TPC Functions in the Immune System

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Contents

Abstract

Two-pore channels (TPCs) are novel intracellular cation channels, which play a key role in numerous (patho-)physiological and immunological processes. In this

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chapter, we focus on their function in immune cells and immune reactions. Therefore, we first give an overview of the cellular immune response and the partaking immune cells. Second, we concentrate on ion channels which in the past have been shown to play an important role in the regulation of immune cells. The main focus is then directed to TPCs, which are primarily located in the membranes of acidic organelles, such as lysosomes or endolysosomes but also certain other vesicles. They regulate Ca^{2+} homeostasis and thus Ca^{2+} signaling in immune cells. Due to this important functional role, TPCs are enjoying increasing attention within the field of immunology in the last few decades but are also becoming more pertinent as pharmacological targets for the treatment of pro-inflammatory diseases such as allergic hypersensitivity. However, to uncover the precise molecular mechanism of TPCs in immune cell responses, further molecular, genetic, and ultrastructural investigations on TPCs are necessary, which then may pave the way to develop novel therapeutic strategies to treat diseases such as anaphylaxis more specifically.

Keywords

Endolysosomes · Mast cells · TPC1 · TPC2

1 Introduction

1.1 Overview on Cellular Immunity

Immune cells develop from hematopoietic stem cells (HSCs) and can be assigned to either the innate or the adaptive immune system (Nicholson 2016; Chaplin 2010). Representative cells of the innate immune system are dendritic cells, macrophages, mast cells, and granulocytes. The adaptive immune system comprises lymphocytes, such as B-cells and T-cells. Natural killer (NK) cells, while counting to lymphocytes, still belong to the innate immune system and are classified as group I innate lymphoid cells (ILCs) (Mazzurana et al. 2018). The first pathogen-associated defense response is initiated by the innate immune system after contact with pathogens, such as bacteria or viruses. Prior to immune system activation, unspecific defense mechanisms, such as the physical barrier of our skin or mucous membranes, protect us from potential infections (Thier et al. 2017; Castelo-Branco and Soveral 2014). Nevertheless, if these barriers are overcome and an inflammatory reaction is triggered, local dendritic cells are the first leukocytes to be activated. In addition, granulocytes, mast cells, and macrophages are primarily attracted. Pathogens can be identified by pathogen-associated molecular patterns (PAMPs) attached to the outer cell membrane of the pathogen (e.g. lipopolysaccharides of the bacterial cell membrane), which can be recognized by pattern recognition receptors (PRR) of granulocytes and macrophages (Triantafilou and Triantafilou 2012; Metcalf et al. 2015; Choudhary et al. 2020). Granulocytes, such as eosinophils, basophils, and neutrophils, are then able to secrete pro-inflammatory mediators to recruit other

leukocytes, while neutrophils, macrophages, and dendritic cells induce PAMP-mediated phagocytosis to digest the pathogen.

The reaction of the adaptive immune system in comparison with the innate immune system is more specific but also more inert. Antigen presenting cells (APCs; e.g., dendritic cells and macrophages) process the pathogens and present their antigens on the cell surface via major histocompatibility complex (MHC) molecules to trigger the response of the adaptive- or specific immune system (Roche and Furuta 2015; Wieczorek et al. 2017). T-cells are subdivided into $CD4^+$- and $CD8^+$ T-cells. $CD4^+$ T-cells activate granulocytes and macrophages and support the activation of B-cells. After the presentation of the antigen on the surface of the, e.g., dendritic cell via MHCII receptors, a specific receptor of the $CD4^+$ T-cell binds. Subsequently both the dendritic cell and the $CD4^+$ T-cell interact, for instance, by secretion of cytokines. Depending on the signaling cascade elicited, the $CD4^+$ T-cell proliferates and differentiates to either pro-inflammatory T-helper- or anti-inflammatory regulatory T-cells (Luckheeram et al. 2012). $CD8^+$ T-cells in turn are cytotoxic to tumor cells or virus infected cells and are activated by antigens presented via MHCI receptors (Kaech et al. 2002; Weninger et al. 2002; Dockree et al. 2017). Furthermore, B-cells are capable of antigen recognition. If the antigen is engulfed by a B-cell, it is presented on MHCII receptors at the cell surface. There, a compatible T-cell receptor (TCR) of a T-helper-cell binds to the presented antigen, which leads to the production of cytokines. Thereby, a B-cell is activated, proliferates, and differentiates to memory B-cells (similar to memory T-cells) and plasma cells. The plasma cell is then producing antibodies, which specifically bind to the antigen of the pathogen (Rodriguez-Pinto 2005; D'Souza and Bhattacharya 2019). Antibodies may now either attract macrophages by opsonization, directly annihilate the pathogen, or activate the complement system and demolish the pathogen by induction of the membrane attack complex (MAC) (Xie et al. 2020). Under certain conditions a class-switch to IgE antibodies is performed which can specifically bind to IgE receptors, Fcε receptors, on mast cells and basophils. Once an allergen binds to and crosslinks specific IgE antibodies, mast cells and basophils are activated to secrete a plethora of preformed pro-inflammatory mediators such as histamine (Evans and Thomson 1972). Histamine is a key effector molecule in allergic reactions and is secreted systemically during an anaphylactic shock (Lindstedt and Kovanen 2006; Bulfone-Paus et al. 2017). Mast cells are essentially present in all organs, especially in close proximity to blood vessels, neurons, and lymphatic vessels, which strategically enables them to distribute local inflammatory signals (Siebenhaar et al. 2018). Aside from IgE antibodies, mast cell activation can be triggered by many other stimuli, including Toll-Like Receptor (TLR) ligands, complement-derived peptides, and neuropeptides (Siebenhaar et al. 2018). Similar to other immune cells in mast cells, depending on the stimulus, diverse patterns of calcium (Ca^{2+}) mobilization and oscillation emerge, triggering subsequent distinct signaling pathways (Chen et al. 2017; Gaudenzio et al. 2016; Vennekens et al. 2007).

