Involvement of the TRPML Mucolipin Channels in Viral Infections and Anti-viral Innate Immune Responses

Giorgio Santoni***, Maria Beatrice Morelli†, Consuelo Amantini‡, Massimo Nabissi†, Matteo Santoni‖ and Angela Santoni†,‡

1 Immunopathology Laboratory, School of Pharmacy, University of Camerino, Camerino, Italy, 2 Immunopathology Laboratory, School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy, 3 Medical Oncology Unit, Hospital of Macerata, Macerata, Italy, 4 Department of Molecular Medicine, Sapienza University, Rome, Italy, 5 IRCCS Neuromed, Pozzilli, Italy

The TRPML channels (TRPML1, TRPML2, and TRPML3), belonging to the mucolipin TRP subfamily, primary localize to a population of membrane-bonded vesicles along the endocytosis, and exocytosis pathways. Human viruses enter host cells by plasma membrane penetration or by receptor-mediated endocytosis. TRPML2 enhances the infectivity of a number of enveloped viruses by promoting virus vesicular trafficking and escape from endosomal compartment. TRPML2 expression is stimulated by interferon and by several toll like receptor (TLR) activators, suggesting a possible role in the activation of the innate immune response. Noteworthy, TRPML1 plays a major role in single strand RNA/DNA trafficking into lysosomes and the lack of TRPML1 impairs the TLR-7 and TLR-9 ligand transportation to lysosomes resulting in decreased dendritic cell maturation/activation and migration to the lymph nodes. TRPML channels are also expressed by natural killer (NK) cells, a subset of innate lymphocytes with an essential role during viral infections; recent findings have indicated a role of TRPML1-mediated modulation of secretory lysosomes in NK cells education. Moreover, as also NK cells express TLR recognizing viral pattern, an increased TLR-mediated activation of cytokine production can be envisaged, suggesting a dual role in the NK cell-mediated antiviral responses. Overall, TRPML channels might play a double-edged sword in resistance to viral infections: on one side they can promote virus cellular entry and infectivity; on the other side, by regulating TLR responses in the various immune cells, they contribute to enhance antiviral innate and possibly adaptive immune responses.

Keywords: TRP channel, mucolipin, innate immunity, viral infection, endolysosome

DISCOVERY AND CHARACTERIZATION OF MUCOLIPIN CHANNELS

The transient receptor potential mucolipin channels (TRPML) are non-selective cation channels that conduct Ca\(^{2+}\) and monovalent cation currents from the lumen to the cytoplasm (1, 2). These channels are tetramers, consisting of proteins with six transmembrane-spanning domains and amino- and carboxy-terminal tails oriented toward the cytosol (3). Each subunit has six...
transmembrane segments (S1–S6) and a pore-loop between S5 and S6, which forms a voltage-sensor-like domain and a pore domain. A long extracellular linker between S1 and S2, “polycystin-mucolipin domain” is identified (4). There are three TRPML subtypes sharing ~40% amino acid sequence identity (2). TRPMLs play a role in membrane trafficking (1, 2, 5), autophagy (6, 7), exocytosis (8), and ion homeostasis (9).

The MCOLN1 gene encoding TRPML1 is located on human chromosome 19. No splicing variants have been found in humans, whereas splice variants were described in mice (10). The MCOLN2 gene encoding TRPML2 is located on human chromosome 1 and only one TRPML2 isoform showing 60% amino acid homology with TRPML1, has been detected in humans. The human MCOLN3 gene maps on the short arm of chromosome 1.

TRPML1 is expressed in a number of tissues including adrenal gland, lung, bladder and placenta as well as in thymus, spleen and immune cells (11–13). Mutations in MCOLN1 gene cause a lysosomal storage disorder called mucolipidosis type IV (MLIV). Over 95% of patients with MLIV have loss of functional mutations in MCOLN1 (11–13). Many patients carry mutations that introduce premature stop signals in MCOLN1, thus the TRPML1 protein is completely absent, or abnormally short and it lacks the ion conducting pore (13–15). Some patients show single point mutations in MCONL1 that maintain the open reading frame but lead to a incorrect location or to the production of a TRPML1 inactive form (11–14, 16–18). TRPML2 mRNA is mainly detected in lymphocytes and other cells of the immune system (19). In addition, TRPML2 was found to be overexpressed in aggressive human glioblastoma (20). TRPML3 is mainly expressed in cochlear and vestibular sensory hair cells and melanocytes (21). Two TRPML3 spontaneous gain-of-function mutations (A419P and I362T) called varitint-waddler mutations cause deafness and coat color dilution in mice (22–26).

