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Chlamydial Infection of Immune Cells: Altered Function and Implications for Disease

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ABSTRACT: *Chlamydia trachomatis* is an obligate intracellular bacterial pathogen that infects the genital and ocular mucosa of humans, causing infections that can lead to pelvic inflammatory disease, infertility, and blinding trachoma. *C. pneumoniae* is a respiratory pathogen that is the cause of 12–15% of community-acquired pneumonia. Both chlamydial species were believed to be restricted to the epithelia of the genital, ocular, and respiratory mucosa; however, increasing evidence suggests that both these pathogens can be isolated from peripheral blood of both healthy individuals and patients with inflammatory conditions such as coronary artery disease and asthma. *Chlamydia* can also be isolated from brain tissues of patients with degenerative neurological disorders such as Alzheimer's disease and multiple sclerosis, and also from certain lymphomas. An increasing number of in vitro studies suggest that some chlamydial species can infect immune cells, at least at low levels. These infections may alter immune cell function in a way that promotes chlamydial persistence in the host and contributes to the progression of several chronic inflammatory diseases. In this paper, we review the evidence for the growth of *Chlamydia* in immune cells, particularly monocytes/macrophages and dendritic cells, and describe how infection may affect the function of these cells.

KEY WORDS: *Chlamydia trachcomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, monocyte, macrophage, dendritic cell, polymorphonuclear leukocyte

I. *CHLAMYDIAE* AND THEIR UNIQUE PLACE IN MICROBIAL EVOLUTION

The *Chlamydiae* are a unique group of obligate intracellular pathogens that are characterized by their biphasic developmental cycle. They are parasites of a wide range of vertebrates, including humans, some arthropod species, and several free-living amoebae. Four families are recognized within the order *Chlamydiales*,[1] with a total of six genera and 13 species (Table I), namely, family *Chlamydiaceae* (*Chlamydia* and *Chlamydophila*), family *Parachlamydiaceae* (*Parachlamydia acanthamoebae*, *Neochlamydia hartmannellae*, *Protochlamydia amoebophilia*), family *Simkaniaceae* (*Simkania negevensis*, *Fritschea bemisiae*), and family *Waddliaceae* (*Waddlia chrondrophila*). More recently, several additional new *Chlamydiae* have been reported. These include *Pisichlamydia salmonis, Rhabdochlamydia*.

The family *Chlamydiaceae* comprises the best-known human and animal chlamydial pathogens. For a long time, the single genus, *Chlamydia*, was the only genus within this family. In 1999, Everrett et al.[2] proposed splitting of this family into two families, *Chlamydia* and *Chlamydophila*, based on a range of phenotypic, bioecological, and genotypic data. Although this subdivision has some merits, it was done before the chlamydial

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TABLE I. Taxonomy of Chlamydiae

Systematics	Natural host	Pathogenicity
Order Chlamydiales		
Family I: Chlamydiaceae		
Genus I: *Chlamydia*		
C. trachomatis	Humans	Ocular, urogenital infections
C. pneumoniae	Humans, horses, koalas	Respiratory, ocular, urogenital infections
C. muridarum	Rodents	Respiratory, ocular, urogenital infections
C. suis	Swine	Enteritis
C. psittaci	Birds	Avian chlamydiosis
C. abortus	Ruminants	Abortion
C. pecorum	Cattle, sheep, koalas	Enteritis, abortion, polyarthritis, ocular, urogenital infections
C. felis	Cats	Ocular, respiratory infections
C. caviae	Guinea pigs	Ocular, urogenital infections
Family II: Parachlamydiaceae		
Genus I: *Parachlamydia*		
P. acanthamoebae	Acanthamoeba	Endosymbiont
Genus II: *Neochlamydia*		
N. hartmannellae	Hartmannella	Parasite
Family III: Simkaniaceae		
Genus I: *Simkania*		
S. negevensis	Humans (?)	Respiratory infections
Genus II: *Fritschea*		
F. bemisiae	Insects	
F. eriococci	Insects	
Genus III: *Rhabdochlamydia*		
Rhabdochlamydia spp.	Woodlice	
Family IV: Waddliaceae		
Genus I: *Waddlia*		
W. chrondrophila	Cattle, fruit bats (?)	Abortion

genome-sequencing projects had been commenced and hence were not informed by the wealth of information in the full genomes. For this reason as well as disagreements between human and animal chlamydiologists, this subdivision into the two genera has not been widely accepted by the chlamydial community.[3] Current knowledge of chlamydial genetics, combined with a consensus within the chlamydial community, is in favor of returning to a single genus model, *Chlamydia*. For the purposes of this review, we will refer to the single genus, *Chlamydia*, within the family *Chlamydiaceae*. When Everett et al.[2] proposed the split into two genera, they also proposed reorganizing the species into nine groups. This nine-species arrangement does make good sense and has been widely accepted by the majority of chlamydiologists. Within the *Chlamydia*/*Chlamydophila* genus, therefore, there are now nine recognized species,

namely, *C. trachomatis*, *C. muridarum*, *C. suis*, *C. psittaci*, *C. pneumoniae*, *C. caviae*, *C. felis*, *C. abortus*, and *C. pecorum*. *C. trachomatis* is a common cause of urogenital infections in humans and is the agent of trachoma, one of the leading infectious causes of blindness worldwide. *C. pneumoniae* is another important human pathogen, mainly causing respiratory infections, and it is suggested that virtually everyone will become infected with *C. pneumoniae* at some stage of their life. There is also strong evidence to implicate *C. pneumoniae* in atherosclerotic cardiovascular diseases[4] as well as some neurodegenerative diseases, such as Alzheimer's disease and multiple sclerosis.[5] Interestingly, *C. pneumoniae* has also been reported from a wide range of animals including horses, koalas and other Australian marsupials, frogs, and other reptiles.[6,7] The other *Chlamydia* species are mainly veterinary pathogens affecting a wide range of hosts including birds, cats, sheep, cattle, pigs, koalas, mice, and guinea pigs.

The second family, *Parachlamydiaceae*, contains two genera, *Parachlamydia* and *Neochlamydia*, which comprise amoebal endosymbionts recovered from both environmental and clinical samples. While *N. hartmannellae* infects and lyses hartmanellae, and thus may be considered as a parasite, the other parachlamydiae seem to have a mutualistic relationship with the acanthamoebal host. Interestingly, *P. acanthamoebae* has been reported to infect humans and cause respiratory disease.[8,9]

The third family, *Simkaniaceae*, comprises two genera (possibly a third), *Simkania negevensis* and *Fritschea bemisiae* (and *F.eriococci*). *S.negevensis* was originally identified as a cell culture contaminant[10] but has also since been indirectly associated with respiratory infections.[11] This organism has several features that differentiate it from the *Chlamydiaceae*. It is resistant to penicillins and shows a longer developmental cycle in vitro, up to two weeks, with a peculiar stationary phase.[12] *S.negevensis* has also been reported to experimentally infect and multiply within Acanthamoeba.[13]

The fourth family in the Order is *Waddlia*, which comprises the unique species and genus, *W.chondrophila*, which has been associated with the induction of abortion in cattle[14] and can also enter and replicate within human macrophages.[15] *W. chondrophila* infection resulted in lysis and death of human macrophages.

A. The Chlamydial Developmental Cycle

The chlamydial developmental cycle consists of two distinct morphological forms, namely, (i) the elementary body (EB), which is the metabolically inactive, extracellular, infectious stage, which is designed for passage between suitable host cells, and (ii) the reticulate body (RB), which is the metabolically active, replicating, intracellular form. The complete developmental cycle takes between 36 and 72 h, depending on the chlamydial species. Chlamydial infection is initiated by attachment of an EB to the host, via as yet uncharacterized receptors. There is evidence, however, for the involvement of heparin sulphate because it, or heparinase treatment, is able to inhibit the infectivity of *C. trachomatis* serovars L2.[16] These molecules are glycosamino glycans (GAGs) that are thought to have an effect on infectivity via nonspecific charge-mediated interactions or via interaction with the major outer membrane protein (MOMP).[17] The process of chlamydial entry into susceptible cells is not fully understood but two mechanisms have been suggested, namely, (i) phagocytosis (microfilament dependent)[18] or (ii) receptor-mediated endocytosis.[19] *Chlamydiae* have been reported to enter the host cell via both mechanisms, depending on the chlamydial strain, the host cell, and other conditions.[20] Once entry occurs, the chlamydial EB becomes internalized in a double-membraned inclusion and, importantly, it is dissociated from the endocytic pathway of the host cell, but does enter early into the anterograde pathway of the Golgi apparatus, avoiding phagolysosomal fusion. In epithelial cells, the inclusion does not acquire lysosomal markers but does fuse with trans-Golgi network-derived sphingolipid-containing secretory vesicles.[21] The inclusion also becomes surrounded with transferrin-containing endosomes very early after infection, although no fusion with these endosomes has been detected. These are clearly key steps in the chlamydial infectious process and undoubtedly define, to some degree at least, which cell types are permissible to chlamydial infection. The double-membraned endosome that develops is termed the chlamydial inclusion and is where chlamydial replication occurs. The chlamydial RBs multiply by binary fission through 8 to 10 rounds of replication, filling up a large part of the expanding inclusion. At this stage, there is a trigger, possibly lack of

contact with the outer membrane as proposed by Hoare et al.,[22] that results in RBs converting back to EBs ready for exit from the host cell. Exit has long been assumed to occur only via a physical lysis process, but recent work by Hybiske et al.[23] has shown that two mechanisms of exit occur. Using a GFP-based approach to visualize inclusions within cells by live fluorescence videomicroscopy, they identified two mutually exclusive pathways. The first mechanism, lysis, consisted of an ordered sequence of membrane permeabilisations, i.e., inclusion nucleus and plasma membrane rupture. The second release pathway was a packaged release mechanism, termed extrusion. This slow process resulted in a pinching of the inclusion, protrusion out of the cell within a membrane compartment, and ultimately detachment from the cell.