1.2 Ion Channels in Immune Cells

Ion channels do not only play an important role in the function of excitable cells, but also in that of non-excitable cells, such as immune cells. The general passage of ions through the plasma membrane and intracellular membranes such as those of mitochondria or endosomes is crucial for cell regulatory processes, immunological function, and cell homeostasis (Arlt et al. 2020; Nadolni and Zierler 2018; Cardoso et al. 2010; and more). This transport of cations (e.g., Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, H^{+}) and anions (e.g., Cl^{-}) is therefore facilitated by the specific conductance of ion channels. In excitable cells, distinct ion channels, for example, regulate the electrical signals of neurons or the contraction of myofibers. In non-excitable cells such as immune cells, however, the function of ion channels is less obvious and less understood. Only few studies have focused on this topic so far, albeit describing important regulatory functions of ion channels in immune cells (Cahalan and Chandy 2009; Feske et al. 2015, 2019; Vaeth and Feske 2018). Ion channels in immune cells regulate the membrane potential as well as intracellular signaling pathways. Members of the transient receptor potential (TRP) cation channel family, for example, are functionally widespread and strongly represented in immune cells. TRPM2 (melastatin subfamily), for instance, is predicted to be a potential target to treat autoimmune central nervous system (CNS) inflammations (Melzer et al. 2012) and the cation channel also plays an important role in macrophage function especially in context with inflammatory diseases (Zhong et al. 2013; Yamamoto et al. 2008). Moreover, TRPM2 limits fever reactions (Song et al. 2016). For TRPM4 in conjunction with potassium channels, Kv1.3 (voltage gated) and $IK_{Ca}3.1$ (Ca^{2+} activated), a regulatory role in the characteristic Ca^{2+} oscillations following T cell activation has been described (Beeton et al. 2001; Feske et al. 2015; Launay et al. 2004). Unfortunately, the availability of specific TRPM4 inhibitors is currently weak and needs further investigations. The kinase-linked TRPM7 cation channel is involved in the function of lymphocytes, mast cells, neutrophils, and macrophages and is therefore an important player in the regulation of the immune system homeostasis (Voringer et al. 2020; Romagnani et al. 2017; Nadolni and Zierler 2018). New TRPM7 kinase inhibitors might be crucial for the treatment of pro-inflammatory diseases, such as allergic hypersensitivity or tissue rejections and graft-versus-host reactions (Nadolni and Zierler 2018; Romagnani et al. 2017; Ryazanova et al. 2014; Mendu et al. 2020; Schappe et al. 2018). The TRPV2 (vanilloid subfamily) ion channel is functionally well established in the mammalian immune system and is also accessible for immunomodulation (Santoni et al. 2013). TRPV2, for example, was found in $CD34^{+}$ HSCs (stem- and progenitor cell cycle regulation via Ca^{2+}), in granulocytes, macrophages, monocytes (stimulation of, e.g., phagocytosis, tumor necrosis, or IgG secretion), and mast cells (protein kinase A-dependent degranulation via Ca^{2+}). Moreover TRPV2 plays an important role in T- and B-lymphocytes, regulating activation and proliferation processes via Ca^{2+} signaling (Santoni et al. 2013).

The L-type voltage-dependent Ca^{2+} channel family comprises three different ion channels that are present not only in excitable cells but also in non-excitable immune cells. $Ca_v1.2$, for example, is expressed in microglial cells. In addition, it has been

reported that blockage of this ion channel aggravates the course of Parkinson's disease (Wang et al. 2019). Deletion of $Ca_v1.3$ was tested in Davenport et al. (2015), in order to target compounds for anti-inflammation and immunomodulation, which are well described for excitable ion channels. Besides that, it was reported earlier that an antibody against $Ca_v1.3$ appears to block IgD-mediated Ca^{2+} responses of isolated rat B-cells (Sadighi Akha et al. 1996). Another L-type Ca^{2+} channel, $Ca_v1.4$, was revealed to play an important role in both, the homeostasis of naïve T-cells and the antigen-induced T cell immune response (Omilusik et al. 2011). The low-voltage-activated T-type calcium channel $Ca_v3.1$ was described in Wang et al. (2016) as a potential regulator for autoimmune responses in immune cells. The authors observed that $Ca_v3.1$-deficient mice were resistant to experimentally induced autoimmune encephalomyelitis (EAE) on the basis of infiltrating T-cells, reducing the production of granulocyte-macrophage colony-stimulating factors (GM-CSFs).

