Immune Evasion Strategies of Pathogens in Macrophages: the Potential for Limiting Pathogen Transmission

Yuwei Ren[1], Faheem Ahmed Khan[1], Nuruliarizki Shinta Pandupuspitasari[1] and Shujun Zhang[1]*

[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agriculture University, Hubei Wuhan 430070, China.
*Corresponding author: Tel: +86 27 87280020, 15071338061; Fax: +86 27 87281813
E-mail: sjxiaozhang@mail.hzau.edu.cn

DOI: http://dx.doi.org/10.21775/cimb.021.021

Abbreviations

Bb: *Borrelia burgdorferi*
CMI: cell-mediated immune responses
CRs: complement receptors
E. faecalis: *Enterococcus faecalis*
EboV: Ebola virus
GPI: glycosylphosphatidylinositol
HIV-1: human immunodeficiency virus type 1
IAV: influenza A virus
IFN: interferon
IKK: I-kappa B kinase
IL-10: interleukin 10
IPS-1: IFN- beta promoter stimulating factor 1
IRF3/7: IFN-regulatory factor 3 and 7
ISGs: interferon-stimulated genes
JEV: Japanese encephalitis virus
LAM: Lipoarabinomannan
LAMP-1: lysosome-associated membrane protein 1
M. tuberculosis: Mycobacterium tuberculosis
M1: classically activated macrophages
M2: alternatively activated macrophages
ManLAM: Mannosylated lipoarabinomannan
MAP: *Mycobacteria avium* subsp. Paratuberculosis
MAPK: mitogen-activated protein kinase
MAVS: mitochondrial antiviral signaling protein
MDA5: melanoma differentiation-associated protein 5
MHC-II: major histocompatibility factor class-II
MR: mannose receptor
MyD88: myeloid differentiation primary response gene 88
NDGA: nordihydroguaiaretic acid
NF-κB: nuclear factor kappa-light chain-enhancer of activated B-cells
NSP1: Nonstructural protein 1
PI3P: phosphatidylinositol- 3-phosphate
PIV5: parainfluenza virus 5
PRRs: pattern recognition receptors
PRRSV: Porcine Reproductive and Respiratory Syndrome Virus
PTMs: posttranslational modifications
RIG-I : Retinoic acid-inducible gene I
RLR: RIG-I like receptors
S. typhimurium: *Salmonella typhimurium*
SHP-1: Src homology region 2 domain-containing phosphase-1
Sifs: *Salmonella*-induced filaments
TBK1: TANK-binding kinase 1
TGFβ: transforming growth factors beta
Th1: T helper type 1
TLR: Toll-like receptor
TNF-α: tumor necrosis factor alpha
TRAF3: TNF receptor associated factor 3
V-ATPase: vacuolar H+-ATPase
VISA: vancomycin intermediate staphylococcus aureus
Vpr: regulatory viral protein
VSV: vesicular stomatitis virus

Abstract

Preventing pathogen transmission to a new host is of major interest to the immunologist and could benefit from a detailed investigation of pathogen immune evasion strategies. The first line of defense against pathogen invasion is provided by macrophages. When they sense pathogens, macrophages initiate signals to inflammatory and pro-inflammatory cytokines through pattern recognition receptors (PRRs) subsequently mediating phagocytosis and inflammation. The macrophage immune machinery classically includes two subsets: the activated M1 and the activated M2 that respond accordingly in diverse immune challenges. The lipid and glycogen metabolic pathways work together with the lysosome to help the mature phagosome to degrade and eliminate intracellular pathogens in macrophages. The viral evasion strategies are even more complex due to the interplay between autophagy and apoptosis. However, pathogens evolve several strategies to camouflage themselves against immune responses in order to ensure their survival, replica-

tion and transmission. These strategies include the muting of PRRs initiated inflammatory responses, attenuation of M1 and/or induction of M2 macrophages, suppression of autophagolysosomal formation, interference with lipid and glycogen metabolism, and viral mediation of autophagy and apoptosis cross-talk to enhance viral replication. This review focuses on pathogen immune evasion methods and on the strategies used by the host against camouflaged pathogens.

Introduction

The discovery of the phagocytosis function of macrophages in 1905 (Nathan, 2008) has resulted in much research on the roles and functions of macrophages in human and animals. Macrophages are now recognized as key players in the innate and adaptive immune responses and their presence is recorded in almost all tissues (Mosser and Edwards, 2008). Macrophages are able to phagocytize, migrate, secret cytokines, present antigens and produce inflammatory responses (Nelson et al., 2012). Once stimulated by viral pathogens, the type I interferon (IFN) expression is induced by Retinoic acid-inducible gene I (RIG-I) signaling and the Toll-like receptor (TLR)-dependent pathways, and is complemented by antiviral cytokines (Takeuchi and Akira, 2010). The internalized pathogens experience a series of steps leading to degradation in macrophages (Kornfeld 1986), that recruits inflammatory molecules around the infected area (Weng and Schuppan 2013; Sanjurjo 2015). The modulation of apoptosis and autophagy is also a typical means of macrophage defense against pathogens (Lai et al., 2015). These actions are intertconnected and are involved in different complex immune responses that cooperate with each other (Galluzzi et al., 2012).

Several bacterial and viral pathogens are able to evade the immune responses and turn the macrophages into a safe haven for replication. A typical example of such interaction is Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) that causes serious reproduction and respiratory problems in sows. PRRSV can inhibit the innate immune response by suppressing type I IFN expression (Sagong and Lee, 2011). *Mycobacteria avium* subsp. Paratuberculosis (MAP) causes chronic intestinal inflammation in cattle. These bacteria can survive and replicate in intestinal macrophages and can prevent recognition by other immune cells (Kuehnel et al., 2001). *Borrelia burgdorferi* (Bb) causes a tick-borne illness called Lyme disease (LD). The pathogen can inhibit the activation and proliferation of macrophages with the help of tick salivary protein (Gwakisa et al., 2001). There are several reports of viral and bacterial pathogens that have evolved to evade the host immune systems specifically by macrophage degradation. This review aims to provide a basic understanding of different evasion mechanisms of bacterial and virus infection in macrophages and methods in which a host can activate its immune responses against camouflaged pathogens.

