.

Evidences for TRP channel expression in T cells

The expression of a growing number of TRP channels has been reported to date in rodent and human T cells [15-17]. In most of these studies, analysis of TRP channel expression at the mRNA level using Northern blot, reverse transcriptase PCR (RT-PCR) or real-time quantitative PCR (q-PCR) techniques was frequently employed. However, analysis of protein expression and channel functionality (i.e., Ca^{2+} signaling and/or electrophysiological data) was not consistently performed. In addition, in many studies T cell lines but not primary T cells were used. Therefore, the functional expression of certain TRP family members in non-transformed cells remains to be determined. Finally, because of the

relatively poor specificity of certain TRP agonists and antagonists, convincing studies rely on genetic approaches and the use of specific TRP knockout mouse or knockdown cells.

Despite the aforementioned limitations in the study of TRP channels in T cells, a certain number of studies have convincingly demonstrated the important role of TRP channels in T cell activation and functions. Among the TRP channels likely to be expressed and functional in T cells, to date the expression of TRPA1, TRPC1/2/3/5/6, TRPV1-6 and TRPM1/2/4/5/6/7 was reported by different investigators (Table 1).

TRPA channels

The TRPA family contains only one member, TRPA1. This ion channel is best known in sensory neurons as a sensor for environmental irritants, inflammatory pain, and itching [18]. However, TRPA1 has a diverse tissue distribution and plays different roles in a variety of non-neuronal cells [2, 18]. TRPA1 expression at the mRNA and protein levels was reported in human secondary lymphoid organs (i.e., spleen and lymph nodes) as well as in a human T cell leukemic cell line (i.e., Jurkat) [19]. However, TRPA1 channel functionality was not investigated in this study. Recently, our group identified the constitutive expression of TRPA1 mRNA and protein in mouse and human primary CD4⁺ T cells. In addition, we demonstrated TRPA1 channel functionality at the plasma membrane of mouse CD4⁺ T cells by electrophysiological and Ca²⁺ imaging techniques. We identified that TRPA1 regulates TRPV1 channel activity in CD4⁺ T cells, and consequently controls CD4⁺ T cell activation and pro-inflammatory responses in models of colitis [Bertin *et al.* Submitted for publication, 2015].

TRPC channels

TRPC channels are subdivided into four subfamilies on the basis of sequence homology and similarities in function: TRPC1, TRPC2 (a pseudogene in human), TRPC3/6/7 and TRPC4/5. All TRPCs form non-selective ion channels with only a very modest Ca²⁺ selectivity [3, 6]. TRPC channels are widely expressed in the nervous system as well as in non-excitable cells [5]. Certain TRPC channels, specifically TRPC1, TRPC3, TRPC5 and TRPC6 are expressed in T cells [15, 20]. TRPC1 and TRPC3 have been proposed to act as store-operated channels and to interact with STIM1 and ORAI1 [21, 22]. Although this remains very controversial [8, 23, 24] and TRPC channels are no longer considered part of the CRAC channel, they may mediate other forms of Ca²⁺ influx relevant for T cell activation [17, 24]. For example, TRPC1 is functionally expressed in a human T cell line (HPB-ALL) and contributes to cannabinoid-induced Ca²⁺ influx in these cells independently of intracellular Ca²⁺ store depletion [25]. In another study, Jurkat cells with mutated TRPC3 channels showed decreased Ca²⁺ influx after TCR stimulation, which was rescued by overexpression of wild-type TRPC3 [26]. Furthermore, TRPC3 mRNA is strongly up-regulated in human primary CD4⁺ T cells following TCR stimulation and siRNA-mediated knockdown of TRPC3 led to a decrease in Ca²⁺ influx and proliferation [17].

Another subfamily member, TRPC5 is upregulated in activated mouse CD4⁺ and CD8⁺ effector T cells and mediates Ca²⁺ influx after binding of surface GM1 ganglioside by

galectin-1 on regulatory T cells (Treg), potentially contributing to Treg-mediated suppression of effector T cell functions in experimental autoimmune encephalomyelitis (EAE) [27] and in the NOD (non-obese diabetic) mouse model of type-1 diabetes [28].

