# *CHLAMYDIA* AND APOPTOSIS: LIFE AND DEATH DECISIONS OF AN INTRACELLULAR PATHOGEN

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Abstract | The chlamydiae are important obligate intracellular prokaryotic pathogens that, each year, are responsible for millions of human infections involving the eye, genital tract, respiratory tract, vasculature and joints. The chlamydiae grow in cytoplasmic vesicles in susceptible host cells, which include the mucosal epithelium, vascular endothelium, smooth muscle cells, circulating monocytes and recruited or tissue-specific macrophages. One important pathogenic strategy that chlamydiae have evolved to promote their survival is the modulation of programmed cell death pathways in infected host cells. The chlamydiae can elicit the induction of host cell death, or apoptosis, under some circumstances and actively inhibit apoptosis under others. This subtle pathogenic mechanism highlights the manner in which these highly successful pathogens take control of infected cells to promote their own survival — even under the most adverse circumstances.

APOPTOSIS
A form of cell death, also known as programmed cell death, which is typically characterized by death receptor ligand or mitochondria-elicited activation of caspase proteases and which leads to nuclear condensation, DNA fragmentation and clearance of the dead cell by surrounding tissue.

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doi:10.1038/nrmicro1007

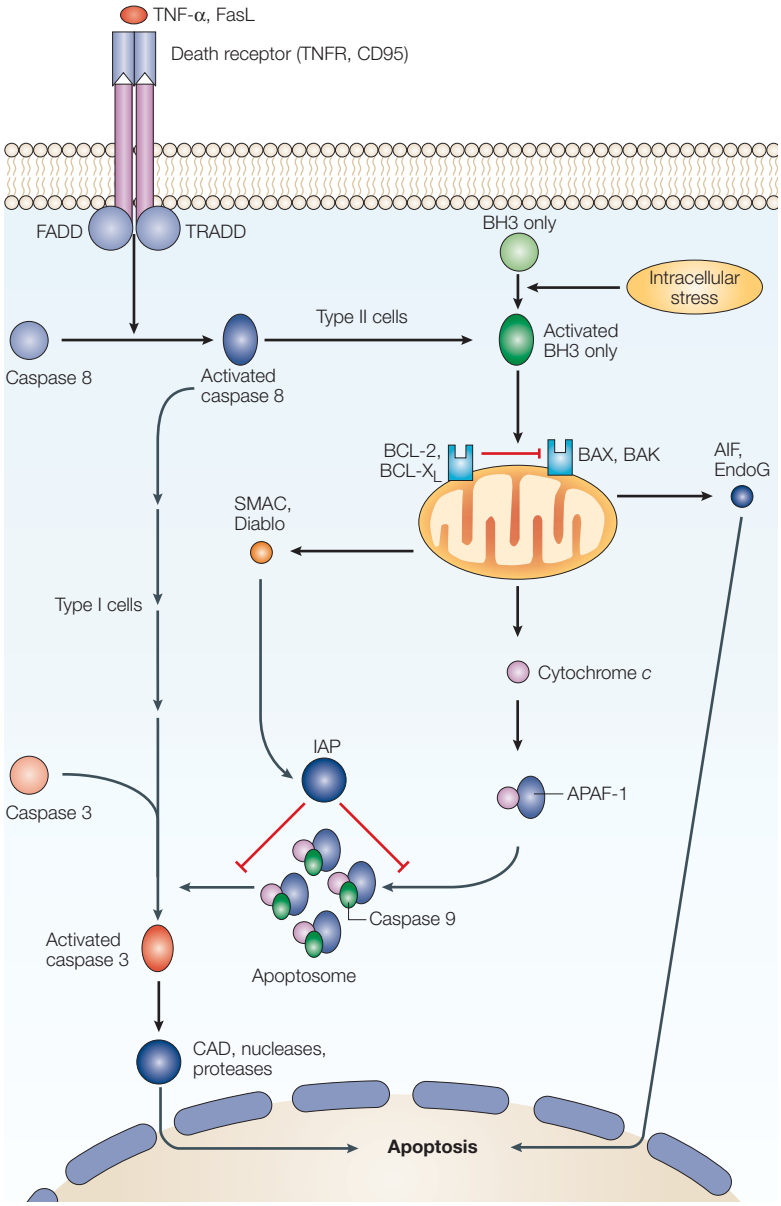
Coordinated programming of various cellular functions is a molecular mechanism that enables multicellular organisms to fine-tune cell division, growth, differentiation and death. Programmed cell death, or APOPTOSIS, is crucial for proper embryogenesis, immune system maintenance and the removal of damaged cells[1], and is a fundamental eukaryotic process that is highly evolutionarily conserved[2–4]. Pathways for apoptosis induction or inhibition originate from both extrinsic and intrinsic signalling systems (BOX 1). Extrinsic signalling pathways[5,6] are initiated by ligand binding to cell-surface receptors (for example, Fas ligand binding to Fas/Apo1/CD95, a cell-surface-signalling molecule). Intrinsic signalling involves the activation and oligomerization of proteins (for example, BAX) that are capable of binding and destabilizing mitochondrial membranes. In most cases, both extrinsic and intrinsic apoptosis require the activation of a family of proteases (CASPASES) and culminate in the participation of nucleases and other destructive enzymes that eliminate cells without eliciting inflammation. Apoptosis is therefore distinct from the more random process of necrotic cell death, which is closely associated with the inflammatory responses that are commonly observed during an infectious disease.

