Role of Endocytosis in TLR Signaling: An Effective Negative Regulation to Control Inflammation

Abstract

Cells take materials from outside without crossing plasma membrane through engulfing them by a process called endocytosis. It can be categorized into phagocytosis, pinocytosis, clathrin and caveolins mediated endocytosis. Toll-like receptor 4 (TLR4) is an innate immune receptor recognizing specific microbial pattern and initiate host response against invading pathogen. Receptor mediated endocytosis (RME) involve in the uptake of ligand activated TLR4 and helps to down regulate the proinflammatory pathway to prevent from septic shock, multiple organ failure and death. Endocytosed TLR4 can activate TIRF-TRAM pathway and produce IRF3 as effector molecule. Later in lysosomes, the endocytosed TLR4 undergoes Triad3A mediated degradation and thereby limiting its further signaling. This review discusses the latest findings on the role of endocytosis and receptor trafficking in TLR4 signaling.

Keywords: Endocytosis; Toll-like receptors; Clathrin; Caveolins; Ubiquitination

Abbreviations: TLR4: Toll-Like Receptor 4; RME: Receptor Mediated Endocytosis; CME: Clathrin Mediated Endocytosis; TGF-β: Transforming Growth Factor β

Introduction

Endocytosis is a process through which cells taking molecules into the cell by bypassing plasma membrane trafficking. The physical and chemical properties of the molecule direct the cell to choose the method of internalization [1]. After endocytosis, they form an independent vesicle in the cytoplasm known as phagosomes, pinosomes, coated vesicles etc. depending on the process of endocytosis. Classically endocytosis can be categorized into four as phagocytosis, pinocytosis, clathrin mediated endocytosis (CME) and caveolae mediated endocytosis [2]. Phagocytosis is a process of internalizing the particulate substrate, which varying in size from 0.05μm to 1.0μm. In pinocytosis, cells engulf lower molecular weight molecules and fluids. Pinocytosis can be further divided into two categories as adsorptive pinocytosis and fluid phase pinocytosis [3]. The mechanism of endocytosis carried out through clathrin coated vesicles is meant for the internalization of antibodies, growth factors, and low-density lipoprotein. Caveolins form small conical invaginations in the plasma membrane, which will uptake the molecules through specific receptor mediated mechanism. Caveolins mediated endocytosis otherwise known as lipid raft /caveolar endocytosis since it carried out through lipid raft regions in most of the times [4-6].

Both clathrin dependent and independent endocytosis play a vital role in cell signaling. A cholesterol-sphingolipids rich region on the plasma membrane known as lipid raft plays significant role in this mechanism. Cell membrane receptors are concentrated in the lipid raft and these lipid rafts are responsible forsignaling molecule assembly and receptor endocytosis. Briefly, membrane proteins categorized in to three groups: ion channel linked receptors, enzyme linked receptors and G protein coupled receptors. Most of the receptors need to form a homo or hetero dimer in order to activate the downstream signaling cascade [7,8].

Upon activation with specific ligand, the receptor recruits their primary adaptor molecules and triggers the activation of effector molecules. These receptors have extracellular, transmembrane and intracellular domains with functional importance. Some receptors endocytosed to the cytoplasm by choosing one of the above endocytic mechanism and in turn change fate of the signaling pathway [6]. Guglielmo and colleagues explained the fate of Transforming Growth Factor β (TGF-β) receptor when it was endocytosed by CME and RME. During the CME, TGF-β activates the smad dependent signaling pathway whereas in RME mediated endocytosis leads to the degradation of TGF-β receptor [9]. Chen and Camilla have shown the dose dependent endocytosis of epidermal growth factor receptor (EGFR). EGFR will choose CME if there is low ligand concentration and tend to undergo RME in the presence of higher dosage [10]. The process of endocytosis regulates the signaling cascade and nutritional uptake of a cell.