Inhibition of PRRs-mediated immune response

Macrophages possess pattern-recognition receptors (PRRs) that recognize pathogens and non-self molecules. Two major distinct PRRs families, the RIG-I like receptors and TLRs serve as essential components of immune responses in macrophages. Upon recognition, macrophages rapidly launch an innate immune response characterized by inducing the expression of type I IFN, interferon-stimulated genes (ISGs), and other proinflammatory molecules to fight against pathogen invasion (Ivashkiv and Donlin, 2014). RIG-I like receptors (RLR) are important for detecting RNA virus in almost all immune cell types, including macrophages, and dendritic cells (Kato et al., 2005). The members of RLR family, RIG-I and melanoma differentiation-associated protein 5 (MDA5)(Figure 1), recognize structurally-distinct dsRNA viruses (Schlee, 2013), where both RIG-I and MDA5 play an important role in sensing RNA viruses, such as flaviviruses, paramyxoviruses, and reoviruses (Goubau et al., 2013). During this process, upon the combination of viral RNA, RIG-I and MDA5 bind to their common downstream adaptor mitochondrial antiviral signaling protein (MAVS)(also known as IFN- beta promoter stimulating factor 1 (IPS-1) or vancomycin intermediate staphylococcus aureus (VISA)), which activates several kinases of I-kappa B kinase (IKK) family through TNF receptor associated factor 3 (TRAF3) and TRAF6. IKK family comprises of IKKα/β/γ/ε and TANK-binding kinase 1 (TBK1), where its activation leads to the phosphorylation of IFN-regulatory factor 3 and 7 (IRF3/7) and nuclear factor kappa-light chain-enhancer of activated B-cells (NF-κB), which then stimulates the IFN transcription (Figure1). Subsequently, IFNs is secreted and binds to the corresponding receptors on the

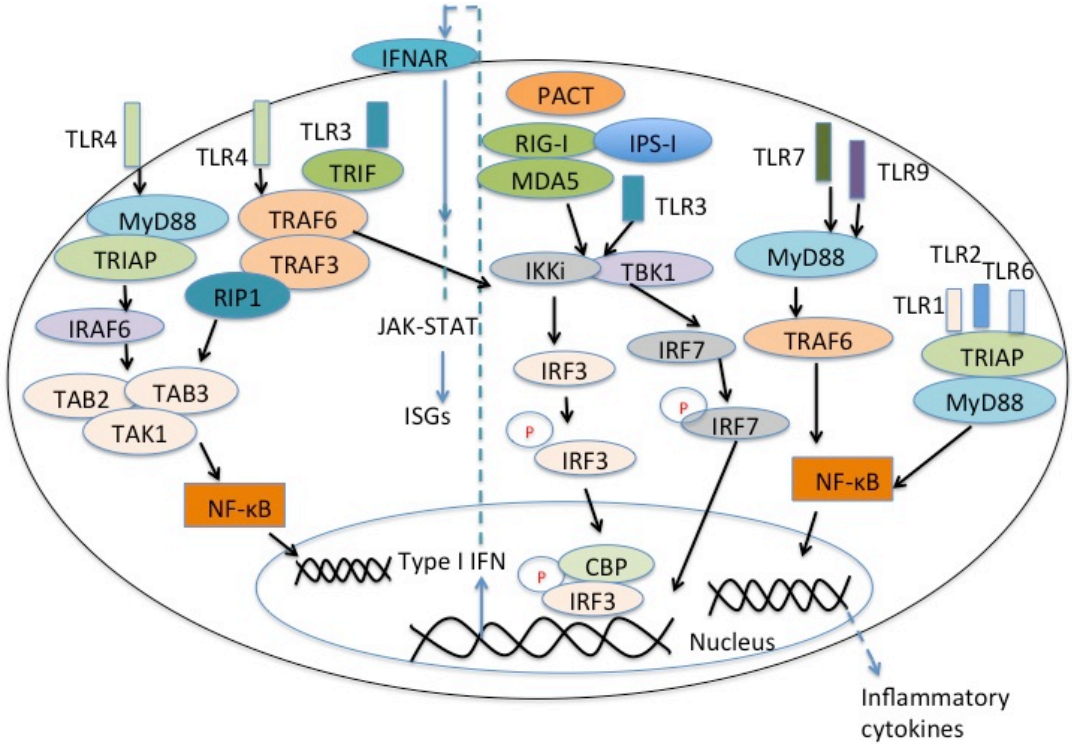

Figure 1. RIG-I and TLRs pathway are two major signaling pathways that mediated type I IFN and inflammatory cytokines expression. RIG-I combines with IPS-1 and recruit IKKi and TBK1, the compounds activate IRF3 and IRF7, leading to their phosphorylated and nuclear translocation, which stimulate type I IFN expression. Secreted type I IFN bind with the receptors IFNAR on the surface of the cell membrane, then promote ISGs expression through JAK-STAT pathway to exert antiviral function. Activation of TLR4 pathway could be mediated by both TRIF and MyD88, which stimulate type I IFN and inflammatory cytokines expression via IRF3 and NF-κB respectively. TLR3 combine with TRIF to activate NF-κB or recruit IKKi and TBK1 to promote type I IFN expression, both TLR7 and TLR9 stimulate NF-κB through recruitment of MyD88, and TLR1, TLR2 and TLR6 could activate by interacting with MyD88 and TRIAP.

cellular membrane and induce the expression of ISGs to stimulate antiviral responses through JAK-STAT pathway (Chiang et al., 2014).

Though PRRs-mediated immune responses important for the clearance of pathogens, several viruses are able to evade immunity to survive and proliferate in macrophages. PRRSV, the causation of PRRS characterized by the serious reproductive failure in pregnant sows and severe respiratory distress in piglets and growing pigs, can inhibit type I IFN expression, especially IFN-α and IFN-β (Sagong and Lee, 2011). Nonstructural protein 1 (NSP1) of PRRSV might be responsible for the inhibition through blocking of IRF3 nuclear translocation (Beura et al., 2010; Sagong and Lee, 2011). Additionally, vesicular stomatitis virus (VSV) infection induces the expression of miR-146a in macrophages, and then miR-146a can function as a negative-feedback regulator of the RLR pathway by targeting TRAF6 (Hou et al., 2009). The increased miR-4661 can decrease IFN-α expression by directly binding to the 3'UTR of IFN-α mRNA during VSV infection (Li et al., 2012). Besides direct regulation by viruses, many viruses have been demonstrated to regulate RLR by post-translational modifications (PTMs). For example, two members of the paramyxovirus family, measles and Nipah viruses, can dephosphorylate RLR and their V protein as act as an IFN antagonist by keeping RLR in an inactive state (Davis et al., 2014). V proteins of parainfluenza virus 5 (PIV5) antagonize MDA5 through interaction with the helicase domain to inhibit its ATPase activity (Motz et al., 2013). Similarly, Ebola virus (EboV) and NS1 of influenza A virus (IAV) specifically block the activation of IFN-I by binding with PACT, which can interact with RIG-I and serve as an important cofactor for the IFN-I re-

sponse (Luthra et al., 2013; Tawaratsumida et al., 2014).

Besides RLRs, Toll like receptor (TLR) also participates in the innate immune response and is widely expressed on the immune cells (Figure 1). In macrophages, TLR4 triggered inflammatory responses via recruitment of the myeloid differentiation primary response gene 88 (MyD88) activates NF-κB when stimulated with LPS (Cheng et al., 2015). At the cellular level, TLR4 regulates macrophage apoptosis and its differentiation to foam cells (Howell et al., 2011; Feingold et al., 2012). Additionally, TLR7 can limit the pro-inflammatory activation induced by TLR2 and TLR4 ligands in macrophages (Salagianni et al., 2012). Based on different recruited adaptor molecules, TLR1, TLR2, TLR4, TLR5, TLR6, TLR7 and TLR9 signaling is directly or indirectly mediated by the MyD88 pathway, whereas TLR3 signaling is achieved through TRIF, and TLR4 regulated type I IFN expression via both MyD88 and TRIF pathways (O'Neill and Bowie, 2007; Barton and Kagan, 2009, Khan et al., 2015). Upon stimulation, the MyD88-dependent pathway results in the inflammatory responses via NF-κB mitogen-activated protein kinase and IFN regulatory factors, cell proliferation and differentiation. The TRIF-dependent pathway also induces the activation of NF-κB and IFN regulatory factors to stimulate type I IFN expression (Kumar et al., 2011)(Figure 1).

