Review

Cell PRESS

Initiation of Infection

Virus interactions with endocytic pathways in macrophages and dendritic cells

Jason Mercer[1] and Urs F. Greber[2]

[1] Eidgenössische Technische Hochschule (ETH) Zürich, Institute of Biochemistry, Schafmattstr. 18, CH-8093, Zürich, Switzerland
[2] University of Zürich, Institute of Molecular Life Sciences, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Macrophages and dendritic cells (DCs) are at the front line of defence against fungi, bacteria, and viruses. Together with physical barriers, such as mucus and a range of antimicrobial compounds, they constitute a major part of the intrinsic and innate immune systems. They have elaborate features, including pattern recognition receptors (PRRs) and specialized endocytic mechanisms, cytokines and chemokines, and the ability to call on reserves. As masters of manipulation and counter-attack, viruses shunt intrinsic and innate recognition, enter immune cells, and spread from these cells throughout an organism. Here, we review mechanisms by which viruses subvert endocytic and pathogen-sensing functions of macrophages and DCs, while highlighting possible strategic advantages of infecting cells normally tuned into pathogen destruction.

The cell biology of virus infection

The use of cell biological and molecular techniques has enabled virologists to understand the interplay between viruses and cells at the heart of the infection process. This is also shown in a recent surge of system-wide approaches, such as high-throughput RNAi screening, functional genomics, or large-scale proteomics (for reviews, see [1–4]). Although these approaches are informing us about interactions between viruses and their host cells, for technical reasons, they have been performed in cell lines that may not be the major targets during natural infections. During the past few years, there has been a push towards novel approaches and the use of primary cell lines or tissue culture systems that more accurately reflect the situation viruses encounter in an organism. This must involve the consideration of phagocytic cells, such as macrophages and DCs. A recent example for a nonautonomous action of macrophages in viral entry has been the finding that apical entry of human adenovirus (HAdV) into polarized epithelial cells is facilitated by macrophage-derived chemokines, such as chemokine (C-X-C motif) ligand 8 coxsackievirus adenovirus receptor (CXCL-8) [5]. These cytokines are released from macrophages upon contacting the virus. On highly polarized epithelial cells, the cytokines trigger a redistribution of the viral entry receptors CAR and integrins from the basolateral to the apical membrane, thereby enabling infection from the luminal side of airways.

The professionals: macrophages and DCs

Why do macrophages and DCs take centre stage for viral infections? These cells are specialized immune cells located throughout the body. By virtue of their fast chemotactic movements to sites of infection, they are often the first to encounter, identify, and engulf invading pathogens [6–10]. They decode the nature of the pathogen, and distinguish between soluble components of pathogens and the agents themselves, hence tuning inflammatory responses [11]. Professional phagocytic cells generate reactive oxygen species highly toxic for pathogens, or they perforate membranes or digest invading pathogens by hydrolytic enzymes.

The immune surveillance function of macrophages and DCs crucially depends on highly active endocytic processes (for reviews, see e.g., [7,12]). This allows them to not only engulf and digest invading pathogens, but also present the products on their cell surface to T lymphocytes using major histocompatibility complex (MHC) molecules (reviewed in [11,13]). T cells themselves produce soluble mediators, such as interferon gamma, which activate and enhance macrophage killing ability [14].

Macrophages and DCs fulfil two major functions in immunity, an innate and an adaptive function. Through efficient ingestion and destruction, pathogens are eliminated from circulation within the body, and second, the displayed antigens act in the initiation of an adaptive immune response. These events involve the production of cytokines, chemokines or reactive oxides, recruitment of other immune cells, and regulation of T cell activity and inflammation in response to the detected pathogen [11,15,16]. In the case of DCs, these functions often overlap between subsets, with classical DCs being more efficient professional antigen-presenting cells (APCs) and plasmacytoid DCs (pDCs), playing the role of the primary producer of type I interferon and proinflammatory cytokines in response to viral infection [17].

Corresponding authors: Mercer, J. (jason.mercer@bc.biol.ethz.ch); Greber, U.F. (urs.greber@imls.uzh.ch).
Keywords: endocytosis; macropinocytosis; receptors; trans-infection; macrophage; dendritic cells.

