Published in final edited form as:

Curr Immunol Rev. 2007 ; 3(1): 31–40. doi:10.2174/157339507779802179.

Host-Cell Survival and Death During *Chlamydia* Infection

Songmin Ying¹, Matthew Pettengill², David M. Ojcius^{*,2}, and Georg Häcker^{*,1}

¹Institute for Medical Microbiology, Technische Universität München, D-81675 Munich, Germany

²School of Natural Sciences, University of California, Merced, CA 95344, USA

Abstract

Different *Chlamydia trachomatis* strains are responsible for prevalent bacterial sexually-transmitted disease and represent the leading cause of preventable blindness worldwide. Factors that predispose individuals to disease and mechanisms by which chlamydiae cause inflammation and tissue damage remain unclear. Results from recent studies indicate that prolonged survival and subsequent death of infected cells and their effect on immune effector cells during chlamydial infection may be important in determining the outcome. Survival of infected cells is favored at early times of infection through inhibition of the mitochondrial pathway of apoptosis. Death at later times displays features of both apoptosis and necrosis, but pro-apoptotic caspases are not involved. Most studies on chlamydial modulation of host-cell death until now have been performed in cell lines. The consequences for pathogenesis and the immune response will require animal models of chlamydial infection, preferably mice with targeted deletions of genes that play a role in cell survival and death.

Keywords

Chlamydia; apoptosis; necrosis; inflammation; danger signals

INTRODUCTION

Chlamydia trachomatis is the most common agent of bacterial sexually-transmitted disease (STD) in the world. In the U.S., 4.2% of young adults are infected [1], with prevalence rates of 5-25% being reported in sexually active teenagers [2,3]. Infection with genital serovars leads to complications such as pelvic inflammatory disease (PID) and infertility. *C. trachomatis* also includes the trachoma serovars, which are the leading cause of preventable blindness worldwide [4]. *Chlamydia pneumoniae* leads to airway infection and has been found in some cases of atherosclerosis [5].

Bacteria of the genus *Chlamydia* have evolved to take advantage of the inherently nutrient-rich environment available within eucaryotic cells. Chlamydiae develop through a biphasic cycle within a membrane-enclosed vacuole in the host cell called an inclusion. Chlamydiae infect initially as metabolically-passive elementary bodies (EB) before maturing into larger metabolically-active reticulate bodies (RB), a process unique to chlamydiae [6]. RB are non-infectious; therefore propagation of infection requires recondensation into EB [6-8]. The timing of this development is crucial: interruption during a stage dominated by RB would prevent completion of the infection cycle. During acute infection, the RB multiply by binary fission to produce many more progeny, up to 1000 from a single inclusion.

* Address correspondence to either author at the School of Natural Sciences, P.O. Box 2039, Merced, CA 95344, USA; Tel: (209) 724-2948; Fax: (209) 724-2912; E-mail: dojcus@ucmerced.edu or Institute for Medical Microbiology, Technische Universität München, D-81675 Munich, Germany; Tel: (49) 89 4140 4134; Fax: (49) 89 4140 4868; E-mail: hacker@lrz.tu-muenchen.de

Chlamydiae depend on eucaryotes not only for sustenance, but for transmission as well. Chlamydiae influence host-cell activities through mostly uncharacterized mechanisms, but the discovery that chlamydiae possess and use a type III secretion system [9-11] suggests that some aspects of the modulation may be caused by secreted chlamydial effector proteins. The expanding chlamydial population can exert biochemical changes at the level of the individual host cell and indirectly through effects on the host organism. The pathology of infection is probably due as much to cytochemical damage induced by infection as an excessive host immune response [12]. An inflammatory response is required for the resolution of primary *C. trachomatis* infection, but chronic inflammation is also responsible for the scarring process observed in trachoma and chlamydial STD. Thus, a number of inflammatory mediators are present during infection, including interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). TNF- α and other inflammatory cytokines may aid in eradicating chlamydial infection, but also promote long-term tissue damage [13,14].

Cells of mucosal tissues infected by *Chlamydia* include epithelial cells, macrophages, fibroblasts and dendritic cells (DC). These cells express Toll-like receptors (TLR) that recognize specific pathogen-associated molecular patterns (PAMP), such as bacterial wall components (lipopolysaccharide (LPS), peptidoglycan (PG), lipoproteins), bacterial DNA, flagellin, and double-stranded RNA. Engagement of TLR by microbial products leads to production of biologically-active mediators, including antimicrobial peptides, cytokines and chemokines that participate in the inflammatory response [15-17]. Chlamydiae have several cell wall and outer membrane components that may be recognized by TLR. Chlamydial LPS and chlamydial heat shock proteins have been reported to be ligands for both the TLR2 and TLR4 receptors [18-24]. However, recent papers describe a dominant role for TLR2 in the recognition of *C. pneumoniae* [25]. Similarly, while both TLR2 and TLR4 are stimulated *ex vivo* during infection of peritoneal macrophages and lung fibroblasts by the mouse pneumonitis agent (MoPn) of *C. trachomatis* (also known as *C. muridarum*), only TLR2 is essential in development of pathology in chlamydial genital tract infection in mice [26].

A predisposition to pathology such as PID has been noted in the past [27], but factors that predispose some individuals to disease and mechanisms by which chlamydiae cause inflammation and tissue damage remain unclear. Although chlamydial interaction with TLR and cytosolic TLR-like receptors undoubtedly plays a role in initiation of pro-inflammatory cytokine and chemokine production, the mode of cell lysis at the end of the infectious cycle is also likely to have a profound effect on the host response. Two main types of cell death have been described, with variations on both extremes. While apoptosis is the predominant form of cell death in physiology [28], cell death in the absence of apoptosis has also been observed. This form is commonly referred to as necrosis, although this concept probably encompasses several forms of cell death caused by different molecular events [29,30]. One occasion where necrosis is found is in situations where a cell attempts to undergo apoptosis but is (for instance, experimentally) prevented from doing so [31].

THE BASICS OF APOPTOSIS

Apoptosis, sometimes called programmed cell death, is a well-conserved process that multicellular organisms use for control of development and homeostasis, as well as the removal of cells recognized by the immune system as being infected or cancerous [28,32,33].

Apoptosis can be induced through both extrinsic (death receptor pathway) and intrinsic (mitochondria pathway) pathways [34-37]. Death receptor pathways are initiated by ligand binding to cell-surface receptors (for example, Fas ligand binding to Fas). Mitochondrial pathway signaling involves the activation of the proteins Bax and Bak that can permeabilize mitochondrial membranes (Fig. 1). In most cases, both of these pathways require the release

of mitochondrial cytochrome *c* and the subsequent activation of a group of cytosolic proteases, called caspases [38]. Cell death can also be induced in the absence of caspases, but authors disagree whether or not this should be called apoptosis [29,39,40]. Usually, the family of caspase proteases causes the death of the cell and the appearance of typical morphological changes such as chromosomal fragmentation and nuclear condensation. Caspase activity has been described as a cascade, with the activation of upstream caspases leading directly to the cleavage and subsequent activation of downstream caspases. Activation of caspase 3, for example, is mediated either by activated caspase 8 during death receptor signaling, or by caspase-9 in the case of signals transmitted through mitochondria (Fig. 1).

The central step of mitochondrial apoptosis is the release of cytochrome *c* into the cytosol, which in turn binds to Apaf-1 and caspase 9 as part of a complex known as the apoptosome, the caspase-activating signaling complex (although many proteins are released from mitochondria during apoptosis and some can probably contribute to cell death and morphological changes, the importance of these proteins is uncertain [41]). The release of cytochrome *c* is governed by Bcl-2 family proteins. This family consists of three groups of proteins: anti-apoptotic Bcl-2 like proteins, pro-apoptotic BH3-only proteins, and the multi-domain Bax/Bak group (Fig. 2). The roles played by these proteins during programmed cell death will be discussed in more detail, as well as the intriguing characteristics of their involvement and outcome during infection of host cells with *Chlamydia*.

