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Killing me softly: chlamydial use of proteolysis for evading host defenses

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Abstract

Chlamydial infections in humans cause severe health problems, including blinding trachoma and sexually transmitted diseases. Although the involved pathogenic mechanisms remain unclear, the ability to replicate and maintain long-term residence in the infected cells seems to significantly contribute to chlamydial pathogenicity. These obligate intracellular parasites maintain a delicate balance between exploiting and protecting their host: they occupy intracellular space and acquire nutrients from the infected cells, but at the same time they have to maintain the integrity of the host cells for the completion of their intracellular growth. For this purpose, chlamydiae hijack certain signaling pathways that prevent the host cells from undergoing apoptosis induced by intracellular stress, and protect the infected cells from recognition and attack by host defenses. Interestingly, one of the strategies that chlamydiae use for these purposes is the induction of limited proteolysis of host proteins, which is the main focus of this article.

The chlamydial lifestyle

There exist multiple chlamydial species, which have a diverse range of tissue tropisms and are involved in various disease processes ¹. *Chlamydia trachomatis* ² and *C. pneumoniae* ³ are human pathogens causing ocular and urogenital tract infections, and respiratory infection, respectively, whereas *C. muridarum* ³ and *C. caviae* ⁴ infect mucosal tissues of mice and guinea pigs; *C. psittaci* mainly affects birds but can also be transmitted to humans ⁵. Despite profound differences in host range, all these parasites display a remarkable similarity in their genome sequences ^{2-4, 6} and possess a conserved intracellular growth cycle with distinct biphasic stages (Figure 1). The infectious particle (also called elementary body or EB) can invade non-phagocytic epithelial cells via induced phagocytosis ^{7, 8}. The EB-laden cytoplasmic vacuole (also called inclusion) migrates to the peri-Golgi region as the EB starts to differentiate into a noninfectious but metabolically active reticulate body (RB) that can undergo rapid replication. The progeny RBs eventually differentiate back to EBs for spreading to other cells. The entire intracellular growth cycle occurs within the initial inclusion that expands to occupy a large portion of the host cytoplasmic space as the parasites replicate. It takes several days for most human chlamydiae to complete a productive infection cycle in cell culture. However, infection in humans can become persistent, during which the RBs, instead of undergoing rapid replication and differentiating into infectious EBs, are limited in numbers and each becomes

the so called aberrant or persistent body with enlarged size and multiple nucleoids. The aberrant body-laden inclusions may persist in the infected hosts for long periods of time⁹. Intracellular survival and growth are considered major contributors to chlamydial pathogenesis¹⁰. Some infected women, if untreated, can develop inflammatory pathologies, including pelvic inflammatory diseases, ectopic pregnancy and infertility^{9, 11}.

Because of their obligate intracellular lifestyle, chlamydiae have evolved strategies for manipulating host cell signaling pathways. After an EB induces its own entry into a non-phagocytic epithelial cell⁷, the EB-containing vacuole diverges from the default endocytic pathway (which would lead to destruction of the microbes) and intercepts with the ER/Golgi secretory pathway¹². The availability, from the mammalian cell cytoplasm, of numerous metabolites not normally found in the external environment has provided selective pressure for chlamydiae to evolve a unique capacity to utilize host metabolic intermediates¹³. Genome sequence analysis²⁻⁴ has revealed that the microbes have not only shortened many of their biosynthetic pathways¹³ but also possess numerous novel genes that might be involved in the manipulation of the host cells¹⁴⁻¹⁷. Chlamydiae can take up host lipid molecules via various strategies including vesicle fusion¹⁸, enzymatic conversion¹⁹ and lipid droplet recruitment^{20, 21}. The expansion of the inclusion in the cytoplasm and the alteration of host signaling pathways during parasite acquisition of nutrients are inevitably destructive to the infected cells. Indeed, infected cells often display altered metabolic²², immunological^{23, 24} and cell biological^{25, 26} characteristics. However, at the same time, the microbes have to maintain the integrity and viability of host cells before completing their own intracellular replication. To achieve this goal, chlamydiae have evolved the ability to both prevent the infected cells from undergoing apoptosis induced by intracellular stress and to protect these cells from recognition and attack by lymphocytes^{14, 23, 24}. Remarkably, the parasites can utilize the degradation of host proteins as a strategy to ensure both intracellular growth and evasion of host defense mechanisms, which is the main focus of this article.