0966-842X/$ – see front matter
 http://dx.doi.org/10.1016/j.tim.2013.06.001

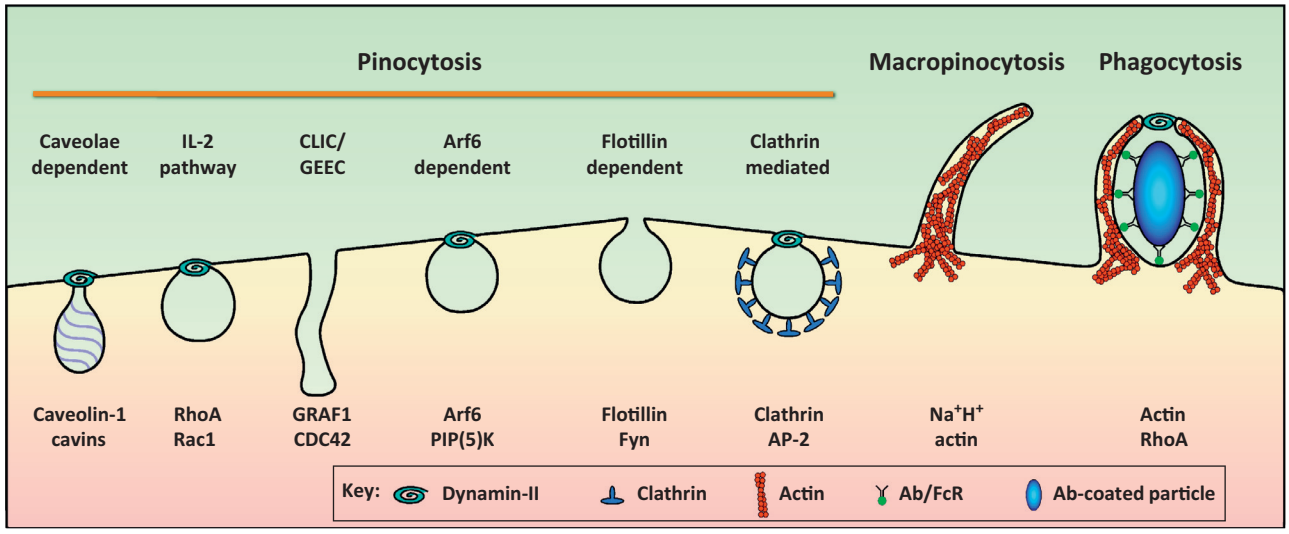

Figure 1. Endocytic mechanisms. The endocytic activities executed by cells are classified into pinocytic, macropinocytic, or phagocytic mechanisms. The various pinocytic mechanisms are classified based on their cellular requirements, that is, dynamin or actin. For each mechanism, the major players are listed (in bold). A major fraction of studies have been conducted to understand clathrin-mediated, macropinocytic, and phagocytic mechanisms in nonprofessional phagocytes; professional phagocytes, however, may use the full range of endocytic mechanisms. Although not fully understood, it is likely that viruses have learned to subjugate the majority of these pathways for infection of both professional and nonprofessional phagocytes. *Abbreviations*: AP-2, adaptor protein 2; RhoA, Ras homolog gene family member A; Rac1, Ras-related C3 botulinum toxin substrate 1; GRAF1, GTPase regulator associated with focal adhesion kinase-1; CDC42, cell division control protein 42 homolog; Arf6, ADP ribosylation factor 6; PIP(5)K, phosphatidyl-inositol-5-phosphate kinase; Fyn, a member of the protein tyrosine kinase oncogene family; Ab, antibody; FcR, fragment constant receptor; IL-2, interleukin-2; CLIC, clathrin-independent carriers; GEEC, GPI-anchored-protein-enriched endosomal compartment.

Endocytic mechanisms in macrophages and DCs

Macrophages and classical DCs, referred to as DCs throughout, are professional phagocytes with exceptionally high endocytic activity. Besides phagocytosis, they carry out pinocytosis, such as clathrin- and non-clathrin-mediated endocytosis and macropinocytosis (Figure 1). These endocytic machineries are elaborate and deeply interconnected with signalling and the physiology of the particular cell type (for excellent reviews, see [18–22]).

The best understood endocytic process is clathrin-mediated endocytosis (CME). It is strictly receptor-dependent and requires the coat protein clathrin, and the large GTPase dynamin for fission and release of clathrin-coated vesicles from the plasma membrane [23,24]. Clathrin-independent mechanisms in turn give rise to distinct endocytic pathways generally independent of dynamin, as schematized in Figure 1 (for reviews, see [25,26]). Some of these pathways have been implicated in repair processes, for example upon membrane insults [27].

Among the clathrin-independent pathways is macropinocytosis, a signal-induced transient actin-dependent process [18,28]. In macrophages and DCs, however, macropinocytosis is wired as an on-going constitutive process [29,30]. It is coupled to the migratory phenotype of these cells and is essential for their immune function. Macropinocytosis is strictly dependent on actin and Na^+/H^+ exchangers. Na^+/H^+ exchangers rectify the cytosolic acidic pH proximal to activated tyrosine kinase receptors near the plasma membrane, and thereby relieve inhibition of guanine nucleotide exchange factors of Rho family GTPase [31].

Phagocytosis is mechanistically similar to macropinocytosis, albeit, unlike macropinocytosis, dependent on dynamin and a close apposition of a multivalent ligand with plasma membrane receptors [28,32,33]. It is key for maintaining tissue homeostasis and balancing innate and adaptive immune responses to bacterial and fungal infections (Box 1). Given that phagocytosis is specified for particles typically larger than 0.5–1 μm [34,35], viruses smaller than 0.5 μm are taken up by endocytosis rather than phagocytosis. Notable exceptions are reports for herpes simplex virus 1 (HSV-1) and Mimivirus [36,37], or HAdV-C2 aggregated by soluble receptor fragments and targeted to the high affinity Fc receptor CD64 of human monocytes [38]. The vacuolar compartments formed during pinocytosis, macropinocytosis, and phagocytosis are classical endosomes, macropinosomes, and phagosomes, respectively. They follow similar maturation programs controlled by Rab GTPases and signalling phosphoinositide lipids (Figure 2) [18,33,39].

Despite the similarities between these processes, we should keep in mind that pinocytosis, macropinocytosis, and phagocytosis are tuned by specific machineries emerging from divergent receptor engagements and responses of subcellular networks [11,40]. This leads to different forms of information processing and physiological reactions.

Virus entry into macrophages and DCs

Virus entry requires distinct cellular processes, typically attachment to receptors, signalling and movements of viruses on the cell surface, endocytic uptake and trafficking, and finally penetration and genome uncoating (Figure 3) [41–43]. Despite their armaments, macrophages and DCs are not immune against viruses, and both DNA and RNA viruses can infect macrophages and/or DCs (Table 1). This is not surprising because viruses have co-evolved with their hosts [44]. Mammals, for example, do not exclusively rely on macrophages and DCs for defence, but possess other potent pathogen killers, such as neutrophils and natural killer cells, which mount rapid responses against infected cells [34,45].