Two types of cells have been identified with regards to the requirement for a mitochondrial amplification loop during apoptosis. In type II cells, apoptosis inducers can activate caspase-3 only through a mitochondrion-dependent step. In type I cells, caspase-8 recruitment and activation by cell surface receptors can lead directly to caspase-3 activation without help from mitochondria [42] (Fig. 1).

REGULATION OF APOPTOTIC SIGNALING BY THE BCL-2 FAMILY

The three groups of Bcl-2 family proteins share one or several Bcl-2 homology domains (BH1, BH2, BH3 and BH4) (Fig. 2). The BH3-only protein group (possessing only BH3 domains) includes at least 8 members [43]. Roles in apoptosis initiation are clearer for some than others, and regulation of their activity has also been shown to be handled differently within the group. Different apoptotic stimuli are processed through different BH3-only proteins; for example, Bim is required for induction of apoptosis *via* microtubule perturbation, and together with Puma plays an essential role during apoptosis upon cytokine withdrawal in lymphocytes [44, 45]. Puma and Noxa are important for cell death induced upon recognition of DNA damage through p53 [46]. Upon cleavage by activated caspase 8, Bid acts as a connecting point between the death receptor pathway and the mitochondria pathway in type II cells. The function of some other BH3-only proteins has not been elucidated, but it is very clear that this group of proteins is required in most cases of apoptosis induction.

The regulation of BH3-only proteins is still under investigation. Bim and Bmf are probably at least in part regulated on a post-translational level by release from their sites of sequestration following their activation [47,48], although a mitochondrial regulatory step at least for Bim also seems to exist [49,50]. Bad is dephosphorylated in the absence of survival signals, allowing it to dissociate from the cytosolic adapter protein 14-3-3 β and migrate to mitochondria [51]. Bid is cleaved proteolytically to form the active tBid (truncated Bid) [52,53], while Puma and Noxa are regulated at a transcriptional level [54-56]. Activation of BH3-only proteins leads to activation of Bax and/or Bak, which cause cytochrome *c* release from mitochondria (Fig. 1). Anti-apoptotic Bcl-2 like proteins (Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A1) can prevent the activation of Bax/Bak, probably through direct binding of BH3-only proteins (for a recent review on the molecular function of BH3-only proteins see [57]).

APOPTOSIS, NECROSIS AND THE IMMUNE SYSTEM

Apoptosis is a “silent” mode of death, where the dead cell disappears without generating much of a response from neighboring tissue. Necrosis, on the other hand, elicits inflammation. Although this probably comprises a large array of molecularly distinct events, necrosis is often defined as any cell death that occurs that is not apoptosis (i. e., it does not involve activation of the apoptotic pathway). During apoptosis, the contents of the dying cell remain in membrane-bounded “apoptotic bodies”, which detach from the dying cell and are subsequently phagocytosed by neighboring cells, including macrophages, dendritic cells and epithelial cells [58-60]. But debris released from necrotic cells is recognized by specific receptors on the surface of macrophages, DC and epithelial cells that promote inflammation. Some of these debris molecules, which are normally present in the cytosol, Golgi apparatus or nucleus, are released into the extracellular space where they act as immune system “danger signals” (DS) [61,62]. Originally, DS were thought to be restricted to molecules of microbial origin, such as LPS and bacterial CpG-DNA (also known as pathogen-associated molecular patterns, or PAMP [15,63]), but recent work has expanded the concept to include host-cell material released from dying or stressed cells, including ATP, adenosine, uric acid, heat-shock proteins and the chromosomal protein, high mobility group box 1 (HMGB1) [64-67]. The difference in outcomes following apoptosis or necrosis are enormous for the immune response, and are especially relevant for their role during infection with microbial pathogens.

The mode of host-cell death could be regulated by either the host or the infectious organism. In the case of infection with different *Chlamydia* species, inflammation is required for elimination of infection, but is also responsible for much of the pathogenesis [68,69]. It would thus appear that survival of the infected cell during chlamydial replication, followed by a noninflammatory, apoptotic death, could be beneficial to both the infectious organism and, to some extent, the host. However, the host would invariably benefit from a premature, apoptotic death of the infected cell, which would limit the ability of the pathogen to proliferate and must therefore be resisted by the pathogen.

CHLAMYDIA INTERFERES WITH HOST-CELL DEATH

Chlamydia has been described many times to either induce or inhibit host-cell death [70,71]. Initially, the apparently contradictory activities of chlamydial infection were viewed as controversial [72], although other intracellular pathogens, such as the herpes simplex viruses [73], were known to exhibit both activities at different stages of the infection cycle. In some cases, it is possible to cleanly separate the two activities. Thus, modified vaccinia virus Ankara blocks apoptosis in infected cells. However, when the antiapoptotic protein FIL is knocked out, the virus induces apoptosis [74-76]. This demonstrates how a pathogen can possess pro-apoptotic activities that are masked by an anti-apoptotic activity that is expressed at the same time.

In the case of chlamydial infections, some of the conflicting interpretations reported in the literature may be due to opposing activities (pro- and anti-apoptotic) being expressed at different levels at different times. Other discrepancies could be due to the use of different chlamydial strains or host-cell types [77]. And the appearance of conflict may have been aided by the fact that, until recently [78,79], separate laboratories were studying either the cell-death inhibiting or inducing activities of chlamydial infection, but not both activities.

Inhibition of Host-Cell Death

The first paper in 1998 described that *C. trachomatis*-infected cells are resistant to numerous experimental apoptotic stimuli, exhibiting inhibition of caspase activation and blockage of cytochrome *c* release from mitochondria [80]. Soon thereafter, we and others demonstrated

that *C. pneumoniae* has a similar effect, being able to block apoptosis as well as cytochrome *c* release induced in infected cells [81,82]. The observation that apoptosis due to death ligands was only blocked in type II cells (apoptotic signals requiring a mitochondrial amplification loop), but not in type I cells (caspase 3 being activated directly by caspase 8), further suggested that the chlamydial anti-apoptotic factor(s) exerts its effect at the level of mitochondria or a pre-mitochondrial step [83]. Since activation of Bax and Bak is required and sufficient to release cytochrome *c* from mitochondria in most cases of apoptosis studied until now, it was not surprising to find that Bax and Bak activation is blocked when *Chlamydia*-infected cells are treated with apoptosis inducers [84-86]. Although infection leads to the transcriptional induction of some cellular anti-apoptotic proteins [87], these changes are probably not responsible for apoptosis resistance, since resistance is also observed when cellular translation is blocked during infection [80]. An explanation for these observations was found by characterizing the upstream members of the Bcl-2 family of proteins. Thus, although transcription levels were unaffected, the BH3-only protein Bim disappeared during chlamydial infection, and its disappearance could be prevented by addition of a proteasome inhibitor [84]. As discussed above, all BH3-only proteins share a small BH3 domain, and deletion studies demonstrated that the BH3 domain is required for targeting for degradation during chlamydial infection [84]. Subsequent studies confirmed and extended these results by showing that all investigated BH3-only proteins are degraded in *Chlamydia*-infected cells [88,89]. Importantly, chlamydial infection is unable to protect against experimental over-expression of active Bim, which still induces apoptosis [84]. Thus, no protection exists in infected cells against active BH3-only proteins, and the prevention of the generation of active BH3-only proteins (as occurs through degradation of BH3-only proteins) is probably a critical mechanism in terms of apoptosis inhibition.

Interestingly, Bid is also a member of the BH3-only protein family, and is normally activated *via* cleavage by caspase-8, generating a truncated form, called tBid. The BH3 domain is hidden in intact Bid, but becomes exposed in tBid [90]. While intact Bid remains unaffected, tBid is targeted for degradation during chlamydial infection [88]. The degradation of tBid thus suggests that the protein-degrading activity of chlamydiae is specific.