TRPML1 is activated by phosphatidylinositol-3,5-biphosphate (PtdIns(3, 5)P2) (15, 21, 27–29). Moreover, TRPML1 has an intraluminal loop whose protonation stimulates channel activation (24, 30, 31). It is inhibited by phosphatidylinositol-4,5-biphosphate (PtdIns(4, 5)P2), sphingomyelins, and lysosomal adenosine (28, 29). PtdIns(3, 5)P2 is able to activate also TRPML2 and TRPML3. Na+ removal or less acidic/neutral pH activate TRPML3 and TRPML2, respectively (32, 33).

Among synthetic activators currently available ML-SA1 activates TRPML1, TRPML2, and TRPML3 in human; ML2-SA1 is TRPML2 specific; MK6-83 activates TRPML1 and TRPML3 (15, 21, 28, 32). There are several synthetic inhibitors (ML-Sls); however, they are unable to discriminate the TRPML isoforms from each other (7, 8). Therefore, PtdIns(3, 5)P2 seems to have a central role in activating the TRPML family. This is a low-abundance endolysosome-specific phosphoinositide, produced by PtdIns (3) P5-kinase (PIKfyve). In the immune response, PtdIns(3, 5)P2 is responsible for the fusion of phagosomes with lysosomes to form phagolysosomes, which are essential for the digestion of engulfed pathogens (11, 12, 21). It should be noted that the phagosome acidification is allowed because PtdIns(3, 5)P2 activates TRPML1 channel by directly binding to its N-terminus (34).

**SUBCELLULAR LOCALIZATION OF TRPML CHANNELS**

TRPMLs primarily localize to vesicles along the endocytosis and exocytosis pathways. TRPML1 is localized in the lysosome-associated membrane protein (Lamp-1)± or Rab7± late endosomal and lysosomal (LEL) compartment (2, 26, 35, 36). Late endosomes have an acidic pH of 5.5–6.0, and lysosomes have a more acidic pH of 4.5–5.0 (37–39), a necessary condition to maintain the activity of lysosomal hydrolases (40). The lysosomal localization of TRPML1 protein is likely mediated by clathrin adaptor AP2-dependent internalization from the plasma membrane and/or AP1/AP3-dependent trafficking from the trans-Golgi network (41). Moreover, TRPML1 functions as a key lysosomal Ca2+ channel controlling both lysosome biogenesis and reformation, crucial events for cellular homeostasis (42). TRPML1 also regulates focal exocytosis and phagosome biogenesis. Phagocytic ingestion of large particles activates a PtdIns(3, 5)P2- and Ca2+-dependent exocytosis pathway necessary for pseudopod extension and for leading to clearance of senescent and apoptotic cells in vivo (8).

Similar to TRPML1, TRPML2, and TRPML3 co-localize with Lamp-1 and Rab7 in the LEL compartment (41). Antigen presentation is central in activating adaptive immunity and is mainly mediated by professional antigen-presenting cells including dendritic cells (DCs) and macrophages. In mouse macrophages TRPML1 co-localizes with the MHC-II molecules (43), and by heteromeric interactions with TRPML2 (44) that also contributes to MHC-II/antigen complex formation. The TRPML2± vesicles colocalize with CD63, Lamp-1 and Lamp-3, and Rab11; they induce accumulation of LysoTracker (3, 45), indicating that a fraction of TRPML2 is present in LEL. Numerous proteins, including MHC-I, CD59, interleukin-2 receptor, β1-integrins, and many glycosylphosphatidylinositol-anchored proteins (GPI-APs), travel along the Arf6-regulated pathway (46–48), and co-localize with TRPML2. In addition, Arf6 mutations induce sequestration of TRPML2, MHC-I, and GPI-APs into the same enlarged vacuolar organelles (49), suggesting that TRPML2 uses the Arf6 pathway to cycle between the plasma membrane and recycling endosomes. TRPML2 overexpression induces a strong activation of Arf6, while the inactive form of TRPML2 (D463D/KK) delays the recycling of internalized GPI-APs back to the plasma membrane (49). TRPML2 has been also suggested to participate in the regulation of the lysosomal compartment of B-lymphocytes (45).

Much less is known about the localization and function of TRPML3. TRPML3 is localized in the ELs, early endosomal (EEs), and plasma membrane compartments. Moreover, TRPML3 regulates endocytosis, membrane trafficking and autophagy (50).