The P2X purinoceptor 7 (P2RX7) belongs to an ATP-gated ion channel family (Di Virgilio et al. 2017). Recently it was shown that P2RX7 antagonists have a negative effect on autoimmune therapy, especially in T follicular helper cells (Tfh)-related autoimmune disease (Felix et al. 2019). Another purinergic receptor for the functionality of immune cells is P2RX1. In Wang et al. (2020), it was suggested that P2RX1 is a potential therapeutic target for the treatment of disordered inflammation of acute pancreatitis. There, it was shown that P2RX1 was the most highly expressed purinergic ion channel in mouse and human pancreas tissue and that genetic ablation of P2RX1 eased the inflammation in caerulein-induced acute pancreatitis in mice. The Leucine-rich repeat containing 8A (LRRC8A) protein is an essential subunit of the volume-regulated anion channel (VRAC) that essentially controls cellular volume. It was reported before that LRRC8A is crucial for the development and functionality of T lymphocytes (Kumar et al. 2014). There, the protein kinase B (mediated) phosphorylation in the thymus of LRRC8A knockout mice was significantly reduced. Therefore, the authors suggested that the protein is of great importance for the development and survival of T-cells. However, it was shown in a follow-up study that LRRC8A-dependent VRAC activity is dispensable for T-cell development and function in specific reference to the VRAC ion channel (Platt et al. 2017).

1.3 Endolysosomal Ion Channels

Endolysosomes, which eponymously arise from the fusion of late endosomes and acidic lysosomes, have a broad functional spectrum from degradation and secretion over repair mechanisms to signaling and energy metabolism (Settembre et al. 2013). Endolysosomal membranes encompass numerous ion channels, such as TRPMLs (TRP mucolipin subfamily), TMEM175 (K^+ channel transmembrane protein 175), BK (voltage-gated big-potassium channel), P2X4 (purinergic receptor), some members of ClCs (chloride channels), the above-described TRPM2 and two-pore channel (TPC) proteins (Grimm et al. 2017; Plesch et al. 2018; Jinn et al. 2017; Cao

et al. 2015; Kanellopoulos et al. 2021; Jentsch and Pusch 2018; Lange et al. 2009; Weinert et al. 2020; and more). The endolysosomal ion channels of the TRPML subfamily are also part of the TRP superfamily (see above). In previous studies, the role of TRPML1 and its responsibility for modulating secretory lysosomes (Cao et al. 2017) and coordinating effector function in NK cells and dendritic cells were shown (Clement et al. 2020; Sun et al. 2022). In contrast to TRPML1, TRPML2 and TRPML3 are more likely to be found in early, endosomal recycling compartments, as these are less acidic and have an almost neutral pH (Plesch et al. 2018). TMEM175 is an endolysosomal potassium channel, which has important functions for the luminal pH stability and the maintenance of the lysosomal membrane potential. There is convincing evidence that this ion channel plays a decisive role in the course of Parkinson's disease and could therefore be a potential therapeutic target for this disease (Jinn et al. 2017).

Ca^{2+}-activated potassium channels (BKs), which are functionally related to the Ca^{2+}-releasing lysosomal channel TRPML1, were investigated in Cao et al. (2015). It was found that Ca^{2+} released via TRPML1 activates BKs. This in turn triggers further lysosomal Ca^{2+} release and facilitates membrane transport. As a result, BK overexpression can compensate for disturbed TRPML1-dependent Ca^{2+} release or disturbed lysosomal storage in cells of Niemann-Pick C1 patients or for other lysosomal storage diseases (Kiselyov et al. 2010).

The purinergic cation channel P2X4, an ATP-gated cation channel, is also mainly found in lysosomes. There, the channel is activated by means of intralysosomal ATP (in its tetraanionic form) when the pH increases above 7.4. It has been shown that P2X4 potentiates P2X7-dependent activation of inflammasomes, leading to an increased release of IL-1β and IL-18 (interleukins). The exact role of P2X4 in various diseases is not yet fully understood. However, in diseases where the activation of the $NLRP_3$ inflammasome (NLR family pyrin domain containing protein 3) is essential, P2X4 has been found to worsen disease progression (Kanellopoulos et al. 2021).

ClCs are manifold and occur either as channels or as Cl^-/H^+-exchangers (Jentsch and Pusch 2018). Within the ClC family, ClC-3 to ClC-7 act as Cl^-/H^+-exchanger in the membrane of endolysosomes. Loss or dysfunction of CLCs may result in severe neurodegenerative diseases (Bose et al. 2021).

Especially and aside from the above-mentioned endolysosomal ion channels, particular attention has been paid to TPCs and their role in immune regulation.

2 TPCs in Immune Cells

2.1 Physiologic Regulation of TPCs

Here, we focus on two-pore channels, in humans and rodents subdivided into TPC1 and TPC2. TPC3 is not expressed in primates or rodents (Brailoiu et al. 2010). The 3-D structure of a TPC ion channel was first observed in *Arabidopsis thaliana* (AtTPC1), using X-ray crystallography at a resolution level of 3.3 Å and 2.87 Å,

