



# Endocytosis and human innate immunity

Xiaofeng Ding<sup>1</sup>, Shuanglin Xiang<sup>1\*</sup><sup>1</sup>Key Laboratory of Protein Chemistry and Development Biology of State Education Ministry of China, College of Life Science, Hunan Normal University, Changsha, P.R. China

## Article Info

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### \*Correspondence:

Dr. Shuanglin Xiang,

Key Laboratory of Protein Chemistry and Development Biology of State Education Ministry of China, College of Life Science, Hunan Normal University, Changsha, P.R. China;

Telephone: 86 731 88872916;

Fax: 86 731 88872792;

E-mail: xshlin@hunnu.edu.cn

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## ABSTRACT

Endocytosis is critical for normal cellular function through clearing foreign materials and protecting the host from pathogen/virus attack. Innate immune cells play important roles in specifically recognizing and degrading microbes by generating phagosomes and phagolysosomes. However, the knowledge of how innate immunity regulates endocytosis *in vitro* and *in vivo* remains limited. In this review, we attempt to systematically and comprehensively summarize our current understanding of endocytosis and the role of Rab GTPases in the innate immune system. Understanding the immunity mechanisms of endocytosis might help develop targeted therapeutics for various applications, including viral inactivation and clearance, pathogen removal and even adjuvant-enhanced antibody responses.

## Endocytosis

Endocytosis is a process in which a cell internalizes non-particulate materials such as proteins by engulfing them in an energy-dependent manner. Endocytosis generally includes pinocytosis (cell drinking), receptor-mediated endocytosis and phagocytosis (cell eating)<sup>1</sup>. Pinocytosis is fluid endocytosis of small particles suspended in extracellular fluid in most mammalian cells, and receptor-mediated endocytosis is ingestion of specific substances that bind to receptor on cell membrane in a clathrin-dependent pathway, while caveolae-, Arf-6, flotillin-1-, CDC42- and RhoA-dependent endocytosis is clathrin-independent<sup>2</sup>. Phagocytosis is generally defined as the uptake of particles (around 1µm or greater in diameter) including foreign pathogens, dead or dying cells, and other particulate debris<sup>3</sup>. Phagocytosis predominantly occurs in professional phagocytes such as macrophages (MΦs), monocytes and neutrophils. The endocytic pathway includes the specific binding of pathogen molecules to surface receptors on phagocyte and induction of actin polymerization, that drives to the material internalization to form a phagosome, fusion with late endosomes and lysosomes to sort them for degradation. Phagocytosis is a front-line defense against pathogen attack, and represents a vital facet of the innate immune response to pathogens<sup>4</sup>.

## Endocytosis and the innate immune response

Endocytosis regulates microbial infection in autoimmune and inflammatory disease and is tightly associated with innate immune cells. Macrophages, neutrophils and dendritic cells (DCs) serve as critical phagocytes to drive the innate immune response. In addition,

innate immune cells produce and release cytokines, which are critical responses to inflammation and infection<sup>5</sup>.

## Macrophages

Macrophages, the principal tissue-resident effector cells of the innate immune system, express receptors to recognize foreign material such as pathogens and viruses<sup>6</sup>. Phagocytic receptors include Fc- and complement-receptors as well as receptors for particle- or pathogen-associated molecular patterns (PAMPs) such as Toll-like receptors (TLRs)<sup>7</sup>. Phagocytic uptake of particles by macrophage cells proceeds as follows: 1) access of particles to the surface of the macrophage membrane, 2) particle recognition by phagocytic receptors on the macrophage membrane, and 3) dynamic changes in membrane structure (protrusion or invagination) (Figure 1). Simultaneously, macrophages orchestrate the inflammatory response through pattern recognition receptor-mediated responses to avoid tissue damage<sup>8</sup>.