## Apoptosis and infectious diseases

***Cell death, infection and inflammation.*** NECROSIS elicits inflammation, whereas the recognition of apoptotic cells leads not only to phagocytosis but also to downregulation of inflammation[7] — via the anti-inflammatory cytokines transforming growth factor-β (TGF-β) and interleukin 10 (IL-10) — due to interaction of the phosphatidylserine (PS) phagocyte receptor with apoptotic cells[8,9]. Intracellular pathogens that are released from infected cells within apoptotic bodies can also be captured and internalized by neighbouring cells. The pathogen therefore benefits from apoptosis in two complementary ways — inhibition of inflammation and enhanced propagation of the infection.

Debris that is released from necrotic cells is not sequestered, but rather is recognized by specific receptors on the surface of macrophages and dendritic cells that promote inflammation. Some of these released molecules, which are normally present in the cytosol, nucleus or Golgi apparatus, enter the extracellular space when a cell undergoes necrotic death and act as immune system 'danger signals'[10,11] to indicate the presence of a damaged cell as a result of infection (FIG. 1). Currently, known danger signals that can be detected by specific

Box 1 | **Extrinsic and intrinsic pathways of apoptosis**




Apoptosis is initiated by sequential activation of a group of proteases called caspases[67], which are so called due to the presence of a cysteine residue in their active sites and an aspartic acid residue in the amino acid sequence of their proteolytic target. The 14 members of this enzyme family are evolutionarily conserved and are present as inactive pro-enzymes until they are activated by apoptotic signalling pathways. Apoptosis requires the participation of initiator (caspases 8 and 9) and effector caspases (caspases 3, 6 and 7) that destroy DNA-repair enzymes and activate nucleases and other enzymes that result in DNA fragmentation (for example, caspase-activated DNAse, CAD), nuclear condensation, membrane blebbing and vesiculation, detachment of apoptotic bodies and exposure of phosphatidylserine on the surface of the apoptotic cell. These events are followed by phagocytosis of the apoptotic cell, resulting in clearance in the absence of inflammation[68].

Intrinsic induction of apoptosis starts within the cell and can occur by direct activation of caspases or a variety of stress-related mechanisms (see figure). The process is initiated by events that alter the integrity of the mitochondrial membrane after the insertion of pro-apoptotic proteins that belong to the BAX family such as BAX and BAK. BAX belongs to the larger BCL-2 family, which includes the anti-apoptotic proteins BCL-2 and BCL-$X_L$. The main function of BCL-2 and BCL-$X_L$ is to suppress the pro-apoptotic function of BAX and BAK. The BAX-family proteins are first activated by proteins that have sequence homology with domain 3 of the BCL-2 family (BH3-only proteins), such as BID, which is activated when cleaved to its truncated form (tBID); or BIK, NOXA, PUMA and BIM, which are sensitive to the integrity of the cytoskeleton or other host-cell structures and initiate the intrinsic pathway[51,69] (see figure). BAX can also be activated by changes in the cytosolic pH or oxidative stress[70–72]. So, the mitochondrion is ideally located to detect metabolic or structural changes in the cytoplasm during microbial infection.