Host defense barriers to chlamydial infection

In order to invade mucosal epithelial cells and to establish long-term infection in mammalian hosts, chlamydiae must overcome various host defense mechanisms belonging to both the innate and the adaptive immunity systems.

Innate immunity

Before entering mucosal epithelial cells for the first time or spreading to neighboring cells, an infectious EB must avoid attack by various anti-microbial factors present in the mucosa, including defensins, lysozymes, complements and natural antibodies, as well as C-type lectins such as surfactant protein D^{27, 28}. These preexisting extracellular defense molecules might be able to inactivate most EBs during a given infection.

Once inside the host cell, chlamydiae face a different set of innate defense mechanisms. They must inhibit the fusion between the EB-containing vacuole (or phagosome) and the lysosomes, which would result in the destruction of the invader²⁹, and they have to acquire nutrients from the host cells¹². However, the precise mechanisms for some of these processes remain unknown.

Inflammation

During chlamydial invasion and intracellular growth, the host innate immunity sensors (or pattern recognition receptors, PRR) can detect the infection by recognizing microbial components (or pathogen-associated molecular patterns, PAMPs). Chlamydial PAMPs such as HSP60 and Mip are recognized by host PRR TLR4³⁰ and TLR2³¹ respectively. The

intracellular PRR NOD1 can also detect the infection, although the corresponding chlamydial ligands have not been identified³². These PRRs, upon ligand binding, can lead to activation of various inflammatory signaling pathways including NF- κ B, NF-IL-6 and MAP kinases.

In chlamydiae-infected cells, the MAP kinase ERK1/2 pathway is activated along the entire infection cycle (Figure 2). Consistent with this, it has been shown that the inhibition of this pathway can suppress chlamydial growth and acquisition of triglycerophospholipids, host cPLA2 activity, and parasite-induced cytokine production^{19,33,34}. It is worth noting that many viruses can also activate host ERK1/2-mediated signaling pathways^{35,36}.

Activation of the ERK1/2/cPLA2 signaling pathway can contribute to inflammation in two ways. First, as a result of cPLA2 activation, arachidonic acid (AA) release is greatly increased in infected cells³³. AA can be converted into prostaglandins, including prostaglandin E2 (PGE2). Remarkably, cyclooxygenase 2 (COX2), which catalyzes the rate-limiting step of AA conversion to prostaglandins, is up-regulated in infected cells³³. PGE2 can both amplify inflammation by inducing the production of cytokines³³ and enhance chlamydial survival by suppressing IFN γ production by NK cells³⁷. Second, the MAP kinase ERK1/2 pathway can also participate in cytokine gene activation. In chlamydiae-infected cells, IL-8 production is dependent on ERK1/2 but not on other MAP kinases³⁴. The IL-8 gene activation also correlates with chlamydiae-induced nuclear localization of intracellular IL-1 α ³⁸. Nuclear IL-1 α is known to promote transcription of inflammatory cytokine genes via an ancient intracellular pathway³⁹. The question is whether the activated ERK1/2 pathway also participates in the promotion of IL-1 α nuclear localization.

Inflammatory responses are meant to strengthen host defense against infection. However, because chlamydiae hide inside the host cells, the induced inflammatory responses can not only fail to effectively clear the infection but also contribute to inflammatory pathologies¹⁰. For example, mice deficient in caspase-1 (which is required for the maturation of IL-1 β , IL-18 and IL-33) were as susceptible to chlamydial infection as wild type mice but developed significantly less severe pathologies in the upper genital tract⁴⁰.

Adaptive immunity

B lymphocytes can secrete IgA antibodies directed to chlamydial antigens for neutralizing extracellular EBs⁴¹, while T lymphocytes can detect the infected cells⁴². CD4+ T cells can enhance the intracellular defense mechanisms to restrict chlamydial replication and to make the uninfected cells more resistant to infection⁴³. CD8+ T cells should be able to induce apoptosis of infected cells so that these cells along with the intracellular chlamydiae can be cleared⁴⁴. Various studies using animal models have shown that both the IgA secreting B cells and IFN γ -producing CD4+ TH1 T cells are the most important adaptive immunity mechanisms in controlling chlamydial infection⁴³ although other immune components also play some roles⁴⁵. It is worth noting that among all the T cell cytokines, IFN γ is the only one that can directly inhibit chlamydial growth⁴⁶⁻⁴⁹.