**GENERAL MECHANISMS FOR VIRUS ENTRY INTO HOST CELLS**

Viruses have developed different mechanisms and molecules to interact with proteins, lipids and sugar moieties expressed on the surface of host cells, which generally trigger virion uptake.
through the endosomal system (51, 52). Different endocytic cell
routes for virus entry have been reported (53–58). Numerous
host factors are involved in the viral uptake, including coat
proteins (clathrin and caveolin), scission factors (dynamin 2),
and regulatory and trafficking factors (Ras, Ras-related C3
botulinum toxin substrate 1, cell division control protein 2
homolog, and phosphatidylinositol 3-kinase, RabGTPases, etc.)
(59). Endocytosis is a dynamic process and involves recycling,
trafficking, maturation and fusion of endocytic vesicles (60).
Viruses that are running the endocytic gauntlet, need to escape
the endosome before being recycled back into the extracellular
space (61–63), or degradation in the lysosome. Thus, enveloped
viruses (e.g., Filoviridae, Arenaviridae, and Orthomyxoviridae)
fuse the viral envelope with an endosome membrane, releasing
their genomic content into the cytoplasm. Non-enveloped
viruses (e.g., Adenoviridae, Parvoviridae, and Picornaviridae)
use membrane-modifying proteins which can physically pierce
the endosomal membrane to allow release of the genomic
content into the cytoplasm and receptor switching to facilitate
the viral endosomal escape (64). Another feature of viral
endosomal penetration is the ability to co-opt membrane damage
responses of the target cell, by recruiting host phospholipases
(Picornavirus) or inducing lysosomal/autophagosomal exocytosis
(Adenovirus) (65).

Among the receptors involved in virus uptake and anti-
viral immune responses, a role for TRPMLs in virus infection
as well as in the activation of innate immune responses has
recently been suggested.

TRPMLs ENHANCE VIRUS INFECTIVITY
BY INCREASING THE TRAFFICKING
EFFICIENCY OF ENDOCYTOSED
VIRUSES

Recently, it has been found that TRPML2 channel is one of
the interferon (IFN)-stimulating genes (ISGs). However, as
several ISGs, TRPML2 enhances the infectivity of the yellow
fever virus, the Zika virus, the influenza A virus (IAV) and
the equine arteritis virus, while no effect on the Venezuelan
equine encephalitis virus, respiratory syncytial virus, or
vesicular stomatitis virus has been reported (65–67). Human
A549 lung adenocarcinoma cells, stably transfected with
TRPML2, result in enhanced IAV infectivity and infectious
virus production. Moreover, knockout of TRPML2 in A549
and U-2 OS osteosarcoma cells caused a reduction of viral
infection. In addition, treatment of THP-1 monocytes
with IFN-γ, poly (I:C) or LPS (68, 69) enhanced TRPML2
protein expression.

Specifically, TRPML2 promotes virus trafficking from early
to late endosomes and causes an enhanced viral release into the
cytosol and a consequent escape from endosomal compartments;
thus, it promotes a productive infection. This process requires
TRPML2 channel activity, but doesn’t involve the antiviral IFN
signaling pathways, and broadly is applied to enveloped RNA
viruses that are transported to late endosomes by infection.

TRPML2 doesn’t modulate antiviral signaling in IFN-responsive
A549 cells: indeed, no differences in MX1, interferon induced
transmembrane protein 3, or Interferon alpha inducible protein
27α as well as IFNB1 and ISG induction were found in TRPML2-
expressing cells infected with either IAV or Sindbis virus. Both
IAV and Sindbis virus infections were enhanced by TRPML2.
Accordingly, no IAV infection has been evidenced in TRPML2-
DD/KK dominant negative mutant stably-transfected A549
cells. Similarly, TRPML3 increased IAV infection in ectopically
expressing cells (65).

Overall, these data suggest that TRPML-mediated increase
of viral infection is not linked to impairment of IFN
or ISG induction.

During virus life cycle, TRPML2 expression increases at
the early, but not at late post-entry stages. No major effects
on adhesion of IAV on A549 cell surface or virus particle
docytosed cells, have been observed, whereas TRPML2
affected virus vesicular trafficking by promoting the efficiency
of IAV trafficking to late endosomes or by preventing virion
degradation (65), with more IAV fused within the endosomes in
ectopic TRPML2-expressing cells. This effect has been evidenced
only with viruses requiring TRPML2-dependent transport to
endocytic carrier vesicles/late endosomes.

