TRPM2 channel and inflammation

Héctor Vivanco-Cid[1], Gabriela Mellado-Sánchez[1], Adriana Sumoza-Toledo[1, *].

1. Laboratorio Multidisciplinario de Ciencias Biomédicas. Instituto de Investigaciones Medico-Biológicas. Universidad Veracruzana campus Veracruz. Iturbide S/N. CP. 91700. Veracruz, Ver., México.

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Address correspondence to Dr. Adriana Sumoza-Toledo, asumoza@uv.mx, Laboratorio Multidisciplinario de Ciencias Biomédicas. Instituto de Investigaciones Medico-Biológicas. Universidad Veracruzana campus Veracruz. Iturbide S/N. Col. Centro CP. 91700. Veracruz, Ver., Mexico.

ABSTRACT

Inflammation is an early mechanism of the immune system to eliminate pathogens and to repair damaged tissue. However, unregulated and persistent inflammation can lead to chronic inflammatory diseases. The transient receptor potential melastatin-2 (TRPM2) channel, a calcium (Ca^{2+})-permeable channel containing intracellular adenosine diphosphate ribose (ADPR) pyrohydrolase activity, has recently been identified as a critical molecular mechanism in reactive oxygen species (ROS)-induced inflammatory process, and thereby, emerged as a potential target for therapeutic intervention. ADPR biding to TRPM2 Nudix-like domain (or NUDT9 homology domain) results in the channel pore opening allowing lysosomal Ca^{2+} release and Ca^{2+} influx into the cells. Ca^{2+} influx via TRPM2 controls ROS-induced nuclear translocation of NF-κB and CXCL2 production in monocytes during inflammation in a chronic experimental colitis mouse model. Moreover, TRPM2 deficient-dendritic cells show defective Ca^{2+} signals and chemotaxis toward CXC12 and CCL21 chemokines. $TRPM2^{-/-}$ mice are also more susceptible to *Listeria monocytogenes* infection. In contrast, TRPM2 is not required for airway inflammation in OVA-induced allergic asthma and in chronic obstructive pulmonary disease in mice. Consequently, TRPM2 appears to play a role in chronic inflammation and might not participate in acute inflammatory responses.

INTRODUCTION

Inflammation is both a mechanism of host defense and a repair mechanism of damaged tissue, generally categorized in acute and chronic inflammation. The acute inflammation is an early mechanism during antigen containment by immune cells and during the reparation of damaged tissue (1). It is characterized by the activation of resident cells (mast cells, macrophages, dendritic cells (DCs), endothelial cells) and by the infiltration of polimorphonuclear neutrophils (PMN) into the inflamed tissue (2). Acute inflammation is also characterized by high levels of innate immune cytokines and chemokines, such as TNF-α, IL-1, IL-6, IL-12 and IL-8, G-CSF, and GM-CSF, respectively (3-5). In contrast, chronic inflammation is a persistent inflammatory response characterized by infiltration of mononuclear cells (macrophages, natural killer (NK) cells, and lymphocytes) and granulocytes (eosinophils and mast cells), being the macrophages and lymphocytes the major components in the infiltrate. The main pro-inflammatory molecules present in chronic inflammatory responses are the reactive oxygen species (ROS), reactive nitrogen species (RNS), IL-2, IL-4, IL-5, IL-7, IL-13, IL-9, IL-10, IL-12, IL-17, IL-21 IFN-γ, TGF-β, and TNF-β (6). Interestingly, some cytokines, such as TNF-α, IL-1 and IL-6 can significantly contribute to both acute and chronic inflammation. Chronic inflammatory conditions can lead to local and systemic alterations; such as, progressive destruction of tissues, tissue remodeling, fibrosis, systemic inflammation, multi-organ failure, cancer development and death (7).