Tseng and colleagues reported that TRPC6 overexpression or antisense downregulation significantly alters the amplitude of phosphatidylinositol 3,4,5-trisphosphate (PIP₃)- and TCR-induced Ca²⁺ responses in Jurkat cells [29]. In addition, silencing of TRPC3 and TRPC6 genes by shRNA abolished Ca²⁺ entry in Jurkat cells in response of certain free fatty acids [30]. Finally, a recent study utilizing a rat model of sepsis, demonstrated that TRPC3 and TRPC6 expression is upregulated in T cells and enhanced T cell apoptosis [31].

TRPV channels

Similarly to the TRPC family, the TRPV family can be divided into two subfamilies based on the structure, function and Ca²⁺ selectivity: TRPV1-4 are non-selective cation channels ($P_{Ca}/P_{Na} \approx 1-10$) whereas TRPV5/6 are exclusively permeable to Ca²⁺ ($P_{Ca}/P_{Na} > 100$) [3, 6]. TRPVs are known to participate in thermosensation (e.g., TRPV1-4) [5], pain perception (e.g., TRPV1) [1] and Ca²⁺ entry in certain types of epithelial cells (e.g., TRPV1/5/6) [32, 33]. TRPV1 can associate with TRPV2 and TRPV3 and widespread interaction has been shown for TRPV1-TRPV4 [6, 34]. Furthermore, TRPV5 and TRPV6 can form heteromeric channel complexes [6, 35]. TRPV1 mRNA and protein expression was reported in primary mouse and human T cells by several different groups [17, 36-40]. TRPV1 mRNA and protein expression was also reported in mouse and rat thymocytes [41, 42]. The authors showed that capsaicin (a specific TRPV1 channel agonist) [43], increases intracellular Ca²⁺ concentration ([Ca²⁺]_i) and induced thymocyte cell death via apoptotic or autophagic pathways in a dose-dependent manner [41, 42]. These results suggested that the TRPV1 channel could influence the development of thymocytes in rodents. A rise in [Ca²⁺]_i and induction of apoptosis in response to capsaicin was also demonstrated in human peripheral T cells and in Jurkat cells [44]. However, although the Ca²⁺ mobilization appeared to be mediated by TRPV1, the induction of apoptosis was TRPV1-independent and was observed in response to prolonged and very high capsaicin concentration exposure (e.g., 200-250 mM) [44]. Functional TRPV1 expression in Jurkat cells was confirmed by another group using both Ca²⁺ imaging and whole-cell patch clamp [45]. Using capsazepine (a TRPV1 channel antagonist) [46], the authors showed that TRPV1 mediates the Ca²⁺ influx rise in response to phytohemagglutinin (PHA, a lectin that binds to glycosylated surface proteins including the TCR, and crosslinks them) and suggested that TRPV1 contributes to physiological TCR-induced Ca²⁺ influx and T cell activation. In line with these observations, our group recently demonstrated that TRPV1 is constitutively expressed in mouse and human primary CD4⁺ T cells where it acts as a non-store operated Ca²⁺ channel and contributes to TCR-induced Ca²⁺ influx [40]. Using *Trpv1*^{-/-} mouse and mice with conditional overexpression of TRPV1 in CD4⁺ T cells, we identified that TRPV1 regulates downstream TCR-induced signaling, cytokine production and the pro-inflammatory properties of CD4⁺ T cells in models of T cell-mediated colitis. We also validated these findings in human primary CD4⁺ T cells using TRPV1-specific siRNA and channel antagonists. Very recently, Majhi *et al.*, confirmed the critical role of TRPV1 in mediating TCR-induced Ca²⁺ influx and cytokine production in mouse and human primary T cells by

using I-RTX (a specific TRPV1 antagonist) [46] and interestingly, also reported the protein expression of other TRPV subfamily members (i.e, TRPV2, TRPV3 and TRPV4) in these cells as well as in Jurkat [47].