Mechanism of receptor mediated endocytosis

Receptor mediated endocytosis is mainly mediated by clathrin. Adaptor proteins like AP-2 binds directly to clathrin and produce a ‘clathrin coat’. The ligand activation of a receptor will result in the binding of activator protein that in turn recruits the clathrin and they form a coated vesicle at the site of activation. Figure 1 explains the mechanism of RME. The vesicle fused with peripheral early endosomes in which the cargo may get processed inside. This fusion involves Rab5 and EEA-1 proteins. It is not clear that how and when the early endosomes transfer the cargo to late endosomes or the former matures to form later, even though they have distinct pH and chemical properties. The fate of the protein inside the endosomes, whether to recycle or degraded...
Endocytosis and regulation of signaling

Receptor activation through ligand binding requires additional adaptor molecules and phosphorylation to carry out the downstream signaling pathway. Endocytosis of the receptors leads to the down regulation of the signaling pathway, and ubiquity mediated degradation will take place in lysosomes or recycle back to plasma membrane from early endosomes. This helps the cell to retain the particular receptor activity simultaneously [15,20].

A well-studied model for RME is EGF receptor endocytosis. Immediately after binding with EGF, the receptor localizes out of the raft area, eventually localized in to clathrin coat, and internalized. In the early endosomes, EGFR interacts with YOTB, Vac1, and EEA1 and forms a ternary protein complex with signal transduction adaptor molecule and EPS15. EGFR recycles back to the plasma membrane or transferred to multi-vesicular bodies for degradation [21-23]. This type of internalization is meant to be a down regulation process for most receptor signaling. Kirchhausen and colleagues have shown the negative regulation of RME in EGFR [24].

Toll-like receptor signaling and endocytosis

Toll-like receptors are the ‘gate keepers’ of the innate immune system. They are responsible for the recognition and response towards invading pathogens [25,26]. There are 13 mammalian TLR family proteins were identified so far. TLRs can be activated by variety of pathogen associated molecular patterns such as LTA, LPS, flagellin, CpG DNA, dsRNA, and ssRNA [27-31]. Upon activation with the ligand, TLRs form homo or heterodimers and recruits its primary adaptor molecules, which will result in a series of cellular and molecular changes inside the cell. TLR signaling pathways follow two types of downstream activation namely, MyD88 dependent and MyD88 independent pathways, which eventually switch on the pro-inflammatory cytokine response through the nuclear translocation of NF-kB and IFN [25,32]. TLRs which are present on the plasma membrane gradually endocytosed and it is found as an important negative regulation in TLR signaling as observed in EGFR previously.

TLR4 recognizes LPS and induces pro-inflammatory pathway via MyD88 mediated activation and nuclear translocation of NF-kB. Another way of TLR4 activation through TRIF-TRAM is introduced during its endocytosis. Continuous exposure to LPS may lead to septic shock and death of host cells, thus the host response should control the process of inflammation through ‘tolerance’. Endocytosis of the receptor and its degradation through ubiquity pathway is one of the negative regulatory mechanisms in LPS induced TLR4 pathway [33]. TRAM is the signaling molecule for TLR4 translocation to endosomes with its bipartite signaling sequence. Kagan et al have explained the difference in host response of membrane localized and endocytosed TLR4 in macrophages [34]. Haselby et al have shown the internalization of TLR4 within 30 min of LPS treatment and a GTPase known as dynamic regulates the process of endocytosis [33]. LPS-CD14-TLR4-MD2 complex is getting internalized to make macrophage desensitized [25,33]. Lee et al. [35] showed the host tolerance of LPS on TLR4 signaling by using TLR4-GFP construct [35]. Suri and colleagues previously reported the presence of TLR4 in the nucleus after stimulating with LPS [36]. The mechanisms and functional aspects of this translocation yet to be explained.

Cellular and functional effects of TLR4 endocytosis

TLR4 is found to activate both MyD88 dependent and independent pathways. But no TLR can activate the MyD88 independent TRIF-TRAM signaling from plasma membrane. This mystery explained after the discovery of TLR4 localization into the endosomes. Internalized TLR4 activates a different pathway in early endosomes and purpose of this internalization is to limit the receptor expression (Figure 2). TLR4 signaling from plasma membrane activates the MyD88 adaptor molecule and leads to the activation of NF-κB and AP-1. Another way of TLR4 activation through TRIF-TRAM is observed in EGFR previously.

Figure 1: Process of receptor mediated endocytosis. Receptor-mediated endocytosis (RME) is triggered by the binding of the specific ligands to host receptors. The ligand attachment to the host receptor induces the binding of an adaptor protein to the receptor cytoplasmic tail. Adaptor proteins bind to clathrin, and the local concentration of adaptor proteins on the inside face of the plasma membrane allows clathrin to multimerize to form characteristic invaginations or Clathrin-Coated Pit. Membrane scission proteins DNM1/Dynamin-1 or DNM2/Dynamin-2 pinch off the CCP from the host membrane thereby releasing the Clathrin-Coated Vesicle.