The main signaling pathways involved in both acute and chronic inflammation include the mitogen activated protein kinase (MAPK), nuclear factor kappa B (NF-κB), Janus tyrosine kinase-signal transducer and activator of transcription (JAK/STAT), and calcineurin/nuclear factor of activated T cells (NFAT). MAPKs are involved in signal transduction of different cytokines, response to microbial components, interferons (IFN), colony stimulating factors (CSF), hormones and environmental stress signals (8). However, NF-κB has been found to be the most important regulator for both inflammation responses. The NF-κB family consists of five members: REL-a (p65), NF-κB1 (p50; p105), NF-κB2 (p52; p100), c-REL and REL-b24. The heterodimer of p50 and p65 is the most common activating form in inflammatory responses (9). NF-κB is activated by a diverse set of stimuli including pro-inflammatory cytokines (TNFα, IL-1 β, IL-6), Toll-like receptors (TLRs) ligands (pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)) and ROS (10, 11). In addition, the JAK-STAT pathway also control inflammation by regulating the production of many cytokines including TNF-α, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, and IL-15. STATs proteins have numerous functions in innate immunity cells such as neutrophils, DCs and macrophages, and therefore control acute inflammatory responses (12). Moreover, NFAT signaling pathway is another important regulator of pro-inflammatory cytokines. In T cells, the downstream target genes of NFAT include IL-2, which regulates development, activation and expansion of T cells. These signaling pathways also control, together with other transcription factors such as T-bet, GATA-3, ROR-γ and FoxP3, the polarization of T cells

toward specific T helper profiles such as Th1, Th2, Th17 and T regs. Recently, studies have shown the role of NFAT signaling in the innate immunity and in the inflammatory responses of granulocytes and DCs (13).

Calcium (Ca^{2+}) release from intracellular stores and Ca^{2+} entry via plasma membrane Ca^{2+} channels are essential mechanisms for the activation and function of immune cells. Therefore, they may regulate the immune response, including inflammation. The transient potential receptor (TRP) melastatin-2 (TRPM2; previously known as LTRPC2 or TRPC7) is a Ca^{2+}-permeable channel containing an adenosine diphosphate ribose (ADPR) pyrophosphatase enzymatic domain (Figure 1) (14). This property of TRPM2 to function as a channel and as enzyme classifies it as “chanzyme”. Only two other channels, TRPM6 and TRPM7, also members of the TRP superfamily, melastatin subfamily, share this dual function although they present kinase activities (15). Recent studies in TRPM2 channel have shown a regulatory role of this protein in migration and cytokine production in monocytes, neutrophils and DCs (14, 15). TRPM2 has also recently been identified as a critical molecular mechanism in reactive oxygen species (ROS)-induced inflammatory process. Nevertheless, the physiological role of TRPM2 is not currently well understood. In this review we discuss the recent findings that suggest a differential role of TRPM2 in acute inflammation and chronic inflammation.

TRPM2 channel

TRPM2 is a TRP-related protein of approximately 170 KDa, expressed in the plasma membrane and lysosomal membrane of several cells, including immune

cells (14, 15). The N-terminus, which is oriented towards the cytoplasm, comprises four melastatin homologous domains (MDH) and a calmodulin (CaM)-biding IQ-like motif. TRPM2 also contains six transmembrane segments (S1-S6), with a loop domain located between S5 and S6, which is involved in the formation of the channel pore (14). Meanwhile, cytoplasmic-oriented TRPM2 C-terminus consist of a TRP box, a coil-coil domain, which has been suggested to be critical for TRPM2 homo-tetrameric assembly, and a Nudix-like domain (or NUDT9 homology domain), which binds with high specificity and hydrolases ADPR to ribose 5-phosphate and adenosine monophosphate (AMP) (Figure 1) (14).

To form a non-selective cation ion channel, TRPM2 proteins typically assemble into homo-tetramers. Binding of ADPR to TRPM2 Nudix-like domain, in synergy with Ca^{2+}, opens TRPM2 ion channel pore allowing the permeation mainly of sodium (Na^{+}) and Ca^{2+}, as well as potassium (K^{+}) and cesium (Cs^{+}) into the cell (Figure 1) (14). In addition to Ca^{2+}, cyclic ADPR (cADPR), hydrogen peroxide (H_2O_2) and nicotinic acid adenine dinucleotide phosphate (NAADP) may directly or indirectly facilitate TRPM2 gating by ADPR. In contrast, AMP and protons regulate it negatively (14).

Recently it has also been suggested that expression of TRPM2 isoforms might regulates its cellular function. Thus, TRPM2 ΔC, which lacks 34 residues (T1292–L1325) in the C-terminus, fails to respond to ADPR but can still be activated by H_2O_2 (16). Other isoform, striatum short protein (SSF)-TRPM2, which consist of TRPM2 transmembrane segments and the C-terminus, also

retains H_2O_2-induced activity (17). In contrast, TRPM2 ΔN, which is missing 20 residues (K538–Q557) in the N-terminus, does not respond to H_2O_2 (16). In addition, a short version of TRPM2 (TRPM2-S), consisting in only the N-terminus and S1-S2, may function as dominant negative inhibitor of TRPM2 activity (18). Interestingly, variants of TRPM2 (melanoma-enriched antisense TRPM2 transcript and a tumor-enriched TRPM2 transcript) have been detected in tumor cells (19).