Although the loss of BH3-only proteins can explain many cases of apoptosis resistance, chlamydiae appear to possess additional strategies for protecting their host-cells against apoptosis. As revealed with host-cells infected at lower multiplicities of infection, chlamydial infection also results in activation of the phosphoinositide-3 kinase (PI3K) “survival pathway” [78]. During infection with *C. trachomatis*, activation of PI3K leads to Akt activation and in turn to phosphorylation of a pro-apoptotic protein, Bad (Fig. 1). Bad belongs to the BH3-only protein family, and is thought to function at the level of mitochondria by binding to some of the anti-apoptotic Bcl-2 family proteins [91]. However, phosphorylated Bad is co-localized with 14-3-3 β on the *C. trachomatis* inclusion, which is recruited *via* a chlamydia-encoded inclusion protein, IncG. Bad is thus sequestered away from mitochondria, where it could induce host-cell death. Interestingly, this effect is observed when cells are infected with *C. trachomatis* but not *C. pneumoniae* [78], because *C. pneumoniae* does not express IncG, nor does it recruit 14-3-3 β to the inclusion [92]. Furthermore, inhibition of PI3K reverses the resistance to apoptosis in *C. trachomatis*- but not *C. pneumoniae*-infected cells [78].

Another pro-apoptotic molecule, protein kinase C δ (PKC δ), a member of the protein kinase c (PKC) family, is found on mitochondria and in the nucleus. During cell death, PKC δ accumulates on mitochondria, where it promotes cytochrome *c* release [93]. It was shown recently that an increase in diacylglycerol levels on the chlamydial inclusion vacuole recruits PKC δ away from its conventional targeting site [94]. Taken together, *Chlamydia* infection leads to inhibition of several mediators of apoptosis at pre-mitochondrial steps, resulting in

BH3-only protein degradation, and Bad and PKC δ recruitment by the chlamydial inclusion, thus promoting survival of the host cell (Fig. 1).

To our knowledge, BH3-only protein degradation has not been described during infection by other pathogens, but several viruses, bacteria, and protozoan parasites are known to enhance host-cell survival through activation of the PI3K pathway [95-102].

The Role of NF- κ B in Apoptosis Inhibition Induced by *Chlamydia*

Members of the transcription factor family, NF- κ B, are key regulators of the expression of many genes involved in immune regulation [103]. NF- κ B is also critically involved in the inhibition of cell death, as demonstrated by the lethal effects of massive liver apoptosis in mice lacking the NF- κ B subunit RelA [104]. Although NF- κ B has transcription-dependent anti-apoptotic activity, the molecular basis for this effect remains elusive. A number of NF- κ B target genes have been identified that could protect against apoptosis, such as the Bcl-2 relative Bcl-X_L and the modulator of caspase-8 activation, cFLIP. However, the link between NF- κ B and anti-apoptosis in individual situations remains difficult to prove [105]. For instance, up-regulation of the member of the Inhibitor of Apoptosis Protein (IAP) family, cIAP2, through NF- κ B has been observed numerous times and has sometimes been equated with protection against apoptosis, especially given that *Drosophila* IAP and their viral counterparts can bind and inhibit caspases. However, recent work shows that human IAP do not block caspases [106], and the reported anti-apoptotic effect of IAP therefore remains unclear.

During chlamydial infection, two questions have been addressed with regard to NF- κ B. First, is NF- κ B activated during infection? Secondly, does it block apoptosis during infection? Diametrically opposed results have been obtained with different cell types. Thus, strong NF- κ B activation has been observed in human monocytes and macrophages [107], endothelial cells [108] and DC [25], but has not been found in epithelial cells [81,109], although NF- κ B activation has been reported for persistent infection of epithelial cells [110]. This cell type specificity is probably due to differential ligation of TLR2 and TLR4 and consecutive NF- κ B activation by chlamydial components on some cells, especially from the innate immune system [19,25].

When activated by *Chlamydia*, does NF- κ B block apoptosis? Experimentally, it is not easy to address this question, since complete inhibition of NF- κ B, at least in macrophages, can kill the cells [111]. However, NF- κ B most likely does not participate in the profound protection against apoptosis that is observed after about one day of infection. Besides the fact that this protection is present in epithelial cells (in the absence of NF- κ B activation), protection has been observed in infected cells treated with cycloheximide, i.e. in the absence of host-cell protein synthesis [80]. It is therefore difficult to see how NF- κ B-induced genes could play a role in resistance against apoptosis. Nonetheless, opposite results have been reported for persistently-infected epithelial cells. Although cIAP2, but not cIAP1, was induced by persistent infection, silencing of either cIAP1 or cIAP2 removed protection against apoptosis of infected cells [110]. The mechanism for cIAP-dependent resistance against apoptosis during persistent infection therefore remains a mystery.

In summary, there is evidence that NF- κ B may be induced and perhaps exert an anti-apoptotic effect in some cell types. However, NF- κ B is not required for the potent anti-apoptotic effect observed in *Chlamydia*-infected cells.

Induction of Host-Cell Death

Despite the ingenious means evolved by chlamydiae to prevent their host cells from dying, old and new studies have demonstrated that cell death is induced at the end of the chlamydial

developmental cycle [79,85,112-118]. However, the molecular mechanism of cell death induced by infection still remains to be fully characterized. Importantly, activation of none of the known caspases has ever been observed during chlamydial infection [70,71,79]. However, either Bax inhibitor-1 or Bcl-2 overexpression can partly revert the death ratio [112], indicating that there may be a mitochondrion-dependent but caspase-independent atypical apoptosis pathway involved in death of *Chlamydia*-infected cells.

Other forms of cell death such as necrosis and a morphologically and biochemically defined form termed aponecrosis were also proposed to describe some of the features of *Chlamydia*-induced host-cell death [85,113]. We have recently analyzed *Chlamydia*-induced cell death in further detail and find several features that are commonly used to classify cells as apoptotic — namely, nuclear condensation and fragmentation, TUNEL staining, and some DNA fragmentation [79]. However, these changes occurred in the absence of activation of the apoptotic pathway. Importantly, there was no detectable release of cytochrome *c* and no activation of effector caspases [79,112]. Paradoxically, despite the absence of cytochrome *c* release, there was less cell death during infection in Bax-deficient [79,119] or Bak-deficient cells [79] than in wild-type controls. Based on these data, *Chlamydia*-induced cell death resembles apoptosis in some ways, but is the result of a mechanistically different process.

However, like other forms of cell death, phosphatidylserine is exposed on the cell surface [119,120], and the nonapoptotic cell death is sufficient to induce uptake of the infected cell by professional phagocytes such as DC [79]. Thus, *Chlamydia*-induced cell death may be relevant for the immune response by transmitting *Chlamydia*-derived antigens to professional antigen-presenting cells. Furthermore, it may impact the innate immune response. For instance, the pro-inflammatory mediator HMGB1 is normally associated with chromatin in viable or apoptotic cells, but becomes released during necrosis [121]. HMGB1 was released during chlamydial infection in an epithelial cell line but not in primary fibroblasts, and HMGB1 was also shed in genital-tract secretions of infected mice [85], suggesting that the form of cell death may depend partially on the host-cell type, but at least some necrosis may occur *in vivo*.

Since the initial description of caspase-independent host-cell death during chlamydial infection [122], a similar type of cell death has been described for cells infected by other pathogens, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, rabies virus, and herpes simplex virus 1 [123-127].

IMMUNOLOGICAL SIGNIFICANCE OF CELL DEATH DURING CHLAMYDIAL INFECTION

Inhibition or induction of host-cell apoptosis has been reported for many viral, bacterial and protozoan pathogens and is thought to play an important role in propagation or pathogenesis of infection [128-130]. There is every reason to believe that the outcome of chlamydial infection will also depend on modulation of host-cell death.