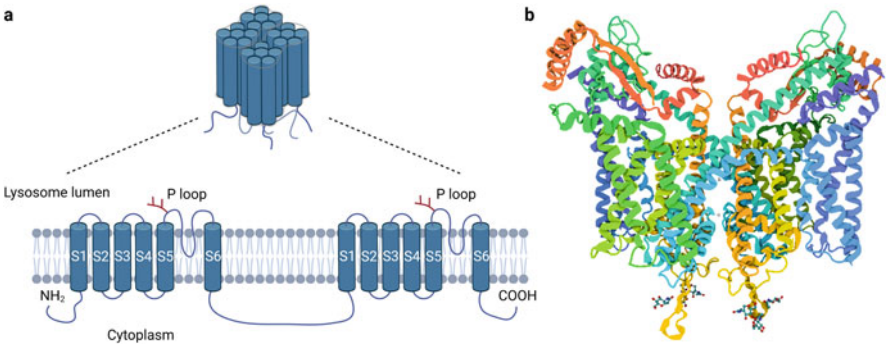

Fig. 1 Graphic illustration and protein architecture of TPC1. (**a**) Schematic visualization of TPC1, which functions as a (homo)dimer with each subunit including six transmembrane domain helices, a pore-forming region between the fifth and sixth segments (S5, S6) and a glycosylation site (red) on each P-loop. (**b**) Corresponding Cryo-EM architecture of TPC1 in *Mus musculus* in the apo state at a resolution of 3.5 Å. The PDB (protein data bank) code 6C96 was used to create the EM architecture. Created with BioRender.com

respectively (Guo et al. 2016; Kintzer and Stroud 2016). Each subunit of a TPC includes two homologous six transmembrane (6-TMs) domain helices, with the pore-forming region located between the fifth and the sixth transmembrane segments (Fig. 1a). At the sour luminal side, additional glycosylation sites (Fig. 1a, red) have been identified (Hooper et al. 2011). More recently, the 3-D structures of mouse TPC1 (MmTPC1; Fig. 1b) and human TPC2 (HsTPC2) were unveiled by the implementation of cryo-electron microscopic single-particle analysis (She et al. 2018, 2019; for futher information on structural details, please see specific book chapters dedicated to TPC1 and TPC2 structures).

Whether TPC1 and 2 can also form heterodimers is still a matter of debate (Rietdorf et al. 2011; Castonguay et al. 2017). Also, their ion conductivity leads to discussions among researchers. Convincing evidence shows that the direct or indirect activation of TPC1 and TPC2 via nicotinic acid adenine dinucleotide phosphate (NAADP) leads to a release of Ca^{2+} from endolysosomes (Brailoiu et al. 2009; Calcraft et al. 2009; Zong et al. 2009; Lin-Moshier et al. 2012; Walseth et al. 2012). A search for NAADP binding sites revealed that NAADP probably does not bind TPCs directly but more likely interacts with accessory proteins (Lin-Moshier et al. 2012; Walseth et al. 2012). More recently, potential candidates for such interacting partners, directly signaling via NAADP binding, have been identified as Sm-like (spliceosomal) protein LSm12 and Jupiter microtubule associated homolog 2 (JPT2) (Zhang et al. 2021; Gunaratne et al. 2021). Zhang and colleagues demonstrated that NAADP receptor proteins are primarily responsible for regulating Ca^{2+} homeostasis in acidic organelles (Zhang et al. 2021). Affinity purification and quantitative proteomic analysis of the interacting proteins of NAADP, TPC1, and TPC2 were carried out. LSm12 was found to be a matching interaction protein. LSm12 was initially identified as an RNA-binding protein (Lee et al. 2017; Fleischer et al. 2006). However, the investigations of Zhang et al. (2021) revealed that LSm12 both

functions as an NAADP receptor and also has a TPC-regulating function. Independently, an NAADP-based photoprobe was used to isolate NAADP-binding proteins from human erythrocytes (Gunaratne et al. 2021). JPT2 was identified as an essential protein for NAADP-based Ca^{2+} signaling.

Aside from NAADP, phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) has been suggested as a potential regulator of TPC channel activity. The activation of TPCs via $PI(3,5)P_2$ is thought to trigger sodium rather than Ca^{2+} currents (She et al. 2018; Gerndt et al. 2020; Wang et al. 2012; Boccaccio et al. 2014).

2.2 TPCs as Pharmacological Targets

The described versatility of TPCs in pathophysiological questions makes these ion channels interesting pharmacological targets. Only recently, two agonists of TPC2, named TPC2-A1-N and TPC2-A1-P, were discovered after monitoring the Ca^{2+} response from TPC2 (Gerndt et al. 2020). There, it was demonstrated that TPC2-A1-P caused Na^+ selective currents and only low Ca^{2+} signals while TPC2-A1-N evoked high Ca^{2+} signals and nonselective cation currents. Thus, it is tempting to speculate that TPC2-A1-N is mimicking the Ca^{2+} mobilizing second messenger NAADP while TPC2-A1-P simulates $PI(3,5)P_2$ activation. Furthermore, the binding sites of the two TPC2-agonists were different and not traceable in TPC1 or TRPML (Gerndt et al. 2020). This indicates that TPC2-A1-P and TPC2-A1-N are highly specific TPC2 agonists and promising future pharmacologic tools. Another promising modulator of TPCs is Ned-19, a selective antagonist of the Ca^{2+} mobilizing second messenger NAADP, which was first detected by a ligand-based virtual screen (LBVS) that was aimed against NAADP (Naylor et al. 2009).