TRPV2 mRNA and protein expression has been reported in mouse and human T cells previously [16, 17, 37-39]. Sauer and Jegla have reported the presence of TRPV2 in Jurkat cells and in primary human T cells in a patent application [48]. They identified that Jurkat cells treated with a dominant negative hTRPV2 or nucleofected with hTRPV2 shDNA display a defect in TCR- or thapsigargin-induced Ca^{2+} mobilization, predominantly during the sustained phase of Ca^{2+} influx. In addition, TRPV2 knockdown Jurkat cells displayed a reduction in Ca^{2+} store release similar to that induced by the knockdown of the lymphocyte-specific protein tyrosine kinase Lck [48]. More recently, Pottosin *et al.*, published a detailed electrophysiological analysis of membrane stretch-activated currents in Jurkat cells and used TRPV2 siRNA to confirm the involvement of the TRPV2 channel in this process [49]. These studies therefore indicate that TRPV2 may play a role in Ca^{2+} signaling in lymphocytes and in its regulation by mechanical stress.

TRPV3 protein expression was recently reported in mouse and human primary T cells as well as in Jurkat [47]. However, in line with observations made by our group and by others, *Trpv3* mRNA [16, 37, 40] and protein [47] are expressed at low level in T cells and *Trpv3*^{-/-} mice don't have any obvious T cell phenotype [Bertin *et al.* Unpublished observations].

In contrast to TRPV3, TRPV4 appear to be present at a higher level in CD4⁺ T cells [16, 40, 47]. Interestingly, Majhi *et al.*, reported a similar role for TRPV4 as for TRPV1 in contributing to TCR-induced Ca^{2+} influx and cytokine production in mouse and human T cells [47]. In accordance with the fact that TRPV1 and TRPV4 can form heteromeric channel complexes [34], these data suggest that inhibition of both TRPV1 and TRPV4 may have a synergistic effect in decreasing TCR-induced Ca^{2+} influx and consequently T cell activation.

The functional expression of the Ca^{2+} -selective TRPV5 and TRPV6 channels was reported in mouse and human primary T cells and in Jurkat cells [15, 50, 51]. However, *Trpv6*^{-/-} mice have no reported immunological phenotype [52] and TCR-induced Ca^{2+} influx in CD4⁺ T cells from *Trpv6*^{-/-} mice is normal [53]. The TRPV6 channel, which is highly permeable to Ca^{2+} , was shown to be triggered by store depletion [51] and, as certain TRPC subfamily members, was considered as a potential molecular entity of the CRAC channel. When a dominant-negative pore-region mutant of TRPV6 was expressed in Jurkat T cells, the CRAC current was found to be reduced [51]. However, the role of TRPV6 as a CRAC channel could not be established [54, 55] as BTP2 (a pyrazole derivative and CRAC channel inhibitor) did not affect the activity of the TRPV6 channel [56]. Recently, it was reported that both TRPV5 and TRPV6 are involved in Ca^{2+} entry and cell cycle progression of human primary T cells and Jurkat cells [50]. The activity of these two channels in Jurkat cells was also shown to be modulated by extracellular pH [57]. However, these conclusions rely on the use of Ruthenium red, a nonspecific channel inhibitor [58, 59]. Further, genetic evidences are still not comprehensive enough to fully demonstrate the physiological importance of TRPV5 and TRPV6 in the context of T cell activation and functions.

TRPM channels

The eight members of the mammalian TRPM subfamily are non-selective cation channels [5] and can be subdivided into two categories according to their Ca^{2+} permeability; practically impermeable (TRPM4/5) and moderately permeable (TRPM1/2/3/6/7/8) to Ca^{2+} [3, 6]. TRPMs are broadly expressed in both neuronal and non-neuronal cells and play diverse roles such as mediating direct Ca^{2+} influx, controlling Mg^{2+} entry and determining the membrane potential [5]. Among them, TRPM2, TRPM4 and TRPM7 appear to be constitutively expressed in CD4^+ T cells [15, 17].