Chlamydiae are obligate intracellular bacteria that can replicate only in the infected host-cell, with the host cell providing protected space and essential nutrients for the bacteria [6,131]. But it still remains to be established how apoptosis modulation could be used to favor or hinder chlamydial infection. It has been proposed that inhibition of apoptosis may allow the *Chlamydia*-infected cell to avoid the cytotoxic mechanisms of the host-immune system [80]; but with the exception of a preliminary report that *Chlamydia*-infected cells are resistant to killing by a cytotoxic T lymphocyte (CTL) cell line [80], all studies have shown that cells infected with *Chlamydia* are sensitive to lysis by *Chlamydia*-specific CTL [132-139]. An obvious alternative would be that any host cell that harbors a large chlamydial inclusion would be prone to die, and the chlamydiae must therefore protect the host cell from succumbing to

stress-induced apoptosis before the chlamydial developmental cycle is complete. In addition, apoptotic or necrotic cells normally expose proteins and lipids on their surface that are recognized as “eat me” signals for ingestion by professional phagocytic cells [59,140,141]. Inhibition of premature host-cell death would thus protect the intracellular pathogen from such an unpalatable fate. Nonetheless, delayed spontaneous apoptosis of *C. pneumoniae*-infected neutrophils [142] may also lead to activation or prolongation of an immune response against the microbial organisms.

Conversely, host-cell death at later times may provide a means for releasing infectious EB at the end of infection cycle. Epithelial cells represent the preferential target cell for chlamydiae, and apoptotic bodies carrying chlamydiae may provide a “Trojan horse” strategy for transmitting the bacteria to new unsuspecting host-cells, as epithelial cells also express receptors that bind apoptotic cells and bodies [58,143]. Consistent with this possibility, chlamydial infection disappears more quickly in Bax-deficient mice than in wildtype mice [119], suggesting a role for Bax-dependent cell death in propagation of the infection. But cell death is not necessarily beneficial to chlamydiae in all cases, since ingestion of infected apoptotic cells by professional antigen-presenting cells such as macrophages and DC could also enhance the adaptive immune response against *Chlamydia*, as apoptotic bodies have already been shown to play a role in cross-priming CTL against mycobacterial infection [144]. Finally, at least some necrotic death most likely takes place even in infected primary cells, which would be detrimental to the efficiency of infection due to the pro-inflammatory effects of necrosis [145,146] (Fig. 3).

It is clear that most individuals mount an efficient immune response against chlamydiae that includes the activation of MHC class I- and class II-restricted T cells [147-152]. Therefore, chlamydial antigens must be delivered to DC and other professional antigen-presenting cells at some stage during the infection. It is possible that some free chlamydiae may be internalized by macrophages and DC, when some RB are released together with EB at the end of the developmental cycle, or when noninfectious EB are produced. However, it also appears likely that infected cells dying during the course of infection may release both antigen and cellular debris, which could then be taken up by DC and used, together with either bacterial or cellular danger signals [153-155], to initiate a T cell response. Although DC can phagocytose chlamydiae and present chlamydial antigens to T cells [156,157], antigens provided by dying cells might be more readily accessible, as apoptotic cells are also transported along the lymphatic vessels and can be processed in lymph nodes [144,158].

In conclusion, there is convincing evidence that chlamydiae can both protect host-cells against apoptosis and induce their subsequent death. The effects of both activities are not always beneficial to the pathogen, as inhibition or induction of host-cell death could also enhance both the adaptive and innate immune response against infection. Most of the studies conducted on chlamydial modulation of host-cell death have been performed in cells infected *in vitro*. Sorting out the consequences of cell death for pathogenesis, the efficiency of infection, and the immune response will require studies with mouse models in which the role of different components of the apoptotic machinery and different immune effector cells are investigated one by one.

REFERENCES

- [1]. Miller WC, Ford CA, Morris M, et al. Prevalence of chlamydial and gonococcal infections among young adults in the United States. JAMA 2004;291:2229–2236. [PubMed: 15138245]
- [2]. Burstein GR, Gaydos CA, Diener-West M, Howell MR, Zenilman JM, Quinn TC. Incident *Chlamydia trachomatis* infections among inner-city adolescent females. JAMA 1998;280:521–526. [PubMed: 9707141]

- [3]. Cohen DA, Nsuami M, Etame RB, et al. A school-based *Chlamydia* control program using DNA amplification technology. *Pediatrics* 1998;101:e1. [PubMed: 9417165]
- [4]. Thylefors B, Negrel AD, Pararajasegaram R, Dadzie KY. Global data on blindness. *Bull World Health Organ* 1995;73:115–121. [PubMed: 7704921]
- [5]. Campbell LA, Kuo CC. *Chlamydia pneumoniae*--an infectious risk factor for atherosclerosis? *Nature Rev Microbiol* 2004;2:23–32. [PubMed: 15035006]
- [6]. Moulder JW. Interaction of Chlamydiae and host cells *in vitro*. *Microbiol Rev* 1991;55:143–190. [PubMed: 2030670]
- [7]. Wyrick PB. Intracellular survival by *Chlamydia*. *Cell Microbiol* 2000;2:275–282. [PubMed: 11207584]
- [8]. Bavoil PM, Hsia R-c, Ojcius DM. Closing in on *Chlamydia* and its intracellular bag of tricks. *Microbiol* 2000;146:2723–2731.
- [9]. Clifton DR, Fields KA, Grieshaber SS, et al. A Chlamydial type III translocated protein is tyrosine-phosphorylated at the site of entry and associated with recruitment of actin. *Proc Natl Acad Sci USA* 2004;101:10166–10171. [PubMed: 15199184]
- [10]. Stephens RS, Kalman S, Lammel C, et al. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. *Science* 1998;283:638–639.
- [11]. Hsia, R-c; Pannekoek, Y.; Ingerowski, E.; Bavoil, PM. Type III secretion genes identify a putative virulence locus of *Chlamydia*. *Mol Microbiol* 1997;5:351–359. [PubMed: 9282747]
- [12]. Bavoil PM, Hsia R-c, Rank RG. Prospects for a vaccine against *Chlamydia* genital disease. I. Microbiology and pathogenesis. *Bull Inst Pasteur* 1996;94:5–54.
- [13]. Ward, ME. Mechanisms of *Chlamydia*-induced disease. In: Stephens, RS., editor. *Chlamydia: intracellular biology, pathogenesis, and immunity*. ASM Press; Washington, D.C.: 1999. p. 171-210.
- [14]. Darville T, Andrews CW, Laffoon KK, Shymasani W, Kishen LR, Rank RG. Mouse strain-dependent variation in the course and outcome of Chlamydial genital infection is associated with differences in host response. *Infect Immun* 1997;65:3064–3073.
- [15]. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* 2002;296:298–300. [PubMed: 11951031]
- [16]. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;2:675–680. [PubMed: 11477402]
- [17]. O'Neill LA. Immunity's early-warning system. *Sci Am J* 2005;292:38–45.
- [18]. Heine H, Muller-Loennis S, Brade L, Lindner B, Brade H. Endotoxic activity and chemical structure of lipopolysaccharides from *Chlamydia trachomatis* serotypes E and L2 and *Chlamydophila psittaci* 6BC. *Eur J Biochem* 2003;270:440–450. [PubMed: 12542694]
- [19]. Bulut Y, Faure E, Thomas L, et al. Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. *J Immunol* 2002;168:1435–1440. [PubMed: 11801686]
- [20]. Ohashi K, Burkart V, Flohe S, Kolb H. Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 2000;164:558. [PubMed: 10623794]
- [21]. Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. *J Immunol* 2000;164:13–17. [PubMed: 10604986]
- [22]. Da Costa CU, Wantia N, Kirschning CJ, et al. Heat shock protein 60 from *Chlamydia pneumoniae* elicits an unusual set of inflammatory responses *via* Toll-like receptor 2 and 4 *in vivo*. *Eur J Immunol* 2004;34:2874–2884. [PubMed: 15368304]
- [23]. Erridge C, Pridmore A, Eley A, Stewart J, Poxton IR. Lipopolysaccharides of *Bacteroides fragilis*, *Chlamydia trachomatis* and *Pseudomonas aeruginosa* signal *via* toll-like receptor 2. *J Med Microbiol* 2004;53:735–740. [PubMed: 15272059]
- [24]. Costa CP, Kirschning CJ, Busch D, et al. Role of Chlamydial heat shock protein 60 in the stimulation of innate immune cells by *Chlamydia pneumoniae*. *Eur J Immunol* 2002;32:2460–2470. [PubMed: 12207330]