Extrinsic initiation of apoptosis occurs by stimulation of sets of surface receptors (Fas/Apo1/CD95 or TNFR) by their cognate ligands. This results in trimerization and docking of proteins containing so-called death domains, which in turn activate large amounts of caspase 8, an initiator caspase, followed directly by cleavage of downstream effector caspases in TYPE I CELLS (see figure). By contrast, TYPE II CELLS produce little active caspase 8 and therefore rely on a mitochondrial amplification step. Caspase 8 cleaves BID into tBID, which allows BAX and BAK to initiate mitochondria-dependent apoptosis. Consequently, overexpression of BCL-2 can inhibit apoptosis in type II cells, but not in type I cells.

Insertion of BAX-family proteins into the mitochondrion results in release of cytochrome *c*, which then forms a complex with a cytoplasmic protein known as apoptotic protease-activating factor 1 (APAF-1). In the presence of ATP/dATP, this complex recruits caspase 9 and oligomerizes in the cytoplasm, forming a structure known as the apoptosome. Like caspase 8 in the extrinsic pathway, the caspase 9-associated apoptosome activates caspase 3 and effector molecules that result in cellular apoptosis (see figure). Simultaneously, apoptosome regulatory proteins such as SMAC or Diablo are also released from the mitochondrion and remove inhibition of the apoptosome pathway due to inhibitors of apoptosis proteins (IAP).

Alternatively, in certain type I cells, caspase 8 activation leads to a caspase cascade that circumvents the mitochondria. In addition, apoptosis-inducing factor (AIF) and endonuclease G (EndoG), both of which are released from the mitochondria, can cleave DNA in the absence of caspase activation.

proinflammatory receptors include ATP, glycosylated proteins with exposed mannose residues, uric acid, heat-shock proteins and the chromosomal protein HMGB1 (REFS 10–14). This strategy has additional implications for protection against infection, as stimulation of at least one of these receptors — a PURINERGIC RECEPTOR for ATP — inhibits infection by the intracellular bacteria *Mycobacterium tuberculosis* and *Chlamydia trachomatis*[15–17], and might have a similar effect on other intracellular pathogens that inhabit membrane-bound vacuoles.

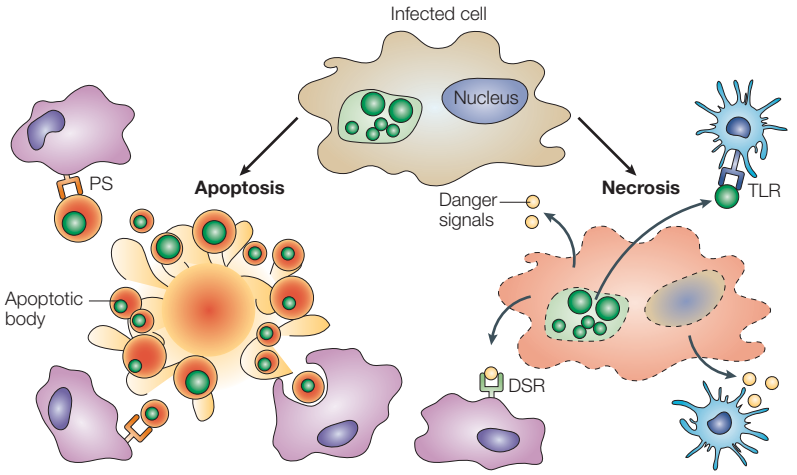



Figure 1 | **Infected cells can die through apoptosis or necrosis, or most likely a combination of both.** Interaction of apoptotic bodies with receptors such as the phosphatidylserine (PS) receptor on phagocytic cells reduces the inflammatory response. Chlamydiae taken up by this mechanism could help to propagate the infection, or the pathogen might be eliminated along with apoptotic bodies. Experimental evidence for either of these possibilities is lacking. Conversely, cells dying through necrosis release molecules that are normally present in the nucleus, intracellular organelles such as the Golgi apparatus, or the cytosol (danger signals, DS), which are recognized by specific receptors (DSRs) that are present on the surface of macrophages and dendritic cells. Ligation of DSRs leads to stimulation of the inflammatory response. Simultaneously, pathogens can be released from necrotic cells. Pathogens can interact directly with Toll-like receptors (TLRs), although pathogens that are coated with host-cell debris might also stimulate DSRs.

CASPASES
Family of cytosolic proteases that contain a cysteine residue within the active site, and which cleave their substrate after an aspartic acid residue. They can be divided into inflammatory caspases, which cleave and activate pro-inflammatory cytokines, and pro-apoptotic caspases, which cleave and activate pro-apoptotic substrates.