Several TLRs function as antiviral and antibacterial mediators in the immune system, for exampe TLR9 recognizes and binds to microbial DNA and provide defense against bacterial infection (Bafica et al., 2005). In contrast, a few TLRs act as immunosuppressive factors to promote pathogen infection. Specifically, several pathogens can suppress type I IFN and pro-inflammatory cytokine expression through TLR signaling. For example, the prevention of IFN-β and pro-inflammatory cytokines occurs by blocking TLR4 mediated MyD88- and TRIF- dependent pathways during *Yersinia pseudotuberculosis* infection (Rosadini et al., 2015). The strategy for *Mycobacterium tuberculosis* survival in macrophages is achieved by the induction of interleukin 10 (IL-10), which is driven by TLR2-dependent pathway (Richardson et al., 2015). IL-10, known as an anti-inflammatory cytokine, is able to inhibit pro-inflammatory cytokines IL-12 and major histocompatibility factor class-II (MHC-II). Mannosylated lipoarabinomannan (ManLAM) expressed by *M. tuberculosis*, also participates in the immune evasion by reducing the TLR-mediated proinflammatory cytokines of macrophages, such as tumor necrosis factor alpha (TNF-α) and IL-12, and increases a tyrosine phosphatase of Src homology region 2 domain-containing phosphase-1 (SHP-1), which suppress macrophage immune responses (Knutson et al., 1998; Nigou et al., 2001). Besides the regulation of proinflammatory cytokines, MAP can also use the TLR2-dependent pathway to inhibit the killing and degradation ability of macrophages through modulation of phagosome acidification and maturation (Weiss et al., 2008). Additionally, *Brucella abortus* adapts to the microenvironment of the alveolar macrophage by modulation of the TLR2-dependent pathway mediated inflammatory cytokines to a modest degree (Ferrero et al., 2014).

Therefore, PRRs expressed on the cell surface of macrophages are important in the recognition of viruses and bacteria, which is the beginning of the stimulation of inflammatory cytokines and antiviral factors expression (Figure 1). However, pathogens are able to escape from the PRRs mediated inflammation by inactivating NF-κB, IRF3/7 and other molecules, as well as the utilization of several receptors to attenuate the inflammation to a modest degree.

Modulation of polarized macrophages

Macrophages can differentiate into distinct functional subsets in response to different inflammatory signals. In different microenvironments, macrophages are mostly segmented into classically activated (M1) macrophages and alternatively activated (M2) macrophages based on their different receptors expression, cytokines production and inflammatory responses (Benoit et al., 2008)(Figure 2). M1 macrophages are activated by bacteria, IFN-γ and other Th1-induced immune factors, and secrete pro-inflammatory cytokines and MHC-II to exert acute immune response, while M2 phenotype macrophages express cytokines such as IL-10, transforming growth factors beta (TGFβ), and mannose receptor (MR), and show anti-inflammatory responses and promote tissue repair and remodeling (Sica et al., 2012). M2 macrophages are composed of a wide spectrum of macrophages that serve various functions, and are divided into M2a, M2b and M2c phenotypes (Mosser and Edwards, 2008; Walker et al., 2015). M1 macrophages are associated with T

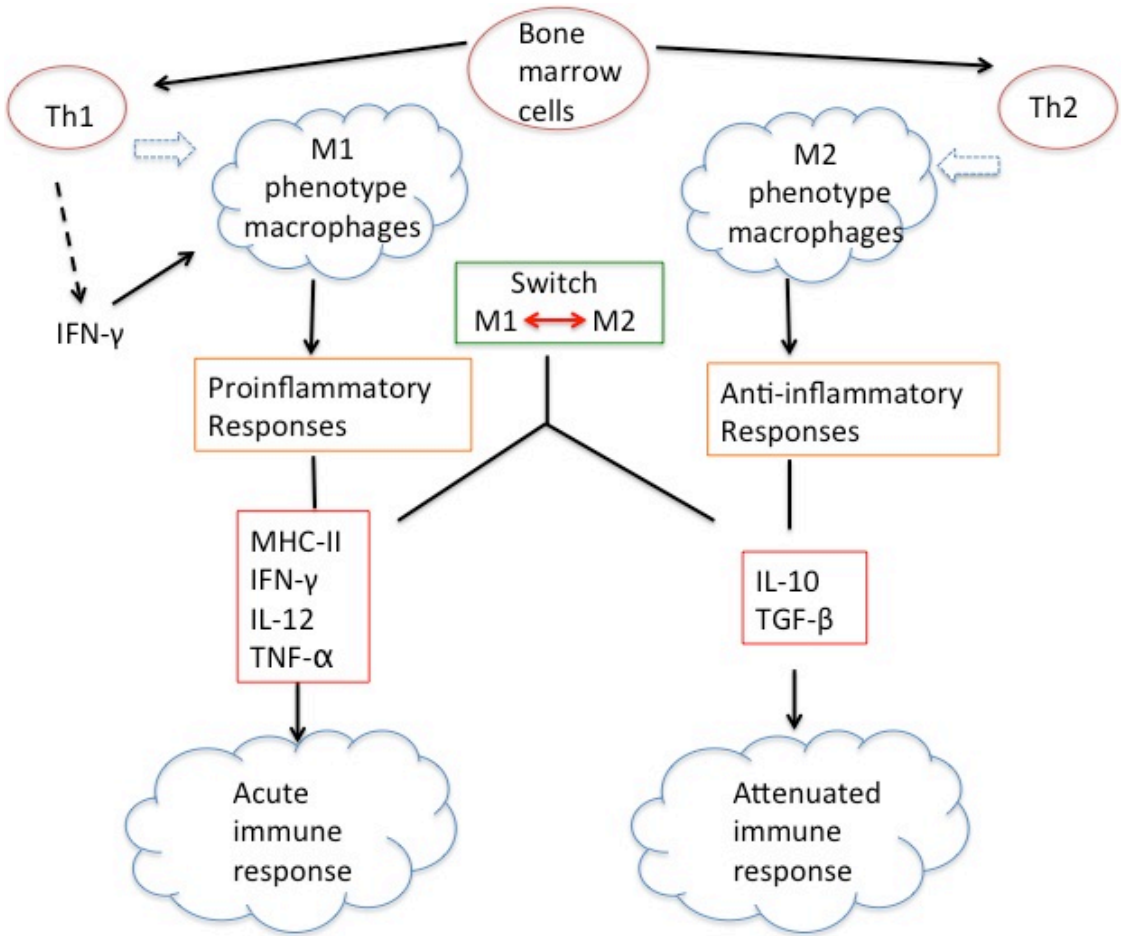

Figure 2. The differentiation of macrophages and related functions. Macrophages are differentiated into two major phenotypes, including M1 and M2 macrophages, which mirror the Th1 and Th2 polarization, and present proinflammatory and anti-inflammatory responses, respectively. M1 macrophages express proinflammatory cytokines, such as MHC-II, IFN-γ, IL-12 and TNF-α to activate immune responses, while M2 macrophages express anti-inflammatory cytokines, such as IL-10 and TGF-β to inhibit immune responses. The two phenotypes of macrophages could switch to each other in different diseases.