- [25]. Prebeck S, Kirschning C, Durr S, et al. Predominant role of Toll-like receptor 2 versus 4 in *Chlamydia pneumoniae*-induced activation of dendritic cells. *J Immunol* 2001;167:3316–3323. [PubMed: 11544320]
- [26]. Darville T, O'Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L, Ojcius DM. Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in Chlamydial genital tract infection. *J Immunol* 2003;171:6187–6197. [PubMed: 14634135]
- [27]. Cohen CR, Brunham RC. Pathogenesis of *Chlamydia* induced pelvic inflammatory disease. *Sex Trans Infect* 1999;75:21–24.
- [28]. Vaux DL, Hacker G, Strasser A. An evolutionary perspective on apoptosis. *Cell* 1994;76:777–779. [PubMed: 8124715]
- [29]. Jäättelä M, Tschopp J. Caspase-independent cell death in T lymphocytes. *Nat Immunol* 2003;4:416–423. [PubMed: 12719731]
- [30]. Leist M, Jäättelä M. Four deaths and a funeral: from caspases to alternative mechanisms. *Nat Rev Mol Cell Biol* 2001;2:589–598. [PubMed: 11483992]
- [31]. Vercammen D, Beyaert R, Denecker G, et al. Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. *J Exp Med* 1998;187:1477–1485. [PubMed: 9565639]
- [32]. Wyllie AH. Apoptosis: an overview. *Br Med Bull* 1997;53:451–465. [PubMed: 9374030]
- [33]. Golstein P, Ojcius DM, Young JD. Cell death mechanisms and the immune system. *Immunol Rev* 1991;121:29–65. [PubMed: 1937533]
- [34]. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770–776. [PubMed: 11048727]
- [35]. Krammer PH. CD95's deadly mission in the immune system. *Nature* 2000;407:789–795. [PubMed: 11048730]
- [36]. Green DR, Reed JC. Mitochondria and apoptosis. *Science* 1998;281:1309–1312. [PubMed: 9721092]
- [37]. Salvesen GS, Duckett CS. Apoptosis: IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002;3:401–410. [PubMed: 12042762]
- [38]. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J* 1997;326:1–16. [PubMed: 9337844]
- [39]. Chipuk JE, Green DR. Do inducers of apoptosis trigger caspase-independent cell death? *Nat Rev Mol Cell Biol* 2005;6:268–275. [PubMed: 15714200]
- [40]. Lieberman J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. *Nat Rev Immunol* 2003;3:361–370. [PubMed: 12766758]
- [41]. Ekert PG, Vaux DL. The mitochondrial death squad: hardened killers or innocent bystanders? *Curr Opin Cell Biol* 2005;17:626–630. [PubMed: 16219456]
- [42]. Barnhart BC, Alappat EC, Peter ME. The CD95 type I/type II model. *Semin Immunol* 2003;15:185–193. [PubMed: 14563117]
- [43]. Willis SN, Adams JM. Life in the balance: how BH3-only proteins induce apoptosis. *Curr Opin Cell Biol* 2005;17:617–625. [PubMed: 16243507]
- [44]. Bouillet P, Metcalf D, Huang DC, et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 1999;286:1735–1738. [PubMed: 10576740]
- [45]. Bauer A, Villunger A, Labi V, et al. The NF-kappaB regulator Bcl-3 and the BH3-only proteins Bim and Puma control the death of activated T cells. *Proc Natl Acad Sci USA* 2006;103:10979–10984. [PubMed: 16832056]
- [46]. Villunger A, Michalak EM, Coultas L, et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* 2003;302:1036–1038. [PubMed: 14500851]
- [47]. Puthalakath H, Huang DC, O'Reilly LA, King SM, Strasser A. The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol Cell* 1999;3:287–296. [PubMed: 10198631]
- [48]. Puthalakath H, Villunger A, O'Reilly LA, et al. Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. *Science* 2001;293:1829–1832. [PubMed: 11546872]

- [49]. Zhu Y, Swanson BJ, Wang M, et al. Constitutive association of the proapoptotic protein Bim with Bcl-2-related proteins on mitochondria in T cells. *Proc Natl Acad Sci USA* 2004;101:7681–7686. [PubMed: 15136728]
- [50]. Gomez-Bougie P, Bataille R, Amiot M. Endogenous association of Bim BH3-only protein with Mcl-1, Bcl-xL and Bcl-2 on mitochondria in human B cells. *Eur J Immunol* 2005;35:971–976. [PubMed: 15724238]
- [51]. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 1996;87:619–628. [PubMed: 8929531]
- [52]. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 1998;94(4):491–501. [PubMed: 9727492]
- [53]. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 1998;94(4):481–90. [PubMed: 9727491]
- [54]. Han J, Flemington C, Houghton AB, et al. Expression of bbc3, a pro-apoptotic BH3-only gene, is regulated by diverse cell death and survival signals. *Proc Natl Acad Sci USA* 2001;98(20):11318–23. [PubMed: 11572983]
- [55]. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell* 2001;7(3):683–94. [PubMed: 11463392]
- [56]. Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 2000;288(5468):1053–1058. [PubMed: 10807576]
- [57]. Labi V, Erlacher M, Kiessling S, Villunger A. BH3-only proteins in cell death initiation, malignant disease and anticancer therapy. *Cell Death Differ* 2006;13(8):1325–38. [PubMed: 16645634]
- [58]. Fadok V, Bratton DL, Rose DM, Pearson A, Ezekewitz RAB, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000;405:85–90. [PubMed: 10811223]
- [59]. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. *Nature* 2000;407:784–788. [PubMed: 11048729]
- [60]. Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. *Nat Rev Immunol* 2002;2:965–975. [PubMed: 12461569]
- [61]. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 2001;13:114–119. [PubMed: 11154927]
- [62]. Matzinger P. The danger model: a renewed sense of self. *Science* 2002;296:301–305. [PubMed: 11951032]
- [63]. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* 2003;21:335–376. [PubMed: 12524386]
- [64]. Di Virgilio F, Chiozzi P, Ferrari D, et al. Nucleotide receptors: an emerging family of regulatory molecules in blood cells. *Blood* 2001;97:587–600. [PubMed: 11157473]
- [65]. Müller S, Scaffidi P, Degryse B, et al. The double life of HMGB1 chromatin protein: architectural factor and extracellular signal. *EMBO J* 2001;16:4337–4340.
- [66]. Sitkovsky MV, Ohta A. The “danger” sensors that STOP the immune response: the A2 adenosine receptors? *Trends Immunol* 2005;26:299–304. [PubMed: 15922945]
- [67]. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 2003;425:516–521. [PubMed: 14520412]
- [68]. Stephens RS. The cellular paradigm of Chlamydial pathogenesis. *Trends Microbiol* 2003;11:44–51. [PubMed: 12526854]
- [69]. LaVerda D, Kalayoglu MV, Byrne GI. Chlamydial heat shock proteins and disease pathology: new paradigms for old problems? *Infect Dis Obstet Gynecol* 1999;7:64–71. [PubMed: 10231012]
- [70]. Fischer SF, Hacker G. Characterization of antiapoptotic activities of *Chlamydia pneumoniae* in infected cells. *Ann N Y Acad Sci* 2003;1010:565–567. [PubMed: 15033792]
- [71]. Byrne GI, Ojcius DM. *Chlamydia* and apoptosis: life and death decisions of an intracellular pathogen. *Nat Rev Microbiol* 2004;2:802–808. [PubMed: 15378044]