Sano *et al.*, published one of the first reports on the role of TRPM2 (formerly LTRPC2) as a Ca^{2+} channel in T cells [60]. Their study demonstrated that intracellular secondary messengers ADP-ribose (ADPR) and nicotinamide adenine dinucleotide (NAD^+) could directly stimulate TRPM2 channel activity to induce Ca^{2+} entry in Jurkat cells. Guse *et al.*, previously demonstrated that a cyclic ADPR antagonist inhibits TCR-induced proliferation and expression of CD25 and HLA-DR in Jurkat cells [61]. Conversely, hydrogen peroxide (H_2O_2) can facilitate ADPR-mediated activation of TRPM2 and may therefore link TRPM2 activation to oxidative stress in various immune cells including T cells [62, 63]. Others showed that Concanavalin A (an antigen-independent mitogen) increases intracellular ADPR concentrations in Jurkat cells and that ADPR mediates Ca^{2+} influx through the TRPM2 channel and induces cell death [64]. In this study, the authors also showed that inhibition of ADPR formation or knockdown of TRPM2 both inhibited TRPM2-mediated Ca^{2+} influx. Another study similarly emphasized on the role of NAD^+ and ADPR in mitogen-induced Ca^{2+} rise in Jurkat and in primary human T cells via TRPM2 [65]. More recently, Melzer and coworkers demonstrated that TRPM2 is expressed in mouse primary CD4^+ T cells and contributes to T cell proliferation and production of pro-inflammatory cytokines after TCR stimulation [66]. The authors showed that the severity of EAE was reduced in *Trpm2*^{-/-} mice and that this protection was associated with impaired TCR-stimulated proliferation as well as IFN- γ and IL-17 production by *Trpm2*^{-/-} CD4^+ T cells.

TRPM4 indirectly regulates Ca^{2+} entry by conducting Na^+ and K^+ currents [3, 6]. Interestingly, TRPM4 has been shown to negatively regulate SOCE in T cells by inducing membrane depolarization and by reducing the driving force for Ca^{2+} entry [67]. Launay *et al.*, first detected TRPM4-mediated currents in Jurkat T cells and showed that downregulation of TRPM4 expression using an siRNA approach, increased PHA-induced Ca^{2+} entry and IL-2 production in Jurkat T cells [67]. More recently, Weber *et al.*, demonstrated that TRPM4 is expressed at different levels in mouse Th cells ($\text{Th2} > \text{Th1}$) and that it differentially regulates Ca^{2+} signaling and NFATc1 subcellular localization in these cells [68]. The authors found that inhibition of TRPM4 expression (via siRNA or a dominant negative construct) in Th2 cells resulted in increased Ca^{2+} influx, NFATc1 nuclear localization and IL-2 production as well as decreased cell motility and IL-4 production. Inhibition of TRPM4 expression in Th1 cells caused a decrease in Ca^{2+} influx and NFATc1 nuclear localization, resulting in an increase in cell motility, and a decrease in IL-2 and IFN- γ production [68]. These *in vitro* studies suggest an important role for TRPM4 in regulating T cell activation and differentiation in Th effector cells. However, *in vivo* validation of a role for TRPM4 in disease models has not yet been reported.

TRPM7 is a Mg^{2+} -permeable, non-selective cation channel required for Mg^{2+} homeostasis in many cell types [69]. Since *Trpm7*^{-/-} mice died prenatally, Clapham and colleagues used Lck-Cre mice to selectively delete TRPM7 in T cells [70]. The resulting mice with conditional deletion of TRPM7 in T cells displayed a severe defect in T cell development in the thymus. The authors noticed a block in transition from the double negative (CD4⁻CD8⁻) to double positive (CD4⁺CD8⁺) stage in *Trpm7*^{-/-} thymocytes. As a result, both the number and the percentage of T cells in the periphery were reduced [70]. However, *Trpm7*^{-/-} T cells did not show any significant defect in Mg^{2+} uptake suggesting that TRPM7 is dispensable for Mg^{2+} homeostasis in T cells and that another mechanism was responsible for the phenotype of these mice. Interestingly, TRPM7 also contains a regulatory serine-threonine kinase domain and is often referred to as a “chanzyme” for its channel-enzyme bifunctionality [71]. A recent study from the same group showed that *Trpm7*^{-/-} T cells are deficient in Fas-mediated apoptosis and that this effect is dependent on TRPM7 activity as a channel rather than a kinase [72]. Therefore, the question arises whether the effect of TRPM7 deletion on T cell development is related to divalent cations other than Mg^{2+} , such as Ca^{2+} or Na^{+} . In line with this idea, TRPM7 was shown to regulate Ca^{2+} oscillations at the uropod of activated human primary T cells and to control their migration [73]. Finally, mice with conditional deletion of TRPM7 in T cells also displayed lung inflammation and pathophysiological similarity to mice in which the Fas receptor is selectively deleted in T cells, further supporting a role for TRP channels as T cell-intrinsic regulators of cell death [31, 41, 42, 44, 64] and inflammation [40, 74-76].