Although the developmental cycle is characteristic of the family *Chlamydiaceae*, and is, in general, highly conserved between chlamydial species, there are differences that are worth noting. There appears to be differences in the attachment, entry, and survival of different species in mouse macrophages.[24] It was shown that *C. trachomatis* will infect mannose receptor–positive mouse macrophages better than mannose receptor–negative cells whereas, in contrast, *C. pneumoniae* will infect mannose receptor–negative cells better than positive ones. *C. psittaci* infected both cells types equally.[24] Some chlamydial species have highly fusogenic inclusions, such as *C. trachomatis*, which results in a single, large inclusion that fills most of the host cell. Other chlamydial species, such as *C. caviae*, the guinea pig strain, produce multilobed, independent inclusions, even when the infection is initiated by a single organism.[25]

B. Chlamydial Persistence

While the progression of EB to RB then back to EB is considered the mainstay of the chlamydial developmental cycle, it has become widely accepted in the last 10 years that a persistent phase also occurs. Chlamydial persistence is defined as a viable but noncultivable growth stage resulting in a long-term relationship with the infected host cell. Chlamydial persistence is best characterized in vitro but there is also significant evidence for its existence in vivo.[26] In vitro, chlamydial persistence is induced by a range of factors that stress the organism in some way, such as (i) amino acid deprivation in general, but tryptophan starvation in particular, (ii) iron depletion, (iii) nonlethal antibiotics, (iv) cytokines such as gamma interferon (IFNγ), (v) phage infection, (vi) heat shock, and (vii) growth in monocytes. In all cases, the developmental cycle becomes stalled and infectious EBs are not produced. The fact that RBs do not convert back to EBs means that when samples from persistent cultures are subcultured, they do not infect the new host cells and are therefore "non-infectious." Instead, the normally round, 0.5–1.0 um RBs, become significantly enlarged and aberrant in shape, when viewed by electron microscopy. Perhaps the best-studied model of in vitro persistence is that induced by treatment with IFNγ. This means of inducing persistence is highly relevant clinically and has been shown to occur via depletion of the host cell tryptophan pool. Indeed, if tryptophan is added back to IFNγ-induced persistent cultures, they will revert to the nonpersistent, infectious state. Gamma interferon–induced persistence can be observed in *C. trachomatis*, *C. pneumoniae*, and *C. psittaci*, although differences do occur between the serovars of *C. trachomatis*.

In contrast to other persistence systems, *Chlamydiae* appear to become spontaneously persistent following infection of monocytes. Cell culture infections of freshly isolated human monocytes were infected with *C. trachomatis* serovars K[27] or *C. pneumoniae*.[28] In both studies, no normal RBs (only aberrant RBs) were observed at any time over the monocyte infection period and chlamydial mRNA continued to be detected, despite a loss of subculture infectivity. These observations suggest that *Chlamydiae* were surviving in a viable but culture-negative state.

In vivo, persistence has been associated with a range of chlamydial diseases such as trachoma, inclusion conjunctivitis of the newborn, genital tract infections, pneumonia, arthritis, and cardiovascular disease.[26] Various characteristics link these in vivo disease states with the well-studied in vitro cell culture models. Among the most convincing lines of evidence for persistence in vivo are observations of aberrant morphological forms in vivo, detection of biochemical markers of persistence, such as heat shock protein 60, and absence of cultivability, recurrences that oc-

cur when reinfection is unlikely and evidence of clinical antibiotic resistance. Atypical, pleomorphic RBs with poorly defined outer membranes were observed in fibroblasts and macrophages in synovial membrane samples from patients with *C. trachomatis*–associated reactive arthritis, despite antibiotic therapy.[29] *C. pneumoniae* forms of similar size to aberrant RBs have also been observed within macrophages in aortic valve samples from patients with degenerative aortic valve stenosis.[30] In addition, mini–*C. trachomatis* forms have been observed in the total ejaculate and expressed prostatic secretion samples from patients with chronic chlamydial prostatitis[31] and in the oviducts of mice experimentally infected with *C. muridarum*.[32] More recently, Pospischil et al.[33] presented some very convincing evidence for persistent forms in pigs infected with *C. suis*. Ultrastructurally, characteristic aberrant bodies were detected in the gut of both experimentally infected and naturally infected pigs.

A hallmark of chlamydial persistence is the ability to detect DNA or chlamydial marker proteins in the absence of viable *Chlamydiae*. Indeed, chlamydial DNA and (heat shock protein 60) HSP60 are often detected in tubal biopsy specimens from culture-negative women with postinfectious tubal infertility following antibiotic treatment.[34] In addition, chlamydial RNA has been detected in the absence of cultivability in experimental trachoma of primates[35] as well as in synovial biopsy samples from patients with reactive arthritis.[36] Because RNA is highly labile, its detection strongly suggests the presence of viable organisms and correlates with similar data indicating viable but culture-negative *Chlamydiae* in vitro.

Experimental and clinical data also provide evidence for reactivating persistent *Chlamydiae* in vivo. Dean et al.[37] showed that *C. trachomatis* infections became reactivated in long-term studies of women with genital tract infections. They demonstrated recurrences of the original ompA genotype over 2 to 4.5 years, despite administration of accepted antibiotic regimens. Similar genotyping data from Dean et al.[37] and Hammerschlag et al.[38] suggested that *C.pneumoniae* can also persist for many years after the initial respiratory infection.

C. Chlamydial Growth: By What Measure?

All chlamydial species are obligate intracellular parasites that infect and replicate inside a variety of cells types including epithelial cells of the female and male reproductive tracts (*C. trachomatis* serovars D–K), the conjunctival epithelium (*C. trachomatis* serovars A–C), and various respiratory tract tissues (*C. pneumoniae*). Due to the absolute requirement of a cellular host for the production of new progeny, it could be argued that the gold standard for demonstration of chlamydial replication would involve isolation of infectious progeny and demonstration of regrowth of *Chlamydia* in new host cells following in vitro culture under appropriate conditions. Although a difficult technique, and not one that could be successfully performed in many laboratories, these methods were used as diagnostic tests for many years. The observation by several groups that chlamydial persistence, described as a "viable but noncultivable growth stage resulting in a long-term relationship with the infected host cells," could be induced in vitro by several methods made culture as a gold standard problematic since persistent forms are, by definition, noncultivable. This has led to the development of several methodologies to demonstrate the existence of *Chlamydia* within tissues, cells, and cell supernatants. These include the use of PCR-based methods to identify primary transcripts from chlamydial rRNA operons and mRNA for chlamydial genes associated with the bacterial protein synthetic system and hence metabolic activity,[36] demonstration by direct fluorescence cytology of the production within the host cell of chlamydial products such as lipopolysaccharide (LPS),[39] or identification of these products in the lysates of infected cells using immunoassays such as ELISA.[40] Although all of these methods are accepted in the literature and are used in many laboratories worldwide, there is not always complete concordance between these methods and the demonstration of infectious progeny by reculturing on appropriate target cells. For example, when cynomolgus monkeys were infected in the conjunctival sac with *C. trachomatis* serovar C, infection was detected one week postinfection by all of the four methods employed (tissue culture, direct fluorescence cytology, PCR, and RNA blotting); however, both the nucleic acid detection methods suggested that *Chlamydia*

were present for longer periods of time than either culture or cytology-based techniques. After a secondary infection, *Chlamydia* were detected in conjunctival swabs and smears for a further five weeks using nucleic acid detection after both culture and fluorescent cytology returned negative results.[35] This study is provided as just one example of the fact that there is not always complete concordance between different methods that are commonly used to measure "chlamydial growth." In the following sections, lack of correlation between methods when identifying chlamydial growth in non-epithelial cell types will be highlighted, but it is probably fair to say that an accepted gold standard for demonstration of viable, replicating *Chlamydia* is still to be agreed on.

II. GROWTH OF CHLAMYDIA IN HUMAN MONOCYTES

The association of *C. trachomatis* infection with reactive arthritis/Reiter's syndrome and *C. pneumoniae* infection with chronic inflammatory conditions such as atherosclerosis/coronary artery disease, asthma, and chronic obstructive pulmonary disease (COPD), and neurological diseases such as Alzheimer's disease and multiple sclerosis has raised the question of how *Chlamydia* are transported from the site of initial infection (the genital tract epithelium or the respiratory tract) to the site of inflammatory disease in the joints, vasculature, or central nervous system (CNS). While it is possible that free EBs may travel through the circulation from the site of initial infection, there is increasing evidence (reviewed later) that monocytes may serve as carriers for both *C. pneumoniae* and *C. trachomatis*. This has led to a large number of studies that have investigated the potential of various chlamydial species to infect monocytes/macrophages and monocyte-derived cell lines in vitro. Most studies have employed monocytes isolated from human peripheral blood by density gradient centrifugation followed by plastic adherence or monocyte/macrophage cell lines such as U-937 or THP-1 and have employed a number of strains of *C. pneumoniae,* and *C. trachomatis* strains including LGV serovars, and *C. psittaci*.

Yong et al.[41] compared the ability of various human biovars of *C. trachomatis* to grow in human mononuclear phagocytes (MNPs) and found that none of the serovars tested were able to grow, as defined by reculture on HeLa cells, in phagocytes cultured for seven days or less prior to infection, and concluded that these cells were effectively microbicidal. However, in vitro culture of MNP for eight or more days prior to infection permitted the growth of the LGV biovar whereas trachoma biovars (Biovars B, C, E, and F) continued to be killed by these cells. Phagolysosomal fusion was not observed in MNPs that had been cultured for eight days or more prior to infection with LGV. Thus, differences in the ability of MNPs to support the growth of *C. trachomatis* are biovar specific and affected by the stage of maturation of the cells. Cells cultured in vitro for up to seven days did not support the growth of any biovar, while cells cultured in vitro for eight days or more could be productively infected with the LGV strain. The authors suggest that this may be due to a loss of oxidative metabolism as the monocytes matured to macrophages. Gerard et al.[42] performed similar studies using *C. trachomatis* serovar K and plastic-adherent human blood monocytes. During 10 days of in vitro culture following infection with *C. trachomatis* serovar K, no infectious EBs, as defined by culture, were detected. However, throughout the 10-day culture period primary transcripts for chlamydial rRNA operons were present as were mRNAs encoding the chlamydial r-proteins S5 and L5, glycl-tRNA synthetase, the 60-kDA cysteine-rich outer membrane protein, and the KDO transferase. The authors also showed that the HSP60 gene product was detectable by Western blot in infected monocytes throughout the 10 day culture period whereas the major outer membrane protein was almost absent. Transmission electron microscopy of infected monocytes revealed single or multiple *Chlamydia* present in aberrant inclusions that appeared not to contain normal reticulate bodies.[27] Chlamydial growth arrest could not be overcome by tryptophan supplementation or neutralization of inflammatory cytokines such as IFNα, IFNβ, or TNFα. The results indicate that in infected monocytes, *Chlamydia* are viable and metabolically active even though EBs capable of infecting epithelial cells are not produced. Schmitz et al.[43] also used *C. trachomatis* serovar K to infect blood monocytes and were unable to demonstrate a productive infection as determined by titration of freeze-thawed and sonicated monocyte preparations on HEp-2

indicator cells. However, chlamydial MOMP antigen, demonstrated by immunofluorescence, was present in infected monocytes for up to 14 days as was chlamydial LPS as measured by enzyme immunoassay. Levels of either MOMP or LPS in infected monocytes never exceeded levels seen at the initiation of infection, even at the highest multiplicity of infection (MOI) used, and both declined over the 14 day culture period. Chlamydial rRNA levels were also determined throughout the 14 days by hybridization with a commercially available DNA probe and although present never exceeded levels seen at initiation of infection and only 10% of input rRNA could be detected at 14 days postinfection. The authors concluded that serovar K does not productively infect blood monocytes but chlamydial antigens such as MOMP and LPS can be detected in cells for extended periods of time. Schrader et al.[44] also infected plastic-adherent blood monocytes with *C. trachomatis* serovar K to investigate the induced expression of host inflammatory genes. In this study, infection of monocytes was assumed but not demonstrated. Of the time points investigated, 4 h and 1 day postinfection were designated as active infection while 7 days postinfection was designated as a persistent infection. Interestingly, many of the genes induced by infection (MIP-1α, MIP-1β, IL-2Rγ) were also induced by culture of monocytes with killed (either heat or UV inactivated) *Chlamydia* making it difficult to attribute the induced gene expression to an active infection.