NECROSIS
An accidental cell death process that is characterized by an accompanying inflammatory response.

PURINERGIC RECEPTORS
A family of receptors that are stimulated by the purine nucleotides — ATP, ADP, AMP and UTP.

TYPE I CELLS
Cells that recruit caspase 8, which results in the subsequent cleavage of caspase 3.

TYPE II CELLS
Cells that activate caspase 3 through a mitochondria-dependent step.

***Infectious agents and apoptotic signalling.*** The influence of infectious agents on apoptosis has been known for nearly two decades. For example, some oncogenic viruses destroy p53, which is an important growth-arrest transcription factor and apoptosis inducer, and thereby increase the neoplastic potential of these viruses by reducing the potential of the host cell for apoptosis[18]. Other viral mechanisms that interfere with apoptosis include the production of inhibitors of the Fas–TNF-receptor-mediated extrinsic pathway (BOX 1), the production of caspase inhibitors, and even the production of proteins with antioxidant activity that provide protection against mitochondrial damage[19]. Viral inhibition of apoptosis has several advantages, including the maintenance of a functional host cell that continues to produce viral products, replicates the viral genome and integrates viral DNA into the host-cell genome. Some viruses express gene products that activate apoptotic signalling by events such as receptor-mediated caspase activation or potentiation of p53 (REFS 20,21). Presumably, viruses that have evolved apoptotic-inducing proteins have done so because these activities are important for viral spread in ways that limit inflammation.

Pathogenic bacteria have also been implicated in modulating host-cell apoptosis. Most often, bacterial pathogens have been shown to elicit a pro-apoptotic response[22–25]. Induction of apoptosis has been linked to the production of bacterial toxins that either target host-cell membranes (for example, those produced by *Helicobacter*, *Staphylococcus* and *Listeria* spp.), inhibit host-cell protein synthesis (for example, *Corynebacterium* and *Shigella* spp.) or secrete effector proteins directly into the host-cell cytoplasm via a type III secretion system (for example *Shigella*, *Salmonella* and *Yersinia* spp.). Inhibition of apoptosis has rarely been reported for bacterial pathogens, although the obligate intracellular pathogen *Rickettsia rickettsii* might inhibit apoptosis via induction of nuclear factor-κB (NF-κB)-mediated events[26]. The rickettsiae probably interfere with apoptosis to encourage a long-term relationship with the invaded host cell and to prevent this protected environment from collapsing.

## Chlamydiae and apoptosis

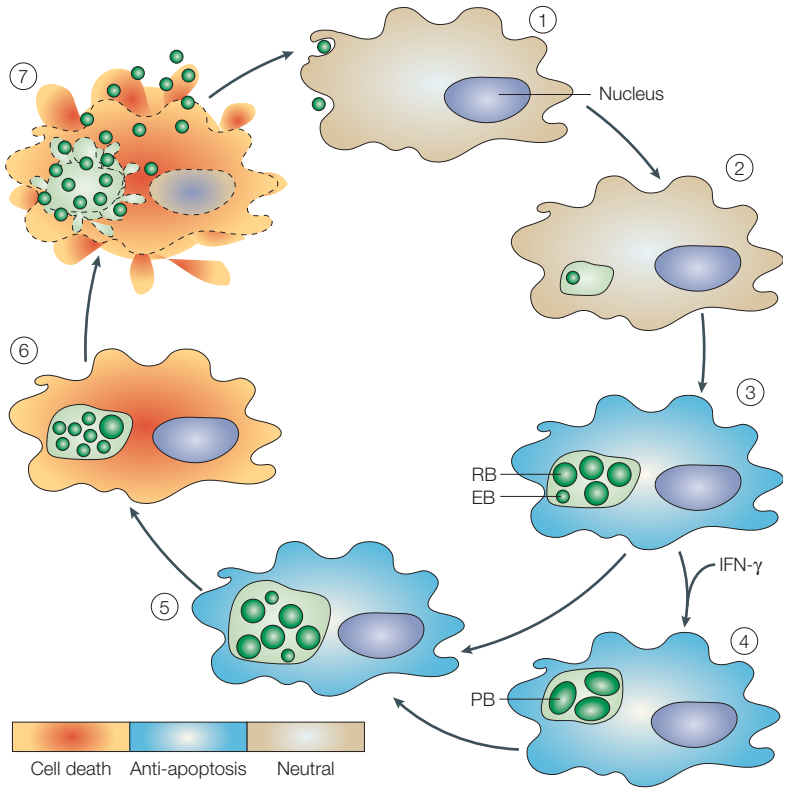
The chlamydiae are also obligate intracellular prokaryotic pathogens (BOX 2), but the effects of the chlamydiae on apoptotic signalling pathways are variable, which makes these microorganisms unique and more complex in their apoptotic signalling patterns than other pathogens (FIG. 2). Under some circumstances these microorganisms induce apoptosis and/or necrosis, but under other circumstances they inhibit apoptosis. The circumstances that dictate whether the chlamydiae inhibit or activate host-cell death reflect several important pathogenic considerations, including whether an acute or chronic infection is in progress and whether intracellular chlamydial growth is programmed to go through a productive infectious cycle or is stalled under non-productive growth conditions (BOX 2).