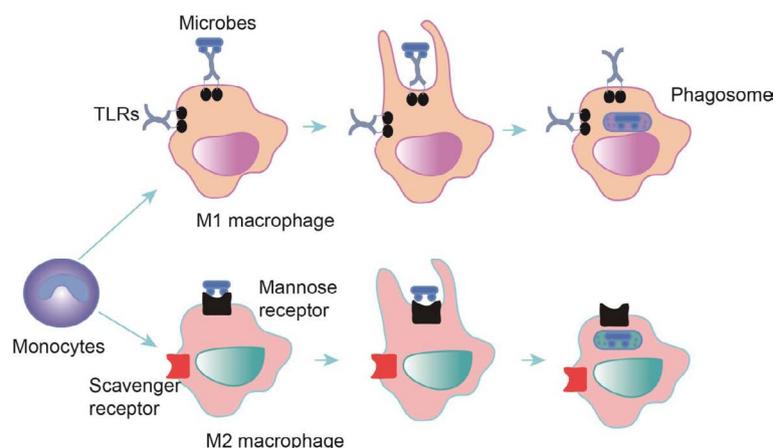
Macrophages were broadly divided into classically (M1) and alternatively (M2) activated. M1 activated macrophages are strongly positive for class II-MHC, present antigen to T lymphocytes that neutralize cells infected by microorganisms. The M1 macrophage is activated by interferon- $\gamma$ , which is produced by stimulated Th1 lymphocytes and Natural Killer (NK) cells<sup>9</sup>. The M2 macrophage is associated with Th2 activation and induced by IL-4<sup>10</sup>. M1 macrophages have a lower endocytic ability compared to M2 macrophages, approximately 20% for M1 compared to 50-100% for M2 macrophages, with IL-10-stimulated M2 macrophages displaying the highest endocytic ability<sup>11, 12</sup>. M1 activation is associated with an increase in HIV-1 endocytosis whereas the opposite effect is observed in M2 macrophage subset (M2a). However, both processes are associated with increases in virus degradation and HIV-1 endocytosis<sup>13</sup>. But pathogens have evolved immune

invasion mechanisms to survive, autophagy *pseudomonas aeruginosa* (PA) induced could reduce the formation of phagosomes and phagolysosomes, suppress bacterial internalization in macrophages<sup>14</sup>. Autophagy is a process of cellular autophagocytosis in times of nutritional stress. Autophagosomes in autophagy have many shared features with phagosomes but are unique. The autophagosome is controlled fusion of subcellular organelles with lysosomes to generate a double-membrane structure<sup>15</sup>.

## Neutrophils and DCs

Neutrophils are very important innate immune cells comprising the first line of innate immune defense against infectious diseases<sup>16</sup>. Neutrophils arrive at sites of inflammation hours before monocytes. Neutrophils also express Fc- and complement-receptors like monocytes to facilitate phagocytic movement and kill ingested bacteria<sup>17</sup>. A neutrophil is equipped with two major pathways for killing: generation of Reactive Oxygen Species (ROS), and degranulation of granules packed with proteases and specific anti-microbial peptides. The active phagocytosis by neutrophils is eventually followed by the formation of neutrophil extracellular traps (NETs) in which externalized chromatin could entangle bacteria<sup>18</sup>. Upon recognition of opsonized *Staphylococcus aureus*, neutrophils internalize the bacterium in a phagosome where secretory granule content is released and ROS are produced<sup>19</sup>. Neutrophils exert mutual cooperation on other innate immune cells, make an important contribution in the activation and recruitment of macrophages at the site of infection or acute inflammation, and generate various chemotactic factors to activate DCs<sup>20, 21</sup>.