Infection of human plastic-adherent monocyte–derived macrophages may also affect their ability to function as antigen-presenting cells. Co-culture of macrophages, infected with *C. trachomatis* serovar K, with autologous PHA-activated T lymphocytes resulted in the death of the activated T cells. Death was by induction of apoptosis and was dependent on the presence of infected macrophages because culture of T cells with chlamydial EBs in the absence of macrophages, or co-culture of T cells with macrophages preincubated with UV-inactivated chlamydial EBs, did not cause T cell death.[45] Induction of T cell apoptosis was partly due to the secretion of TNFα by infected macrophages but also involved mechanisms that required direct cell-to-cell contact.[46] The authors suggested that this could explain why persistently infected macrophages can escape T cell surveillance and why T cell responses are reduced during persistent chlamydial infection.

Because *C. pneumoniae* infection has been potentially linked with atherosclerosis, Alzheimer's disease, multiple sclerosis, and macular degeneration, there have been many studies investigating the growth of this chlamydial species in monocytes. [28,47–51]. Airenne et al.[28] infected MNP with *C. pneumoniae* Kajanni 7 at a MOI of 10. Staining of cells three days postinfection (PI) with a genus-specific antibody suggested that the cells were heavily infected with 90% of the monocytes containing multiple inclusions. Inclusions in monocytes were smaller and contained fewer chlamydial particles than in HL cells. However, mechanical disruption of monocytes three days postinfection followed by reculture on fresh HL cells revealed no growth in HL cells despite the high levels of apparent infection of monocytes as determined by immunofluorescence. In infected monocytes, transcripts for chlamydial 16S rRNA and Crp60 were present for at least three days postinfection and HSP60 for seven days. Infected monocytes retained the ability to activate CD4 T cells to proliferate, suggesting that at least some phagolysosomal fusion had occurred allowing chlamydial antigens to access the MHC class II pathway. The data suggest that *C. pneumoniae* can form inclusions, albeit aberrant, in monocytes and that metabolic activity is detectable for three to seven days even though infectious EBs are not produced. Geng et al.[48] also infected plastic-adherent monocytes with *C. pneumoniae* strain TW 183 and found that the infected cells were resistant to apoptosis induction by 8-methoxypsoralen and hypercin whereas cells incubated with heat-inactivated *Chlamydia* were not protected from apoptosis. Immunofluorescent staining of infected MNP with a commercially available monoclonal antibody against the major outer membrane protein (MOMP) revealed that only a small percentage of MNP contained *Chlamydia* although this may be explained by the low MOI used in the studies. Infected monocytes secreted both IL-12 and IL-10 and neutralization of IL-10 abolished the resistance to apoptosis. No attempt was made in this study to identify active chlamydial metabolism or the development of infectious EBs. Productive infection of plastic-adherent blood monocytes with *C. pneumoniae* Kajanni 6 has, however, been demonstrated[50] but production of

EBs capable of infecting HL cells required that the MNP be incubated in vitro for at least three days prior to infection. Viable *Chlamydiae* were not recovered from monocytes infected immediately after isolation. This is consistent with the findings of Yong[41] who showed that human MNP cultured in vitro for eight days supported the growth of *C. trachomatis* LGV strains whereas *Chlamydia* would not grow in freshly isolated MNP. Poikonen et al.[47] were also able to infect blood monocytes that had been matured by two weeks of in vitro culture with *C. pneumoniae* K7. They showed that susceptibility to infection was highly variable ranging from 0 to 630 *C. pneumoniae*/human genome equivalents in blood monocytes isolated from different individuals. These studies suggest that freshly isolated blood monocytes are resistant to productive *C. pneumoniae* infection, as defined by reculture on HL or other cell lines. However, if monocytes are cultured on plastic for three days or more, they become susceptible to infection. Although infection of monocytes may not be productive, the limited infection that does occur can affect antigen processing, cytokine production, and susceptibility to apoptosis.

While blood-derived MNP provide a ready source of human macrophages for infection studies, the cells most likely to be infected following respiratory *C. pneumoniae* infection are alveolar macrophages (AMs), cells that have been shown to be different from circulating monocytes/macrophages.[52] Nakajo et al.[53] found that AMs from nonsmoking adults were able to kill *C. trachomatis*, both the LGV serovar and a clinical cervical isolate, following infection of AM at a MOI of 1 or 10. By 48 hours postinfection, no viable *Chlamydia*, as determined by culture on McCoy cells, could be recovered from AM lysates. These studies did not, however, look at killing of *C. pneumoniae*, but show that AMs readily kill *C. trachomatis*. Redecke et al.[54] isolated AM from healthy male nonsmokers by bronchoalveolar lavage and plastic adherence. AMs were infected with *C. pneumoniae* CWL-029 at MOI of 4 and 40. Infection was determined by fluorescent cytology to identify intracellular *Chlamydia* and was evident for up to 120 h postinfection, although the presence of infectious progeny by cell culture was not demonstrated. Infection was associated with a dose-dependent release of reactive oxygen species, production of TNFα, IL-1β, and IL-8, and upregulation of HLA-DR expression. Similar staining of AMs from patients with chlamydial pneumonia was demonstrated. Gaydos et al.[55] showed that viable *C. pneumoniae* 2023, as determined by reculture on HEp-2 cells, could be recovered from infected AM for at least 96 h postinfection. AM were infected after three days adherence to plastic tissue culture plates. Although viable progeny were recovered, the levels of *Chlamydia* never exceeded the numbers used to infect AMs at the initiation of infection. Thus, although chlamydial numbers did not increase following growth in AMs, viability of the infecting inoculum was retained for sufficient time to permit transport of *Chlamydia* from the lung to other tissues.

A. Growth of Chlamydia in Human Monocyte/Macrophage Cell Lines

1. THP-1 and U-937 Cells

Studies with monocyte/macrophage–derived cell lines such as THP-1 and U-937 have also revealed differences in susceptibility to infection with *C. trachomatis* serovar L2.[56] *C. trachomatis* grew actively in THP-1 cells but not in U-937 cells. Both cell lines, however, contained viable *Chlamydia*, as determined by the expression of unprocessed 16S rRNA, indicating that both cell lines were persistently infected, even though viable EBs were only recovered from THP-1 cells. This persistent infection of both cell lines spontaneously resolved after two months in culture even in the absence of antibiotic treatment. Of particular interest was the finding that both THP-1 and U-937 cells became persistently infected following co-culture with *Chlamydia*-infected HeLa cells, but not by direct infection in vitro, producing EBs that were capable of productively infecting HeLa target cells. These data suggest that infection acquired through interaction of monocytes with infected epithelial cells may not only be different to direct infection of these cells by centrifugation in vitro, but also may promote persistence and transfer of infection to anatomical sites distant from the infected epithelium. Another study using the U-937 cell line[57] demonstrated that chlamydial DNA encoding the major outer membrane (MOMP) could be detected in U-937 cells for 150 days after infection with *C. tracho-*

matis L2. Chlamydial LPS was also detectable in the medium of infected U-937 cells for 150 days, whereas chlamydial LPS was only detectable for 14 days following infection of U-937 cells with *C. pneumoniae* TW183. Gaydos et al.[55] infected U-937 cells that had been adhered by culture with myristate phorbol acetate with multiple strains of *C. pneumoniae* and were able to recover viable *Chlamydia* capable of infecting HEp-2 cells out to 96 h postinfection for three of the four strains tested. Yields of recoverable *Chlamydia* were, however, 2–4 logs lower than when these same strains were passaged through HEp-2 cells. Yamaguchi et al.[58] used a number of human cell lines, including THP-1, to investigate susceptibility to infection with *C. pneumoniae* TW183. All of the three cell lines tested [Molt 4 (T cell), P3HR1 (B cell), and THP-1 (monocyte)] supported the growth of *C. pneumoniae* as determined by both LPS ELISA and reculture of cell lysates on HEp-2 cells. At 24 and 48 h postinfection, there was at least a 10-fold increase in infectious progeny from all three cell lines while infection of HEp-2 cells at the same MOI (10:1) resulted in more than a 100-fold increase. Morphologically, the inclusions in the cell lines were small compared to those in HEp-2 cells and contained small irregular RBs. The same group[49] showed that infection of THP-1 monocytic cells with *C. pneumoniae* induced differentiation of these cells into macrophages as demonstrated by changes in cell morphology, increased phagocytosis of polystyrene beads, and increased expression of ICAM-1. Treatment of cells with heat-killed organisms did not cause increased phagocytic activity or morphological changes but did result in increased surface expression of ICAM-1. Similarly, infection of peripheral blood monocytes with *C. pneumoniae* also increased the phagocytic activity of these cells in two or three donors tested, although incubation with heat-killed *Chlamydia* also increased phagocytic activity of human MNPs.

2. Mono Mac 6 Cells

Heinemann et al.[59] used another human monocytic cell line, Mono Mac 6, to demonstrate that *C. pneumoniae* TW-183 could productively infect monocytes/macrophages. At 96 h PI, there was a 10-fold increase in infectious *C. pneumoniae* as determined by culture on HeLa cells. At the MOI used (10), approximately one in six Mono Mac 6 cells contained inclusions at 48 h PI. The inclusions showed a typical morphology containing all developmental forms of *Chlamydia* including RBs in the process of binary fission as well as EBs. Infection was sustained in the cells for at least two weeks; however, at this time, the viability of the cells had dropped to 20%, suggesting that infection was cytotoxic. The authors concluded that the Mono Mac 6 cell line is more susceptible to infection than U-937 cells because it has a more mature and differentiated phenotype. This hypothesis is consistent with the several studies showing that although freshly isolated peripheral blood monocytes cannot be infected with *Chlamydia*, these cells are readily infected following a period of in vitro culture to differentiate these cells to macrophages.[41,47,50]

3. The Affect of Chlamydial Infection on Cytokine Secretion, Apoptosis, and NF-κB Activation

Infection of Mono Mac 6 cells with *C. pneumoniae* also resulted in the secretion of cytokines TNFα, IL-6, and IL-1β by infected cells. Production of TNFα was rapid, peaking at 8 h postinfection and reaching levels reported to actually inhibit *C. trachomatis* infection of HEp-2 cells.[60] Infection of Mono Mac 6 cells with *C. pneumoniae* also induced rapid activation of NF-κB, which was maximal at 1 h postinfection.[61] Pretreatment of cells with inhibitors of NF-κB prior to infection resulted in activation of caspase-3 and increased levels of apoptotic cell death in infected monocytes/macrophages.[61] These studies suggest that activation of NF-κB in *C. pneumoniae*–infected Mono Mac 6 cells may protect against apoptosis and, if this occurs in monocytes in vivo, could contribute to the systemic spread of *Chlamydia* from the site of initial infection.