These seemingly contradictory responses might not be as unusual as they initially appear. First, there are several different chlamydial BIOVARS, and distinctions based on the characteristics of these biovars are possible. Second, chlamydiae cause disease in many different species, and pro-apoptotic responses in one host species might not be applicable for responses that are elicited in another, even if the disease pathogenesis is similar. Third, the intracellular growth cycle of the chlamydiae is complex and several growth options are possible, depending on the host-cell type, the particular environmental conditions in the host cell and the nature of the tissue that is being affected. It is possible that apoptotic activity is controlled to some extent by the intracellular growth status of the chlamydiae, which can be influenced by any or all of these considerations.

***Protection against apoptosis.*** Several studies have shown that epithelial cells or monocytes that are infected with *C. trachomatis* or *Chlamydia pneumoniae* are protected against mitochondrion-dependent cell death, but not death once the effector caspase 3 is activated[27–32]. Release of cytochrome *c* from the mitochondria is required for activation of caspase 3 via the APOPTOSOME PATHWAY, and *Chlamydia* infection inhibits cytochrome *c* release in cells treated with different apoptosis inducers.

Interestingly, infection of monocytes by *C. pneumoniae* leads to activation of the transcription factor NF-κB[33]. NF-κB is better known for its role in inducing the expression of genes that are involved in inflammatory responses via Toll-like receptor (TLR) signalling, but its activation is also associated with resistance to apoptosis[34]. Experiments with inhibitors of NF-κB activation indicate that at least part of the resistance to apoptosis in

Box 2 | ***Chlamydia* and chlamydial diseases**




The chlamydiae are ubiquitous pathogens. In humans, *Chlamydia trachomatis* is a pathogen of mucosal surfaces that is responsible for ocular (serovars A, B, Ba and C) and genital tract (serovars D–K and L1–L3) infections[73]. Chronic sequelae are common. Trachoma is a blinding manifestation of chlamydial ocular infection and, in women, pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility can occur after chlamydial infection of the lower genital tract. Reactive arthritis can also be seen in individuals who are genetically predisposed to this condition. *Chlamydia pneumoniae* is a frequent cause of community-acquired pneumonia, and although its involvement in chronic sequelae is not firmly established, it is frequently present in atherosclerotic lesions in coronary and carotid arteries and might therefore have a causal involvement in heart disease and stroke[74].

Definitive pathogenesis and virulence investigations for the chlamydiae have been hampered because methods for gene transfer have not yet been developed for these microorganisms. Despite this critical experimental limitation, a great deal of information has been generated on intracellular chlamydial growth and development, and the effects of chlamydial infection on host-cell physiology[75]. The organism undergoes an orderly alternation between a metabolically inactive, highly infective form — the elementary body (EB) — and a metabolically active intracellular growth stage form — the reticulate body (RB). Intracellular growth and development occur within the confines of a membrane-bound vesicle or inclusion (see figure).