DCs represent a class of professional antigen-presenting cells whose primary function is to present antigens to the immune system, not to clear invading microorganisms



**Figure 1. Phagocytosis in macrophages**

Monocytes differentiate into M1 and M2 macrophages, there are different stimuli for two types-IFN $\gamma$  (M1), IL-4, IL-13 and IL-10 for M2. Macrophages express specific receptors for bacterial binding, which initiates the release of cytokines. Macrophages engulf and digest bacteria to form phagosome, and phagosome is fused with a lysosome to generate phagolysosomes. Microbes are killed by enzymes and other chemicals. Indigestible materials are discharged by exocytosis.

as professional phagocytes. Mature DC lose phagocytic capacity and become potent antigen-presenting cells<sup>22</sup>. But immature DCs are highly phagocytic, efficiently and continuously sampling the antigenic content of their environment by phagocytosis of particulates. Unlike in normal tissues, DCs rather than macrophages with high MHC II levels are the major phagocytic cell in immune destruction and eventual regression of skin tumors<sup>23</sup>.

### Other innate immune cells (Mast cells, NK cells, Gamma delta T cells and eosinophils)

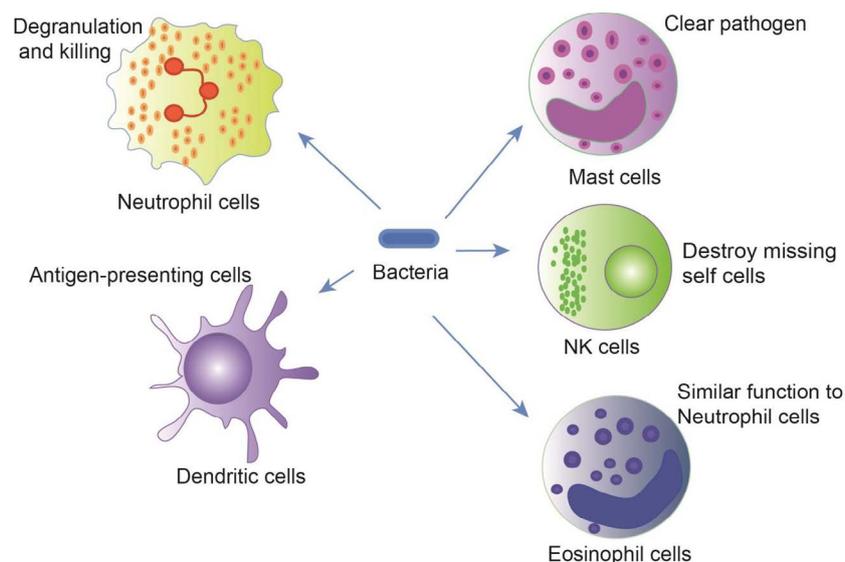
In addition, there are other innate immune cells, including Mast cells (MCs), NK cells, Gamma delta T ( $\gamma\delta$ T) cells and eosinophils (Eos). MCs are associated with wound healing and defense against pathogens. When activated, MCs detect pathogens and send danger signals and rapidly releasing characteristic granules and cytokines to promote pathogen-specific clearance and simultaneously recruit neutrophils and macrophages<sup>24</sup>. NK cells are a critical kind of cytotoxic lymphocyte to the innate immune system. NK cells recognize virus-infected cells dependent on activating KIR/Ly49 and NKG2D receptors<sup>25</sup> and destroy compromised host cells known as "missing self cells"<sup>26</sup>. Epstein-Barr virus (EBV) infection is cleared in humans by strong NK cell and possible  $\gamma\delta$ T cell responses<sup>27</sup>.  $\gamma\delta$ T cells are an important subset of the innate immunity population of T cells *in vivo*, and account for 5-15% of T cells in peripheral blood. The largest subset of human  $\gamma\delta$  T cells is the V $\gamma$ 2 (alternate V $\gamma$ 9) V $\delta$ 2 subset, large numbers of V $\gamma$ 9/V $\delta$ 2 T cells respond within hours to common molecules produced by microbes<sup>28</sup>. Eosinophilic phagocytosis is accompanied by degranulation and involves lysosomal enzymes, similar

function to neutrophil cells. However, phagocytosis by eosinophils occurs less efficiently than neutrophil phagocytosis. Whereas the mechanism of phagocytosis by eosinophils was mediated by complement receptor 1 (CD35), that of neutrophils was modulated by CD16 and CD32<sup>29</sup>. Finally, these innate immune cells synergistically function to form the sentinels at the potential portals of microbial entry<sup>30</sup> (Figure 2).