In this regard, a rare genetic variant of human TRPML2,
which induces a lysine/glutamine or arginine change at 370 aa
in TM3 and TM4 domains of TRPML2 protein (MCOLN2-
K370Q), failed to enhance TRPML2-mediated viral infection
when ectopically expressed. Intriguingly, the frequency of this
variant is rather low (about 3%), but increases (11%) in some
African populations (65).

TRPML2 CHANNEL TRIGGERS
ANTI-VIRAL INNATE IMMUNE
RESPONSES

Innate immune activation is based on the ability of the host
to recognize pathogens through specific pathogen recognition
receptors such as TLR, NOD-like receptors, lectin-like receptors
and RIG-1 receptors. Engagement of these receptors activates
the production of cytokines, chemokines, and interferons
that by binding to their cognate receptors, signal through the
JAK-STAT pathways and transcriptionally induce hundreds
of ISGs (66, 67). Recent evidence indicates that TRPML2 is
expressed at low levels in resting RAW 264.7 macrophages,
but its expression is strongly induced upon TLR activation,
with no effect on TRPML1 or TRPML3 (68). These data
have been also confirmed in bone marrow and alveolar
macrophages as well as in microglia from mice treated
with a panel of TLR activators, including zymosan (TLR2
ligand), PolyI:C (TLR3 ligand), LPS (TLR4 ligand), R-848
(TLR7/8 ligand), and Imiquimod (TLR7 ligand). Endogenous
TRPML2 co-localizes with perinuclear vesicles that also
contain the transferrin receptor and likely correspond to
recycling endosomes.

It is therefore interesting to consider the implications of
TRPML2 up-regulation during in vivo viral infections, when IFN
is produced and triggers a number of responses. In non-immune cells, basal or IFN-induced TRPML2 expression may lead to enhanced viral uptake thus promoting virus infection. However, in immune cells expressing higher levels of basal TRPML2 (68, 70, 71), TRPML2-mediated increased viral uptake also results in increased PAMP receptor engagement and activation, stronger immune response, and subsequently improved viral clearance.

In this regard, apilimod, an inhibitor of PIKfyve by functioning as activator of the TRPML2 channels, blocks the entry and the infection of the Ebola virus and the Marburg virus in Huh 7 liver, in Vero E6 kidney cells and in human primary macrophages. Apilimod also blocked Ebola-glycoprotein-virus like particle (VLP) entry and VLP infection (72).

Infection of mouse bone marrow derived macrophages (BMDM) with the intracellular Mycobacterium smegmatis induces TRPML2 expression, suggesting that TRPML2 up-regulation, occurs not only in response to purified TLR ligands but also to live pathogens. In addition, TRPML2 knocked-out mice treated for 24 h with LPS showed reduced expression of chemokine (C-C motif) ligand (CCL) 3, CCL5, chemokine (C-X-C motif) ligand 2 and intercellular adhesion molecule 1. Moreover, the amount of secreted CCL2 a chemokine released via the early/recycling endosomal was significantly reduced in the supernatants from LPS-treated TRPML2−/− BMDM. Similarly, ML2-SA1 a new TRPML2 agonist, stimulated CCL2 release by LPS-activated WT but not TRPML2−/− macrophages (73). ML2-SA1 treatment also promoted macrophage migration (32), and macrophage and neutrophil migration, in response to LPS, was reduced in TRPML2 knocked-out mice (68).

**INVolVEMENT OF TRPML1 IN THE REGULATION OF TLR RESPONSES IN DC: POTENTIAL ROLE IN THE ANTIVIRAL ADAPTIVE IMMUNITY?**

Dendritic cells play an important role in the beginning of specific immune responses. Immature DCs patrol the tissues and check for the antigen presence by continuously internalizing extracellular material mainly via micropinocytosis (32, 74). Sensing of pathogen/danger signals triggers the DC maturation, reduces the antigen uptake, and up-regulates the membrane expression of CC-chemokine receptor 7 that binds to CCL21 and CCL19 chemokines, driving DCs to lymph nodes, where they present the antigen to T cells (75, 76). Recent evidence indicates that activating the TRPML1-transcription factor EB (TFEB), by regulating TRPML1 gene expression, allows DCs to switch from a tissue-patrolling mode to a fast migratory mode in order to reach the lymph nodes (77). Upon microbial sensing, lysosomal calcium is released by TRPML1, which in turn activates myosin II at the cell rear, promoting fast and directional migration. Lysosomal calcium also induces the activation of TFEB, that at steady state is phosphorylated by the mammalian target of rapamycin complex 1 (mTORC1) and remains in the cytosol (78), but due to the dephosphorylation induced by the TRPML1-mediated calcium efflux-activated calcineurin (79), TFEB translocates into the nucleus where it regulates the TRPML1 expression. Of interest, sensing of bacterial or viral products also induced the TFEB translocation from the cytosol to the nucleus with consequent expression of a network of genes involved in lysosome activity, biogenesis, and secretion (80–83).