Intracellular chlamydiae communicate with host cells by introducing proteins into the inclusion membrane and secreting effector proteins into the host-cell cytoplasm. This probably occurs via a type III secretion apparatus. Productive infections involve: invasion of susceptible host cells (1,2); differentiation of EBs into RBs within the internalized vesicle (3); intracellular growth and division of RBs in the inclusion (5); followed by differentiation of RBs into EBs and escape of EBs from the host cell (6,7). Modification of this intracellular development cycle can occur under a variety of altered environmental conditions (for example, activation of host cells by the $T_H1$ cytokine interferon-γ) so that RBs cease to divide, do not differentiate into EBs, and maintain a more stable association with the infected host cell in the form of persistent bodies (PBs) (4). This reversible growth option is referred to as persistence and is important in the pathogenesis of chronic chlamydial infections[38,39]. The growth status of chlamydiae (early versus later; productive versus persistent) influences whether the infected cells receive no apoptotic signals (beige), anti-apoptotic signals (yellow) or signals that lead to cell death (orange). It is difficult to know with certainty how pro-apoptotic and anti-apoptotic effects correlate with the many clinical manifestations and various chlamydial diseases. A working hypothesis that could link apoptotic events and disease-specific scenarios is that chlamydiae-induced apoptotic activity is associated with acute manifestation of disease, whereas inhibition of apoptosis is integral to chronic disease states. Experimental resolution of these hypotheses will require careful evaluation of experimental *in vivo* models for apoptotic induction or inhibition. Figure modified with permission from REF. 76 © (2003) Elsevier.

BIOVARS
The phenotypical distinction of bacteria within the same species based on biological tests such as simple biochemical and/or enzymatic differences.

APOPTOSOME PATHWAY
A pathway of caspase activation that requires release of cytochrome *c* from the mitochondria.

PLAQUE ASSAY
An assay that allows visualization of the cytopathic effect of viruses or bacteria in a monolayer of host cells. The plaque centre lacks cells due to infection-induced lysis.

*C. pneumoniae*-infected cells might be due to infection-mediated activation of NF-κB[33]. However, the extent to which NF-κB-dependent gene transcription contributes to resistance to apoptosis in *C. trachomatis*-infected cells is unknown. Clearly, differential expression patterns of TLRs in epithelial cells compared with macrophages could markedly influence the responses that are seen at mucosal surfaces. Importantly, protection of *C. trachomatis*-infected cells is not affected by treatment with cycloheximide, which is an inhibitor of host-cell protein synthesis[28]. So, although cells that are infected with *C. trachomatis* or *C. pneumoniae* can upregulate the expression of genes encoding either pro-apoptotic (the apoptosis inducer EPHA2 and the Fas activator EGR1) or anti-apoptotic (IAP (inhibitors of apoptosis proteins) homologues and the BCL-2 family member MCL-1) mediators[35–37], it is not known how upregulation of these genes affects modulation of apoptosis during *Chlamydia* infection.

Chlamydial inhibition of apoptosis could represent a mechanism that has evolved to help establish chronic infections. Chronic *Chlamydia* infections are thought to involve a non-productive growth state[38,39] that predicts a long-term stable association with each infected host cell. Under these conditions *in vivo*, active maintenance of host-cell viability and longevity would be advantageous, and inhibition of apoptosis would be a way by which this could be achieved.

***Cell death due to the infection itself.*** Cytolytic activity associated with *Chlamydia* infection was first described more than 30 years ago[40–44], and is the basis for the PLAQUE ASSAY that is still used to measure chlamydial growth[45]. However, the mechanism of cell death was not