TRPM2 signaling in immune cells

Expression of TRPM2 has been described so far in the plasma membrane of neutrophils, T cells and monocytes, and in lysosomes of DCs (14, 15). On the other hand immune cells may produce ADPR, which is considered the main activating molecule of TRPM2, via CD38 and CD157 and by activation of poly(ADPR)-polymerase/poly(ADP-ribose) glycohydrolase (PARP/PARG) pathway during DNA repair, replication, and transcription (15). However, the mechanisms that link these pathways to TRPM2 are still unclear. The ectoenzymes CD38 and CD157 uses β-NAD^+ as a substrate to catalyze the production of ADPR, cADPR, and NAADP; however, these metabolites are produced extracellularly and it is still not known how they might cross the plasma membrane and acts on the cytosolic Nudix domain of TRPM2 (15). Interestingly, CD38 knock-out neutrophils shows defects in chemotaxis linked to an impaired Ca^{2+} entry, similarly to $TRPM2^{-/-}$ neutrophils both stimulated with fMLP, a bacterial peptide (20).

Phagocytes also produce H_2O_2 during the respiratory burst. The external stimulation of human U937 monocyte cell line with H_2O_2 causes activation of TRPM2 in their plasma membrane and consequently Ca^{2+} entry into the cell, contributing to Ca^{2+}-dependent tyrosine PYK2 activation, amplification of ERK signaling via Ras GTPase, translocation of NFκB and production of CXCL8 cytokine (20). These findings were also confirmed in $TRPM2^{-/-}$ mouse monocytes, which were unable to mobilize extracellular Ca^{2+} in respond to H_2O_2 and to produce CXCL2 cytokine, a mouse CXCL8 homolog (20). The production of other cytokines such as IL-1β, CCL-1, CCL-2, CCL-3, CCL-4, CCL-21, and CXCL12 were not affected in the absence of TRPM2 (20). Furthermore, $TRPM2^{-/-}$ neutrophils show impaired *in vitro* chemotaxis toward fMLP but migrate normal toward CXCL2, CCL-1, CCL-3, and CCL5. $TRPM2^{-/-}$ monocytes also migrate normal toward CCL-1, CCL-2 and CCL-5 (20). Interestingly, DCs express TRPM2 only in lysosomes (21). In DCs, lysosomal TRPM2 function as a Ca^{2+} release channel, although it also indirectly regulates Ca^{2+} entry when immature and mature DCs are stimulated with chemokines CXCL12 and CCL19 (21). Immature and mature $TRPM2^{-/-}$ DC shows also impaired chemotaxis towards CXCL12 and CCL19, respectively. Furthermore, a lower percentage of the population of DCs from $TRPM2^{-/-}$ mice expresses costimulatory molecules, such as CD80, CD86, MHC-II and CD83 upon cellular activation with TNF-α and CpG (21). However, the signaling pathways involved are unknown.

Role of TRPM2 in acute and chronic inflammation processes.

TRPM2 channel has been associated to different pathological conditions, including bipolar disorder, diabetes, chronic colitis, oxidative stress, ischemia and cancer (14). However, the molecular mechanisms that lead to the activation of TRPM2 and therefore development of these diseases are widely unknown. Yamamoto et al, using a dextran sulfate sodium (DSS)-induced colitis inflammation model, demonstrated that TRPM2 controls the severity of inflammation and ulceration of the colon by affecting the production of IFN-γ, IL-12, CXCL2 and subsequent infiltration of neutrophils (20). Interestingly, $TRPM2^{-/-}$ mice are particularly susceptible to infection to Listeria monocytogenes due an impaired production of IFN-γ and IL-12 (22). Furthermore, in $TRPM2^{-/-}$ mouse model of sciatic nerve injury-induced neuropathic pain, the neutrophil infiltration was diminished as consequent of a reduction in CXCL2 chemokine production (23). These findings are indicative of a role for TRPM2 in regulating IFN-γ, IL-12, CXCL2 expression. TRPM2 also plays a protective role in lipopolysaccharide (LPS)-induced lung inflammation mouse model by preventing ROS production in neutrophils (24). Contrariwise, recent studies in chronic obstructive pulmonary disease in mice exposed to ozone, LPS and tobacco smoke (25) and OVA-induced mouse allergic asthma showed no role for TRPM2 in airway inflammation (26). These findings are highly suggestive of a role for TRPM2 in chronic inflammation but not in acute inflammation, probably by regulating the production of cytokines.