Citation: Balachandran Y (2016) Role of Endocytosis in TLR Signaling: An Effective Negative Regulation to Control Inflammation. MOJ Immunol 3(6): 00111. DOI: 10.15406/moji.2016.03.00111
Gene expression profiling studies have revealed that the long-term exposure to LPS will lead the production of IFN-β [38].

The activation of interferon pathway leads to the production of IFN-β, IP10, ISG15 etc. and will result in the activation of JAK-STAT pathway [40]. TLR4 endocytosis also helps in the coordination between innate and adaptive immune systems. Degraded antigen bound TLR4 presented by class II MHC receptor and T helper cells recognize them to activate adaptive immune response [37].

**Summary and Future Prospects**

TLR4 activates two different pathways in which TRIF-TRAM mediated signaling refers the negative regulatory. Endocytosis of the receptor and ubiquity mediated degradation regulate an important role in negative regulation of signaling. Transfer of endocytosed TLR4 to the lysosomes and its degradation conflicts with the data showing their nuclear translocation and this differential immune response still need explanation.

**Acknowledgement**

Author expresses immense thanks to Dr. Baljit Singh, Dr. William Trimble, and Dr. Stacy Anderson for their guidance, advices and directions throughout.

**References**

1. Kumari S, Mg S, Mayor S (2010) Endocytosis unplugged: multiple ways to enter the cell. Cell Res 20(3): 256-275.
2. Mukherjee S, Ghosh RN, Maxfield FR (1997) Endocytosis. Physiol Rev 77(3): 759-803.
3. Besterman JM, Low RR (1983) Endocytosis: a review of mechanisms and plasma membrane dynamics. Biochem J 210(1): 1-13.
4. Hansen CG, Nichols BJ (2009) Molecular mechanisms of clathrin-independent endocytosis. J Cell Sci 122: 1713-1721.
5. Kirchhausen T (2009) Imaging endocytic clathrin structures in living cells. Trends Cell Biol 19(11): 596-605.
6. Polo S, Di Fiore PP (2006) Endocytosis Conducts the Cell Signaling Orchestra. Cell 124(5): 897-900.
7. King N, Hittinger CT, Carroll SB (2003) Evolution of Key Cell Signaling and Adhesion Protein Families Predates Animal Origins. Science 301(5631): 361-363.
8. Schlessinger J (2000) Cell Signaling by Receptor Tyrosine Kinases. Cell 103(2): 211-225.
9. Di Guglielmo GM, Le Roy C, Goodfellow AE, Wrana JL (2003) Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover. Nat Cell Biol 5(5): 410-421.
10. Chen H, De Camilli PD (2005) The association of epsin with ubiquitinated cargo along the endocytic pathway is negatively regulated by its interaction with clathrin. Proc Natl Acad Sci U S A 102(8): 1731-1736.
11. Aniento F, Gruenberg J (1995) Membrane transport from early to late endosomes. Cold Spring Harb Symp Quant Biol 60: 205-209.
12. Chavrier P, Parton R, Hauri H, Simons K, Zerial M, et al. (1999) Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. Cell 62(2): 317-329.
13. Ghosh R, Maxfield F (1995) Evidence for nonvectorial, retrograde transferring trafficking in the early endosomes of Hep2 cells. J Cell Biol 128(4): 549-561.
14. Gorvel J, Chavrier P, Zerial M, Gruenberg J (1999) rab5 controls early endosome fusion in vitro. Cell 64(5): 915-925.
15. Liu J, Shapiro JI (2003) Endocytosis and Signal Transduction: Basic Science Update. Biol Res Nurs 5(2): 117-128.
16. Raiborg C, Bache K, Gillooly D, Madshus I, Stang E, et al. (2002) Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. Nat Cell Biol 4(5): 394-398.
17. Kaksonen M, Sun Y, Drobne DG (2003) A pathway for association of receptors, adaptors and actin during endocytic internalization. Cell 115(4): 475-487.
18. Ferguson SM, Brasnjo G, Hayashi M, Wolff M, Collesi C, et al. (2007) A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. Science 316(5824): 570-574.
19. Miaczynska M, Stenmark H (2008) Mechanisms and functions of endocytosis. J Cell Biol 180(1): 7-11.
20. Strange K, Willingham M, Handler J, Harris HJ (1988) Apical membrane endocytosis via coated pits is stimulated by removal of antidiuretic hormone from isolated, perfused rabbit cortical collecting tubule. J Membr Biol 103(1): 17-28.
21. Howe CL (2005) Modeling the signaling endosome hypothesis: Why a drive to the nucleus is better than a (random) walk. Theor Biol Med Model 2: 43.
22. Katzmann DJ, Wendland B (2005) Analysis of Ubiquitin-Dependent Protein Sorting Within the Endocytic Pathway in Saccharomyces cerevisiae. Methods Enzymol 399: 192-211.
23. Le Roy C, Wrana JL (2005) Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling. Nat Rev Mol Cell Biol 6(2): 112-126.
24. Kirchhausen T (2002) Clathrin adaptors really adapt. Cell 109(4): 413-416.
25. Akashi S, Saitoh S, Wakabayashi Y, Kikuchi T, Takamura N, et al. (2003) Lip polysaccharide interaction with cell surface Toll-like receptor 4-MD-2: Higher Affinity than that with MD-2 or CD14. J Exp Med 198(7): 1035-1042.