helper type 1 (Th1) responses, that serve cell-mediated immune responses (CMI), and produce IFN-γ, IL-2, TNF-α, and IFN-γ to kill and clear the intracellular pathogens and foreign materials (Sica et al., 2012). Th2 cells guide M2 induced-attenuated generation of IL-4, IL-5 and IL-10 and down-regulate the expression of TLR2 and IL-12 (Galli et al., 2011). However, there is no consensus that whether these subsets of macrophages exist as simple functional states or distinct phenotypes, but it is confirmed that one function of the macrophage can switch into another and functional changes can occur during disease development (Galli et al., 2011)(Figure 2). However, the release of pro-inflammatory cytokines, such as TNF-α, IL-12, IL-23, can in turn regulate T cell polarization and macrophage differentiation. The feedback product of Th1 cells, IFN-γ, drives the shape of M1 macrophages and stimulate their antimicrobial activity, whereas the suppression of IFN-γ and the activation of transforming growth factor beta (TGFβ) is considered as a switch from Th1 to Th2 type immune response, resulting in the ineffective clearance of intracellular mycobacterial infections (Ma et al., 2015).

Several bacteria can affect the polarization of macrophages. During MAP infection, the target macrophages express a high level of IL-10 but a low level of IFN-γ (Magombedze et al., 2015), suggesting that MAP inhibited the M1 macrophage state and sustained the cell at the M2 state for its own survival. ManLAM from *M. tuberculosis* was shown to be involved in the inhibition of IFN-γ-induced macrophage activation and the stimulation of TGFβ, which led to the switch from M1 to M2 (Adams et al., 1993; Takeuchi et al., 1998). However, the LPS polarized macrophage type is a controversy. LPS infection is associated with the WNT pathway-mediated switch of macrophage phenotype, which results in β-catenin activation and accumulation but does not show inflammatory cytokine production (Thiele et al., 2001). However, LPS can induce IL-1β and IL-6 expression to skew macrophage polarization towards the M1 phenotype (Bode et al., 2012). This undefined differentiation might be due to the dynamic changes of microenvironment with LPS infection. As well as bacteria, most monocytotropic viruses may affect macrophage polarization that in turn leads to the selection between immunosuppression and immunopathology. Human immunodeficiency virus type 1 (HIV-1) infection is able to drive cells toward a M1-like status, which increases production of M1-related markers such as CCL3 and CCL5 and decreases secretion of M2-associated cytokines including CD206 and IL-10. However, the HIV-1 polarized M1-like macrophages possess less antimicrobial activity and cause more serious inflammation than typical M1 macrophages (Alfano et al., 2013; Cassetta et al., 2013). Several viruses, including hepatitis C, measles virus and PRRSV have been demonstrated to up-regulate IL-10 expression of monocytic cells, and increased IL-10 secretion was detected during the period of viremic persistence caused by HIV-1 infection (Richter et al., 2013; Boehler et al., 2014; Zdrenghea et al., 2015).

Pathogens are able to take advantages of various functions performed by different phenotypes of macrophages (Figure 2). In particular, M1 guides acute inflammatory responses, and secretes IFN-γ, IL-2 and TNF-α factors that contribute to the clearance of intracellular pathogens, while M2 drives attenuated inflammation and expresses IL-10 and TGFβ, which inhibit the immune responses in macrophages and are beneficial for the survival of pathogens. Thus pathogens tend to drive the differentiation of macrophages into the M2 phenotype and weaken the inflammation response.

Regulation of the phagosomal maturation

In general, following phagocytosis by the macrophages, bacteria can fuse with the cell membrane to form bacteria-containing phagosomes. These undergo a step-wise development through progressive changes in the membrane, to combine with early endosomal vesicles; Rab5 (a small GTPase) is a marker of this step. After a series of steps they become acidic, resulting in the departure of Rab5 and acquirement of Rab7, another GTPase, to form the late phagosome, which then fuses with lysosome to create phagolysosome, generating a mature microenvironment in the macrophage that finally kills and destroys bacteria (Koul et al., 2004)(Figure 3). Furthermore, lysosome plays many essential roles within cells, such as antigen presentation, inflammatory responses, signal transduction and autophagy, that contribute to immune responses of pathogens degradation and clearance (Parkinson-Lawrence et al., 2010). However, several pathogens, such as *M. tuberculosis*, a major cause of intestinal disease mortality around the world, can disrupt the phagosome maturation and inhibit the phagosome-lysosome fusion to avoid being killed by the macrophages (Goren et al., 1976). In particular, the cholesterol of *M. tuberculosis* can inhibit phagosome maturation by inhibiting the activation of Rab 7 (Huynh et al., 2008). Similarly, *M. tuberculosis*, a major cause of bovine bowel diseases, is able to segregate early endosome from the late endosomal network to inhibit macrophage maturation, and stay safely in early endosome, but it still can communicate with the intracellular microenvironment and acquire nutrients from outside the phagosomes (Russellet al., 2010).

Several receptors are used by pathogens to inhibit the fusion of phagosome and lysosome. Mycobacteria gain entry into the macrophages with the assistance of several cell-surface molecules, including complement receptors (CRs) 1, 3, 4, mannose receptors and Fcγ receptors (Ernst, 1998). CRs is commonly used by several bacteria as the engagement of CRs prevents the production of reactive oxygen intermediates that inhibit bacterial survival by suppressing the recruitment of NADPH oxidase to phagosomes (Caron and Hall, 1998; Hellwig et al., 2001). However, although CR3 is important for bacterial