Recent studies[62] showing that active *C. trachomatis* L2 infection causes the selective downregulation of tumor necrosis factor receptor 1 (TNFR1) on the surface of infected cells, including U-937 monocytic cells, may offer an explanation as to why monocyte/macrophage cells can be productively infected in the presence of cytokines known to

enhance bactericidal activity. TNFR1 in infected cells accumulated at the chlamydial inclusion and was shed by infected cells into the culture supernatant. Receptor shedding required activation of the MEK-ERK pathway and the metalloproteinase TACE (TNFα converting enzyme). Interestingly, *C. psittaci* infection, or exposure to inactivated *Chlamydia*, also modulates the expression of TNFα receptors on HeLa cells.[63] Negating the microbicidal effects of TNFα production through downregulation of TNFR1 expression may be one of several strategies employed by *Chlamydia* to enable growth in monocyte/macrophage cells and possibly other cell types. Other strategies may involve the inhibition of apoptosis, thereby increasing the life span of the infected monocyte/macrophage and the chance for spread of infection and the induction of monocyte maturation into macrophages, which may more readily support chlamydial growth.

B. Growth of *Chlamydia* in Murine Macrophages and Cell Lines

1. Peritoneal Cavity Macrophages

Early studies using mouse thioglycolate–activated peritoneal cavity macrophages (PerCMs)[64] showed that both *C. trachomatis* serovar B and L2 were cytotoxic for these cells at high MOI. Approximately half of cultured PerCM were killed after four to six hours of culture with serovar B (MOI 30) or L2 (MOI 250). Toxicity was reduced to background levels when the MOI was reduced 10-fold. To affect cytotoxicity, phagocytosis of *Chlamydia* was required although replication was not. At lower MOI, growth of the L2 serovar in PerCMs was significantly greater than the trachoma serovar,[65] consistent with the infection of macrophages seen in lymphogranuloma venereum. Studies by Wyrick[66,67] used *C. psittaci* to infect PerCMs and showed that at low MOI (≤1) *Chlamydia* were able to infect and grow in these cells while at higher MOI (100) the *Chlamydia* were toxic for PerCMs. The toxicity of *Chlamydia* for PerCMs at high MOI was abolished by heat treatment of the *Chlamydia*. Collectively, these studies showed that PerCMs could be infected by *C. trachomatis* and *C. psittaci* at low MOI but that both species were toxic at higher MOIs.

2. Alveolar Macrophages

The targets of infection with *C. pneumoniae*, a respiratory pathogen, are alveolar macrophages, cells that have been shown to be different from tissue macrophages.[52] Haranaga et al.[68] therefore used a cell line derived from murine alveolar macrophages (MH-S cells) and primary mouse alveolar macrophages to determine if these cells could support the replication of *C. pneumoniae* AR-39. Both the cell line and primary alveolar macrophages contained chlamydial inclusions, as determined by fluorescence cytology, when stained 48 h after being infected at an MOI of 1. However, reculture of lysates of infected MH-S cells on HEp-2 cell monolayers showed that IFU numbers did not increase above levels present at the initiation of infection, despite the fact that chlamydial LPS levels in extracts of infected primary macrophages and MH-S cells, as determined by ELISA, increased significantly over 48 h. Expression of HSP60 mRNA, but not *omcB* mRNA, was also increased 48 h postinfection. Interestingly, both primary alveolar macrophages and MH-S cells secreted high levels of TNFα when infected with viable *Chlamydia* and neutralization of TNF resulted in increased numbers of cells positive for chlamydial inclusions. Neutralization of TNF also resulted in increased production of HSP60 mRNA and LPS in infected MH-S cells. These data suggest that growth of *Chlamydia* in murine alveolar macrophages, at least as far as production of infectious progeny, is restricted and that the production of TNFα by infected macrophages may be one of the mechanisms that restrict growth. Unfortunately, the studies by Haranaga[68] did not look at expression of TNF receptors on MH-S cells to determine if these are downregulated by infection, as is seen in human U-937 cells following infection with *C. trachomatis*.[62]

3. Bone Marrow–Derived Macrophages

Bone marrow–derived macrophages (BMMs) grown from bone marrow precursors by culture in macrophage colony stimulating factor (M-CSF) represent another source of mouse macrophages that have been used for growth studies. Infection of BMMs with *C. pneumoniae*[69] resulted in expression of IFNγ mRNA and protein early

after infection. Production of IFNγ was transient and required both bacterial and host cell protein synthesis, was independent of IL-12 production, and was regulated by IL-10. Production of type 1 interferons (IFNα/β) was also required for IFNγ production, and both types of interferons were essential for control of *C. pneumoniae* infection in BMMs, which was mediated by inducible NO synthase (iNOS). Interferon production by infected BMMs is regulated by IRAK4 signaling and involves activation of NF-κB[70] as well as a TLR4-MyD88-IFNαβ-STAT1–dependent pathway.[71] Thus, although BMMs can be infected with *C. pneumoniae*, as demonstrated by the production of progeny capable of infecting HL cells,[69] following infection, BMMs rapidly activate interferon-mediated protective mechanisms to limit further intracellular bacterial growth.

C. *Chlamydia Pneumoniae* Infection Affects Macrophage Adherence and Migration

In addition to providing a means of transport out of the lungs, infection of macrophages with *C. pneumoniae* also affects the adherence and migration properties of macrophages facilitating accumulation in plaques and potentially transmigration of the blood-brain barrier. Infection of human peripheral blood monocytes with *C. pneumoniae* (TW-183) enhanced monocyte adhesion to aortic endothelial cells in a time- and dose-dependent manner compared to noninfected monocytes.[72] Culture of monocytes with heat- or UV-treated *Chlamydia* also increased monocyte adherence, and treatment of monocytes with purified *Chlamydia* LPS alone was sufficient to increase adherence to aortic endothelial cells,[72] suggesting that actual infection of macrophages may not be necessary for increased adherence. Kaul[73] also found that infection of human blood monocytes with *C. pneumoniae* (VR1310) increased adherence to human coronary artery endothelial cells (HCAECs) and human coronary smooth muscle cells (HCSMCs). The presence of chlamydial inclusions in infected monocytes was demonstrated by fluorescent cytology. In this study, however, culture of monocytes with heat-killed *Chlamydia* did not result in enhanced adhesion to HCAECs or HCSMCs. May et al.[74] infected the mouse macrophage cell line ANA-1 with *C. pneumoniae* (CM1) for 48 h before infected and noninfected macrophages were fluorescently labeled and infused into the right jugular vein of naive C57 mice. *Chlamydia*-infected ANA-1 cells showed both enhanced rolling on, and adherence to, the vessel wall compared to noninfected macrophages. Infection of human monocytic Mono Mac 6 cells with *C. pneumoniae* also enhanced in vitro adherence of these cells to human umbilical vein endothelial cell monolayers (HUVECs) and transmigration through monolayers of endothelial-like ECV604 cells.[74] When human blood monocytes were infected with either *C. pneumoniae* or *C. trachomatis* (L2) such that 30% of cells were positive for chlamydial inclusions, only cells infected with *C. pneumoniae* showed increased adhesion to HUVEC monolayers. Equivalent adherence of *C. trachomatis*–infected monocytes only occurred when 70% of the cells were infected.[74] Cell adhesion was inhibited by antibodies against VLA-4, LFA-1, MAC-1, or urokinase receptor.[74] In these studies, polymyxin B did not affect *C. pneumoniae*–induced adhesion, indicating that chlamydial LPS was not involved in upregulation of adhesion. This is in contradiction to the findings of Kalayoglu,[72] which showed that LPS did increase blood monocyte adhesion.

In vitro studies using PerCM from green fluorescent protein (GFP) transgenic mice infected with *C. pneumoniae* AR-39 demonstrated that infected macrophages adhered better than control macrophages to endothelial cells.[75] The number of macrophages adhering to endothelial cell monolayers correlated with MOI used. Ex vivo studies also showed that infected macrophages adhered better than uninfected macrophages to aortas from both normal and hyperlipidemic mice. Adherence of infected macrophages to aortas from ICAM-1 knockout mice, however, was not increased, compared to noninfected macrophages showing that ICAM-1 is important for adherence of *Chlamydia*-infected macrophages to the endothelium.

Chlamydial infection may also increase adhesiveness of macrophages by directly affecting lipid metabolism and hence the physiochemical properties of the macrophage plasma membrane.[76] *Chlamydia* are believed to acquire certain essential lipids from the host eukaryotic cell[77–79] since the chlamydial genome does not appear to provide for a bacterial source of these lipids. *C. pneumoniae*

infection of THP-1–derived macrophages has been shown to deplete membrane cholesterol, phosphatidylinositol, and cardiolipin but increase phosphatidylcholine. This resulted in increased membrane fluidity, enhanced macrophage fragility, and increased macrophage adherence to endothelial cells even in the presence of inhibitors of adhesion molecules,[76] suggesting that host macrophage membrane changes resulting from infection contribute to chlamydial dissemination.

C. pneumoniae infection of THP-1 monocytes also stimulates monocyte transendothelial migration through monolayers of human brain endothelial cells (HBMECs).[80] *C. pneumoniae* were also able to infect HBMECs and maximal monocyte migration was observed when both the monocytes and HBMECs were infected with *C. pneumoniae*. Migration into the endothelial monolayer involved infection-induced upregulation of LFA-1, VLA-4, and MAC-1 on the monocytes, and VCAM-1 and ICAM-1 on the endothelial cells.[80] These studies suggest that chlamydial effects on both the local vasculature and circulating monocytes may contribute to inflammation in central nervous system and breaching of the blood-brain barrier.