Despite these powerful host defense mechanisms, acute chlamydial infection (if not treated) can persist in some infected hosts, leading to inflammatory pathologies including infertility and ectopic pregnancy^{9,11,50}. Thus, understanding the mechanisms of chlamydial immune evasion might provide important information for developing new therapies to attenuate these pathologies.

Degradation of host proteins for evading host defense

A bioinformatics analysis of the *C. trachomatis* genome identified more than two dozens of ORFs encoding proteins with potential proteolytic activity². Additionally, a substrate-oriented

functional assay unveiled a novel serine protease, designated as chlamydial proteasome/protease-like activity factor (CPAF)¹⁴. Besides their roles in chlamydial biology, some of these proteases might also be used to target host cell proteins since some proteins are cleaved and/or degraded in infected cells. The attacked host proteins include the transcriptional factors USF-1²³, RFX5²⁴, NF- κ B^{51, 52} and HIF-1⁵³, the BH3-only proteins (proapoptotic members of the Bcl-2 family)⁵⁴⁻⁵⁶, the DNA repairing enzyme PARP (Poly-(ADP-ribose) polymerase)⁵⁷, cyclin B1⁵⁸, various cytoskeleton proteins such as keratin 8, keratin 18 and vimentin⁵⁹⁻⁶¹, golgin-84⁶² and even cell surface proteins CD1d⁶³ and nectin-1⁶⁴. Degradation of these and other yet to be identified host proteins might allow chlamydiae to effectively manipulate host cell signaling pathways for both evading host defense and supporting their own intracellular growth (Table 1).

Evading immune recognition

Extracellular pathogens can be easily attacked by both humoral and cellular immune effector mechanisms such as defensins or complement-mediated lysis, antibody-mediated neutralization and phagocyte-mediated digestion. However, chlamydiae as well as other successful intracellular pathogens can hide in privileged intracellular compartments to make themselves invisible to these immune mechanisms. In particular, chlamydiae restrict themselves within a cytoplasmic inclusion that neither become acidic nor fuse with host cell lysosomes²⁹, which makes it difficult for host MHC class II molecules to capture peptides derived from live EBs. However, the inhibition of lysosomal fusion is only restricted to vacuoles containing live EBs²⁹, and infected cells are still able to carry out lysosomal processing of other antigens, including dead chlamydiae. Indeed, there is a high probability for a given host cell to be infected with live EBs and simultaneously internalize chlamydial antigens when a neighboring infected cell is burst to release both live EBs and a large amount of bacterial noninfectious materials. Epitopes processed from the endocytosed noninfectious antigens can be presented to CD4+ T cells by the infected cells. In addition, chlamydial proteins secreted into the inclusion membranes and host cell cytosol by live EBs can be presented to CD8+ T cells.

Protecting the infected cells from detection by lymphocytes is essential for the parasite to safely complete intracellular replication. This is because immune recognition of the infected cells can lead to total destruction of both the host cells and the intracellular microbes. As a strategy for evading host immune recognition, chlamydiae can inhibit IFN γ -inducible MHC class II expression²³, which might affect antigen presentation to CD4+ T cells. Chlamydiae also suppress both constitutive and IFN γ -inducible MHC class I expression²⁴, which might reduce the recognition of the infected cells by CD8+ T cells. The parasite achieves these goals by secreting CPAF into the host cell cytoplasm, leading to degradation of the host transcription factors RFX5 and USF-1, which are required for MHC gene activation¹⁴. Other mechanisms can also be used by the microbe to regulate the MHC antigen expression on the infected cells. For example, the infected cells were found to secrete IFN β that is known to block IFN γ -inducible MHC class II expression⁶⁵.

Evading immune effector mechanisms

Besides evading immune recognition, many intracellular pathogens have also evolved strategies for escaping immune effector mechanisms to double ensure that they can survive in their infected hosts for long periods of time. One efficient host defense mechanism is apoptosis, or programmed cell death, which plays a critical role in both innate and adaptive immunity against intracellular pathogens. It is noticeable that many viruses have evolved strategies to counteract host cell apoptosis⁶⁶.