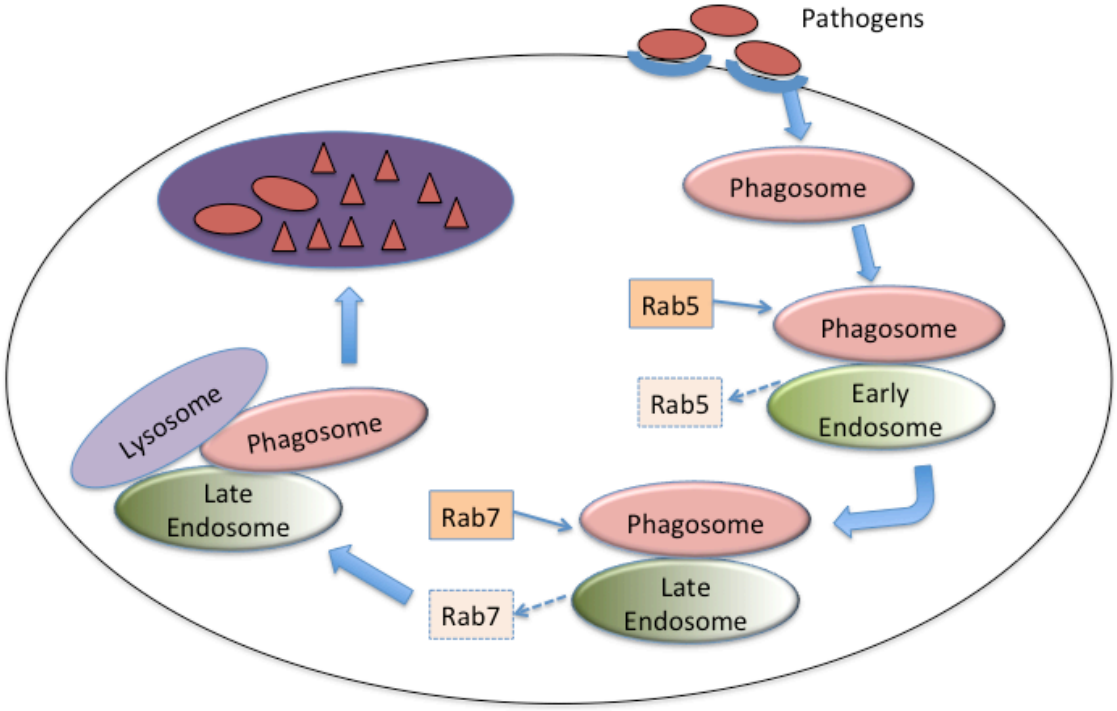

Figure 3. The process of macrophage mature and pathogen degradation. Pathogens are recognized by receptors on the macrophages, and internalized with and wrapped up by plasma membrane to form phagosome, which combines with early endosome, and Rab5 (a small GTPase) is a marker of this step. Then the compounds become acidification, and experience the departure of Rab5 and acquirement of Rab7 to combine with late phagosome. When phagosome and late endosome move to lysosome vesicle, the macrophages begins to mature, and pathogens would be digested in the compound.

phagocytosis, it may not directly mediate the intracellular survival and replication of mycobacteria but instead trigger an anti-inflammatory response (Melo et al., 2000). In addition, similar pathogenic molecules of pathogens may have diverse impacts on host cells leading to the inhibition of immune responses. ManLAM from *M. bovis* BCG exert an anti-inflammatory role, leading to inhibition of TNFα release in a CR3-dependent manner (Driss et al, 2012). Moreover, ManLAM expressed by *M. tuberculosis* is released during the interaction of *M. tuberculosis* and phagosomes leads to interference with phagolysosomal fusion (Hayakawa et al., 2007). Another type of receptor, mannose receptor, is also essential for the internalization of bacteria by macrophages. Lipoarabinomannan (LAM) is one of the key virulent components of *M. tuberculosis*. It interacts with the cell membrane of host macrophages and this incorporation of LAM and cell membrane can inhibit phagosomal maturation via the mannose-dependent method (Welin et al., 2008). Though the phagocytic vesicles shaped from the interaction of ManLAM and mannose receptor are less likely to mature (Kang et al., 2005), it is still under debate whether the inhibition of phagosomal maturation is caused by the internalization of LAM into membrane rafts of macrophage via its glycosylphosphatidylinositol (GPI) anchor at the cell membrane level or via inhibition of intracellular p38 mitogen-activated protein kinase (MAPK) activation (Vergne et al., 2004; Welin et al., 2008). The inhibition of phagosomal maturation might be partly mediated by the suppression of p38 MAPK and subsequent failure of recruiting early endosomal antigen 1, which is required for the delivery of lysosomal vesicle to the mature phagosome (Vergne et al., 2004). In contrast, it has been indicated that the inhibition of phagosomal maturation by ManLAM is dependent on the GPI anchor of ManLAM insertion into the raft of cell membrane, rather than the activation of p38 MAPK (Welin et al., 2008).

Besides mycobacteria, several viruses can also interfere with phagosomal maturation. For instance, porcine bone marrow-derived macrophages infected by PRRSV show decreased phagosomal maturation through Fc-receptor-mediated phagocytosis, leading to ineffective immune response (Chaudhuri et al., 2015). HIV-1 also impaired phagosomal maturation to cause dysfunction of macrophages that ineffectively respond to the stimulation of phagocytic and clear bacteria. Specifically, regulatory viral protein (Vpr) of HIV-1 is the key for interfering with phagosome maturation. Vpr is able to alter the localization of the microtubule by interacting with its critical compartments, which affects the microtubule-dependent endocytic trafficking of the phagosome, resulting in the failure of phagosomal maturation and also altered phagolysosome biogenesis (Dumas et al., 2015).

The fusion of phagosome and lysosome is the key step for macrophage maturation, which has a dominating influence in the next step of pathogen digestion and clearance in the phagolysosomal compound (Figure 3). However, several viruses and bacteria create effective strategies to inhibit the formation of phagolysosome. Specific receptors from host cells and virulent molecules of pathogens cooperate to inhibit macrophage maturation. Besides inhibiting the intracellular recognition of phagosome in macrophages, interference with the movement of phagosome trafficking is another way to prevent the interaction with lysosome.

Modulation of cellular metabolism

Glucose and lipids are two critical types of nutrient sources for cellular metabolism and functions (Lu et al., 2014). Glucose is generated from the process of glycogen breakdown and is a prevalent fuel that can be used as an energy source in metabolism and synthesis pathways of all mammalian cell types, including fuel macrophages to activate their immune responses (Chawla et al., 2011). Additionally, lipid bodies, also called lipid droplets, are lipid-rich organelles in the cytoplasm that regulate the storage and metabolism of neutral lipids (Martin and Parton, 2006; Bozza and Viola, 2010). Lipid is also an important component of the mycobacterial cell wall that provides unique advantages for bacterial survival and replication in the host environment, and acts as a permeability barrier against the entry of protons thus maintaining a constant pH in the bacteria (Tessema et al., 2001; Vergne et al., 2004; Thirunavukkarasu et a., 2014). Host abundant proteins related to immune regulators are composed of a variety of lipids, of which the pattern is altered based on different stages of the disease, and these adapt to the host immune responses (Rocha-Ramírez et al., 2008). In macrophages, glycogen and lipid storage are also called lysosome storage because glycogen in macrophages is frequently accumulated not in the cytosol but rather in a granule surrounded by a membrane called a lysosome (Hers, 1965), which was first described in 1955 as a granule containing lysosomal hydrolases (De Duve et al., 1955). Furthermore, the lysosome plays an essential role in the macrophage; macromolecules and pathogens are transported toward the lysosome for degradation via either the phagocytosis pathways from the extracellular environment or from the cytosol (Kornfeld, 1986; Lim et al., 2015). Therefore, lysosome storage disorder can cause a wide range of abnormalities in signaling pathways, including calcium homeostasis, lipid metabolism and vesicle trafficking (Ballabio et al., 2009)(Figure 4). Moreover, the progressive enlargement of glycogen-filled lysosomes can be dysfunctional and release toxic substances into the cytoplasm (Zhou et al., 2011). Fusion with autophagosome is prevented due to the inhibition of acidification, leading to autophagic vacuoles in the neutrophil cells and macrophages, and may result in the inhibition of pathogen killing (Ballabio et al., 2009). Moreover, a wide range of proteins are found in lipid bodies, not only proteins related to lipid transport and metabolism, but also several proteins linked with cellular membrane function, such as GTPases of the Rab family and kinases. Therefore, lipid bodies may have a broad range of functions, including cellular lipid transmission and metabolism, membrane repair, vesicle trafficking, intracellular signaling, as well as cell-to-cell communication (Fujimoto et al., 2004; Imanishi et al., 2004; Liu et al., 2004).