D. Induction of Macrophage Foam Cell Formation by *Chlamydia pneumoniae*

Foam cell formation is the hallmark of the early stages of atherosclerosis. Atheroma macrophages accumulate excess cholesterol that is esterified and stored in the cytoplasm in lipid-filled vacuoles giving rise to the foamlike appearance of these cells. Epidemiological studies have linked atherosclerosis with *C. pneumoniae* infection and in recent years there is increasing evidence that infection of macrophages and monocyte/macrophage cell lines with *C. pneumoniae* in the presence of low-density lipoprotein (LDL) promotes foam cell formation.[81–85] Foam cell formation is induced in human monocyte-derived macrophages,[81] the mouse macrophage cell line (RAW-264.7),[82] and mouse peritoneal cavity macrophages[84] by direct infection with *C. pneumoniae* in the presence of LDL. Chlamydial LPS[82] and UV-killed *C. pneumoniae* also induced foam cell formation, indicating that active chlamydial infection is not essential for cholesterol accumulation. Foam cell formation is mediated by Toll-like receptor 2 (TLR2)[83] and TLR4 signaling[84] and used both the MyD88-dependent and MyD88-independent pathways that involved the activation of both NF-κB and IRF3.[84] *C. pneumoniae* infection of THP-1–derived macrophages, in the presence of LDL, also increased the expression of acyl-coenzyme A: cholesterol acyltransferase-1 (ACAT-1) mRNA and protein.[85] ACAT-1 is the key intracellular microsomal enzyme that catalyzes the formation of cholesterol esters in many cell types.[86]

E. *Chlamydia* and Monocytes/ Macrophages: Summary

The most consistent finding from infection studies with monocytes/macrophages and cell lines derived from these cells is that chlamydial replication, as measured by isolation of EBs capable of infecting epithelial targets, is limited, with the majority of studies recovering less viable *Chlamydia* from infected macrophages than was used to initiate the infection. At high MOIs, many chlamydial species are also cytotoxic for macrophages. Maturation of blood monocytes to macrophages, by in vitro culture on plastic, in a medium containing fetal calf serum (FCS) may increase susceptibility to infection, and *C. trachomatis* L2 and *C. pneumoniae* are more likely to establish infections of macrophages than other *C. trachomatis* serovars. Infected monocytes/macrophages contain small aberrant inclusions, exhibit chlamydial transcription profiles associated with persistent infection, and contain chlamydial LPS and HSP60, often for extended time periods. Infected cells appear to be resistant to apoptosis, and both infection and exposure to killed *Chlamydia* result in the secretion of a range of cytokines. Infection may also affect macrophage function, including phagocytic ability, antigen presentation, lipid metabolism leading to foam cell formation, and adhesion properties for vascular endothelium and transmigration of the blood brain barrier.

III. GROWTH OF *CHLAMYDIA* IN DENDRITIC CELLS

Dendritic cells are key cells for the activation of naive CD4 T cells and the subsequent adaptive

immune responses that are key for both protective immunity against *Chlamydia* infection as well as the immunopathology that can develop following natural infection. A number of studies in recent years have shown that some human and mouse dendritic cells can also be infected with *Chlamydia*.[39,87–90]

A. *C. pneumoniae* Infection of Human Monocyte-Derived Dendritic Cells

Using dendritic cells derived from human monocytes by culture with GM-CSF and IL-4 (MoDC), Wittkop et al.[88] were able to infect these cells with a clinical isolate of *C. pneumoniae*. Beginning 2 h postinfection, chlamydial particles were found in MoDC and up to 25 days postinfection fluorescent chlamydial particles identified by staining with FITC anti-MOMP antibodies were detectable. The inclusions inside MoDC were smaller than inclusions inside HEp-2 cells. On day seven, postinfection inclusions containing EBs and RBs were easily identified by electron microscopy. Lysates of MoDC infected at MOIs of 0.5 and 2 contained chlamydial particles capable of productively infecting HEp-2 cells. After infection at these low MOIs, infectious *Chlamydia* were recovered for 19–25 days postinfection of MoDC from three different donors. In contrast, if MoDC were infected at a higher MOI of 10, no infectious progeny were detected beyond 10 days postinfection, suggesting that the course of infection is determined in part by the initial infectious dose. Although infectious progeny were recovered, numbers never exceeded the numbers used to initially infect MoDC, showing that although productive, infection is limited compared to infection of HEp-2 cells. Up to 14 days postinfection, transcripts from chlamydial genes encoding products involved in cell division and energy production (dnaA, ftsK, and tal) were detected by RT-PCR, indicative of active chlamydial metabolism. Further evidence of productive infection of MoDC was provided by the demonstration that infected MoDC were able to infect macrophages in a transwell co-culture system that prevented direct cell-cell contact.[91] *C. pneumoniae* inside infected MoDC were labeled with the red fluorescent dye ceramide. After three to five days, co-culture red-labeled *Chlamydia* were detected in macrophages in the lower compartment of the transwell system. Importantly, *Chlamydia* able to productively infect HEp-2 cells were recovered from both MoDC and the macrophages. In another study[92] using MoDC as targets for *C. pneumoniae* infection, recoverable *Chlamydia* capable of infecting Hep-2 cells were recovered from lysates of infected MoDC for between 24 and 72 h postinfection, depending on the donor; however, as with the findings of Wittkop,[88] the numbers of recoverable *Chlamydia* never exceeded the number used to initiate infection of MoDC, suggesting only a limited duration of productive infection, or survival of some of the initial inoculum. Chlamydial transcripts for the 16S rRNA, groEL-1, and OmvB genes were detected by RT-PCR for several days postinfection and chlamydial LPS was detectable by immunofluorescence for up to 28 days. Chlamydial infection induced the activation of MoDC and also the secretion of IFNγ. Although this cytokine is important for protective immunity against chlamydial infection, antibody-mediated neutralization of IFNγ did not enhance chlamydial infection of MoDC.[92]

B. *C. trachomatis* Infection of Human Monocyte-Derived Dendritic Cells

Because C. *trachomatis*–infected dendritic cells have been detected in synovial tissues from patients with reactive arthritis, Matyszak et al.[39] infected MoDC with *C. trachomatis* L2. Entry of *Chlamydia* was mediated by attachment to heparin sulfates and could be blocked by heparin. Inhibition of micropinocytosis did not prevent uptake of *Chlamydia* by MoDC. At 24 h postinfection, MoDC were fixed with paraformaldehyde, with and without saponin, then stained with anti-*Chlamydia* antibodies. Without saponin permeabilization, only 4% of cells stained positive whereas almost 70% of cells stained positive following treatment with saponin, showing that at 24 h PI, MoDC contained intracellular *Chlamydia*. *Chlamydiae* were contained in distinct vacuoles that did not develop into characteristic inclusion bodies. In this study, the presence of viable *Chlamydia* or bacterial metabolism were not assessed. *C. trachomatis*–infected MoDC secreted IL-12 and TNFα, but not IL-10, and were able to support the proliferation and expansion of both CD4

and CD8 T cells, demonstrating that infection of MoDC results in chlamydial antigen entry into both the MHC class II and class I presentation pathways. Gervassi et al.[87] were able to infect human MoDC with both *C. trachomatis* serovar E and L2. Infection was demonstrated by immunofluorescence and by reculture of EBs from infected DC on HeLa cells. Serovar L2 resulted in higher rates of infection of MoDC than serovar E at similar MOI. Both infection and exposure to heat-inactivated EB resulted in upregulation of CD54 and CD80 on MoDC and induced secretion of IL-6, IL-8, IL-12p70, and TNFα. Interestingly, however, only actual infection of MoDC, but not exposure to killed *Chlamydia*, resulted in secretion of IL-1β and IL-18, and the processing of each of these cytokines from their precursors required infection-induced activation of caspase-1 that was not induced by exposure to killed EBs.[87] Thus, the phenotype of MoDC activated by either live or inactivated EBs was similar, but cytokine secretion profiles differed between MoDC infected with live *Chlamydia* and those exposed to heat-inactivated EBs.

C. *C. muridarum* Infection of Mouse Bone Marrow–Derived Dendritic Cells

Mouse bone marrow–derived dendritic cells (BMDCs), grown by culture of bone marrow precursors in GM-CSF, either alone or in combination with IL-4, and murine DC cell lines such as JAWS II or DC2.4 have also been used as targets to study the growth of *Chlamydia*.[89,90,93] Most of these studies have used the mouse-adapted strain of *Chlamydia, C. muridarum*. Infection of BMDCs at an MOI of 1 or 3 resulted in approximately 10% of BMDCs showing atypical inclusions that stained with antibody against chlamydial MOMP. Reculture of BMDC lysates on HeLa cells showed that DCs contained very low levels of cultivable bacteria. Numbers of recoverable bacteria from 10^6 BMDCs was only 300–900 at nine days postinfection, although only 20% of BMDCs were still alive at this time. Death of infected BMDCs was due to induction of apoptosis.[93] These data show that BMDCs support only low-level growth of *C. muridarum*.[89] However, intranasal inoculation of naive mice with BMDC, infected with *Chlamydia* nine days previously, resulted in weight loss and lung infections in recipient mice, showing that *Chlamydia* surviving in BMDCs at nine days postinfection can cause mouse lung infection. Infected BMDCs were also able to process and present antigen to *Chlamydia*-specific CD4 T cells isolated from mice that had been previously infected with *C. muridarum*.[89] The JAWS II cell line derived from C57BL/6 BMDCs is also able to support the growth of *C. muridarum*[93]; however, levels of recoverable *Chlamydia* from JAWS II cells were 10-fold greater than that from an equivalent number of BMDCs, suggesting the cell line was more supportive of chlamydial growth. *Chlamydia* infection of both BMDCs and the JAWS II cell line resulted in activation of cells as shown by upregulation of CD40, CD86, and MHC class II and secretion of IL-12. JAWS II cells pulsed with *Chlamydia* were also able to support proliferation and IFNγ secretion by *Chlamydia*-immune CD4 T cells in a manner similar to *Chlamydia*-infected BMDCs.[93]

Adoptive transfer of either BMDCs or JAWS II cells, pulsed with either live or UV-killed *Chlamydia,* to naive mice was also able to provide protection against respiratory challenge. In both cases, DCs pulsed with live *Chlamydia* provided greater protection against weight loss. Other groups have also used *Chlamydia*-primed DCs to elicit protective immunity in mice[94–96] and have shown that production of IL-12 by primed DCs is essential for protection. Furthermore, suppression of IL-10, or using DCs from IL-10 knockout mice, further enhances protective immunity.[95] Although exposure of DCs to inactivated *Chlamydia* and infection with live *Chlamydia* both cause maturation of DCs, the phenotype of the activated DCs differs with each stimulus.[97,98] BMDCs exposed to live *Chlamydia* expressed high levels of MHC class II, CD80, CD86, CD40, and ICAM-1 and secreted high levels of TNFα and IL-12, whereas BMDCs exposed to UV-inactivated EBs expressed low levels of CD40 and CD86 but increased CD80, MHC class II, and ICAM-1 and secreted lower levels of inflammatory cytokines. BMDCs pulsed with live EBs were better at supporting proliferation of *Chlamydia*-specific CD4 T cells than BMDCs pulsed with inactivated EBs and also provided better protection of naive hosts following adoptive transfer and challenge.[97] Exposure to live versus inactivated *Chlamydia* also resulted

in marked differences in CXC chemokine expression profiles.[98]

Chlamydia-infected BMDCs have also been used as a tool to identify potential protective chlamydial antigens capable of activating a strong CD4 Th1 response.[99,100] Isolation and sequencing of peptides eluted from class II MHC molecules of *Chlamydia*-infected BMDCs has identified several novel peptide antigens that can partially protect mice against chlamydial infection. These studies suggest that a combination of antigens naturally presented on MHC class II as a result of infection of DCs and using adjuvants that mimic the immune response elicited by adoptive transfer of infected DCs may provide a pathway toward a successful chlamydial vaccine for humans.