A profound chlamydial antiapoptotic activity was first reported in 1998⁶⁷ and subsequently confirmed in many laboratories under different experimental conditions^{25, 56, 68-71}. Although there were also reports describing a chlamydial proapoptotic activity⁷², it seems that most of the apoptotic cells detected in infected cultures were not successfully infected⁷³. It is worth noting that chlamydiae have been known to induce an immediate cytotoxicity when added in extremely high numbers (MOI of 50 or higher) to cell cultures^{74, 75}, but this is a different process from the pro- or anti-apoptotic activities detected in cultures that have been productively or persistently infected at lower MOIs. More importantly, the chlamydial antiapoptotic activity was correlated with inhibition of caspase 3 activation⁶⁷, blockade of mitochondrial cytochrome c release⁶⁷, inhibition of Bax/Bak activation⁷⁶ and lack of NF- κ B activation^{52, 77}.

Finally, recent studies have shown that the intracellular stress sensor molecules such as Puma and Bim are degraded in cells infected by chlamydiae^{54, 56}. These BH3-only proteins are normally sequestered in various intracellular organelles in the cytoplasm. Upon detecting overwhelming intracellular stress signals, these BH3-only proteins can migrate to mitochondria and activate the multi-domain proapoptotic Bax and Bak to overcome the inhibition of anti-apoptotic Bcl-2 proteins to launch apoptosis. Interestingly, CPAF is both necessary and sufficient for degrading the BH3-only domain proteins in chlamydiae-infected cells⁵⁵. Thus, CPAF-mediated degradation of host proteins contributes to evasion of both immune recognition and effector mechanisms, making this protease a bona-fide virulence factor. However, one must realize that the CPAF-mediated mechanism cannot account for all the chlamydial antiapoptotic activity. For example, CPAF is only made and secreted at about 16 hours after infection⁷⁸, and it is not known how the parasite prevents host cell apoptosis before CPAF is secreted. It is equally unclear how the microbe inhibits the apoptosis induced by death receptors⁶⁷, since BH3-only proteins are not required for apoptosis induced by death receptors such as Fas in some cell types.

Evasion of inflammation

As described in a previous section, chlamydial infection can activate inflammation and induce the production of a wide variety of inflammatory cytokines, including IL-1, IL-6, IL-8 and TNF α ^{38, 79}. Since NF- κ B can activate many of these cytokine genes and often plays a dominant role in the induction of inflammation, it was expected that chlamydial infection would induce NF- κ B activation. However, no significant NF- κ B activation is detected in infected cells^{52, 77}. Instead, the microbe appears to use the MAP kinase pathway to activate inflammatory responses, probably because this is also useful for nutrient acquisition. Therefore, the question is how the parasite manages to silence the NF- κ B inflammatory pathway. Examination of infected cells revealed that NF- κ B p65 was cleaved into two major fragments (p40 and p20), and the chlamydial tail-specific protease (Tsp) was found to be responsible for the cleavage⁵². Since the N-terminal fragment p40 maintains the ability to interact with I- κ B α (a cytoplasmic inhibitor of NF- κ B) and to bind to DNA but lacks transactivation ability, overexpression of the p40 might block the residual full length p65-mediated response via a dominant negative effect⁵². The biological relevance of this finding was further demonstrated in an experiment showing that expressing chlamydial Tsp in mammalian cells alone can block NF- κ B pathways⁵².

Promoting expansion and integrity of the chlamydial inclusion

Expansion of the chlamydial inclusion in the host cell cytoplasm is necessary to accommodate the rapid replication of the parasite. Epithelial cells, the natural target of chlamydiae, are enriched with cytoskeletal structures including the rigid intermediate filaments (IF) for maintaining cell shape. Therefore, the microbe has to overcome the host cell cytoskeletal restriction for expanding its inclusion, while at the same time maintain the integrity of the

apparently fragile inclusion (note that these inclusions can quickly lose its integrity upon microinjection^{80, 81}). How is this accomplished? The discovery of chlamydial cleavage of cytokeratin 8⁶⁰ and other cytoskeletal proteins^{59, 61} might provide partial answers to the question. The parasite can use CPAF to cleave off both head and tail domains of cytoskeletal proteins, and the residual rod domain might have a dominant negative effect on the polymerization of the residual cytoskeletal proteins; this might lead to loosening up portions of the cytoskeleton surrounding the inclusions. The residual levels of IF along with other cytoskeletal structures such as filamentous actin or stress fibers might be required for maintaining the integrity of both the infected cells and the rapidly expanding inclusions⁵⁹.