- [72]. Fields KA, Hackstadt T. The Chlamydial inclusion: escape from the endocytic pathway. *Annu Rev Cell Dev Biol* 2002;18:221–245. [PubMed: 12142274]
- [73]. Galvan V, Roizman B. Herpes simplex virus 1 induces and blocks apoptosis at multiple steps during infection and protects cells from exogenous inducers in a cell-type-dependent manner. *Proc Natl Acad Sci USA* 1998;95:3931–3936. [PubMed: 9520470]
- [74]. Fischer SF, Ludwig H, Holzapfel J, et al. Modified vaccinia virus Ankara protein F1L is a novel BH3-domain-binding protein and acts together with the early viral protein E3L to block virus-associated apoptosis. *Cell Death Differ* 2006;13(1):109–18. [PubMed: 16003387]
- [75]. Postigo AC, Cross JR, Downward J, Way M. Interaction of F1L with the BH3 domain of Bak is responsible for inhibiting vaccinia-induced apoptosis. *Cell Death Differ* 2006; in press.
- [76]. Wasilenko ST, Banadyga L, Bond D, Barry M. The vaccinia virus F1L protein interacts with the proapoptotic protein Bak and inhibits Bak activation. *J Virol* 2005;79:14031–14043. [PubMed: 16254338]
- [77]. Miyairi I, Byrne GI. *Chlamydia* and programmed cell death. *Curr Opin Microbiol* 2006;9:102–108. [PubMed: 16406838]
- [78]. Verbeke P, Welter-Stahl L, Ying S, et al. Recruitment of BAD by the *Chlamydia trachomatis* vacuole correlates with host-cell survival. *PLoS Pathogens* 2006;2:e45. [PubMed: 16710454]
- [79]. Ying S, Fischer S, Pettengill M, et al. Characterization of host cell death induced by *Chlamydia trachomatis*. *Infect Immun* 2006;74:6057–6066. [PubMed: 16940144]
- [80]. Fan T, Lu H, Shi L, et al. Inhibition of apoptosis in *Chlamydia*-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. *J Exp Med* 1998;187:487–496. [PubMed: 9463399]
- [81]. Fischer SF, Schwarz C, Vier J, Hacker G. Characterization of anti-apoptotic activities of *Chlamydia pneumoniae* in human cells. *Infect Immun* 2001;69:7121–7129. [PubMed: 11598088]
- [82]. Rajalingam K, Al-Younes H, Muller A, Meyer TF, Szczepek AJ, Rudel T. Epithelial cells infected with *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*) are resistant to apoptosis. *Infect Immun* 2001;69:7880–7888. [PubMed: 11705971]
- [83]. Fischer SF, Harlander T, Vier J, Hacker G. Protection against CD95-induced apoptosis by Chlamydial infection at a mitochondrial step. *Infect Immun* 2004;72:1107–1115. [PubMed: 14742558]
- [84]. Fischer SF, Vier J, Kirschnek S, et al. Chlamydia inhibit host cell apoptosis by degradation of proapoptotic BH3-only proteins. *J Exp Med* 2004;200:905–916. [PubMed: 15452181]
- [85]. Jungas T, Verbeke P, Darville T, Ojcius DM. Cell death, BAX activation, and HMGB1 release during infection with *Chlamydia*. *Microbes Infect* 2004;6:1145–1155. [PubMed: 15488733]
- [86]. Xiao Y, Zhong Y, Greene W, Dong F, Zhong G. *Chlamydia trachomatis* infection inhibits both Bax and Bak activation induced by staurosporine. *Infect Immun* 2004;72:5470–5474. [PubMed: 15322047]
- [87]. Hess S, Rheinheimer C, Tidow F, et al. The reprogrammed host: *Chlamydia trachomatis*-induced up-regulation of glycoprotein 130 cytokines, transcription factors, and antiapoptotic genes. *Arthritis Rheum* 2001;44:2392–2401. [PubMed: 11665982]
- [88]. Ying S, Seiffert B, Häcker G, Fischer SF. Broad degradation of pro-apoptotic BH3-only proteins during infection with *Chlamydia trachomatis*. *Infect Immun* 2005;73:1399–1403. [PubMed: 15731037]
- [89]. Dong F, Pirbhai M, Xiao Y, Zhong Y, Wu Y, Zhong G. Degradation of the proapoptotic proteins Bik, Puma, and Bim with Bcl-2 domain 3 homology in *Chlamydia trachomatis*-infected cells. *Infect Immun* 2005;73:1861–1864. [PubMed: 15731089]
- [90]. McDonnell JM, Fushman D, Milliman CL, Korsmeyer SJ, Cowburn D. Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. *Cell* 1999;96(5):625–34. [PubMed: 10089878]
- [91]. Chen YL, Law PY, Loh HH. Inhibition of PI3K/Akt signaling: an emerging paradigm for targeted cancer therapy. *Curr Med Chem Anticancer Agents* 2005;5:575–589. [PubMed: 16305480]
- [92]. Scidmore MA, Hackstadt T. Mammalian 14-3-3b associates with the *Chlamydia trachomatis* inclusion membrane via its interaction with IncG. *Mol Microbiol* 2001;39:1638–1650. [PubMed: 11260479]

- [93]. Li L, Lorenzo PS, Bogi K, Blumberg PM, Yuspa SH. Protein kinase Cdelta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. *Mol Cell Biol* 1999;19(12):8547–8558. [PubMed: 10567579]
- [94]. Tse SM, Mason D, Botelho RJ, et al. Accumulation of diacylglycerol in the *Chlamydia* inclusion vacuole: possible role in the inhibition of host cell apoptosis. *J Biol Chem* 2005;280:25210–25215. [PubMed: 15863503]
- [95]. Ruhland A, Leal NK, Kima PE. *Leishmania* promastigotes activate PI3K/Akt signalling to confer host cell resistance to apoptosis. *Cell Microbiol* 2006; in press.
- [96]. Yilmaz O, Jungas T, Verbeke P, Ojcius DM. Activation of the phosphatidylinositol 3-kinase/Akt pathway contributes to survival of primary epithelial cells infected with the periodontal pathogen *Porphyromonas gingivalis*. *Infect Immun* 2004;72:3743–3751. [PubMed: 15213114]
- [97]. Aoki M, del P, Cano RC, Pellegrini AV, et al. Different signaling pathways are involved in cardiomyocyte survival induced by a *Trypanosoma cruzi* glycoprotein. *Microbes Infect* 2006;8:1723–1731. [PubMed: 16815723]
- [98]. Rajala MS, Rajala RV, Astley RA, Butt AL, Chodosh J. Corneal cell survival in adenovirus type 19 infection requires phosphoinositide 3-kinase/Akt activation. *J Virol* 2005;79:12332–12341. [PubMed: 16160160]
- [99]. Mannova P, Beretta L. Activation of the N-Ras-PI3K-Akt-mTOR pathway by hepatitis C virus: control of cell survival and viral replication. *J Virol* 2005;79:8742–8749. [PubMed: 15994768]
- [100]. Lee CJ, Liao CL, Lin YL. Flavivirus activates phosphatidylinositol 3-kinase signaling to block caspase-dependent apoptotic cell death at the early stage of virus infection. *J Virol* 2005;79:8388–8399. [PubMed: 15956583]
- [101]. Portis T, Longnecker R. Epstein-Barr virus (EBV) LMP2A mediates B-lymphocyte survival through constitutive activation of the Ras/PI3K/Akt pathway. *Oncogene* 2004;23:8619–8628. [PubMed: 15361852]
- [102]. Street A, Macdonald A, Crowder K, Harris M. The Hepatitis C virus NS5A protein activates a phosphoinositide 3-kinase-dependent survival signaling cascade. *J Biol Chem* 2004;279:12232–12241. [PubMed: 14709551]
- [103]. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004;25(6):280–8. [PubMed: 15145317]
- [104]. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 1995;376(6536):167–170. [PubMed: 7603567]
- [105]. Karin M, Lin A. NF-kB at the crossroads of life and death. *Nat Immunol* 2002;3:221–227. [PubMed: 11875461]
- [106]. Eckelman BP, Salvesen GS. The human anti-apoptotic proteins cIAP1 and cIAP2 bind but do not inhibit caspases. *J Biol Chem* 2006;281:3254–3260. [PubMed: 16339151]
- [107]. Wahl C, Oswald F, Simnacher U, Weiss S, Marre R, Essig A. Survival of *Chlamydia pneumoniae*-infected Mono Mac 6 cells is dependent on NF-kappaB binding activity. *Infect Immun* 2001;69:7039–7045. [PubMed: 11598079]
- [108]. Krull M, Klucken AC, Wuppermann FN, et al. Signal transduction pathways activated in endothelial cells following infection with *Chlamydia pneumoniae*. *J Immunol* 1999;162:4834–4841. [PubMed: 10202027]
- [109]. Xiao Y, Zhong Y, Su H, Zhou Z, Chiao P, Zhong G. NF-kappa B activation is not required for *Chlamydia trachomatis* inhibition of host epithelial cell apoptosis. *J Immunol* 2005;174:1701–1708. [PubMed: 15661934]
- [110]. Paland NR, Rajalingam KM, Machuy NS, Zcepek AW, Wehr WR, Rudel TNF-kB and inhibitor of apoptosis proteins are required for apoptosis resistance of epithelial cells persistently infected with *Chlamydia pneumoniae*. *Cell Microbiol* 2006; in press.
- [111]. Pagliari LJ, Perlman H, Liu H, Pope RM. Macrophages require constitutive NF-kappaB activation to maintain A1 expression and mitochondrial homeostasis. *Mol Cell Biol* 2000;20:8855–8865. [PubMed: 11073986]