26. Beutler B, Rietschel ET (2003) In innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 3(2): 169-176.

27. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, et al. (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303(5663): 1526-1529.

28. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, et al. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410(6832): 1099-1103.

29. ALEXOPULOU L, HOLT AC, MEDZHITOV R, FLAVELL RA (2001) Recognition of double-stranded RNA and activation of NF-kB by Toll-like receptor 3. Nature 413(6857): 732-738.

30. Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A, et al. (2003) Toll-like Receptor 9-mediated recognition of Herpes Simplex Virus-2 by Plasmacytoid Dendritic Cells. J Exp Med 198(3): 513-520.

31. Osamu Takeuchi SS, Takao H, Katsuki H, Kiyoshi T, Zhongyun D, et al. (2002) Cutting Edge: Role of Toll-Like Receptor 1 in Mediating Immune Response to Microbial Lipoproteins. J Immunol 169(1): 10-14.

32. O'Neill LA, Fitzgerald KA, Bowie AG (2003) The Toll-IL-1 receptor adaptor family grows to five members. Trends in Immunol 24(6): 286-289.

33. Husebye H, Halaas O, Stemmark H, Tunheim G, Sandanger O, et al. (2006) Endocytic pathways regulate Toll-like receptor 4 signaling and link innate and adaptive immunity. EMBO J 25(24): 683-692.

34. Kagan JC, Su T, Horng T, Chow A, Akira S, et al. (2008) TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-β. Nat Immunol 9(4): 361-368.

35. Lee Y, Sohn W, Kim D, Kwon H (2004) NF-kappa B- and c-Jun-dependent regulation of human cytomegalovirus immediate-early gene enhancer/promoter in response to lipopolysaccharide and bacterial CpG-oligodeoxynucleotides in macrophage cell line RAW 264.7. Eur J Biochem 271(6): 1094-1105.

36. Suri SS, Jamardhan KS, Parbhakar O, Caldwell S, Appleyard G, et al. (2006) Expression of Toll-like receptor 4 and 2 in horse lungs. Vet Res 37(4): 541-551.

37. Mc Gettrick AF, O'Neill LA (2010) Localisation and trafficking of Toll-like receptors: an important mode of regulation. Curr Opin Immunol 22(1): 20-27.

38. Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, et al. (2002) IRF3 Mediates a TLR3/TLR4-Specific Antiviral Gene Program. Immunity 17(3): 251-263.

39. Hiscott J, Alper D, Cohen L, Leblance J, Sportza L, et al. (1989) Induction of Human Interferon Gene Expression Is Associated with a Nuclear Factor That Interacts with the NF-kB Site of the Human Immunodeficiency Virus Enhancer. J Virol 63(6): 2557-2566.

40. Greenhill CJ, Rose-John S, Liissila R, Ferlin W, Ernst M, et al. (2010) IL-6 Trans-Signaling Modulates TLR4-Dependent Inflammatory Responses via STAT3. J Immunol 864(5): 1199-1208.