In Calcraft et al. (2009), TPCs first have been shown to comprise a family of NAADP receptors, which are expressed in endolysosomal membranes. In particular, TPC2 supports the NAADP-induced Ca^{2+} release from lysosomes, which is additionally reinforced by inositol 1,4,5-triphosphate receptors (IP_3R). If IP_3Rs are blocked or if the ER Ca^{2+} stores are emptied by thapsigargin, a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), it weakens the above-mentioned reactions to NAADP. As mentioned above, JPT2 was identified as an essential protein for NAADP-TPC-based Ca^{2+} signaling (Gunaratne et al. 2021). In addition, JPT2 was responsible for the translocation of a SARS-CoV-2 pseudovirus through the endolysosomal system. The authors therefore postulated that JPT2 not only belongs to the NAADP receptor complex and thus mediates Ca^{2+} signaling via TPCs, but also has a decisive role in the inception of SARS-CoV-2 viruses. Another synonym for JPT2 is HN1L (hematological and neurological expressed 1-like protein). Under this alias, it was discovered by Roggenkamp et al. (2021) that deletion of the protein suppresses the development of Ca^{2+} microdomains in Jurkat- and primary rat T-cells and consequently reduces global Ca^{2+} signal transmission. This is in line with the results obtained in Gunaratne et al. (2021). Therefore, it seems likely that NAADP does not bind to TPCs directly, but indirectly via certain adaptors in a protein complex (see Fig. 2).

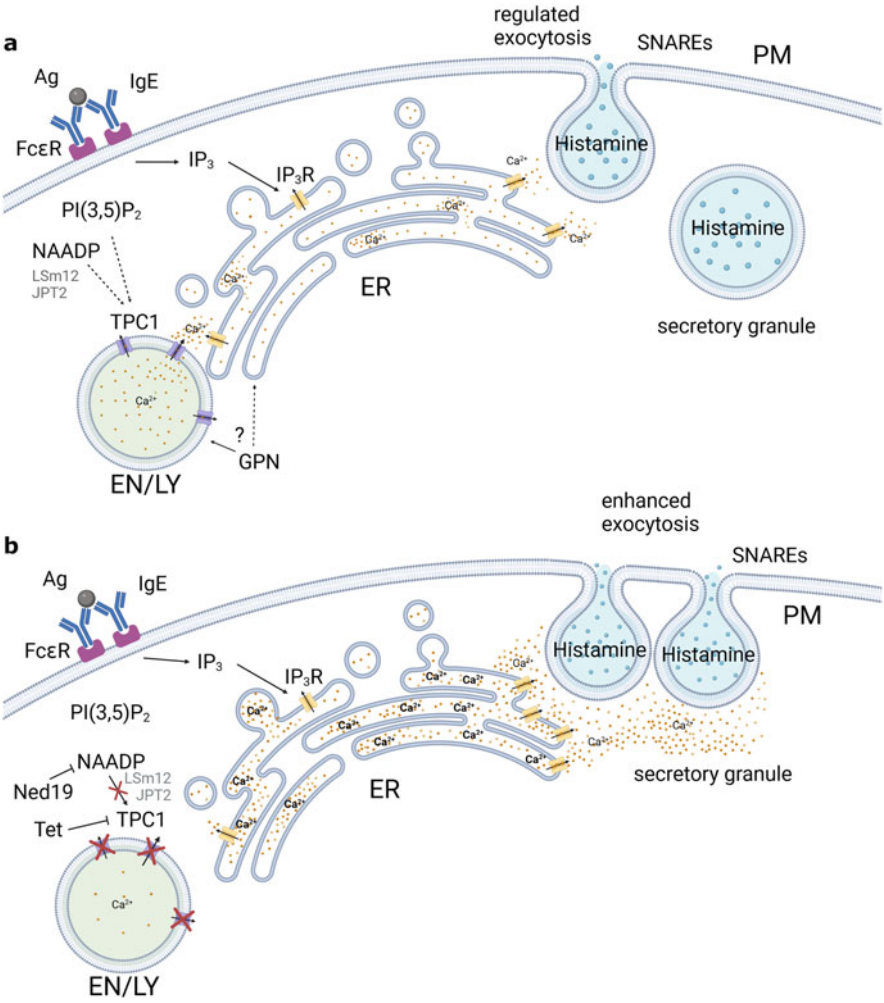

Fig. 2 Graphic model of the regulation of Ca^{2+} homeostasis by TPC1 in mast cells. (**a**) The stimulation of FcεR by IgE crosslinking leads to the activation of phospholipase C and the formation of IP_3. Upon IP_3 binding, the IP_3R in the ER membrane opens and releases Ca^{2+} from the ER. Endolysosomal Ca^{2+} release regulated by TPC1 triggers the release of Ca^{2+} from the ER, which leads to a re-uptake of Ca^{2+} into the endolysosome. In mast cells, the tightly regulated Ca^{2+} release from the ER results in a controlled exocytosis of histamine. $PI(3,5)P_2$ and NAADP (via LSm12 and/or JPT2) are both potential regulators of TPC1 channel activity. (**b**) If TPC1 is blocked by tetrandrine or Ned-19 (indirectly via NAADP), Ca^{2+} accumulates within the ER and an augmented ER-Ca^{2+} release occurs upon IP_3R stimulation. This results in an enhanced exocytotic release of histamine from mast cells. The functional role of GPN on ER and/or endolysosomes is not yet fully clarified. PM: plasma membrane; FcεR: high affinity receptor of the Fc region of IgE; IgE: Immunoglobulin E; EN/LY: endolysosome; ER: endoplasmic reticulum; IP_3R: inositol 1,4,5-triphosphate receptor; SNARE: soluble NSF attachment protein receptor. NAADP: nicotinic acid adenine dinucleotide phosphate; LSm12: Sm-like (spliceosomal) protein 12; JPT2: Jupiter microtubule associated homolog 2; $PI(3,5)P_2$: phosphatidylinositol 3,5-bisphosphate. Created with BioRender.com