Bacteria may change the host lipid metabolism to evade the immune response. In the *M. tuberculosis* infected macrophages, *M. tuberculosis* can inhibit phagosome-lysosome fusion by directly integrating bacterial lipids into the phagosome membrane, which destroys the structure of the membrane and interferes with its biological function (Karakousis et al., 2004). *M. tuberculosis* can suppress membrane repair by preventing the transmission of lysosomal or Golgi-derived

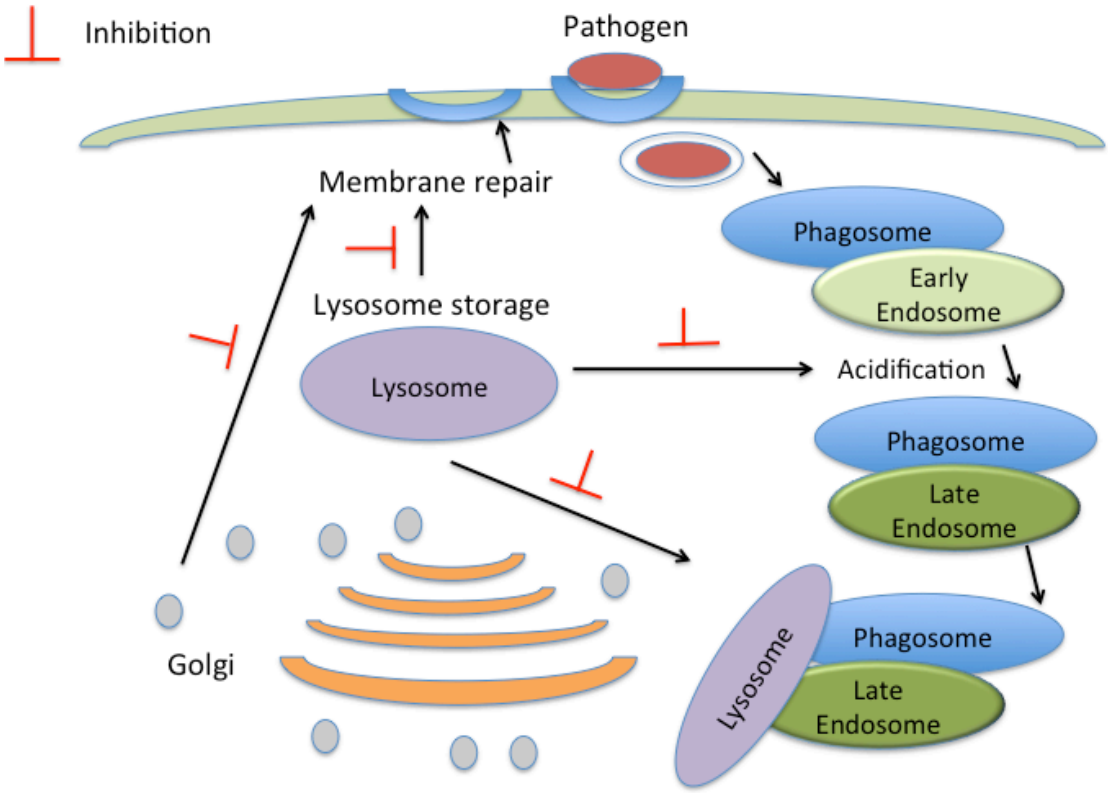

Figure 4. Lysosome storage could cause numerous problems. Membrane repair following the pathogen internalization is inhibited by preventing the transmission of lysosomal or Golgi-derived lipid vesicles to the macrophage plasma membrane, and the acidification of phagosome and early endosome is suppressed, which directly affects the development of late endosome, leading to the failure of combination of phagosome and lysosome.

lipid vesicles to the macrophage plasma membrane, leading to defects in endocytosis (Divangahi et al., 2009). Furthermore, *M. tuberculosis* is able to inhibit the fusion of phagosome and lysosome in macrophages by removing an important membrane-trafficking regulatory lipid, phosphatidylinositol-3-phosphate (PI3P), from the endosomal membrane (Vergne et al., 2005; Divangahi et al., 2009). Besides disrupting host lipid metabolism, bacteria are able to adapt to the intracellular environment by restoring host-derived lipid for their own advantage. For example, *Mycobacterium* is able to utilize host-derived lipid to modify the cell envelope, leading to a type of mimicry by appearing as "self component" in macrophage, to make the bacteria less susceptible to the immune response than directly exposed in host cells (Sturgill-Koszycki et al., 1994). Bacteria also acquire host-derived lipid from outside of the pathogen-containing vacuolar, which is necessary for its survival and replication. *Salmonella typhimurium* can gain sufficient membrane from host cells for intravacuolar survival and replication. However, unlike other internalized particles that undergo progressive interaction with early and late endosome sequentially in macro-phages, *S. typhimurium* resides in the specific tubules called *Salmonella*-induced filaments (Sifs) which fuse with Rab7 but not Rab5, thus *S. typhimurium*-containing vacuoles alter the cellular trafficking pathway and interact with late endocytic compartments (Brumell et al., 2001).

For many bacteria, the phagosomal environment is hostile for their survival due to the toxic substances of oxygen and nitrogen species, lysosomal hydrolases and an increased acidification (Rohde et al., 2007). Most microbes will be killed in the acidic, hydrolytically competent environment of macrophages. However, several pathogens, such as *M. tuberculosis*, can subvert

the normal maturation process of the phagosome by sustaining its pH at 6.4 to prevent the acidification of phagosome (Rohde et al., 2007; Welin et al., 2008). The endosome-lysosome development is associated with lysosome vacuoles that are composed of a series of hydrolytic enzymes. These exert their catabolic activity completely in an acidic environment. A highly acidic environment of pH less than 5.0 is responsible for sending bacteria to the lysosome (Abramovitch et al., 2011). It has been shown that the pH of the bacteria-containing phagosome is substantially higher than than that of killed pathogens or uninfected granules (Sturgill-Koszycki et al., 1994; Tsukano et al., 1999), and that the acidification is achieved via the activation of the vacuolar H^+-ATPase (V-ATPase) that pumps protons into the vacuole using energy from ATP (IP et al., 2010). *Yersinia pseudotuberculosis* can block the phagosome maturation by inhibiting V-ATPase activation (Tsukano et al., 1999). V-ATPase does not promote the formation of phagolysosome as phagolysosomal fusion in mice is not inhibited by the knock-out of the V-ATPase compound (Kissing et al., 2015), thus the mechanism for intracellular acidification is still undefined and requires further investigation.

Metabolism is a complicated and important aspect of macrophages. Lipid and glycogen metabolism can affect the survival of pathogens and host cells. Besides providing essential materials for host cells and pathogen replication, lipid and glucose breakdown from glycogen are also involved in other functions, such as membrane repair, vesicle trafficking, and transmission of Golgi-derived lipid vesicles (Figure 4). As lysosome storage caused by glycogen and lipid metabolic problems would directly affect immune responses in the macrophage, pathogens can develop strategies to interfere with cellular metabolism. Additionally, hydrolytic enzymes and V-ATPase that function in acidification are also utilized by pathogens to change the intracellular microenvironment and inhibit acidification. Therefore, cellular metabolism, including lipid and glycogen metabolism and acidification, are utilized by pathogens in immune evasion.