Closing remarks

TRPM2 channel is a versatile channel, which can regulate Ca^{2+} entry and Ca^{2+} release. It is widely expressed in the cells, including immune cells, in which the regulation of intracellular Ca^{2+} is very important for activation, migration and function. Although the recent findings have advance the understanding of the role of this protein in the context of the immune system, future work will focus in signaling pathways involved in the activation of TRPM2 in immune cells and to distinguish a differential role of TRPM2 in chronic inflammation and acute inflammation.

Competing interest statement

The authors have no conflicting financial interests.

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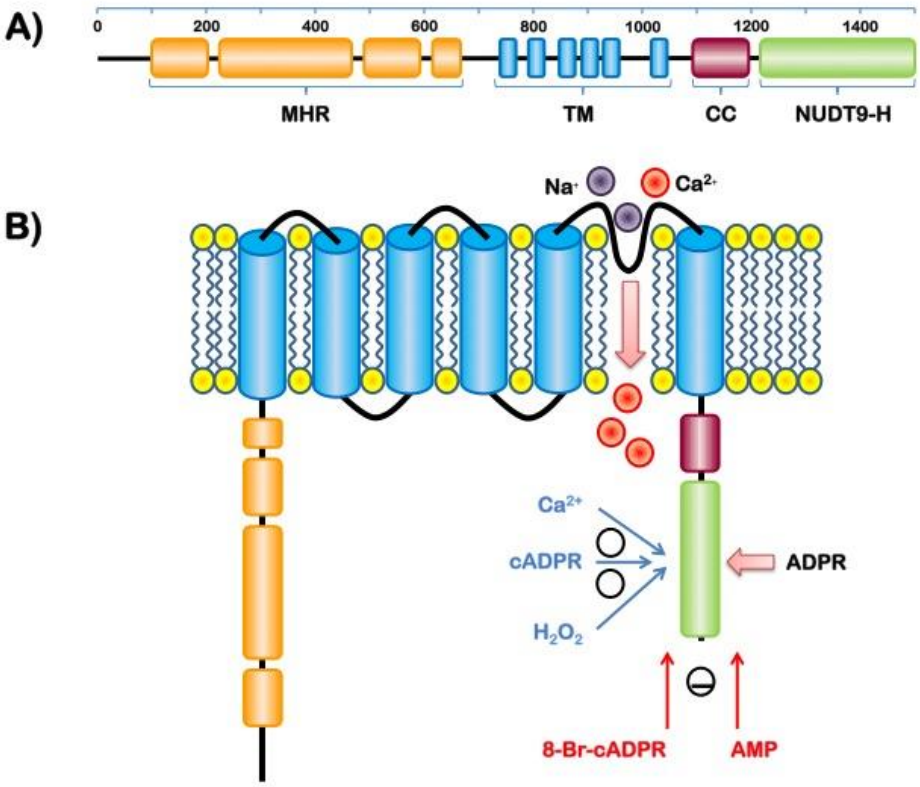
Figure legends

Figure 1. TRPM2 protein structure.

A) TRPM2 protein. The channel's N-terminal has four homologous regions (MHR), followed by six transmembrane segments (TM: S1–S6). The pore-forming loop domain locates between S5 and S6. The C-terminus contains a TRP box and a coil–coil domain (CC), and a C-terminal adenosine diphosphate ribose (ADPR) pyrophosphatase domain (Nudix-like domain or NUDT9 homology domain, NUDT9-H). **B)** TRPM2 channel topology. ADPR binds to the TRPM2 NUDT9-H region allowing calcium (Ca^{2+}) and sodium (Na^{+}) influx. The NUDT9-H enzymatic activity hydrolyses ADPR to ribose 5-phosphate and adenosine monophosphate (AMP). ADPR biding to the NUDT9-H region is facilitated by hydrogen (H_2O_2), cyclic ADPR (cADPR) and Ca^{2+}. AMP and 8Br-cADPR act as a negative regulator of TRPM2 gating by ADPR.



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