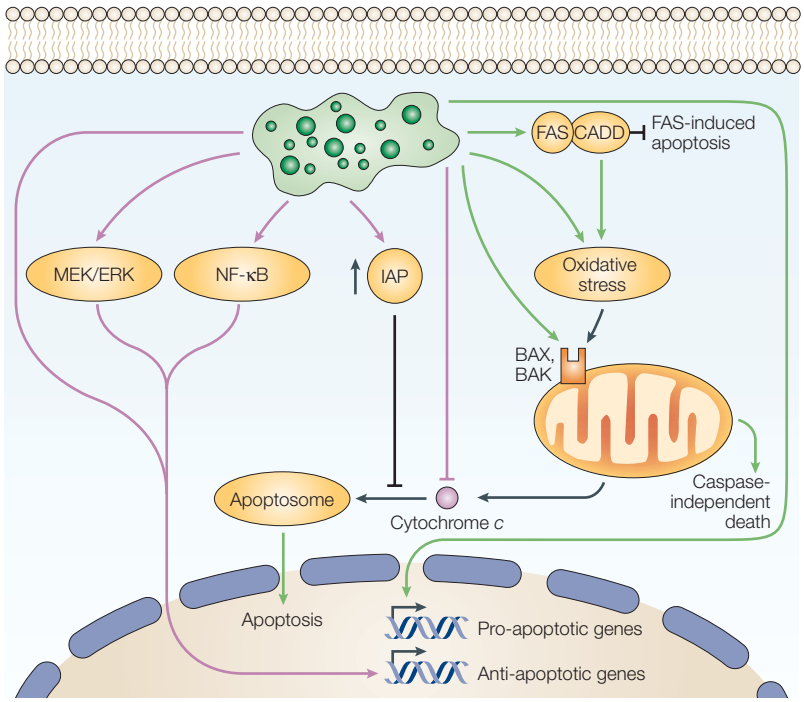



Figure 2 | **The chlamydiae have the ability to both activate and inhibit apoptotic signalling pathways in eukaryotic host cells.** Inhibition of apoptosis could be achieved by several different mechanisms, including inhibiting the release of cytochrome *c* from the mitochondria, activation of the transcription of anti-apoptotic genes, including via the nuclear factor-κB (NF-κB) and the ERK/MEK mitogen-activated protein kinase (MAPK) signalling pathways, and upregulation of inhibitors of apoptosis proteins (IAP), which could inhibit the apoptosome pathway, as shown by the inhibitory black arrow. Activation of apoptosis could also occur by different mechanisms including activation of BAX and activation of the transcription of pro-apoptotic genes such as those that encode EPHA2 and the Fas activator EGR1. Bioinformatic analysis has also revealed the presence of CADD (*Chlamydia* protein associating with death receptors), which has both a death-domain-like region that could interact with Fas and an oxidoreductase domain, suggesting that it could modulate Fas-induced apoptosis or induce cell death through oxidoreductase activity. Anti-apoptotic and pro-apoptotic mechanisms are represented by pink and green arrows, respectively. Black arrows represent the normal cell reactions.

characterized until more recently[46–48]. Infected cells have characteristics of apoptotic death but none of the known caspases are involved[47,49]. In the absence of caspase activation, what mechanism could account for death of infected cells? An increasing number of studies demonstrate that some types of cell death can occur without the involvement of caspases[50–52]. Indeed, overexpression of either of the pro-apoptotic proteins BAX and BAK results in cell death even when caspases are inhibited[53,54], and BAX is activated at the end of the developmental cycle in cells that are infected with *Chlamydia psittaci* or some strains of *C. trachomatis*. Apoptosis after BID cleavage is inhibited in infected cells, so it is likely that BAX activation is also inhibited in infected cells that are treated with external apoptosis inducers[77]. BAX activation at the end of the developmental cycle would therefore have to be independent of truncated BID (tBID) and perhaps other BH3-ONLY PROTEINS. However, *Chlamydia* infection is also known to increase mitochondrial metabolism and oxidative stress[55–57], which could perhaps lead to BAX activation independently of 'classic' BH3-only proteins such as BID, PUMA or BIM (BOX 1).

A potential biological function for BAX-dependent cell death was suggested by the observation that after two infection cycles fewer chlamydiae were obtained from *BAX*-deficient cells than from wild-type cells[58]. Therefore, BAX-dependent cell death might allow chlamydiae to exit from infected cells and initiate a new infection cycle in neighbouring cells. These cell-culture results were confirmed in an *in vivo* model of genital infection in which it was demonstrated that infection by *C. trachomatis* lasts longer in wild-type mice than in *BAX*-deficient mice[58]. Although these results are consistent with a role for BAX-dependent death in chlamydial propagation, further studies will be required to determine if a BAX deficiency might also allow immune effector cells to survive longer, thereby eliminating the infection more efficiently.

Cell death during infection by some *Chlamydia* strains has features that are characteristic of both apoptosis and necrosis[58]. *Chlamydia*-induced necrosis might be associated with a heterogeneous family of CLOSTRIDIAL CYTOTOXIN HOMOLOGUES that are encoded by the genomes of *C. trachomatis* and *C. psittaci*[59]. The balance between apoptotic and necrotic cells might also be affected by the host-cell type, as primary fibroblasts are more sensitive to apoptosis during *Chlamydia* infection than the HeLa epithelial cell line[60]. HeLa cells constitutively express oncogenes from the human papilloma virus that might partially inhibit apoptosis via their effect on p53 (REF. 61). Although fibroblasts are not preferential host cells for *Chlamydia*, the results highlight the need to characterize host-cell survival and death using physiologically relevant primary cells from tissues that are infected by *Chlamydia* spp.