Regulation of apoptosis and autophagy

Apoptosis and autophagy are both essential for the removal of unwanted fractions and foreign pathogens to maintain cellular homeostasis. Apoptosis is identified as a mode of genetically programmed cell death, which normally removes old and damaged cells without harming adjacent cells (Hellwiget al., 2011)(Figure 5a). During pathogen infection, the host may trigger the apoptosis process to reduce the amount of pathogen-containing cells in the early infected stage, while inhibiting apoptosis of infected cells to prevent the release of progeny virus and subsequently control the replication of pathogens. The early apoptosis of porcine alveolar macrophages results in the limitation of avian influenza virus replication (Chang et al., 2015). In contrast, induced apoptosis by the release of new progeny virus via viral p10 protein can be suppressed by the interaction of host cellular lysosome-associated membrane protein 1 (LAMP-1) and p10 protein (Wu et al., 2016). A rather more complex case is when macrophages are infected by two or more pathogens. *Salmonella enterica* and noroviruses, two major causes of gastroenteritis, have been shown to antagonize each other with respect to the development of programmed cell death. In particular, murine norovirus can stimulate apoptosis of macrophages, however, this stimulation was blocked by the subsequent bacterial infection, despite no impact on virus replication (Agnihothram et al., 2015).

More importantly, apoptosis does not function alone to determine the fate of pathogens, autophagy has been shown to cooperate with apoptosis in a complex interplay. Autophagy refers to a series of non-specific catabolic processes in which any cellular or foreign material is delivered to the lysosome for degradation (Ward et al., 2016)(Figure 5b). Considering the fundamental role of lysosome in the autophagic pathway by fusion with the autophagosome and degradation of unwanted material, it is reasonable to connect lysosome storage with autophagy. When lysosome storage disorder occurs, this can interfere with the autophagic pathway by inhibiting the incorporation of multiple endosomal and autophagosomal vesicles into the lysosomal vacuoles (Lieberman et al., 2012; Lim et al., 2015). Both the formation of autophagolysosome and phagolysosome are related with the lysosme of macrophages. The phagolysosome is independent of the autophagic machinery while autophagolysosome is dependent (Klionsky et al., 2014). As the lysosomal system is considered to being at the hub of the metabolic process (Figure 5b), and the formation of autophagolysosome by fusion of autophagosome and lysosome is necessary for degrading

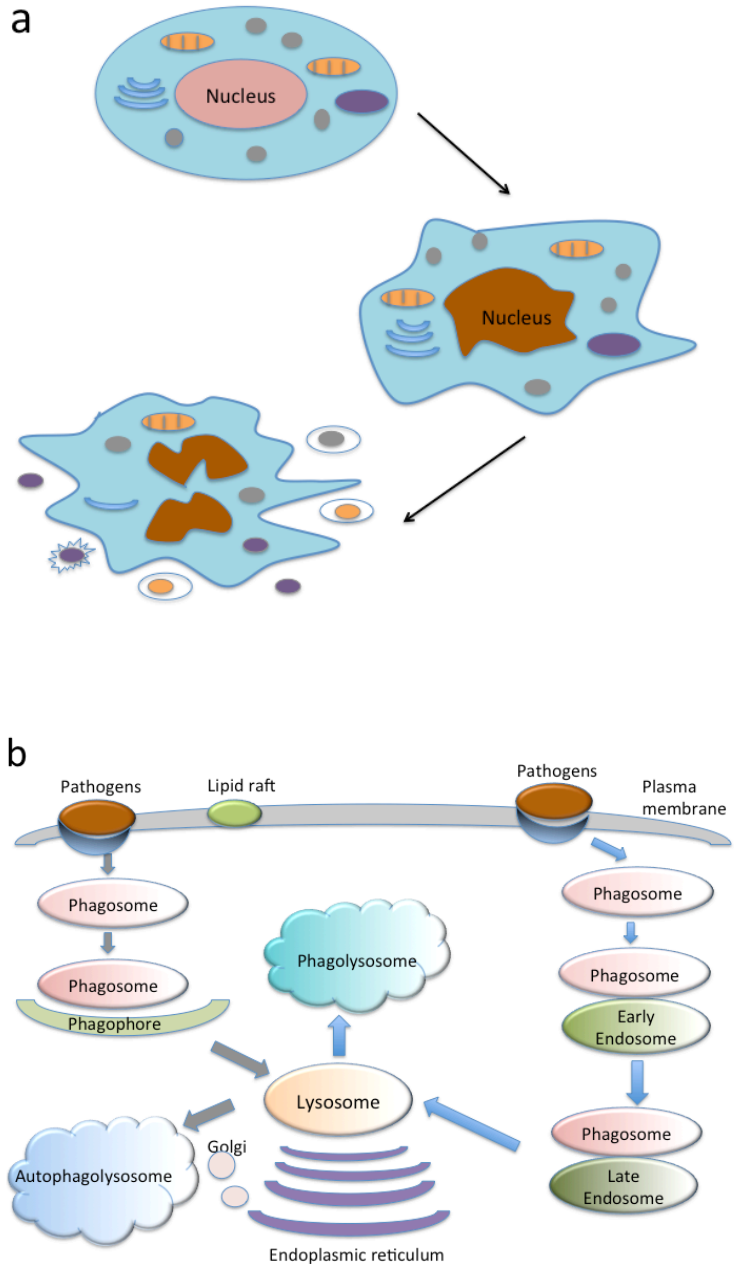

Figure 5. The process of apoptosis and autophagy. (a) The apoptosis is a series of programmed cell death process. The nucleus become compact and the cytoplasm clump together, and then the nucleus break and apoptotic bodies develop gradually. (b) Pathogens are internalized and form the vacuole of phagosome, which combine with early endosome, and interact with late endosome to form a compound that fuses with lysosome to create a place called phagolysosome for digesting pathogen. In autophagic pathway, internalized phagosome is surrounded by phagophore, and the fusion of phagosome and lysosome results in the formation of autophagolysosome to digest pathogen. Lysosome is considered as the hub site for the phagosomal mutation, which is necessary for the formation of phagolysosome and autophagolysosome and degradation of pathogen.

pathogens (Klionsky et al., 2014), failure to digest and recycle pathogens in the lysosome leads to an inefficient autophagosomal process that can result in persistent infection and serious disease. In some circumstances, autophagy directs cellular death by collaborating with apoptosis, or functions in a back-up manner when apoptosis is defective. Autophagy can serve as a cell survival signaling pathway via inhibiting apoptosis (Eisenberg-Lerner et al., 2009). Therefore, the cross-talk between apoptosis and autophagy is extremely complicated, and the interaction is mainly dependent on the dynamic intracellular actions of host response to pathogen invasion.