Promoting chlamydial uptake of Golgi-derived lipids

Chlamydiae can acquire sphingomyelin⁸² and cholesterol⁸³ from the Golgi exocytic pathways, and host kinases sensitive to rottlerin inhibition appear to be required for this process⁸⁴. However, the precise mechanism on how Golgi-derived lipids are transported to the inclusion remains unclear. A recent study from Meyer's group shows that chlamydial proteases can target the Golgi apparatus to facilitate the uptake of Golgi-derived lipids⁶². This finding has provided a mechanistic link between the acquisition of Golgi-derived lipids and a long standing observation that Golgi apparatus closely associates with the inclusions and forms ministacks around them^{38, 82}. In infected cells, golgin-84 is cleaved, which triggers Golgi fragmentation and the formation of ministacks surrounding the inclusions. The recruitment of the fragmented Golgi around inclusions seems to be necessary for both acquisition of nutrients and maturation of the microbe⁶². Although it is still unknown how the fragmented Golgi is recruited, and what protease is responsible for the cleavage of golgin-84, the fact that the parasite can hijack the entire organelle via its proteolytic force re-highlights the importance of proteolysis as an strategy to manipulate host signaling pathways.

Concluding remarks and future directions

The degradation of such a broad spectrum of host proteins is likely accomplished via multiple pathways. First, it is possible that chlamydial infection could induce modification of host proteins and make the modified proteins more susceptible to host proteosomal degradation. Although this is a common viral strategy^{85, 86}, there is still lack of biochemical evidence for chlamydiae to use such a pathway. Second, infection could activate host proteolytic systems that are normally silenced in uninfected cells. This is definitely a testable hypothesis in need to be pursued. Third, the chlamydial genome appears to encode several dozens of predicted proteases², many of which might participate in the degradation of host proteins. However, so far only Tsp and CPAF have been demonstrated to be able to cleave host proteins. Finally, only CPAF has been shown to target multiple host proteins, suggesting that this protease might play a dominant role in the process. The recently unveiled crystal structure of CPAF suggests that it might have a broad substrate specificity, since the contacts between a putative substrate peptide and the active site are mainly via hydrophobic interactions⁸⁷, which might enable CPAF to cleave many different types of proteins. However, not all accessible host proteins are degraded by CPAF in infected cells²³. How does this protease recognize and attack certain proteins and not others? To address this question we need to understand how CPAF is activated and secreted in the infected cells.

Since degradation of host proteins, due to either direct attack by chlamydial proteases or by activation of host proteolytic systems, can occur both inside cells during infection and outside cells during sample processing (after cell lysis), it is extremely important to distinguish between intracellular and extracellular proteolysis. Obviously, only the degradation occurring inside infected cells would be biologically relevant to the study of the mechanisms of host protein degradation by chlamydiae. Thus, caution should be taken to block all proteolytic activities when harvesting and analyzing cellular samples.

The extensive cleavage of host proteins in infected cells has inevitably alerted us to question the fate of the degradation products. Host protein degradation might be intended to (i) enrich the nutritional supply in the infected cell cytosol for chlamydial uptake or (ii) alter host cell signaling pathways for protecting the intracellular parasite. The question boils down to which is more important for chlamydial survival: food or war. Without a lengthy debate on the rather philosophic question, one can hypothesize that the degradation products might effectively serve as nutrients. This hypothesis is supported by the fact that the chlamydial genome appears to encode multiple amino acid and oligopeptide transporters², which might equip the microbe with the tools for taking in nutrients and building blocks from host cells. Although chlamydial proteases might not directly generate oligo-peptides and single amino acids from host proteins, the host proteosomal systems might be able to do the follow-up job by chewing up the damaged proteins. Furthermore, most chlamydiae-targeted proteins appear to be constitutively expressed in the host cells. It makes more sense to target preexisting and abundant proteins for the purpose of generating food. It would be much more efficient to target less abundant and transiently expressed proteins for the purpose of inactivating the targets' function. Interestingly, *Protochlamydia amoebophila* UWE25, a member of the phylum Chlamydiae and obligate intracellular symbiont of a free-living amoeba, also encodes a CPAF homolog⁸⁸. Since there is no need to protect the infected amoeba from lymphocyte detection and attack, one can speculate that the protochlamydial CPAF might help UWE25 to fight off the amoeba's digestive power and to generate nutrients for UWE25. Finally, the extent of host protein degradation seems to correlate with the speed of chlamydial growth. In most cases, host protein degradation reaches the highest level at middle cycle when parasite biosynthesis and replication peak. Whether host protein-derived amino acids can indeed end up in chlamydial proteins requires experimental demonstration. The degradation of host proteins might even allow the endoparasite to literally eat its way out since chlamydiae-mediated lysis of host cells is also dependant on a proteolytic activity⁸⁹. It will be interesting to test whether CPAF plays any role in chlamydial exiting and spreading.