***Search for chlamydial apoptosis modulators.*** A bioinformatic search for chlamydial genes encoding potential apoptotic mediators has led to the identification of '*Chlamydia* protein associating with death receptors' (CADD), which is present in the genomes of *C. trachomatis* and *C. pneumoniae* and is expressed in chlamydial-infected cells[62]. CADD has a DEATH-DOMAIN-like region that allows it to interact with Fas, and Fas is recruited towards the *Chlamydia* inclusion, which indicates that CADD might inhibit Fas-induced apoptosis. However, transient expression of CADD in different uninfected cells results in apoptosis[62]. As CADD seems to be an oxidoreductase[63], it is tempting to speculate that CADD could either induce apoptosis via its interaction with Fas, or cause reactive oxygen species to accumulate, leading to necrosis. In this scenario, the host-cell environment and host-cell anti-apoptotic factors would determine the balance between apoptosis and necrosis. However, the role of CADD during infection with whole chlamydiae still needs to be evaluated.

Studies showing that *Chlamydia* infections have an inhibitory effect on apoptosis consistently provide evidence that the presence of chlamydiae interferes with mitochondria-based signalling. So, in a manner similar to the effects of the BCL-2 family of inhibitory factors in type II cells, *Chlamydia* infection inhibits

BH3-ONLY PROTEINS
Proteins that contain a BCL-2 homology (BH) 3 domain, but not the other BH domains usually found in BCL-2 family proteins. The BH3 domain is required to inhibit the activity of pro-survival proteins related to BCL-2.

CLOSTRIDIAL CYTOTOXIN HOMOLOGUES
Cytopathic toxins, found in many bacteria, which inactivate host-cell proteins that regulate actin polymerization, causing the cells to round up.

DEATH DOMAIN
A region of limited homology consisting of about 80 residues close to the intracellular carboxyl terminus of some cell-membrane receptors that is essential for the receptors to generate a signal leading to apoptosis.

apoptosis pathways that require a mitochondrial amplification step, but not if a caspase cascade is activated directly[29]. It is therefore possible that the chlamydiae produce and secrete a BCL-2-like protein. Alternatively, as phosphorylation can modify the anti-apoptotic activity of BCL-2 and BCL-$X_L$, infection might induce the activation of pre-existing anti-apoptotic BCL-2-like proteins in the host cell, or the inactivation and/or degradation of pro-apoptotic proteins[77]. Genomic searches[29] have so far failed to identify a chlamydial BCL-2 homologue, but this does not exclude the possibility that a functional chlamydial homologue without sequence homology might have a similar role. Finally, potentially anti-apoptotic signalling pathways, such as the MEK/ERK pathway, are activated during infection[64], and might also contribute to resistance to apoptosis.

If secreted chlamydial gene products contribute to the induction or inhibition of apoptosis, it is important to also note that chlamydiae seem to have a type III secretion system, the genes of which are differentially regulated during intracellular growth[65]. If some genes are more important during persistent growth, and other genes function mainly during productive growth, then examination of the secreted effector proteins that are associated with each group might shed light on how chlamydiae activate apoptosis under some sets of growth conditions and inhibit it under others.

## Apoptosis and chlamydial disease

It is clear that apoptosis has a direct role in many infectious diseases, especially those caused by viruses[19], intracellular protozoans[66] and intracellular bacteria[22]. It is also clear that for many of these microbial pathogens, apoptotic signalling is driven by the pathogen and not by the host cell. So, induction or inhibition of apoptosis during infections could identify important virulence factors for these microorganisms. Work on the mechanistic studies of apoptosis has provided the impetus to determine whether secreted chlamydial gene products interact physically with host-cell proteins. These types of investigations have helped to identify chlamydial CADD and should provide a means to further understand other virulence factors, their function and the role of differential gene expression by chlamydiae in a variety of host cell types under different growth conditions[58].

The study of apoptosis will continue to help us understand chlamydial virulence factors, mechanisms of disease pathogenesis and requirements for effective immune responses. Results from these studies will hopefully contribute to the development of improved diagnostic methods, the development of better chemotherapeutics against chlamydiae and vaccine design. In these ways, work on apoptosis and chlamydiae can impact on both our basic knowledge and its practical applications.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### Acknowledgements
Work in the authors' laboratories is supported by grants from the Public Health Service. We thank O. S. Mahdi for her help in organizing and critiquing the manuscript.

### Competing interests statement
The authors declare no competing financial interests.

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