- [112]. Perfettini JL, Reed JC, Israël N, Martinou JC, Dautry-Varsat A, Ojcius DM. Role of Bcl-2 family members in caspase-independent apoptosis during *Chlamydia* infection. *Infect Immun* 2002;70:55–61. [PubMed: 11748163]
- [113]. Dumrese C, Maurus CF, Gygi D, et al. *Chlamydia pneumoniae* induces apoptosis in human aortic smooth muscle cells. *BMC Microbiol* 2005;5:2. [PubMed: 15663783]
- [114]. Banks J, Eddie B, Schachter J, Meyer KF. Plaque formation by *Chlamydia* in L cells. *Infect Immun* 1970;1:259–262. [PubMed: 16557725]
- [115]. Friis RR. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. *J Bacteriol* 1972;180:706–721. [PubMed: 4336694]
- [116]. Todd WJ, Storz J. Ultrastructural cytochemical evidence for the activation of lysosomes in the cytotoxic effect of *Chlamydia psittaci*. *Infect Immun* 1975;12:638–646. [PubMed: 1172486]
- [117]. Wyrick PB, Brownridge EA, Ivins BE. Interaction of *Chlamydia psittaci* with mouse peritoneal macrophages. *Infect Immun* 1978;19:1061–1067. [PubMed: 565339]
- [118]. Chang GT, Moulder JW. Loss of inorganic ions from host cells infected with *Chlamydia psittaci*. *Infect Immun* 1978;19:827–832. [PubMed: 640730]
- [119]. Perfettini JL, Ojcius DM, Andrews CW, Korsmeyer SJ, Rank RG, Darville T. Role of proapoptotic BAX in propagation of *Chlamydia muridarum* (the mouse pneumonitis strain of *Chlamydia trachomatis*) and the host inflammatory response. *J Biol Chem* 2003;278:9496–9502. [PubMed: 12509420]
- [120]. Lupu M, Ojcius D, Perfettini JL, Patry J, Dimicoli JL, Mispelter J. Changes of sodium ion compartmentalization in biological systems due to pathological states. A noninvasive NMR study. *Biochimie* 2003;85:849–861. [PubMed: 14652174]
- [121]. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191–195. [PubMed: 12110890]
- [122]. Ojcius DM, Souque P, Perfettini JL, Dautry-Varsat A. Apoptosis of epithelial cells and macrophages due to infection with the obligate intracellular pathogen *Chlamydia psittaci*. *J Immunol* 1998;161:4220–4226. [PubMed: 9780196]
- [123]. Zhou G, Roizman B. Wild-type herpes simplex virus 1 blocks programmed cell death and release of cytochrome c but not the translocation of mitochondrial apoptosis-inducing factor to the nuclei of human embryonic lung fibroblasts. *J Virol* 2000;74:9048–9053. [PubMed: 10982349]
- [124]. Mitchell L, Smith SH, Braun JS, Herzog KH, Weber JR, Tuomanen EI. Dual phases of apoptosis in pneumococcal meningitis. *J Infect Dis* 2004;190:2039–2046. [PubMed: 15529270]
- [125]. Haslinger-Löffler B, Wagner B, Bruck M, et al. *Staphylococcus aureus* induces caspase-independent cell death in human peritoneal mesothelial cells. *Kidney Int* 2006;70:1089–1098. [PubMed: 16871245]
- [126]. Sarmiento L, Tsegai T, Dhir V, Fu ZF. Rabies virus-induced apoptosis involves caspase-dependent and caspase-independent pathways. *Virus Res* 2006;121:144–151. [PubMed: 16814422]
- [127]. Lee J, Remold HG, Jeong MH, Kornfeld H. Macrophage apoptosis in response to high intracellular burden of *Mycobacterium tuberculosis* is mediated by a novel caspase-independent pathway. *J Immunol* 2006;176:4267–4274. [PubMed: 16547264]
- [128]. Weinrauch Y, Zychlinsky A. The induction of apoptosis by bacterial pathogens. *Annu Rev Microbiol* 1999;53:155–187. [PubMed: 10547689]
- [129]. Kwak, Y Abu; Gao, L-Y. The modulation of host cell apoptosis by intracellular bacterial pathogens. *Trends Microbiol* 2000;8:306–313. [PubMed: 10878765]
- [130]. Hacker G, Kirschnek S, Fischer SF. Apoptosis in infectious disease: how bacteria interfere with the apoptotic apparatus. *Med Microbiol Immunol (Berl)* 2006;195:11–19. [PubMed: 16086183]
- [131]. McClarty G. Chlamydiae and the biochemistry of intracellular parasitism. *Trends Microbiol* 1994;2:157–164. [PubMed: 8055179]
- [132]. Beatty PR, Rasmussen SJ, Stephens RS. Cross-reactive cytotoxic T-lymphocyte-mediated lysis of *Chlamydia trachomatis*- and *Chlamydia psittaci*-infected cells. *Infect Immun* 1997;65:951–956. [PubMed: 9038302]
- [133]. Beatty PR, Stephens RS. CD⁸⁺ T lymphocyte-mediated lysis of *Chlamydia*-infected L cells using an endogenous antigen pathway. *J Immunol* 1994;153:4588–4595. [PubMed: 7963531]