In summary, chlamydiae appear to use a basic strategy (degradation of host proteins) for ensuring its own intracellular replication while maintaining the integrity of the infected host cells for long periods of time. However, emphasizing the role of host protein degradation does not in any way mean that this is the only major chlamydial strategy for immune evasion. On the contrary, the parasite has probably evolved other strategies, most of which we still do not know. Further research on the biology of these microorganisms will likely unveil new mechanisms and, hopefully, will result in novel therapeutic approaches to treat chlamydial pathologies.

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Glossary

Arachidonic acid

a 20-carbon fatty acid that humans use to synthesize regulatory molecules such as prostaglandins. Prostaglandins can exhibit various hormonal effects.

BH3-only proteins

members of the Bcl-2 family but only containing the Bcl-2 homology domain 3. These proteins can convert cytoplasmic stress into an apoptotic signal by antagonizing the function of the anti-apoptotic Bcl-2 family members Bcl-2 and Bcl-xL.

CD1d

a protein belonging to the CD1 (cluster of differentiation 1) family related to the MHC class I molecules. CD1 molecules expressed on the surface of antigen-presenting cells are involved in the presentation of lipid antigens to Natural Killer T (NKT) cells that produce both IFN γ and IL-4.

cPLA2

a calcium-dependent cytosolic phospholipase that can be activated by MAP kinase ERK1/2 to convert triglyceride phospholipids into lysophospholipids.

Defensins

a group of small cationic, microbicidal peptides that contain six cysteines in disulfide linkage and form a critical component of innate immunity against infection by bacterial, fungal and enveloped viral pathogens.

Golgin-84

an 84-kDa Golgi membrane protein involved in the tethering of vesicles in the Golgi apparatus.

MAPK or MAP kinases

mitogen-activated protein kinases. They consist of three major types: p38, JNK (Jun-N-terminal kinase) and ERK1/2 (extracellular signal regulated protein kinases). Each type responds to different stimuli and leads to activation of different groups of genes, although there is always redundancy among these MAPK and other signaling pathways.

Nectin-1

a member of a sub-family of immunoglobulin-like adhesion molecules, and a component of adherens junctions (protein complexes occurring at cell-cell junctions). Pathogens like HSV (Herpes Simplex Virus) can use nectin-1 as a cellular receptor.

NF- κ B

a nuclear factor initially discovered for its ability to transactivate the gene of the immunoglobulin light chain kappa (κ). It regulates the expression of many genes, especially those related to host defenses. NF- κ B is activated by signal-induced degradation of its cytoplasmic inhibitor I- κ B.

PARP

poly-(ADP-ribose) polymerase, a DNA repairing enzyme. It is cleaved into characteristic fragments during apoptosis.

RFX-5

a member of the nuclear regulatory factor X (RFX) family that influences MHC class II gene expression.

USF-1

a member of the upstream transcription factor family that participates in activation of MHC class II genes.

Figure 1.

Chlamydial life cycle and the inflammatory processes associated. **(i)** Chlamydiae are obligate intracellular bacterial pathogens with a life cycle including two cell types: elementary bodies (EBs), which are infectious and invade the host cells, and reticular bodies (RBs), which are not infectious and undergo duplication inside a cytoplasmic vacuole (or inclusion). RBs differentiate back to EBs for exiting the infected cells and then enter adjacent new target cells, starting a new cycle of infection. This productive infection is observed in cell cultures⁹⁰. **(ii)** However, during infection in humans, chlamydiae can enter a persistent infection status in which RBs do not undergo rapid duplication, instead becoming enlarged bodies each with multiple nuclei. Although it is not known how the parasites enter or exit the infection status, it is believed that the persistent infection can significantly contribute to the inflammatory pathologies induced by chlamydiae⁹. **(iii)** Both productively and persistently infected cells can release large amounts of inflammatory cytokines that can induce and sustain tissue damage and inflammatory responses at the site of infection^{38, 79}. The lysis of productively infected cells might further amplify the inflammation. Due to the inability of the inflammation to clear the intracellular infection, the net result may be tissue damage and fibrosis of the tissue^{9, 10}.