Our own recent studies[90] suggest that *Chlamydia* infection of DCs may subvert their antigen-presenting function to promote nonprotective Th2 immunity. Infection of BALB/c BMDCs with *C. muridarum* at an MOI of 2 resulted in approximately 60% of cells staining positive for chlamydial inclusions after 36 h. Viable *Chlamydiae* capable of infecting McCoy cells were recovered from lysates of infected BMDCs. Infected BMDCs expressed lower levels of CD80 and increased CD86 compared to noninfected BMDCs, and infection did not increase MHC class II levels. All three markers (MHC II, CD80, and CD86) were upregulated on LPS-activated BMDCs as expected. Infected BMDCs secreted increased levels of TNFα, IL-6, IL-10, IL-12, and IL-13 compared to noninfected BMDCs. IL-10 secretion by infected BMDCs was significantly greater (> threefold) than in cultures of LPS-stimulated BMDCs used as positive controls. Of particular interest, however, was the effect chlamydial infection had on the antigen-presenting function of BMDCs. Infected BMDCs pulsed with OVA peptide induced five- to sixfold greater proliferation of OVA-specific transgenic DO11.10 CD4 T cells than noninfected BMDCs pulsed with OVA peptide. Furthermore, DO11.10 T cells activated by *Chlamydia*-infected BMDCs were strongly Th2 biased, secreting high levels of IL-10 and IL-13 while IFNγ secretion was reduced more than 20-fold compared to D10 cells that were cultured with noninfected BMDCs.[90] Intratracheal adoptive transfer of *Chlamydia*-infected BMDCs, but not noninfected BMDCs, to naive mice also caused increased airways hyperreactivity, an animal model of asthma, in recipient mice. This was due mainly to increased IL-13 production by infected BMDCs. These data suggest that *Chlamydia* infection of BMDCs allows the pathogen to deviate the induced immune response from a protective Th1 to a nonprotective Th2 response (in BALB/c mice) and that in a setting of allergic airways inflammation this may lead to an exacerbation of the asthmatic phenotype.[90] Interestingly, all of the studies showing that adoptive transfer of *Chlamydia*-pulsed DCs induced protective immunity against chlamydial infection[93–96] used mice on a C57BL/6 background while our studies used BALB/c mice, suggesting that host genetic background influences the outcome of chlamydial infection of dendritic cells, such that atopic individuals may respond in a way that leads to chronic infection and exacerbation of allergic disease. Allergic airways diseases (AADs) such as asthma typically develop in early life and chlamydial infection of the airways is increasingly linked to the development of asthma in children.[101] Early life immunity is dominated by Th2 responses, which suggests that chlamydial infections of atopic individuals may reinforce or enhance the Th2 phenotype of early life to promote the development of asthma.[101] Because DCs play pivotal roles in the induction of adaptive responses and may deviate immune responses from a Th1 to Th2 phenotype in certain circumstances or susceptible groups, they are likely to be important in the induction of *Chlamydia*-associated asthma in early life. The immune system of early life is plastic and may be educated by infectious exposure. Therefore, chlamydial infections that reinforce DC-mediated Th2 responses may have permanent effects on immunity and promote lifelong susceptibility to Th2-mediated diseases such as asthma.

D. Summary: Chlamydial Infection of Dendritic Cells

Several studies of DC susceptibility to chlamydial infection have isolated *Chlamydia* capable of infecting epithelial cells from infected DCs. In most cases, however, the amount of *Chlamydia* recovered from DCs did not exceed the amount of *Chlamydia* used to establish the initial infection. Adoptive transfer of infected DC to naive mice did however result in a chlamydial respiratory

infection and infected DCs were able to infect macrophages in a transwell culture system. Small irregular inclusions can be detected in DCs and a transcript profile suggestive of active infection was noted in some studies. Use of a low MOI led to prolonged presence of inclusions whereas at higher MOIs infections were of shorter duration and may have been cytotoxic for DCs. Infection of DCs resulted in altered expression of surface markers associated with antigen presentation (CD40, CD80, CD86, MHC-II) and increased cytokine secretion. Both the phenotype of infected DCs and the cytokine profile differed from that of DCs exposed to killed *Chlamydia*. Furthermore, infected DCs were better able to induce proliferation of CD4 and CD8 T cells in vitro and to elicit protective immunity following adoptive transfer to mice than DCs activated by culture with inactivated *Chlamydia*. Infection of DCs from BALB/c mice resulted in DCs that induced strong Th2 immunity and suppressed Th1 development, implying that not only are the *Chlamydia* able to actively infect DCs, but may also be actively altering their antigen-presenting functions. *Chlamydia*-infected DCs also exacerbated allergic airways disease when adoptively transferred to naive mice, suggesting that chlamydial infection of DCs in atopic individuals might reinforce the Th2 phenotype, resulting in exacerbation of allergic disease and persistent chlamydial infection.

IV. CHLAMYDIAL INFECTION OF OTHER LEUKOCYTE SUBSETS

In addition to infection of monocytes/macrophages and dendritic cells, there have been reports of chlamydial infection of neutrophils, mast cells, and T lymphocytes.

A. Neutrophils

Register[103] demonstrated that both *C. trachomatis* and *C. psittaci* were rapidly internalized in an opsonin-independent manner by human polymorphonuclear leukocytes (PMNs). At an MOI of 1, approximately 60% of *C. trachomatis* were internalized by 15 min. While most internalized *Chlamydia* appeared to be destroyed within peroxidase-positive phagolysosomes,[104] both *C. psittaci* and *C. trachomatis* capable of infecting McCoy cells could still be recovered from PMN lysates at 10 h postinfection. Because PMNs are short-lived cells, it was suggested that the 3–7% of *Chlamydia* that remained viable at 10 h could be able to infect macrophages on engulfment of dying PMNs or potentially epithelial cells if EBs were released from dying PMNs. Another study[105] showed that *C. pneumoniae* were also rapidly internalized by human PMNs in an opsonin-independent manner. These studies showed that following internalization, *Chlamydia* were not killed but actually survived and multiplied within infected PMNs. Infection was determined by staining of cells with anti-*Chlamydia* monoclonal antibodies (Mabs) and also by analysis of the amount of chlamydial LPS within infected PMNs. As measured by LPS ELISA, there was a fivefold increase in chlamydial load in PMNs four days after infection. Importantly, infection delayed the apoptotic cell death of PMNs. While noninfected PMNs became apoptotic (annexin V and TUNEL staining) within 10 h, infected PMNs survived for up to 90 h. The antiapoptotic effect was associated with lower levels of procaspase-3 processing and consequently lower caspase 3 activity in infected PMNs.[105] Chlamydial LPS was a major bacterial component responsible for the observed antiapoptotic effects in addition to increased production of IL-8, an antiapoptotic cytokine, by infected PMNs. This study suggests that even though *C. pneumoniae* respiratory infection elicits a massive influx of PMNs, *Chlamydia* can extend the life span of PMNs by modulating apoptosis, thereby providing a suitable host cell for infection and bacterial multiplication. In addition to providing a permissive host cell for chlamydial replication, recent ultrastructural studies of *C. caviae* infection in the guinea pig conjunctival infection model suggest that PMNs may also play an active role in detaching infected epithelial cells from the underlying epithelium,[106] providing another mechanism for facilitating the spread of infection to new tissue sites.

B. Mast Cells

Because a significant number of asthmatics with chlamydial infections have increased numbers of mast cells in their airways,[107] the mast cell has

been investigated as another potential host cell for *Chlamydia*. Culture of peripheral blood-derived cultured human mast cells with *C. pneumoniae*[108] induced message expression and secretion of IL-8, TNFα, and MCP-1. Induction of these inflammatory cytokines was reduced when mast cells were cultured with UV-inactivated bacteria. Administration of live *Chlamydia* to apolipoprotein E–deficient mice also resulted in accumulation and activation of mouse aortic mast cells in vivo. No attempt however was made to isolate recoverable *Chlamydia* from mast cells, or to demonstrate direct infection using immunocytology in either study however, so with regard to infection of mast cells the data are still not definitive.

C. T lymphocytes

Infection of human peripheral blood lymphocytes, depleted of plastic-adherent cells, with *C. pneumoniae* was demonstrated in four of 11 donors at three days postinfection by staining of inclusions with a genus-specific anti-*Chlamydia* Mab.[40] The frequency of lymphocytes harboring inclusions, the number of inclusions per cell, and the inclusion size were all less than those seen in HEp-2 cells infected at the same MOI. Similarly, cultures of mouse spleen cells depleted of plastic-adherent monocytes also contained cells that harbored FITC-labeled inclusions three days postinfection. When mouse lymphocytes were enriched for T cells (> 95% CD3+) prior to infection, many T lymphocytes also contained chlamydial inclusions at three days. Chlamydial LPS, as determined by ELISA, also increased in mouse lymphocyte cultures over the three day infection period, as did the amount of *omp1* DNA.[40] Infection of the human T cell line Molt 3 with *C. pneumoniae* also resulted in increased numbers of cells harboring chlamydial inclusions and increased levels of chlamydial LPS in cultures over a three day infection.[40] Despite the demonstration of inclusions and increased levels of LPS in lymphocytes and Molt 3 cells, the authors were unable to isolate *Chlamydia* able to infect HEp-2 cells from lysates of lymphocytes or T cell lines, except when these were prepared immediately after lymphocyte infection. This further highlights the lack of correlation between methods used to identify chlamydial "infection" and suggests that the chlamydial developmental cycle may not be completed in lymphoid cells.