Toll like receptors play a crucial role in the early host detection of invading viruses (84–87). In particular, TLR-7 and TLR-9 recognize single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA), respectively, in the endolysosomes (84–86). A recent report demonstrates the involvement of TRPML1 in TLR7-mediated DC responses by facilitating ssRNA trafficking into lysosomes. TRPML1−/− DCs showed impaired TLR7 responses to ssRNA, while a mucolipin agonist specifically enhanced TLR7 responses to ssRNAs. In addition, the inhibition of PtdIns(3, 5)P2 generation, that binds directly to TRPML1 and induces the Ca2+ release, completely inhibited TLR7 responses to ssRNA in DCs (88). Confocal analyses showed that ssRNA transportation to lysosomes in DCs was impaired by a PIKfyve inhibitor as well as by the lack of TRPML1. Moreover, in TRPML1−/− bone marrow derived-DCs (BM-DCs) RNA transportation to lysosomes was more severely impaired than DNA transportation. TLR9 responses to CpG-A were also significantly impaired in TRPML1−/− Bone Marrow-conventional DCs (BM-cDCs) and plasmacytoid-DC (pDCs) by the PIKfyve inhibitor, suggesting that TRPML1 has a role in CpG-A transportation to lysosomes. However, CpG-A transportation to lysosomes was only transiently halted in TRPML1−/− BM-cDCs, suggesting a redundant role of TRPML1 in this pathway. Conversely, TLR9 responses to CpG-B were not altered in TRPML1−/− BM-cDCs and pDCs (77, 88). Impaired TLR7 and TLR9 responses in TRPML1−/− BM-cDCs stimulated with ssRNA or CpG-A was associated with reduced IL-6, TNF-α and IFN-α production. In addition, the PIKfyve inhibitor induced an impairment of TLR7 responses to ssRNA in BM-pDCs, while only reduced production of IFN-α in response to TLR9 stimulation by CpG-A was observed.

A role for TRPML channels in CpG-A transportation is not limited to TRPML1, but it has been described also for TRPML2 and TRPML3 (88).

Collectively, these findings suggest that TRPML channels, by enhancing TLR responses and promoting DC maturation/activation, play a critical role in stimulating antiviral adaptive immune responses. In this regard, it has recently been discovered that TRPML2 increases the expression of B7 costimulatory molecules on DC via TFEB activation and simultaneously induces CD8 T cell proliferation and cytolytic activity in an antigen-specific manner (89).

**TRPML1 AND TRPML2 REGULATE OF NK CELL FUNCTIONS: A DUAL ROLE IN ANTIVIRAL NK CELL RESPONSES?**

Natural killers are a subset of innate lymphoid cells that have the ability to recognize and eliminate infected cells. Moreover, they can secrete anti-viral cytokines such as IFN-γ and TNF-α and chemokines to recruit and instruct other immune cell types.
Recent findings demonstrated that TRPML1 is expressed at mRNA level in both educated (KIR+) and not educated (KIR−) NK cell subsets. Interestingly, pharmacological inhibition of PtdIns(3, 5)P2 synthesis, or genetic silencing of TRPML1, resulted in enlargement of lysosomal compartment, increased granzyme B (GZB), and enhanced specific degranulation and IFN-γ production. On the contrary, stimulation of NK cells with the TRPML agonist, MK6-83, induced the loss of GZB and decreased degranulation and IFN-γ production in response to K562 cells. Overall, these findings suggest an important role of TRPML1-mediated modulation of secretory lysosomes in NK cell education (96). Recent evidence reported that NK cells also express high levels of TRPML2, which further increase during NK cell differentiation. Silencing of TRPML2 leads to slight enhanced NK cell degranulation and to the production of IFN-γ (96).

Finally, although no evidence is available in the literature so far, a role for TRPML in promoting NK cell-mediated TLR responses to viral patterns can be envisaged (97).

Collectively, these findings suggest that TRPML channels negatively affecting NK cell education and promoting TLR activation, play a dual role in NK cell-mediated antiviral responses.