Figure 2.

The role of host kinase pathways in chlamydial acquisition of lipids and induction of inflammation. Chlamydiae can acquire lipids from host cells by converting glycerophospholipids into lysophospholipids through activation of host Raf/MEK/ERK/cPLA2 pathway (i) 19, intercepting Golgi-derived exocytic vesicles by cleaving golgin-84 (ii) 62, activating kinases that are sensitive to rottlerin inhibition (iii) 84, inducing fusion with CD63-positive vesicles (iv) 18 and recruiting lipid droplets (LD) (v) 20. U0126, an inhibitor of MEK1/2, can inhibit the ERK/cPLA2 pathway and the acquisition of glycerophospholipids by the microbe (vi) 19. The endoparasites modify host-derived glycerophospholipids by replacing the straight chain fatty acid with a branched chain fatty acid at the sn2 position (i, vii). They synthesize the branched fatty acid chain using glucose (GLC) to build the main carbon chain, and use the branched side chain of isoleucine (ILE) to seal the carbon chain. Thus, one can conveniently quantitate the acquisition of host glycerophospholipids by monitoring the incorporation of H^3 -labeled ILE into chlamydial lipids 19. Rottlerin (iii) is a plant-derived inhibitor targeting various mammalian kinases, including protein kinase C delta (PKC δ), and it can inhibit sphingomyelin trafficking from Golgi to the parasite inclusion 84. However, it is not known whether rottlerin exerts its inhibitory effect via inhibition of PKC δ . Importantly, rottlerin also blocks chlamydial replication. Since it is known that blocking sphingomyelin trafficking is not enough for inhibiting chlamydial growth, rottlerin must block multiple and redundant pathways; these might include the fusion with CD63-positive vesicles, the

recruitment of lipid droplets, and cPLA2-catalyzed lysophospholipid generation. Interestingly, the activation of host kinases for acquisition of lipids might also contribute to the induction of inflammation. Many inflammatory cytokines including IL-8 and IL-6 are indeed produced by chlamydiae-infected cells ^{38, 40, 79}. ERK1/2-mediated pathway might directly or indirectly (via promoting nuclear localization of Pro-IL-1 α , precursor of IL-1 α) participate in the activation of inflammatory genes, since U0126 can significantly inhibit the cytokine production **(vi)** ³⁴. However, the effect of rottlerin on inflammation has not been investigated. In addition, the cleaved arachidonic acid can be converted into PGE2 by the action of COX2. PGE2 can further enhance inflammation by promoting the activation of inflammatory genes **(viii)** ³³.

Table 1
Degradation of host proteins as a strategy for intracellular survival

Chlamydial proteases	Targeted host proteins	Cellular function affected	Potential consequences
CPAF	USF-1, RFX5	MHC gene activation	Evasion of immune recognition ^{23, 24}
CPAF	BH3-only proteins	Activation of apoptosis in response to intracellular stress	Inhibition of apoptosis ⁵⁵
CPAF	CD1d	Antigen presentation to NKT ^a	Evasion of NKT recognition ⁶³
CPAF	Keratin 8, keratin18, vimentin	Cell structure and other processes	Promotion of inclusion expansion and maintenance of inclusion integrity ^{59, 60}
CPAF	HIF-1	Response to low oxygen levels	Evasion of stress responses triggered by low oxygen levels ⁵³
CPAF	Nectin-1	Cell-to-cell adhesions	Aid to chlamydial dissemination ⁶⁴
Tsp	NF-κB	Activation of the expression of genes encoding proinflammatory proteins	Evasion of inflammatory responses ⁵²
Unknown	Cyclin B1	Cell mitosis	Inhibition of host cell division ⁵⁸
Unknown	Golgin-84	Golgi structure	Golgi fragmentation to promote chlamydial acquisition of host lipids ⁶²
Unknown	Unknown	Maintenance of cell integrity?	Promotion of chlamydial exit ⁸⁹

^a NKT, Natural killer T cell.