Recently it was also reported that the dipeptide glycyl-I-phenylalanine 2-naphtylamide (GPN) releases Ca^{2+} from the ER, but not from lysosomes (Atakpa et al. 2019). Normally, when GPN is cleaved by the lysosomal enzyme cathepsin C, the lysosomal membrane should be ruptured (Berg et al. 1994). However, in the latter study it was shown that GPN caused a sustained increase in lysosomal pH, a brief increase in cytosolic pH, and a brief increase in cytosolic Ca^{2+} concentration without rupturing the lysosome and without cathepsin C playing a role. It was further postulated that increasing the cytosolic pH increases the release of Ca^{2+} from the ER. Accordingly, the authors concluded that Ca^{2+} release from the ER is not exclusive due to IP_3- and ryanodine receptors, but that a cytosolic pH increase due to GPN has the same effect. Furthermore, based on these results, it was assumed that GPN is not exclusively selective for lysosomes. In another study (Yuan et al. 2021) it was shown that GPN triggered a rapid increase in the cytosolic pH, but only resulted in a slow Ca^{2+} signal. The results were compared with NH_4Cl and the V-type ATPase inhibitor bafilomycin A1. The pH increase triggered by NH_4Cl was even more pronounced compared with GPN. However, NH_4Cl did not change the Ca^{2+} signal. Bafilomycin A1 increased the lysosomal pH and also increased the Ca^{2+} signal. However, the effect on Ca^{2+} was also selectively inhibited with chronic use. It was also found that GPN blocks Ca^{2+} reactions that were triggered by the NAADP-like agonist TPC2-A1-N. It was therefore assumed that GPN-evoked Ca^{2+} signals are more likely to be related to pH increase in the lysosome than in the cytosol, as these are presumably dependent on the lysosomal Ca^{2+} release. Accordingly, the conclusion that the Ca^{2+} signals triggered by GPN are more likely to come from acidic organelles is plausible. The role of GPN in lysosomes has also been extensively discussed by Morgan et al. (2020). There, the authors reflected on the study of Atakpa et al. (2019) and made comparisons with previous literature relating to the topic (Jadot et al. 1984; Kilpatrick et al. 2016; Thurston et al. 2012; and more). In this context, two fundamental questions remained unanswered for the authors: Is Ca^{2+} released from the ER after Ca^{2+} has been released from the lysosomes? And can the release of Ca^{2+} from the ER be triggered by cytosolic alkalinization? In summary, Morgan et al. (2020) considered the results of Atakpa et al. (2019) on the function of GPN in connection with lysosomal Ca^{2+} as an important addition to the previous understanding of the functional interplay between ER and lysosomes. However, there are currently no adequate tools and methods to fully explain the function of GPN.

3 Two-Pore Channels in Immune Regulation

3.1 Function of TPCs in Innate Immune Cells

In macrophages isolated from mice, TPC2 activation by means of $PI(3,5)P_2$ leads to an increase in lysosomal exocytosis. Activation by NAADP, however, reduces lysosomal exocytosis due to a change in vesicular pH (Gerndt et al. 2020). This contrasts previously published results obtained with cytotoxic $CD8^+$ T-cells (CTLs)

reviewed below (Davis et al. 2012). Therefore, the effect of TPC2 activation in distinct immune cell types needs further clarification.

In murine mast cells TPC1, but not TPC2, has been described as a potential regulator to keep intracellular Ca^{2+} levels in equilibrium (Arlt et al. 2020). Due to the IgE antibody-mediated activation of Fcε receptors, IP_3Rs are activated, which release Ca^{2+} from the ER. The resulting increase in cytosolic free Ca^{2+} concentration triggers exocytosis of secretory granules. It was presumed that TPC1 is mainly located at the contact sites between the endolysosomes and the ER to facilitate the uptake of Ca^{2+} from the ER (Arlt et al. 2020). These contact sites have been described before (Kilpatrick et al. 2017). Nevertheless, an exact identification or classification of these contact sites could not be made until now. It is assumed that these contact sites are essential to adjust the Ca^{2+} concentration in the ER. If TPC1 is absent or blocked, the Ca^{2+} concentration within the ER is augmented. Consequently, the Ca^{2+} release upon IP_3R activation is enhanced, which ultimately results in an increased release of histamine (Arlt et al. 2020). A similar concept has already been discussed previously and also states that the interaction between ER and endolysosomal compartments has an important influence on intracellular Ca^{2+} buffering and signaling (Patel and Docampo 2010). A broad overview of local and global Ca^{2+} signaling using TPCs has been summarized in Zhu et al. (2010).

