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Inducible expression of MCOLN2 in immune cells.

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Mucolipins (or TRPMLs) constitute a family of endosomal cation channels with homology to the transient receptor potential superfamily. In mammals, the mucolipin family includes three members, mucolipin-1, -2, and -3 (MCOLN1-3). MCOLN1 and -3 have been well characterized. However, the cellular function of MCOLN2 remains elusive. Consistent with the proposed role of MCOLNs in Ca^{2+}-dependent membrane trafficking, we have previously described that recombinant MCOLN2 enhances recycling of internalized GPI proteins back to the cell surface in HeLa cells. To further corroborate MCOLN2 function in a more physiologically relevant cell type, we analyzed MCOLN2 expression in a series of mouse tissues and organs by RT-PCR. We found that MCOLN2 was expressed predominantly in lymphoid and kidney organs. Notably, expression of MCOLN2 was tightly regulated at the transcriptional level. While MCOLN2 was present at low levels in resting RAW 264.7 macrophages, its expression was induced over 10 fold in response to Toll-like Receptors (TLRs) activation. In contrast, the levels of MCOLN1 and MCOLN3 did not change upon TLR activation. To confirm the upregulation of MCOLN2 in response to TLR activation in primary cells we isolated bone marrow macrophages, alveolar macrophages, and microglia from mice and treated them with a panel of TLR activators, including Zymosan (TLR2 ligand), PolyIC (TLR3 ligand), LPS (TLR4 ligand), R-848 (TLR7/8 ligand), and Imiquimod (TLR7 ligand). In all cases, we observed a significant increase in the levels of MCOLN2 upon TLR activation as assessed by RT-PCR, western-blot, and immunofluorescence. Endogenous MCOLN2 co-localized to perinuclear vesicles that also contain transferrin receptor and likely correspond to recycling endosomes. This is in clear contrast with MCOLN1 and MCOLN3 that mainly localize to the late and early endosomal pathway, respectively. Overall, our data reveal interesting differences in the regulation and distribution of the members of the MCOLN family and suggest a possible role of MCOLN2 in innate immune response.

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Distinct Intracellular Trafficking Patterns of Host IgG by Herpes Virus Fc-Receptors.

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Members of both alpha and beta herpes viruses affects 50–98% of people around the world. They cause severe symptoms in congenitally infected newborns, a lifelong latent infection that is lethal in immuno-compromised individuals, and are associated with several types of cancer. Human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) viruses express proteins (HCMV gp68 and gp34; HSV-1

gE-gI) that function as Fc receptors (FcRs) by binding to the Fc regions of human IgG. In addition to binding free IgG, these viral FcRs can bind to IgG complexed with an antigen to form an antibody bipolar bridged (ABB) complex. Although HCMV gp68 and HSV-1 gE-gI have an overlapping binding site on Fc, the finding that the gp68/Fc interaction is stable at pH values between 5.6 and 8.1 but that gE-gI binds only at neutral or basic pH suggests distinct pH-based downstream events after IgG is internalized via receptor-mediated endocytosis into intracellular compartments. Here we developed a cell-based in vitro model system to define the fates of ABB complexes formed by the two types of viral FcRs. We found that alpha (HSV-1) and beta (HCMV) herpes virus FcRs displayed distinct intracellular trafficking patterns to target internalized ligands: HSV-1 gE-gI dissociates from its IgG-antigen ligand in acidic endosomal compartments and recycles back to the cell surface, whereas HCMV FcRs (gp68) are transported together with IgG-antigen complexes to lysosomes for degradation. In both cases, anti-viral IgGs and their viral targets are selectively degraded, a potential immune evasion strategy allowing herpes viruses to escape from IgG-mediated immune responses.

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Differential regulation of organelle dynamics by tubulin acetylation in polarized epithelia.

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Intracellular trafficking is coordinated by the actin and microtubule cytoskeletons and associated motor proteins. Organelle-specific recruitment of motor proteins is accomplished in part by post-translational modifications of α-tubulin such as acetylation and detyrosination. Here, we used high-speed live imaging to monitor organelle dynamics in the prototypical polarized MDCK cell line and in adult primary retinal pigment epithelial (RPE) monolayers. A key function of the RPE is the daily phagocytosis and degradation of shed photoreceptor outer segments, necessary for photoreceptor health and for vision. Over a lifetime, this high metabolic activity leads to the accumulation of visual cycle by-products called lipofuscin bisretinoids within the RPE endo-lysosomal system. Lipofuscin bisretinoids have been implicated in the pathogenesis of numerous retinal diseases including age-related macular degeneration, the most common cause of vision loss among older adults today. We have shown that bisretinoids trap cholesterol and bis(monoacylglycero)phosphate, an acid sphingomyelinase cofactor in RPE lysosomes. Acid sphingomyelinase activation increases cellular ceramide, which promotes tubulin acetylation on stabilized microtubules. Live imaging using spinning disc confocal microscopy revealed that long-range displacement of LC3-labeled autophagosomes and LAMP2-labeled late endosomes/lysosomes (LE/Lys) is significantly impaired in cells with abnormally acetylated microtubules. This results in incomplete autophagic flux and accumulation of canonical autophagic substrates. Calcium-induced fusion of LE/Lys with the plasma membrane is critical for membrane repair and removal of cellular debris. In RPE with lipofuscin bisretinoids or in MDCK with excess cholesterol, we observed decreased LE/Lys exocytosis after exposure to calcium ionophores or pore-forming toxins.