The association between pathogens and apoptosis or autophagy in macrophages is also complex. Pathogens may either suppress cell apoptosis and autophagy to maintain macrophages as a place for survival and replication, or adapt to the hostile microenvironment of macrophages by resisting apoptosis and autophagy. *Enterococcus faecalis* has been reported to be able to survive and replicate in macrophages. This might be achieved through the resistance of acidification and autophagy. As none of the *E. faecalis* vacuoles were delivered into autophagosomes, it appears that autophagy may not participate in the elimination of intracellular *E. faecalis* (Zou and Shankar, 2015). In contrast, Japanese encephalitis virus (JEV) infection promots the formation of autolysosomes *in vivo*, which is important for the induction of virus replication (Jin et al., 2013), and *Pseudomonas aeruginosa* can also survive by enhancing macrophage autophagy (Deng et al., 2015). However, the impact of autophagy on *M. tuberculosis* is complicated. It has been reported that enhanced autophagy is capable of promoting *M. tuberculosis* survival (Kumar et al., 2015), whereas nordihydroguaiaretic acid (NDGA) and α-mangostin-induced autophagy can inhibit the replication of *M. tuberculosis* in infected macrophages derived from the human THP-1 cell line (Guzmán-Beltrán et al., 2015). Furthermore, the impact on apoptosis and autophagy exerted by PRRSV might be different *in vivo* and *in vitro* and change with various target cells. PRRSV can stimulate autophagy to promote virus replication (Liu et al., 2012; Sun et al., 2016), but the stimulation was incomplete since the formation of autophagosome was activated while the fusion between autophagosome and lysosome was inhibited. This attributed to the accumulation of autophagosomes that function as a safe haven for virus replication (Sun et al., 2016). Additionally, it has been reported that the induction of PRRSV replication by cell apoptosis occurred in MARC-145 cells (Ge et al., 2015). The HP-PRRSV HuN4 strain caused both apoptosis and autophagy in bystander cells of thymic epithelial cells, and also induced autophagy in thymus cells of infected piglets (Wang et al., 2015). Other studies showed that autophagy and apoptosis could both be stimulated in PRRSV-infected MARC-145 cells (Li et al., 2016).

Both apoptosis and autophagy influence pathogen survival. Apoptosis is commonly inhibited by pathogens since it directly determines the fate of intracellular pathogens by destroying the safe haven for pathogen survival (Figure 5a), and the development of autophagy is restricted to the early stage by pathogens as autophagosome function as a safe location for virus replication (Figure 5b). However, the interaction between apoptosis and autophagy is complicated and varies in infections by different pathogens. Their cross-talk can be diverse even when challenged by the same pathogen. Apoptosis and autophagy are involved in immune evasion strategies and regulated by pathogens.

The interaction between immune evasion strategies and limit of pathogen transmission

The consequence of the immune response is dependent on the interaction between the host immune system and pathogen infection. The invasion of viruses and bacteria trigger host innate immune responses and subsequently develop to adaptive immune responses, which degrade and eliminate particles of pathogens. However, several pathogens are capable of disrupting the homeostasis through a series of immune evasion strategies. A typical example is that *M. tuberculosis* hides in intestinal macrophages and remains asymptomatic for several years and bursts out suddenly with serious clinical symptoms. PRRSV also survives and replicates in alveolar macrophages for a long period and transmits to other susceptible cells. It is important to the host to restrain the transmission of pathogens to new host cells by clearing pathogens in the infected cells. Plenty of evasion strategies have been developed by pathogens, including the inhibition of proinflammatory cytokine expression and M1 phenotype macrophages differentiation, induction of lysosome storage to interfere with phagosomal maturation,

and the modulation of autophagy and apoptosis to enhance replication. Hunting immune evasion strategies of pathogens provides us with a new perspective for limiting pathogen transmission. It is of high importance to trigger and increase host immune responses prior to the elicitation of the immune evasion by pathogens, including the increase of host pro-inflammatory cytokine expression in macrophages, resolving the metabolic problems and stimulating phagosomal maturation, and promoting the speed of degradation and elimination of foreign particles. An intimate knowledge of the mechanisms of hiding and incubation could contribute to the control of pathogen infection. For this reason the investigation of the interaction between host immune responses and strategies of immune evasion is the key for limiting pathogen transmissions to new host cells.

Conclusion

Macrophages are one of the most important immune components. In different diseases, macrophages initiate diverse measures to fight against pathogens. PRRs on the surface of the cell membrane, differentiation of polarized macrophages, maturation of phagosomes, lipid and glycogen metabolism, apoptosis and autophagy all participate in the immune response. Meanwhile, pathogens exploit several strategies to escape being killed by macrophages. They gain access to macrophages through PRRs and inhibit the PRRs-mediated production of inflammatory cytokines, suppress polarized M1 phenotype macrophages to decrease the proinflammatory response and activate polarized M2 phenotype macrophages to increase the anti-inflammatory response, inhibit phagosomal maturation to prevent degradation by phagolysosome, induce lysosome storage to result in dysfunction of vesicle trafficking and membrane repairing, and modulate apoptosis and autophagy to create a safe place for replication. The immune evasion strategies work together to help viruses and bacteria to hide and incubate in macrophages and wait for an appropriate chance to release and infect other cells. Taken together, the investigation of the immune evasion strategies provides a new insight that could help limit the transmission of pathogens to new host cells.

Acknowledgements

Financial assistance from the Natural Science Foundation of China (31572367, 31272427), Hubei Project (2015BHE009) are greatly appreciated.

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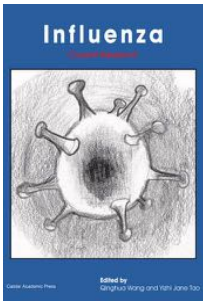

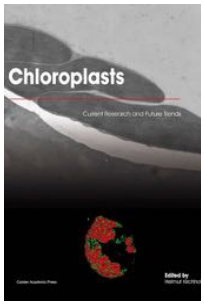

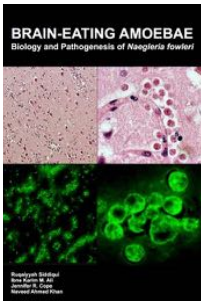

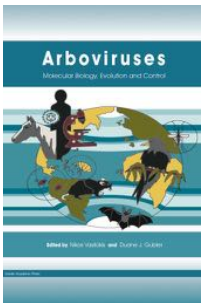

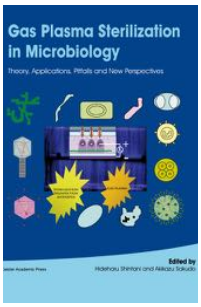

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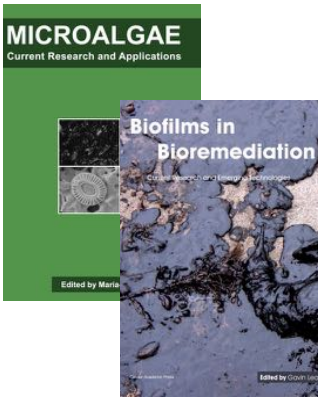

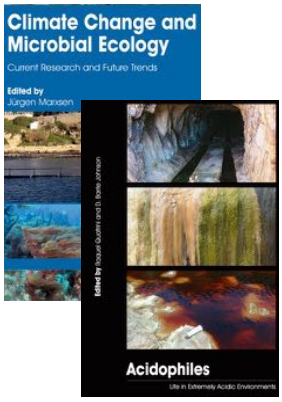

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