A recently published study focused on allergic and anaphylactic reactions, for which the incidence has increased enormously in the last few decades (Arlt et al. 2020). In particular, mast cells and basophil granulocytes should be mentioned here as effector cells that release histamine and thereby promote allergic reactions. The study bridges the gap between TPC1 on the basis of systemic anaphylaxis in vivo and the mast cell function, ex vivo. The experiments were implemented using mice with TPC1 deficiency, which showed increased systemic anaphylaxis (e.g., lower body temperature after IgE stimulation compared to wild-type controls). A pharmacological inhibition or a genetic knockout of TPC1 increased the release of histamine in primary mast cells due to an increased release of Ca^{2+}. Figure 2 schematically shows the importance of TPC1 with regard to organellar Ca^{2+} homeostasis and IgE-induced exocytosis in a mast cell. It is assumed that TPCs can regulate the Ca^{2+} buffering capacity of endolysosomes. If FcεR is stimulated by means of IgE crosslinking, phospholipase C activation occurs and, consequently, the second messenger IP_3 is formed. Subsequently, IP_3R within the ER membrane is opened, which triggers the release of Ca^{2+} from the ER. If the ER and endolysosome are in close proximity, a feed-forward-loop occurs, involving TPC1 activity. The Ca^{2+} from the endolysosome stimulates the release of Ca^{2+} from the ER and this in turn causes a renewed uptake of Ca^{2+} into the endolysosome (Fig. 2a). Arlt et al. (2020) additionally suspected that there are more contact sites between the ER and the endolysosome if TPC1 is active. If TPC1 is absent (e.g., knockout) or if the channel is blocked, e.g., by tetrandrine or trans-NED-19 (via NAADP), there is an increased outflow of Ca^{2+} from the ER, which would otherwise be balanced by the endolysosome via TPC1 feed-forward activity. Ultimately, this leads to increased exocytosis due to the Ca^{2+} imbalance (Fig. 2b). Based on the results of Arlt et al. (2020), it is alluring to postulate that TPC1 could be a potential drug target against

allergic hypersensitivity. The work of Arlt et al. (2020) was recently discussed in another work by Patel and Malmberg (2020). Above all, the question of how TPC1 actually controls the Ca^{2+} content remained unanswered. The authors referred to an earlier work with a TPC1 knockdown in SKBR3 cells (Brailoiu et al. 2009). There, however, the Ca^{2+} release from the ER via IP_3 remained unchanged. The authors (Patel and Malmberg 2020) therefore suspected that the effect of the TPC1 knockout could be cell-type specific.

3.2 Function of TPCs in Adaptive Immune Cells

In $CD8^+$ cytotoxic T lymphocytes (CTL) TPC activation triggers the release of perforin and granzyme B from the cytolytic granules into the extracellular space. Additionally, it was shown that TPC1 and TPC2 were also present within the immunological synapse, which is formed between CTLs and corresponding target cells (Davis et al. 2012). However, it has not yet been investigated whether TPC activation could enhance CTL-mediated antitumor responses or whether it could be exploited for the development of antiviral therapies. CTLs are essential effector cells in the fight against cancerous cell growth as well as viral infections. On the other hand, TPCs have been shown to regulate the uptake of certain viruses, including the Ebola-virus, into a host cell, with TPC inhibition having beneficial effects (Gunaratne et al. 2018; Ou et al. 2021; Grimm and Tang 2020). Some of the proposed antiviral therapies, therefore, suggest inhibiting TPCs (Sakurai et al. 2015; Gunaratne et al. 2018; Castonguay et al. 2017). Most recently, it was reported that TPC2 is also responsible for the virus entry of SARS-CoV-2 into HEK 293-cells stably expressing hACE2 (HEK 293/hACE2) and that the inhibition of TPC2 via the plant alkaloid and TPC2 blocker tetrandrine weakens the entry of SARS-CoV-2 (Grimm and Tang 2020; Ou et al. 2021). Further research will be necessary to clarify the potential of TPCs as pharmacological targets for antiviral therapies.

Another interesting aspect is the role of TPCs in $CD4^+$ T-cells, which is not yet fully understood. It has already been shown that no significant effect on the release of endolysosomal Ca^{2+} by NAADP was observed in human $CD4^+$ Jurkat T-cells (Dammermann and Guse 2005). However, the function of TPCs in terms of Ca^{2+} regulation and signaling over time still has to be evaluated. The importance of long-lasting Ca^{2+} signals for T-cell activation is well established (Gwack et al. 2008; Kaufmann et al. 2016; Vaeth et al. 2016; Weidinger et al. 2013). It is therefore conceivable that TPCs are involved in the regulation of Ca^{2+} signals in $CD4^+$ T-cells as well. Like in the process described in mast cells (see above; Arlt et al. 2020), TPCs could represent gate openers to regulate Ca^{2+} shifts between ER and endolysosomal compartments, resulting in an effective buffer system to control cytosolic Ca^{2+}. Taken together, future studies will be necessary to fully understand the functional role of TPCs in lymphocytes.

3.3 Modulation of TPCs in Immune Cell Signaling

Also in the crosstalk between innate and adaptive immunity, TPCs seem to play a role. He et al. (2020) recently demonstrated that inhibition of TPCs in APCs promotes the expansion of tumor necrosis factor receptor 2 (TNFR2)-expressing $CD4^+Foxp3^+$ regulatory T-cells (T_{regs}). Pharmacologic inhibition of TPCs via tetrandrine increased the expression of transmembrane TNF in APCs, which in turn resulted in an increased number of T_{regs}. Since T_{regs} can regulate autoimmune reactions, this has been suggested as an important therapeutic approach. In their study, they showed that the increased number of T_{regs} led to a diminished colon inflammation in a mouse colitis model.