D. Glial Cells, Astrocytes, and Neuronal Cells

The recent linking of *C. pneumoniae* infection with neurological conditions such as multiple sclerosis[109] and Alzheimer's disease[110] has stimulated studies of chlamydial infection of CNS-derived cells such as glial cells and astrocytes. Boelen et al.[111,112] used murine astrocyte (C8D1A), neuronal (NB41A3), and microglial (MMC BV-2) cell lines to determine susceptibility to infection with *C. pneumoniae* (TWAR 2043). Infection was measured by immunofluorescence, real-time PCR (ompA), and culture of supernatants from cell lines on HEp-2 indicator cells. Immunofluorescent staining of chlamydial inclusions suggested that chlamydial replication in astrocytes and neuronal cells was comparable to that in HEp-2 cells (60–70% of cells contained inclusions at 24 h postinfection) whereas chlamydial growth in MMC BV2 microglial cells was greatly reduced (<5% of cells contained inclusions at 24 h) and inclusions were virtually absent at 72 h postinfection. In MMCs, the levels of chlamydial DNA did not increase above levels present at the initiation of infection whereas chlamydial DNA increased 100- to 1000-fold in astrocytes, neuronal cells, and epithelial cells. Supernatants were collected from all four cell types one week postinfection and inoculated onto HEp-2 monolayers. Similarly high levels of infectious *Chlamydia* were recovered from HEp-2 cells and astrocytes, significantly less from neuronal cells, and almost no infectious progeny were found in the culture supernatants of infected MMCs. Chlamydial infection of astrocytes and neuronal cells, but not MMCs, also increased the number of necrotic cells. These data suggest both astrocytes and neurons are susceptible to infection, produce viable progeny, and are prone to infection-induced necrotic cell death whereas MMCs, cells thought to be derived from macrophages, are highly resistant to infection. Another study with the neuroblastoma cell line SK-N-MC however showed that *C. pneumoniae*–infected neuronal cells were resistant to staurosporine-induced apoptosis and the authors suggested that this could exacerbate

chronic infections in the brain.[113] Although only small numbers of MMCs contained inclusions, exposure of these cells to *C. pneumoniae* resulted in the secretion of MCP-1, IL-6, TNFα, and IL-1β. Exposure of neurons to supernatants of infected MMCs, but not mock-infected MMCs, resulted in significant neuronal cell death. Neutralization of IL-6 and TNFα in supernatants of infected MMCs reduced neuronal cell death by approximately 50%.[111] Thus MMCs, although resistant to chlamydial infection, respond by secreting proinflammatory cytokines that may play a role in neurodegeneration. Infection of another mouse microglial cell line (EOC 20) with *C. pneumoniae* (TW 183) also resulted in many cells containing inclusions that stained positive with a chlamydial LPS Mab and increasing levels of bacterial 16S rRNA and omcB transcripts.[114] Lysates of this microglial cell line however did contain chlamydial EBs that productively infected HEp-2 cells, and by 24 h postinfection there was an eight- to ninefold increase in infectious progeny. Infection of microglial cells rapidly induced expression of TNFα mRNA, as well as mRNA for IL-10 and IL-12. Infection of microglial cells also induced the selective expression of matrix-metalloproteinase-9 (MMP-9) mRNA but not transcripts for MMP-2 and tissue inhibitor of metalloprotease-1 (TIMP-1). Both TNFα and MMP-9 are believed to be involved in damage to the myelin sheath.[115,116] Human astrocyte and microglioma cells lines (U-87 MG and CHME-5, respectively) have also been infected with *C. pneumoniae* AR-39.[117] Both cell types displayed inclusions that were indistinguishable from those in HEp-2 cells, and real-time PCR analysis showed that the accumulation of chlamydial DNA over time was identical to that seen in infected HEp-2 cells. Patterns of chlamydial gene transcription in astrocytes, microglia, and HEp-2 cells were characteristic of an acute rather than persistent infection.[117] Collectively, these studies show that astrocytes in particular can be infected with *C. pneumoniae* and, importantly, that although microglial cells may or may not be productively infected, they do create a proinflammatory environment that may contribute to neurodegenerative diseases.

V. CHLAMYDIAL INFECTION OF IMMUNE CELLS IN DISEASE

If chlamydial infection of immune cells plays a role in diseases such as atherosclerosis, asthma, Alzheimer's disease, and multiple sclerosis, then cells infected with *Chlamydia* should be identifiable in patients with these conditions.

A. Coronary Artery Disease

Using PCR-based methods, *C. pneumoniae* has been detected in peripheral blood mononuclear cells isolated from patients undergoing abdominal aortic aneurysm surgery,[118] in PBMCs of 72% of patients with symptomatic carotid atherosclerotic disease but only 30% of patients with asymptomatic disease,[119] in CD14+ peripheral blood monocytes from patients with unstable angina or myocardial infarction,[120] in circulating leukocytes of 20% of patients undergoing surgery for carotid stenosis, aneurysm, or peripheral vascular disease but also 13% of normal control subjects,[121] in PBMCs of 59% of patients admitted for coronary angiography but also in 46% of age-matched controls,[122] in plastic-adherent PBMCs, and CD3+ cells from coronary artery disease patients and normal blood donors,[123] in PBMCs from 8.8% of men with coronary artery disease but in only 2.9% of controls,[124] and in 8.9% of PBMCs from normal blood donors.[125] From the above studies it is difficult to conclude that detection of chlamydial DNA in circulating blood cells can be used as a predictor of coronary artery disease (CAD) due to the detection of *Chlamydia* in control groups in many of the studies. It has been suggested that measurement of *C. pneumoniae* bacterial load in peripheral blood may be useful in assessing infection in CAD patients[126] and another study concluded that *C. pneumoniae* DNA in PBMCs is a predictor of CAD in men;[124] however, a recent systematic review of 18 relevant studies[127] concluded that *C. pneumoniae* DNA detection was associated with CAD in unadjusted case-control studies but that adjustment for confounders such as smoking or season and standardization of laboratory methods would be needed to confirm this association. *Chlamydia* have also been detected in 25% of PBMCs from normal blood donors by immunofluorescent microscopy of blood smears[128] with neutrophils,

eosinophils/basophils, and monocytes all found to stain positive with anti-*Chlamydia* EB antiserum. Lysis of PBMCs and culture of lysates on J774 monolayers demonstrated that PBMCs from 25% of donors contained infectious *Chlamydia* able to infect this murine macrophage cell line, providing evidence that infected circulating blood cells are a potential source of transmission of infection.

B. *C. pneumoniae* and Asthma

C. pneumoniae was detected by immunofluorescent staining in smears of bronchoalveolar lavage (BAL) cells isolated from pediatric asthma patients by bronchoscopy.[129] Morphological observation suggested that inclusions were present in alveolar macrophages and lymphocytes as well as epithelial cells. BAL cells isolated from 67% of pediatric asthma patients were also positive by PCR (16S rRNA) and in 33% of patients lysates of BAL cells contained infectious *Chlamydia* that were able to infect human or mouse monocyte/macrophage cell lines (THP-1 and J774A.1).[129] Studies by the same group also detected *C. trachomatis* in bronchoalveolar lavage fluid (BALF) obtained from pediatric patients with respiratory infections.[130] *C. trachomatis* DNA in BALF decreased with age whereas *C. pneumoniae* DNA generally increased with age.[130] Collectively, these studies suggest that viable *C pneumonia* and *C. trachomatis* occur frequently in children with chronic respiratory infections and may be more prevalent in asthma patients. The role of persistent chlamydial infection in adult-onset asthma and in exacerbation of asthma in adult asthmatics is presently unclear, although *C.pneumoniae* can certainly persist for many years after the initial respiratory infection.[37,38]

C. *Chlamydia (Chlamydophila) psittaci* and Lymphomas

Psittacosis is a zoonotic disease caused by *C. psittaci* and is usually contracted through contact with poultry or psittacine birds such as cockatoos, parrots, and parakeets.[131] Recent evidence has suggested that *C. psittaci* infections may be associated with some non-Hodgkin's lymphomas, particularly ocular adnexal lymphomas (OALs).[132–136] *C. psittaci* DNA was detected by PCR in 80% of OAL samples and all samples were negative for *C. trachomatis* and *C. pneumoniae*.[132] None of the 20 non-neoplastic orbital biopsies tested were positive for *C. psittaci*. Of the patients with positive lymphomas, 43% also had *C. psittaci* DNA detectable in their PBMCs while all healthy blood donor controls were negative.[132] In a follow-up study,[134] 74% of OAL patients were positive for *C. psittaci* by PCR, immunohistochemistry, and immunofluorescence. Furthermore, laser-capture microdissection was used to show that monocytes/macrophages were the cells infected with *C. psittaci* and intact EBs were demonstrated in these cells by electron microscopy. *C. psittaci* DNA was also demonstrated in some non-OAL lymphomas, both nodal and extranodal, and seemed to be more common in large B cell lymphomas of the skin and Waldeyer's ring. Viable *C. psittaci*, able to productively infect J774A.1 monocyte/macrophage cell monolayers, was isolated from PBMCs and/or conjunctival swabs of 25% of ocular adnexal MALT lymphomas (OAMLs) in a prospective case-control study by this group.[136] All patients with positive cultures had a *C. psittaci*–positive lymphoma and *C. psittaci* was not recovered from any of the normal healthy blood donors used as controls.[136] The study authors also provided epidemiological data showing that OAML is more common in rural areas in individuals who have prolonged contact with animals that may carry *C. psittaci* and who also have a history of chronic conjunctivitis. Treatment of OAL/OAML with doxycycline, an antibiotic known to be effective against *Chlamydia*, resulted in complete and partial remissions in up to 64% of patients, even in some patients with relapsed disease and regional lymphadenopathies.[135,137] It should be noted however that lymphoma regression was also observed in patients with *C. psittaci* DNA-negative tumours.[137] Not all studies, however, have supported a link between *C. psittaci* infection and OAL[138,139] and it has been suggested that geographic regional differences may occur or that methodological differences in detection of *C. psittaci* may contribute to the discrepant data.[140] Notwithstanding the still controversial nature of the association of chlamydial infection with lymphoma, the studies by Ferreri et al.[132] in the Italian population provide compelling evidence that chronic *C. psittaci* infection of monocytes/

macrophages may play a role in the progression of OAL in some populations. The authors pointed to the role of *H. pylori* infection in gastric MALT lymphomas as a precedent for chronic bacterial infections playing a role in cancer development and progression, and the evidence presented for a role of *C. psittaci* infection in OAL certainly justifies further investigation.

D. Lymphogranuloma Venereum, *Chlamydia*, and Monocytes

Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by *C. trachomatis* serovars L1, L2, and L3 and primarily infects the lymphatics rather than being confined to the epithelium. The LGV serovars have a trophism for lymphoid cells and evidence suggests that macrophages may be the target cells of *C. trachomatis* infection in LGV.[141] In this study, viable *C. trachomatis* LGV serovars were cultured from patient lymph nodes. There is recent evidence of the re-emergence of LGV, due predominantly to the L2 serovar, in men who have sex with men (MSM) (reviewed in Ref. 142). It is unknown if persistent chlamydial infections play a role in LGV disease; however, due to the fact that persistent infections and infections in macrophages are more resistant to antibiotic therapy, and the documented increase in LGV infections, further study of these infections may be warranted.

E. Alzheimer's Disease and Multiple Sclerosis

Chlamydia pneumoniae has certainly been demonstrated by PCR in the brain tissue of 20/27 Alzheimer's disease (AD) patients but only 3/27 controls[143] and cultures of *Chlamydia* from two AD brain samples identified these as *C. pneumoniae*[144] with brain-derived isolates more closely related to respiratory isolates than vascular strains of *C. pneumoniae*. Immunohistochemical analysis showed that astrocytes, microglia, and neurons were all host cells for *Chlamydia* in AD brain[143] and this is supported by studies with cell lines as described earlier. Furthermore, inclusion morphology, quantitation of infectious yield, and transcript profile were suggestive of an active rather that persistent infection, although one study using cell lines suggested that microglial cells may be less susceptible to infection than astrocytes or neurons.[112] In addition, intranasal infection of normal BALB/c mice with *C. pneumoniae* induces the deposition of Alzheimer-like amyloid plaques in the brains of these animals with the density, size, and number of plaques increasing as the infection progressed.[145] Plaques in mouse brain occurred in areas where plaque deposition/pathology occurs in human AD brain.[145] This study, however, did not identify if any particular cell type was preferentially infected. Multiple sclerosis is another neurodegenerative disease where *C. pneumoniae* infection is believed by some to play a role. Several studies have attempted to identify *C. pneumoniae* and parachlamydial DNA in the CSF of MS patients.[146–153]. The results to date have been equivocal with some studies reporting a correlation with MS as opposed to other neurological diseases or with active disease, while other studies showed no association. Thus, while the data supporting a link between *Chlamydia* infection and AD is stronger than that supporting a relationship with MS, it is clear that cell lines characteristic of cell types found in the brain can be infected with *Chlamydia* and that there is PCR-based evidence of chlamydial DNA in the CSF of patients with a number of neurological diseases. It would be premature, however, to assert that chlamydial infection, either acute or persistent, is a cause of any of these conditions.