- [134]. Gervassi AL, Probst P, Stamm WE, Marrazzo J, Grabstein KH, Alderson MR. Functional characterization of class Ia- and non-class Ia-restricted *Chlamydia*-reactive CD8⁺ T cell responses in humans. *J Immunol* 2003;171:4278–4286. [PubMed: 14530352]
- [135]. Kim SK, Angevine M, Demick K, et al. Induction of HLA class I-restricted CD8⁺ CTLs specific for the major outer membrane protein of *Chlamydia trachomatis* in human genital tract infections. *J Immunol* 1999;162:6855–6866. [PubMed: 10352308]
- [136]. Popov I, Cruz CS Dela, Barber BH, Chiu B, Inman RD. The effect of an anti-HLA-B27 immune response on CTL recognition of *Chlamydia*. *J Immunol* 2001;167:3375–3382. [PubMed: 11544328]
- [137]. Saren A, Pascolo S, Stevanovic S, et al. Identification of *Chlamydia pneumoniae*-derived mouse CD8 epitopes. *Infect Immun* 2002;70:3336–3343. [PubMed: 12065471]
- [138]. Starnbach MN, Bevan MJ, Lampe MF. Murine cytotoxic T lymphocytes induced following *Chlamydia trachomatis* intraperitoneal or genital tract infection respond to cells infected with multiple serovars. *Infect Immun* 1995;63:3527–3530. [PubMed: 7642287]
- [139]. Wizel B, Starcher BC, Samten B, et al. Multiple *Chlamydia pneumoniae* antigens prime CD8⁺ Tc1 responses that inhibit intracellular growth of this vacuolar pathogen. *J Immunol* 2002;169:2524–2535. [PubMed: 12193722]
- [140]. Henson PM, Bratton DL, Fadok VA. Apoptotic cell removal. *Curr Biol* 2001;11:R795–R805. [PubMed: 11591341]
- [141]. Fadok VA, Henson PM. Apoptosis: giving phosphatidylserine recognition an assist--with a twist. *Curr Biol* 2003;13:R655–R657. [PubMed: 12932346]
- [142]. van Zandbergen G, Gieffers J, Kothe H, et al. *Chlamydia pneumoniae* multiply in neutrophil granulocytes and delay their spontaneous apoptosis. *J Immunol* 2004;172:1768. [PubMed: 14734760]
- [143]. Monks J, Rosner D, Geske F Jon, et al. Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. *Cell Death Diff* 2005;12:107.
- [144]. Winau F, Weber S, Sad S, et al. Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* 2006;24:105–117. [PubMed: 16413927]
- [145]. Huynh M-LN, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF- β 1 secretion and the resolution of inflammation. *J Clin Invest* 2002;109:41–50. [PubMed: 11781349]
- [146]. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF- β , PGE2, and PAF. *J Clin Invest* 1998;101:890–898. [PubMed: 9466984]
- [147]. Rottenberg ME, Rothfuchs AC Gigliotti, Gigliotti D, Svanholm C, Bandholtz L, Wigzell H. Role of innate and adaptive immunity in the outcome of primary infection with *Chlamydia pneumoniae*, as analyzed in genetically modified mice. *J Immunol* 1999;162:2829–2836. [PubMed: 10072530]
- [148]. Bachmaier K, Penninger JM. *Chlamydia* and antigenic mimicry. *Curr Top Microbiol Immunol* 2005;296:153–163. [PubMed: 16323424]
- [149]. Pinchuk I, Starcher BC, Livingston B, et al. A CD8⁺ T cell heptaepitope minigene vaccine induces protective immunity against *Chlamydia pneumoniae*. *J Immunol* 2005;174:5729–5739. [PubMed: 15843575]
- [150]. Gervassi AL, Grabstein KH, Probst P, Hess B, Alderson MR, Fling SP. Human CD8⁺ T cells recognize the 60-kDa cysteine-rich outer membrane protein from *Chlamydia trachomatis*. *J Immunol* 2004;173:6905–6913. [PubMed: 15557186]
- [151]. Starnbach MN, Loomis WP, Ovendale P, et al. An inclusion membrane protein from *Chlamydia trachomatis* enters the MHC class I pathway and stimulates a CD8⁺ T cell response. *J Immunol* 2003;171:4742–4749. [PubMed: 14568950]
- [152]. Williams DM, Grubbs BG, Pack E, Kelly KA, Rank RG. Humoral and cellular immunity in secondary infection due to murine *Chlamydia trachomatis*. *Infect Immun* 1997;65:2876–2882. [PubMed: 9199462]

- [153]. Lich JD, Arthur JC, Ting JP-Y. Cryopyrin: in from the cold. *Immunity* 2006;24:241–243. [PubMed: 16546091]
- [154]. Mariathasan S, Weiss DS, Newton K, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 2006;440:228–232. [PubMed: 16407890]
- [155]. Meylan E, Tschopp J, Karin M. Intracellular pattern recognition receptors in the host response. *Nature* 2006;442:39–44. [PubMed: 16823444]
- [156]. Ojcius DM, de Alba Y Bravo, Kanellopoulos JM, et al. Internalization of *Chlamydia* by dendritic cells and stimulation of *Chlamydia*-specific T cells. *J Immunol* 1998;160:1297–1303. [PubMed: 9570547]
- [157]. Matyszak MK, Young JL, Gaston JS. Uptake and processing of *Chlamydia trachomatis* by human dendritic cells. *Eur J Immunol* 2002;32:742–751. [PubMed: 11870618]
- [158]. Bertho N, Adamski H, Toujas L, Debove M, Davoust J, Quillien V. Efficient migration of dendritic cells toward lymph node chemokines and induction of T(H)1 responses require maturation stimulus and apoptotic cell interaction. *Blood* 2005;106:1734–1741. [PubMed: 15899913]

Fig. (1).

The role of BH3-only proteins in resistance to apoptosis of cells infected with *Chlamydia*. BH3-only proteins are activated by extrinsic stimuli and trigger mitochondrial apoptosis by activating Bax/Bak, a process that may depend on Bcl-2-like proteins and is inhibited by high levels of these molecules. Chlamydiae interfere with the mitochondrial pathway of apoptosis, probably by targeting BH3-only proteins for degradation or inactivation, such as phosphorylation of Bad. Chlamydial infection does not inhibit death receptor-induced activation of caspase-8 in type I cells, which do not require an “amplification” loop in mitochondria, but lead to caspase-8 activation, and in turn, caspase-3 activation directly. All other cells rely on tBid-mediated release of cytochrome *c* and subsequent activation of caspases, both of which are inhibited by chlamydial infection. Significantly, chlamydial infection does not inhibit apoptosis when it is experimentally induced by over-expression of active Bim, demonstrating that chlamydiae inhibit only mitochondrion-dependent apoptosis.

Fig. (2).

Domain structure and function of Bcl-2 family proteins. Bcl-2 family proteins are clustered into three groups, based on both structure and function. The anti-apoptotic proteins usually contain all four Bcl-2 homology (BH) domains. Pro-apoptotic Bax and Bak (also known as multi-domain proteins) contain BH-domains 1-3 and mediate cytochrome *c*-release from mitochondria. Mitochondrial apoptosis is initiated by activation of one or several BH3-only proteins, which share homology only in the short BH3-domain. The known mechanisms for activating BH3-only proteins are indicated.

Fig. (3).

Effects of epithelial cell death on transmission of infection and activation of both the innate and adaptive immune response of the host. Infection by many intracellular pathogens leads to death of the host cell, most likely through a combination of apoptosis and necrosis. Necrosis leads to release of infectious pathogen, which can activate Toll-like receptors (TLR) on antigen-presenting cells (APC) and therefore stimulate the innate immune system. Concomitant release of danger signals (DS) such as ATP and uric acid and their binding to danger signal receptors (DSR) on APC leads to maturation of dendritic cells (DC) and secretion of pro-inflammatory cytokines by DC and other APC. Most pathogens express adhesins that can associate with adhesion receptors on neighboring cells, resulting in transmission of infection. Cells dying through apoptosis release apoptotic bodies that can contain viable pathogens or pathogen-derived antigens. APC that phagocytose the apoptotic bodies can process microbial antigens and present them on their surface *via* major histocompatibility complex (MHC) molecules, thus stimulating an adaptive immune response. Viable pathogens in apoptotic bodies can also be phagocytosed by new host cells, and thereby contribute to propagation of the infection through a “Trojan horse” strategy.