TRPP and TRPML channels

The TRPP and TRPML constitute two small subfamilies of non-selective cation channels that are predominantly expressed intracellularly [77]. TRPMLs play a critical role in endolysosomal trafficking and function [78] and genetic defects in TRPML1 and TRPML3 lead to a variety of cellular phenotypes including neurological and neurosensory deficiencies in humans and in animal models [77]. In addition, human TRPML2 has been shown to co-localize with major histocompatibility complex class I (MHC-I) and CD59 antigen [79], whereas mouse TRPML1 co-localizes with MHC-II in macrophage cell lines [80]. A role for TRPML1 and TRPML2 in vesicular transport in B cells has also been reported [81]. However, to the best of our knowledge their expression hasn't been reported to date in T cells and therefore remains to be investigated.

Modes of activation of TRP channels in T cells

TRP channels can respond to stimuli ranging from changes in temperature, pH, osmolarity, $[Ca^{2+}]_i$, to natural product compounds, pro-inflammatory agents, and various endogenous or exogenous stress mediators [5]. TRP channels are polymodal receptors and can therefore be activated via diverse mechanisms, some through direct binding of agonists (e.g., TRPV1 by capsaicin) [43], following receptor stimulation in a PLC- (e.g., TRPC1, TRPC4, TRPC5) or DAG- (e.g., TRPC3, TRPC6, TRPC7) dependent way [82, 83]. Temperature can also activate the thermosensitive TRP channels (e.g., TRPA1, TRPV1-4, TRPM8) [84] and store-depletion is discussed to be playing a role in modulating the activity of certain TRPC channels. Several studies have reported that STIM1 and ORAI1 can directly interact with

TRPC channels, specifically with TRPC1 and TRPC3 [21, 22]. However as mentioned before, this remains a controversial discussion [8, 23, 24, 82].

The activity of certain TRP channels can also be modulated by cellular kinases (e.g., PKA, PKC, Src) [85, 86]. For example, our results suggested that phosphorylation by Lck is the gating mechanism of the TRPV1 channel in CD4⁺ T cells upon TCR stimulation [40, 87]. Tyrosine phosphorylation by Lck may activate TRPV1 downstream of TCR engagement by several potential mechanisms: (i) directly as it was shown for Src-mediated activation of TRPV1 [88] and other TRP family members such as TRPV4 [89], TRPV6 [90], TRPC3 [91, 92], TRPC6 [93] and TRPM2 [94]; (ii) indirectly by lowering its activation threshold (i.e., sensitization) to endogenous agonists (e.g., DAG) [95]; or (iii) by inducing TRPV1 translocation from intracellular pools to the plasma membrane and increasing the surface density of TRPV1 channels [96-98].

Another mode of regulation of TRP channel activities resides in their multimerization capacity. Most TRPs can form heteromultimeric channel complexes with another subfamily member (e.g., TRPC1/5, TRPC4/5, TRPV5/6) [3, 5, 6, 35] or with a member of a different subfamily (e.g., TRPA1/V1) [99-101], creating a variety of chimeric channels with unique properties, as compared to homomultimers, where each TRP channel within a same heteromultimeric complex can influence each other's activity. For example, we recently identified that TRPA1 and TRPV1 are co-expressed in CD4⁺ T cells and that TRPA1 restrains TRPV1 channel activity in these cells. Indeed, *Trpa1*^{-/-} CD4⁺ T cells display augmented TRPV1 channel activity and consequently, increased TCR-induced Ca²⁺ influx, T cell activation and pro-inflammatory capacities [Bertin *et al.* Submitted for publication, 2015].