Exosomes are a type of important membrane vesicles 30-100 nm in diameter and contain various molecular constituents including protein, mRNA and miRNA, transfer molecules from one cells to another via membrane vesicle trafficking, thereby influencing the immune system<sup>31</sup>. Exosomes enter phagocytic cells via phagocytosis, move to phagosomes and further sort into phagolysosomes, in an actin- and phosphatidylinositol 3-kinase (PI3K)-dependent way<sup>32</sup>. Exosomes released from HBV-infected hepatocytes stimulated NK cells and macrophages in innate immune response against HBV<sup>33</sup>. Exosomes derived from bacterially infected macrophages could stimulate macrophages and neutrophils to secrete proinflammatory mediators, and DCs-derived exosomes are also powerful immunoregulators<sup>34</sup>. Hence, the exosome uptake through phagocytosis plays important roles in exosome-cell interactions and intracellular trafficking pathway, and shows new light on a novel class of drug delivery systems.

### Interplay between endocytosis proteins Rab and innate immunity

About 20 Rab proteins among over 70 mammalian Rab GTPases are regulators of endocytosis. The Rab5 subfamily (Rab5, Rab21, and Rab22) are localized to early endosomes



**Figure 2. Phagocytosis in main innate immune cells**

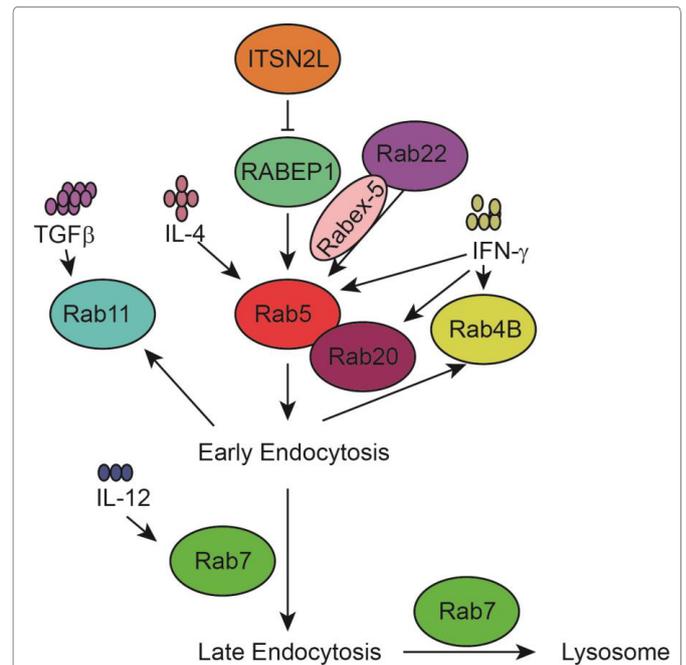
The innate immune cells include mast cells and natural killer cells, phagocytes (monocytes, macrophages and dendritic cells), and the granulocytes (neutrophils, eosinophils and basophils). Each cell type is equipped with different mechanisms to attack and eliminate pathogens from the host, but these innate immune cells could synergistically function to combat microbial entry.