In Müller et al. (2021b), the role of TPC1 and TPC2 in epidermal growth factor receptor (EGFR) uptake and degradation (and/or recycling) in different TPC knockout cells was investigated. It was shown that an increased presence of activated EGFRs in the endolysosomal system due to the genetic inactivation of TPCs affects EGFR signaling and EGFR de novo synthesis. Furthermore, it was shown that increased basal phospho-c-Jun levels contributed to high EGFR expression. It was reported before that the MAP kinase-activated transcription factor c-Jun regulates EGFR transcription (Fang et al. 2014). An important role of TPCs in EGFR transport regulation, signaling, and expression is therefore very likely.

Grossmann et al. (2021) recently summarized the effects of a genetic TPC deletion on endocytosis, the processing of various surface receptors and the uptake and effect of bacteria and viruses. Furthermore, the review focused even more on the effects of TPC inactivation on receptor expression and receptor signaling. A detailed RNA sequence analysis using TPC1-knockout fibroblasts was used to identify numerous changes in the expression levels of surface receptors and signaling proteins. Different classes of receptors such as TGF, EGF, insulin, and other proteins important for endocytosis were highlighted in the review.

In a recently published study (Müller et al. 2021a) it was shown that a knockout of TPC2 reduces the proliferation of cancer cells and their energy metabolism in vitro. It was also observed that in vivo tumor growth was inhibited in the case of TPC2 knockouts. The study also focused on the alkaloid tetrandrine, which is already known as a TPC inhibitor (Arlt et al. 2020; Sakurai et al. 2015). Müller et al. (2021a), however, developed analogs of tetrandrine. These were classified as more potent, less toxic, and more suitable for tumor therapy. The authors concluded that TPC2 plays an important role in cancer therapy and that tetrandrine analogs are pharmacologically relevant for appropriate treatment.

Recently endolysosomes were investigated in the context of phagocytosis and a novel signaling pathway was suggested (Davis et al. 2020). The focus was on the idea that FcγR activation mobilizes NAADP and consequently opens TPCs. The Ca^{2+} released by the TPC then activates calcineurin and dephosphorylates it. The result is an activation of the GTPase dynamin-2, which is essential for phagocytosis. The authors pointed out that it is not only global Ca^{2+} signaling, but much more local endolysosomal nanodomains that are of significant relevance for phagocytosis.

Similarly, in the above-mentioned study on mast cells, FcεR stimulation may cause NAADP-dependent TPC1 activation, resulting in local Ca^{2+} release and a feed-forward IP_3R-mediated global Ca^{2+} signaling, leading to exocytosis. Here, the lack of the inter-organellar Ca^{2+} exchange and local release contributes to the enhanced exocytotic phenotype observed in TPC1 knockout mast cells (see Fig. 2).

Taken together, it seems likely that TPC1 and TPC2 are nonredundant endolysosomal ion channels that might signal differently in distinct cell types, depending on where they are located or which accessory proteins are expressed. The function of TPCs in immune cells has only recently been reviewed in terms of regulation of inter-organellar Ca^{2+} homeostasis (Steiner et al. 2022).

4 Outlook

In recent years, numerous studies have contributed to the fact that the physiological function of TPCs is better understood and that related pathologies could be treated more specifically (Arlt et al. 2020; Castonguay et al. 2017; Zhu et al. 2010; and more). Nevertheless, there are still gaps that need to be filled in order to better understand the physiological and pathophysiological role of TPCs in immune function. For example, the molecular activity of certain tools, such as GPN, must first be better understood in order to draw final conclusions about TPCs (Morgan et al. 2020; Atakpa et al. 2019). Furthermore, the question of the cell specificity of TPC1 has not yet been fully clarified (Patel and Malmberg 2020). There are still open questions regarding the expression and function of TPCs in distinct immune cells. For instance, the precise role of TPC1 and TPC2 in primary $CD4^+$ (T-helper) and $CD8^+$ (CTL) T lymphocytes remains to be established. Moreover, the exact localization of TPCs in distinct intracellular vesicles, such as granula in mast cells and granulocytes or lysosome-related vesicles in NK cells or CTLs, needs to be clarified.

It has already been mentioned that a genetic modification or a pharmacologic inhibition of TPC1 triggered an increased anaphylactic immune response in mice (Arlt et al. 2020). In the latter study, as well as in many other studies, there is, however, a lack of ultrastructural basis that underlies these processes. There is great potential for ultrastructural, analytical, electron microscopic (EM) methods to shed light on the ultrastructural darkness. Contact areas between ER and endolysosomal compartments were previously analyzed with 2D EM methods in regard to their Ca^{2+} exchange potential (Kilpatrick et al. 2017). For further investigations, however, a 3-dimensional spectrum of EM methods is indispensable in order to understand the actual scope of the interorganellar interaction areas. In addition to the visual representation of EM methods (2D & 3D), there are also numerous analytical EM methods for the detection of elements (e.g., energy dispersive X-ray analysis, electron energy loss spectroscopy), as well as the immunocytochemical marking of interacting molecules identifying signaling complexes, that need to be considered for future questions. We suggest that the combination of modern EM methods with physiological, molecular biological and immunological techniques could further

elucidate whether TPCs are indeed promising pharmacological targets for, e.g., the treatment of allergic hypersensitivity.

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