In conclusion, TRP channels in T cells, such as in other cell types, appear to be regulated via several different mechanisms and to operate either as: (i) primary detectors (ligand-gated ion channel) of chemical and physical stimuli, (ii) as secondary transducers (downstream of a receptor e.g., TCR) of cell activation, or (iii) as ion transport channels.

Functional Roles of TRP Channels in T cells

Involvement in physiological T cell activation

The increase in [Ca²⁺]_i is fundamental for T cell activation and biological functions [8, 9, 102, 103]. Like in other non-excitable cells, TRP channels in T cells contribute to Ca²⁺ influx either directly as Ca²⁺ permeable channels at the plasma membrane (e.g., TRPC1/3, TRPV1/4/5/6, TRPM2) or indirectly by controlling the membrane potential and thus, the driving force for Ca²⁺ entry through other Ca²⁺ entry channels (e.g., TMRP4) [3, 5, 12-14]. As mentioned above, several TRP channels appear to regulate the Ca²⁺ influx induced in response to TCR stimulation and therefore participate in the activation of T cells in physiological conditions. However, genetic evidence for the physiological roles of most TRP channels in T cells is still lacking in thoroughness. In comparison to CRAC, TRP channels may have mostly modulatory and homeostatic roles in T cells, which may be important when the immune system is challenged but not necessary apparent under normal homeostatic conditions in unchallenged mice. By mediating SOCE, CRAC is largely

accepted as the major source of Ca^{2+} influx in T cells with certain TRP channels such as TRPC1, TRPC3 and TRPM4, acting as regulators of this process [8, 23, 24, 53]. For example, TRPM4 negatively regulates SOCE and plays an important physiological role in T cells by preventing Ca^{2+} overload [67]. Other TRP channels such as TRPV1, TRPV2 and TRPV4 may act independently of CRAC as non-store operated channels in T cells [40, 47, 48] but nevertheless appear to contribute to the physiological Ca^{2+} influx following TCR stimulation. Despite the significant advances in the understanding of Ca^{2+} signaling in T cells, further studies are needed to determine the relative contribution of TRP channels to the overall Ca^{2+} entry process that follows engagement of the TCR [103].

Certain TRP channels may also play an important physiological role in T cells by modulating T cell activation in response of temperature changes. Although the effect of temperature on the immune response is well documented [104-107], the exact molecular mechanisms are poorly understood [108]. The expression of thermosensitive TRPs such as TRPA1, TRPV1-4 and TRPM2 in T cells suggest that these channels may play a role in the effect of temperature changes on T cell activation and ensuing immune responses.

Finally, by regulating Ca^{2+} signals in T cells, TRP channels may be important regulator of cell survival and cell death. Although an entry of Ca^{2+} is vital for cell functions and so are necessary for cell survival, an excess of $[\text{Ca}^{2+}]_i$ can have detrimental effects. Recently it has become clear that cellular Ca^{2+} overload, or perturbation of intracellular Ca^{2+} compartmentalization, can cause cytotoxicity and trigger cell death via apoptotic, necrotic or autophagic pathways [109]. As documented for TRPV1 [41, 42] and TRPM2 [64] in response to chemical or oxidative stress respectively, and for TRPM4 in response to SOCE [67], TRP channels may play a critical role in the process of Ca^{2+} overload in T cells under physiological and pathological conditions.