**CONCLUSION**

Several evidences indicate that TRPMLs play a crucial role in membrane trafficking, autophagy, exocytosis and ion homeostasis. Thanks to these functions, TRPML1 and TRPML2 have been found to be involved in the entry and trafficking of virus by promoting virus infectivity and productive infection. Conversely, these receptors are also expressed on innate immune cells where they stimulate the transport of viral patterns and therefore the cognition by their respective receptors present in the endosomal compartment. In DCs, this results in enhanced TLR responses that lead to increased DC maturation, production of IFNs, inflammatory cytokines/chemokines and migration with consequent activation of the anti-viral adaptive immune responses (Figure 1). Recent findings indicate that on NK cells, which also express TRPML1 and TRPML2 as well as the TLRs recognizing the viral nucleic acids, TRPML1 impairs NK cell education and functional activity by modulating the secretory lysosomes, thus suggesting a dual role in the NK cell-mediated antiviral responses.

For what concern TRPML3 role in infections, it has been demonstrated in bladder epithelial cells that TRPML3, by mediating efflux of Ca^{2+} ions from lysosomes, promotes the expulsion of exosome-encased bacteria (98). However, little is known about its functions in viral infections. At this regard, findings showed that TRPML3 increased IAV infections in ectopically expressing cells (65). Moreover, it has been recruited in the autophagosome upon induction of autophagy (50) and this suggests that it could participate to the xenophagy. Regarding the viral infections, autophagy can be either pro-viral or anti-viral. Some virus exploit the autophagy machinery for their intracellular survival, while other express specific protein to evade autophagy and propagate in host cells (99). Thus, an important role of TRPML3 in viral infections cannot be excluded; however, additional findings are required to further clarify this issue.

**AUTHOR CONTRIBUTIONS**

GS and AS drafted the manuscript. GS conceived and designed the study. MM, CA, MN, and MS critically revised the manuscript.
ACKNOWLEDGMENTS