and plasma membrane and regulate early endocytosis and signal transduction. Rab5 has a critical role in controlling both phagosome-early endosome fusion and the maturation of phagosomes into degradative compartments. In one respect, IL4 and 6 upregulate Rab5 expression<sup>35</sup>. IL-4 also prolongs the retention of Rab5 on phagosomes in a PI3K-dependent manner<sup>36</sup>. In the other respect, macrophages stimulated with IFN- $\gamma$  show an increase of Rab5a and partner Rab20 expression in phagosome maturation<sup>37, 38</sup>. Moreover, IFN- $\gamma$  produced by NK and NKT cells is critical for combating viral and bacterial infections and contributes to macrophage activation by increasing phagocytosis and producing cytokines<sup>39</sup>. Rab22 recruits Rabex-5 (a Rab5 GDP/GTP exchange factor (GEF)) to early endosomes for activation of Rab5 and stimulation of early endosome fusion<sup>40</sup>. Rab proteins interact with different effectors and regulators, and RABEP1 (Rab GTPase-binding effector protein 1) functions as a vital regulator and molecular switch for Rab5 function. We previously found that Intersectin 2 long isoform (ITSN2L) interacts with RABEP1 and stimulates the degradation of RABEP1 to regulate endocytosis and endosome trafficking<sup>41</sup>. Therefore, we speculate that cytoskeletal protein ITSN2L might regulate central Rab5 by inhibiting the function of regulator RABEP1 to interfere with early endocytosis and innate immune response, which will be further investigated in our future studies.

The early endosome segregate molecules are sorted to plasma member through fast (Rab4, Rab14, Rab15) and slow (Rab11a, Rab15, Rab22a) recycling routes<sup>42</sup>. GTPase subsets (Rab14, Rab20, Rab22a, Rab32, Rab34, Rab38, Rab39, Rab43) interact to regulate phagosome formation<sup>42, 43</sup>. Rab32 formed a persistent complex with two interacting proteins, Prohibitin (PHB) and PHB2, to encompass bacteria during early phagosome formation<sup>44</sup>. Rab7 and Rab34 regulates the fusion with late endocytic compartments in late phagosomes<sup>45</sup>. IL-12 is naturally produced by DCs, macrophages, neutrophils, and human B-lymphoblastoid cells in response to antigenic stimulation<sup>46</sup>. IL-12 then upregulates Rab7 expression by activating p38 mitogen-activated protein kinase (p38 MAPK) in intracellular trafficking<sup>47</sup>. Taken together, a network of Rab cascades regulate certain critical steps of innate immune responses. Rab5 and Rab7 are the best characterized Rab proteins in endocytosis (Figure 3), the way that most of GTPases function in pathogen control by immune cells remains poorly defined.

### Perspectives

Endocytosis has been studied for over a century since Metchnikoff put it forward in 1882. Thus, it has become clear that endocytosis regulates normal cell physiology linked with important innate immune responses. But many aspects are not yet well described. Especially, (1)



**Figure 3. Rab5/7 proteins and immune factors in endocytosis**

Early endocytosis is mainly regulated by Rab5. RABEP1 functions as a vital regulator for Rab5. ITSN2L interacts with RABEP1 and stimulates RABEP1 degradation. Rab22 recruits Rabex-5 to early endosomes for activation of Rab5. IFN- $\gamma$  increases the expression of Rab5 and partner Rab20 in phagosome maturation. Indigestible molecules can return to the plasma membrane via fast (Rab4B) or slow (Rab11) recycling routes. Late endosome and phagosome fusion with lysosomes is promoted by another critical Rab GTPase, Rab7, which is induced by IL-12.

to understand many disease pathology it is necessary to know more about the basic cell biology of endocytosis. The dynamics interplay between basic science and disease-targeted research is expected to yield new findings and novel therapies in future. (2) More studies are still required to define endocytosis using genomics and proteomics techniques, and high-throughput screen signal pathways in Rab cascades and innate immune signaling to identify specific targets and interactions for important innate immune responses and immune evasion of many pathogens. (3) With the rapid development of advanced technology, it is possible to intensively investigate endocytosis from *in vitro* studies to *in vivo* models. There are only a few examples about the effect of the immune-regulatory role on endocytosis *in vivo*<sup>48, 49</sup>. We expect that a better understanding of the regulation of endocytosis on innate immune response may provide a basis for the development of immunotherapeutic approaches to design new drugs or approved existing drugs to disrupt pathogen growth or infection and inhibit chronic viral infections.

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### Conflict of interest

There is no conflict of interest.

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