Involvement in pathological T cell activation

Defects in ion channel function have been linked to various diseases, so-called channelopathies. However, among the 28 mammalian TRP channels, relatively few have been directly connected to human diseases [110]. As mentioned above, it is now clear that several TRP channels are functionally expressed in T cells and their activation contributes to T cell activation including proliferation, differentiation into effector cells and release of specific inflammatory cytokines, suggesting a potential role for these ion channels in immune-related diseases [4, 111]. Changes in TRP channel expression or function in T cells may be associated with a variety of autoimmune and chronic inflammatory diseases, such as asthma [112-114], inflammatory bowel disease [40, 76, 115, 116], rheumatoid arthritis [117, 118], multiple sclerosis [27, 66, 119], type-1 diabetes [4, 28], lupus [120], and psoriasis [4] but *in vivo* studies focusing on the role of TRP channels in T cells are limited and up to now restricted to TRPV1 [40], TRPC5 [27], TRPC6 [112], TRPM2 [66] and TRPM7 [70, 72]. The above-mentioned studies therefore suggest that certain TRP channels could represent new drug targets for the management of various T cell-mediated diseases. In addition, such as other molecules interfering with Ca^{2+} signaling in T cells (e.g., cyclosporin A and FK506, two calcineurin inhibitors), certain TRP channels modulators may have potential therapeutic

applications in organ transplantation, where T cells are key players in the process of graft rejection and transplantation tolerance [121].

Conclusions

It is becoming clear that T cell functions are regulated by a network of different ion channels including CRAC, TRPs, voltage-gated Ca^{2+} (Cav) channels, P2X receptors, Ca^{2+} -activated K^{+} channels (KCa) and voltage-gated K^{+} (K_v) channels [12-14, 102]. However, compared to the CRAC channel, the contribution of other families of ion channels to TCR-induced Ca^{2+} influx and T cell functions has not been investigated as comprehensively.

In this review, we attempted to summarize the recent studies that demonstrate the functional expression and the critical role of TRP channels in T cells. Despite the increasing number of studies reporting the expression of various TRP channels at the mRNA and/or protein level in T cells, only a few have demonstrated the functionality of TRP channels in primary T cells. In addition, reports using conditional mice with T cell-specific deletion of *Trp* genes are restricted until now to TRPM7 [70, 72] and most studies have employed T cells isolated from mice with ubiquitous inactivation of individual *Trp* genes in which the observed phenotype may potentially be affected by developmental defects or compensatory upregulation of other genes in adult animals. Therefore, more *in vivo* studies with conditional TRP-deficient mice are needed in addition to the use of si/shRNA-mediated knockdown strategies in *in vitro* experiments with primary T cells in order to unambiguously demonstrate the cell-intrinsic role of TRP channels in T cells. In spite of these limitations, the most important conclusion of this review is that several TRP channels are functionally expressed in T cells and contribute to T cell activation under physiological and pathological conditions. However, how TRP channels function in T cells and how they interact with other family members and with other channels (e.g., CRAC channel) remain poorly understood. Future studies will be needed to explore the complex interplay between ion channels in T cells and to identify the precise role of each channel during T cell development and in the different effectors T cell subsets.

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Abbreviations

Ca²⁺	Ca ²⁺ imaging
CD3⁺	primary CD3 ⁺ T cells
CD4⁺	primary CD4 ⁺ T cells
CD8⁺	primary CD8 ⁺ T cells
EP	electrophysiology
IB	immunoblot
IHC	immunohistochemistry
LN	lymph nodes
Mg²⁺	Mg ²⁺ imaging
NB	northern blot
PBMC	peripheral blood mononuclear cells
q-PCR	quantitative PCR
RT-PCR	conventional reverse transcription PCR
SB	southern blot
SP	spleen