We thank Oliviero Marinelli and Federica Maggi for their assistance.
37. Xu H, Martioina E, Szabo I. Organellar channels and transporters. *Cell Calcium*. (2015) 58:1–10. doi: 10.1016/j.ceca.2015.02.002
38. Kiseliov KK, Ajiuwa M, Rybalchenko V, Patel S, Muhallem S. The intracellular Ca\(^{2+}\) channels of membrane traffic. *Channels (Austin)*. (2012) 6:343–51. doi: 10.4186/chann.2012.3
39. Patel S, Cai X. Evolution of acidic Ca\(^{2+}\) stores and their resident Ca\(^{2+}\)-permeable channels. *Cell Calcium*. (2015) 57:222–30. doi: 10.1016/j.ceca.2014.12.005
40. Appelqvist H, Wäster P, Kågedal K, Ollinger K. The lysosome: from waste bag to cell organelle. *J Mol Cell Biol.* (2007) 8:54. doi: 10.1186/1471-2121-8-54
41. Zeevi DA, Lev S, Frumkin A, Minke B, Bach G. Heteromultimeric TRPML channel assemblies play a crucial role in the regulation of cell viability models and starvation-induced autophagy. *J Cell Sci.* (2010) 123:3112–24. doi: 10.1242/jcs.067330
42. Song Y, Dayalu R, Matthews SA, Scharenberg AM. TRPML cation channels regulate the specialized lysosomal compartment of vertebrate B-lymphocytes. *Eur J Cell Biol.* (2006) 85:1253–64. doi: 10.1016/j.ejcb.2006.08.004
43. Brown FD, Rozelle AL, Yin HL, Balla T, Donaldson JG. Phosphatidylinositol 4,5-bisphosphate and Arf6-regulated membrane traffic. *J Cell Biol.* (2001) 154:1007–17. doi: 10.1083/jcb.200103107
44. Mercier J, Schelhaas M, Helenius A. Virus entry by endocytosis. *Cell Calcium*. (2007) 8:54. doi: 10.1186/1471-2121-8-54
45. Zila V, Difato F, Klimova L, Huerfano S, Forstova J. Involvement of microtubular network and its motors in productive endocytic trafficking of mouse polyomavirus. *PLoS One*. (2014) 9:e96922. doi: 10.1371/journal.pone.096922
46. Imaizumi BA, Dermody TS. Transport to late endosomes is required for efficient reovirus infection. *J Virol.* (2012) 86:8346–58. doi: 10.1128/JVI.01010-12
47. Grove J, Marsh M. The cell biology of receptor-mediated virus entry. *J Cell Biol.* (2011) 195:1071–82. doi: 10.1083/jcb.201108131
48. Jae LT, Brummelkamp TR. Emerging intracellular receptors for hemorrhagic fever viruses. *Trends Microbiol.* (2015) 23:392–400. doi: 10.1016/j.tim.2015.04.006
49. Rinkenberger N, Schoggins JW. Mucolipin-2 cation channel increases trafficking efficiency of endocytosed viruses. *MBio*. (2018) 9:e2314–7. doi: 10.1128/mBio.02314-17
50. Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol*. (2011) 1:519–25. doi: 10.1016/j.coviro.2011.10.008
51. Schoggins JW. Interferon-stimulated genes: roles in viral pathogenesis. *Curr Opin Virol*. (2014) 6:60–6. doi: 10.1016/j.coviro.2014.03.006
52. Sun L, Hua Y, Vergara-Auregui S, Diab HI, Puertollano R. Novel role of TRPML2 in the regulation of the innate immune response. *J Immunol.* (2015) 195:4922–32. doi: 10.4049/jimmunol.1500163
53. Lanford RE, Guerra B, Lee H, Chavez D, Brasky KM, Bigger CB. Genomic response to interferon-alpha in chimpanzees: implications of rapid downregulation for hepatitis C kinetics. *Hepatology.* (2006) 43:961–72. doi: 10.1002/hep.21167
54. Valadez JA, Cuijungco MP. PAX5 is the transcriptional activator of mucolipin-2 (MCL021) gene. *Gene*. (2015) 555:194–202. doi: 10.1016/j.gene.2015.11.003
55. Cuijungco MP, Silva J, Habibi A, Valadez JA. The mucolipin-2 (TRPML2) ion channel: a tissue-specific protein crucial to normal cell function. *Pflugers Arch.* (2016) 468:177–92. doi: 10.1007/s00424-015-1732-2
56. Nelson EA, Dyall J, Hoenen T, Barnes AB, Zhou H, Liang JY, et al. The phosphatidylinositol-3-phosphate 5-kinase inhibitor apilimod blocks filoviral entry and infection. *PLoS Negl Trop Dis.* (2017) 11:e0005540. doi: 10.1371/journal.pntd.0005540
57. Galianeo A, Davis LC. Revealing the secrets of secretion. *elife*. (2018) 7:e43512. doi: 10.7554/elife.43512
58. Sallusto F, Cell M, Danielli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med.* (1995) 182:389–400. doi: 10.1083/jem.182.3.389
59. Martin-Fontecha A, Sebastiani S, Hopken UE, Ugucioni M, Lipp M, Lanzavecchia A, et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. *J Exp Med.* (2003) 198:615–21. doi: 10.1083/jem.20030448
60. Weber M, Hauschild R, Schwarz J, Moussion C, de Vries I, Legler DF, et al. Intestinal dendritic cell guidance by haptotactic chemokine gradients. *Science.* (2013) 339:328–32. doi: 10.1126/science.1228456
61. Bretou M, Sáez PJ, Sanséau D, Maurin M, Lankar D, Chabaud M, et al. Lysosome signaling controls the migration of dendritic cells. *Sci Immunol.* (2017) 2eaak9573. doi: 10.1126/sciimmunol.aak9573
62. Settembre C, Zoncu R, Medina DL, Di Paola S, Peluso I, Armani A, De Stefani D, Venditti R, et al. A lysosome-to-nucleus signaling mechanism senses and regulates the lysosome via miTOR and TFEF. *EMBO J.* (2012) 31:1095–108. doi: 10.1038/emboj.2012.32
63. Medina DL, Di Paola S, Peluso I, Armani A, De Stefani D, Venditti R, et al. A lysosomal calcium signaling regulates autophagy through calcineurin and TFEF. *Nat Cell Biol.* (2015) 17:288–99. doi: 10.1038/ncb3114
64. Visvikis O, Iluegbu N, Labed SA, Luhachack LG, Alves A-MF, Wollenberg AC, et al. Intracellular host defense requires TFEF-mediated transcription of cytoprotective and antimicrobial genes. *Immunity.* (2014) 40:896–909. doi: 10.1016/j.immuni.2014.05.002
65. Samie M, Cresswell P. The transcription factor TFEF acts as a molecular switch that regulates exogenous antigen-presentation pathways. *Nat Immunol.* (2015) 16:729–36. doi: 10.1038/ni.3196
82. Santoni, M., Palmieri, M., di Ronza, A., Medina, D.L., Valenza, M., Gennarino, V.A., et al. A gene network regulating lysosomal biogenesis and function. *Science*. (2009) 325:473–7. doi: 10.1126/science.1174447

83. Settembre, C., Di Malta, C., Polito, V.A., Garcia Arencibia, M., Vetrini, F., Erdin, S., et al. TFEB links autophagy to lysosomal biogenesis. *Science*. (2011) 332:1429–33. doi: 10.1126/science.1204592

84. Beutler, B., Jiang, Z., Georgel, P., Crozat, K., Croker, B., Rutschmann, S., et al. Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. *Annu Rev Immunol*. (2006) 24:353–89. doi: 10.1146/annurev.immunol.24.021605.090552

85. Kaisho, T., Akira, S. Toll-like receptor function and signaling. *J Allergy Clin Immunol*. (2006) 117:979–88. doi: 10.1016/j.jaci.2006.02.023

86. Kawai, T., Akira, S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*. (2010) 11:373–84. doi: 10.1038/ni.1863

87. Gasteiger, G., D’Osualdo, A., Schubert, D.A., Weber, A., Bruscia, E.M., Hartl, D. Cellular innate immunity: an old game with new players. *J Innate Immun*. (2017) 9:111–25. doi: 10.1159/000453397

88. Li, X., Saitoh, S-I., Shibata, T., Namamura, N., Fukui, R., Miyake, K. Mucolipin 1 positively regulates TLR7 responses in dendritic cells by facilitating RNA transportation to lysosomes. *Int Immunol*. (2015) 27:83–94. doi: 10.1093/intimm/dxu086

89. Ma, J., Wei, K., Zhang, H., Tang, K., Li, F., Zhang, T., et al. Mechanisms by which dendritic cells present tumor microparticle antigens to CD8\(^{+}\) T cells. *Cancer Immunol Res*. (2018) 6:1057–68. doi: 10.1158/2326-6066.CIR-17-0716

90. Wende, H., Colonna, M., Ziegler, A., Volz, A. Organization of the leukocyte receptor cluster (LRC) on human chromosome 19q13.4. *Mamm Genome*. (1999) 10:154–60. doi: 10.1007/s003359900961

91. Billadeau, D.D., Leibson, P.J. ITAMs versus ITIMs: striking a balance during cell regulation. *J Clin Invest*. (2002) 109:161–8. doi: 10.1172/JCI14843

92. Bastidas-Legarda, L.Y., Khakoo, S.I. Conserved and variable natural killer cell receptors: diverse approaches to viral infections. *Immunology*. (2019) 156:319–28. doi: 10.1111/imm.13039

93. Pisciotta, S., Pirooz, G., Piccoli, M., F dealti, L., Santoni, A., Palmieri, G. p38 MAPK activation controls the TLR3-mediated up-regulation of cytotoxicity and cytokine production in human NK cells. *Blood*. (2004) 104:4157–64. doi: 10.1182/blood-2004-05-1860

94. Sivori, S., Falco, M., Della Chiesa, M., Carlomagno, S., Vitale, M., Moretta, L., et al. CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors and dendritic cells. *Proc Natl Acad Sci USA*. (2004) 101:10116–21. doi: 10.1073/pnas.0403744101

95. Adib-Conquy, M., Scott-Algara, D., Cavaillon, J-M., Souza-Fonseca-Guimaraes, F. TLR-mediated activation of NK cells and their role in bacterial/viral immune responses in mammals. *Immunol Cell Biol*. (2014) 92:256–62. doi: 10.1038/icb.2013.99

96. Goodridge, J.P., Jacobs, B., Saetersmoen, M.L., Clement, D., Hammer, Q., Clancy, T., et al. Remodeling of secretory lysosomes during education tunes functional potential in NK cells. *Nat Commun*. (2019) 10:514. doi: 10.1038/s41467-019-08384-x

97. Clement, D., Zandstra-Krokeide, S., Skarpbn, E., Goodridge, J., Malmberg, K-J. Role of the mucolipin calcium channel family in vesicular trafficking and natural killer cell function. *Proceedings of the 18th Meeting of the Society for Natural Immunity (NK2019); Sept 30–Oct 3 Luxembourg, abstract n.17. Luxembourg*. (2019), 46 p.

98. Miao, Y., Li, G., Zhang, X., Xu, H., Abraham, S.N. A TRP channel senses lysosome neutralization by pathogens to trigger their expulsion. *Cell*. (2015) 161:1306–19. doi: 10.1016/j.cell.2015.05.009

99. Paul, P., Munz, C. Autophagy and mammalian virus: roles in immune response, viral replication and beyond. *Adv Virus Rev*. (2016) 95:149–95. doi: 10.1016/bs.avir.2016.02.002

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Copyright © 2020 Santoni, Morelli, Amantini, Nabissi, Santoni and Santoni. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*