Table 1
Expression of TRP channels reported to date in T cells

Channel	Cell type	Species	Detection methods	References
TRPA1	SP, LN, Jurkat	Human	NB, IB, IHC	[19]
	CD4 ⁺	Mouse, Human	q-PCR, IB, IF, Ca ²⁺ , EP	[Bertin <i>et al.</i> Submitted]
TRPC1	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	CD4 ⁺ , Jurkat	Human	RT-PCR, q-PCR	[17]
	HPB-ALL, Jurkat	Human	RT-PCR, Ca ²⁺	[25]
TRPC2	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
TRPC3	Jurkat	Human	SB, NB, IB, Ca ²⁺ , EP	[26]
	CD4 ⁺ , Jurkat	Human	RT-PCR, q-PCR	[17]
	Jurkat	Human	RT-PCR, q-PCR, Ca ²⁺	[30]
	PBMC	Rat	IB, Ca ²⁺	[31]
TRPC5	CD4 ⁺ , CD8 ⁺	Mouse	RT-PCR, IF, Ca ²⁺	[27, 28]
TRPC6	PBMC, Jurkat	Human	RT-PCR, IB, Ca ²⁺	[20]
	Jurkat	Human	RT-PCR, Ca ²⁺	[29]
	Jurkat	Human	RT-PCR, q-PCR, Ca ²⁺	[30]
	PBMC	Rat	IB, Ca ²⁺	[31]
TRPV1	PBMC	Rat	RT-PCR	[36]
	CD3 ⁺	Human	q-PCR	[37, 39]
	PBMC	Human	RT-PCR, IF	[38]
	CD4 ⁺ , Jurkat	Human	RT-PCR, q-PCR	[17]
	CD4 ⁺ , Jurkat	Mouse, Human	q-PCR, IB, IF, FACS, Ca ²⁺ , EP	[40]
	CD4 ⁺ , Jurkat	Mouse, Human	IF, Ca ²⁺	[47]
	Thymocytes	Rat	q-PCR, IB, IF, FACS, Ca ²⁺	[41, 42]
	CD3 ⁺ , Jurkat	Human	Ca ²⁺ , EP	[44, 45]
TRPV2	PBMC	Human	RT-PCR, IF	[38]
	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	CD3 ⁺	Human	q-PCR	[37, 39]
	CD4 ⁺ , Jurkat	Human	RT-PCR, q-PCR	[17]
	CD4 ⁺ , Jurkat	Mouse, Human	IF	[47]
	CD4 ⁺ , Jurkat	Human	q-PCR, Ca ²⁺	[48]
	Jurkat	Human	EP	[49]
	CD4 ⁺ , Jurkat	Human	EP	[49]
TRPV3	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	CD3 ⁺	Human	q-PCR	[37, 39]
	CD4 ⁺ , Jurkat	Mouse, Human	IF	[47]
TRPV4	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	CD3 ⁺	Human	q-PCR	[37, 39]
	CD4 ⁺ , Jurkat	Mouse, Human	IF, Ca ²⁺	[47]
TRPV5	CD3 ⁺ , Jurkat	Human	q-PCR, IB, EP	[50]

Channel	Cell type	Species	Detection methods	References
TRPV6	Jurkat	Human	IF, Ca ²⁺ , EP	[57]
	Jurkat	Human	RT-PCR, q-PCR, Ca ²⁺ , EP	[51]
	CD3 ⁺ , Jurkat	Human	q-PCR, IB, EP	[50]
TRPM1	Jurkat	Human	IF, Ca ²⁺ , EP	[57]
	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	CD4 ⁺ , Jurkat	Human	RT-PCR, q-PCR	[17]
TRPM2	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	Jurkat	Human	EP	[62]
	Jurkat	Human	IB, IF, Ca ²⁺ , EP	[64]
TRPM4	CD3 ⁺ , Jurkat	Human	IB, Ca ²⁺ , EP	[65]
	CD4 ⁺	Mouse	RT-PCR, IF	[66]
	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
TRPM5	Jurkat	Human	RT-PCR, IB, Ca ²⁺ , EP	[67]
	CD4 ⁺	Mouse	IB, Ca ²⁺	[68]
	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
TRPM6	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
	CD4 ⁺ , Jurkat	Human	RT-PCR, q-PCR	[17]
	SP, LN, CD4 ⁺	Mouse	RT-PCR	[16]
TRPM7	Thymocytes, CD3 ⁺	Mouse	RT-PCR, IB, Mg ²⁺ , EP	[70]
	CD3 ⁺ , Jurkat	Mouse, Human	q-PCR, IB, EP	[72]
	CD3 ⁺	Human	IF, EP	[73]