F. Reactive Arthritis

Although a number of bacterial species have been associated with reactive arthritis (RA), epidemiological studies have identified *Chlamydia* as the most common triggering agent of RA in Western countries (reviewed in Refs 154 and 155). Approximately 1–3% of patients with a genital *Chlamydia* infection go on to develop *Chlamydia*-induced arthritis (CIA). *C. trachomatis* migration from the genital epithelium to the affected joint is through circulating monocytes/macrophages.[155] There is increasing evidence of the persistence of bacterial products in synovial fluid and viable, metabolically active organisms can exist for extended periods in the joint, usually in monocytes/macrophages and the synovial membrane. Morphological[156] and

gene expression studies[36] suggest that chlamydial infections in the joint are persistent. Although not completely understood the aetiopathogenesis of CIA is believed to be due to a combination of (i) a cytokine imbalance following infection in a susceptible host (association with HLA-B27), (ii) failure to clear the triggering chlamydial infection, (iii) persistence of chlamydial antigen, (iv) molecular mimicry, where immune responses against host HSP60 are triggered by high levels of chlamydial HSP60 expression that are characteristic of persistent infections, and (v) altered gene expression patterns in host cells within the joint including upregulation of growth factors, apoptosis-related genes, and adhesion molecules.[155] The presence of persistently infected monocytes/macrophages in the joint may explain why antibiotic therapy has not been successful.[157] This may also explain why infection persists and escapes T cell surveillance since studies have shown that *Chlamydia*-infected macrophages induce death by apoptosis in activated T lymphocytes,[45,46] providing a potential mechanism for evasion of the host immune response.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

Compared to growth in epithelial cell lines such as Hep-2 cells, McCoy cells, and HeLa cells, the growth of all *Chlamydia* species in various immune cells almost never exceeds the number of infection forming units (IFUs) that are used to initiate the infection. If isolation of infectious EBs (that are able to infect traditional epithelial cell targets) is used as the criteria for "infection," then in many cases no infectious *Chlamydia* are recovered. In many immune cell types, however, evidence of some chlamydial replication, as evidenced by the presence of inclusions, intracellular accumulation of chlamydial antigens such as LPS and transcription of replication-associated chlamydial genes can be demonstrated and this has led to the conclusion that in most immune cells infections are persistent and that the developmental cycle does not proceed to completion such that fully infectious EBs are not produced (summarized in Table II). The outcome of infection can be influenced by several factors including the infectious dose used and also the stage of development of a particular cell lineage. Early studies with peritoneal macrophages and PMNs demonstrated that at high MOIs, many chlamydial species were cytotoxic for both these cell types, while at lower MOIs cells could become persistently infected and in some cases fully infectious EBs were recovered. In most of the studies in the literature, only a single or at most two infectious doses are used and this is perhaps a shortcoming that may have given an incomplete picture of the ability of a particular cell to support infection. In addition, the fact that there are almost no data available on the infectious dose required to initiate a genital or respiratory infection in a human host makes it difficult to predict from in vitro studies if a chlamydial infection will follow an acute course or become persistent. Furthermore, it is not presently possible to quantitate *Chlamydia* numbers in affected tissues such as blood vessels, fallopian tubes, or conjunctival tissues so that correlations can be made with in vitro studies.

Many immune cells develop along defined developmental pathways from hemopoietic stem cells to fully differentiated end-stage cells. It cannot be assumed that cells of a particular lineage will show the same susceptibility to infection at all developmental stages. This is well demonstrated in studies with cells and cell lines of the monocyte/macrophage lineage where blood monocytes or early monocyte-like cell lines are less susceptible to infection than more mature macrophages. Indeed, in many studies maturation of monocytes by plastic adherence for three to eight days increased the susceptibility of these cells to infection with various chlamydial species. Further studies comparing susceptibility of classically activated versus alternatively activated macrophages and myeloid dendritic cells versus plasmacytoid dendritic cells, for example, will be required to fully define the infection potential of these cell lineages.

Another issue that should be considered is how representative the infection techniques employed in in vitro studies are of infection in vivo. In most in vitro studies, lysates or supernatants of infected immune cells are cultured on epithelial cell monolayers to determine the presence of infectious *Chlamydia*. Although this approach has demonstrated the lack of infectious *Chlamydia* in many immune cell types, co-culture systems of infected immune cells with other cell types have demonstrated that infection is transferred to the

TABLE II. Summary of Current Known *Chlamydia* Infection of Immune Cells

Cell type	*Chlamydia* sp.	Growth status	Disease/immunological impact
Monocytes/macrophages			
U-937	*C. trachomatis*	Persistence/survival (long term recorded)[%] (unless infected by coculture from HeLa, then active growth)	
	C. pneumoniae	Persistence/survival (up to 14 days)	
THP-1	*C. trachomatis*	Active growth[#]	
THP-1 (also Molt 4; T cell, and P3HRI; B cell)	*C. pneumoniae* TW183	Active growth	• Able to induce differentiation of THP-1s to macrophages • Migration—human brain endothelial monolayers
MNP	*C. trachomatis* LGV biovars	LGV Biovars, active growth only after > eight days MNP growth	
	C. pneumoniae Kajanni 7	Persistence/survival or active only after > two weeks MNP growth	• Enhanced adhesion to artery endothelia • Increased adhesion to HUVEC • Migration thriough epithelia monolayer
	C. pneumoniae Kajanni 6	Active infection only after > three days MNP growth	
	C. pneumoniae TW-183		
Plastic-adhered monocytes	*C. trachomatis* K	Persistence/survival	• Adherence to human umbilical vein endothelia
	C. pneumoniae TW 183		
Mono Mac 6	*C. pneumoniae* TW-183	Active infection	
Alveoloar macrophages	*C. trachomatis*	Lethal	
	C. pneumoniae CWL-029	Persistence/survival	
Murine peritoneal macrophages	*C. trachomatis*	Active growth (LGV serovars, less growth trachoma serovar) (low MOI only, high MOI cytotoxic)	
	C. psittaci	Active growth (low MOI only, high MOI cytotoxic)	
	C. pneumoniae AR-39		• Adherence to endothelia • Foam cell formation
Murine alveolar macrophages	*C. pneumoniae*	Persistence/survival	

(continued)

TABLE II. Continued

Bone marrow derived macrophages (murine)	*C. pneumoniae* Kajaani	Limited active growth	• Foam cell formation
Dendritic Cells (DCs)			
Monocyte derived DCs	*C. pneumoniae*	Low active growth (low MOI) and long term (28 days) persistence/survival	
	C. trachomatis	Viability unknown; able to infect	Infected DCs identified in synovial tissue from reactive arthritis
Mouse bone marrow–derived DCs	*C. muridarium*	Persistence/survival but able to propagate infection	• Subversion of antigen presenting function, bias to Th2 immunity • Allergic airway diseases
Neutrophils	*C. trachomatis*	Persistence/survival	
	C. pneumoniae	Active growth, prolong cell life	Potential role in spreading infection by detaching epithelia (?)
Mast cells	*C. pneumoniae*	Unknown growth status	Able to alter mast cell immune secretion profile
T lymphocytes	*C. pneumoniae*	Persistence/survival	
CNS-derived cells			
Microglial cell (MMC BV-2)	*C. pneumoniae* (TWAR 2043)	Resistant to infection	Potential for role in neurodegeneration
Microglial cell (murine; EOC 20)		Active growth	
Astrocytes (murine; C8D1A)		Active growth	
Neuronal cells (murine; NB41A3)		Active growth	
Human astrocytes (U-87)	*C. pneumoniae* AR-39	Active growth	Potential proinflammatory role in neurodegenerative diseases
Human microglioma cells (CHME-5)		Active growth	

[#] Active growth: recovery of viable EBs at a higher level than infected

[%] Persistence/survival: internalized into cell but nonlethal and/or evidence of longer-term infection by microscopy or detection of bacterial RNA and/or proteins, may not recover viable EBs

target cell. For example, infected dendritic cells are able to infect monocytes, and THP-1 and U-937 monocytic cell lines become infected by co-culture with infected HeLa cells and go on to produce infectious EBs, whereas infection of these monocytic cell lines with purified *Chlamydia* did not result in a productive infection. Most in vitro studies also employ epithelial cells grown as monolayers on tissue culture flasks or plates. The same cells grown as polarized epithelial layers on transwell systems are more representative of epithelial barriers and it has been demonstrated in many studies that receptor expression[158] and cytokine secretion,[159] to name but two functions, differ greatly at the apical and basolateral surfaces of cells grown as polarized monolayers, differences that would not be seen in normal monolayer cultures. It is not unreasonable, therefore, to expect that binding and internalization of *Chlamydia* may also differ in cells grown as polarized monolayers.

The increasing number of in vivo studies demonstrating infected immune cells, monocytes in particular, in peripheral blood of both normal blood donors and patients with conditions such as CAD, together with the in vitro infection study data, suggest that immune cells are not a significant host cell type for chlamydial replication but serve more as a "Trojan horse" for dissemination of *Chlamydia* from the site of initial infection (genital and respiratory epithelium) to distant tissue sites such as blood vessels, joints, and lymph nodes.

As well as using these cells as a free ride to other tissues, *Chlamydia* species can modulate the function of infected cells in order to avoid the host immune response and to facilitate entry into tissues such as the CNS. Chlamydial infection of both PMNs and monocytes delays or prevents apoptosis in these cells. In the case of infected PMNs, apoptosis is delayed long enough (96 h) such that *C. pneumoniae* is able to complete at least one developmental cycle. Infected PMNs can then be phagocytosed by alveolar macrophages and transported from the lung to other tissues or, alternatively, EBs released by PMNs can infect epithelial cells in the lung. Infection of monocytes results in the modulation of receptors for TNFα, IL-1β, and IFNγ potentially protecting infected monocytes from the host innate immune response. Infected monocytes also induce apoptotic cell death in activated T lymphocytes, a function that is mediated in part by the production of TNFα. This may allow chlamydial infections to persist by avoiding T cell surveillance. Thus, *Chlamydia*-infected monocytes produce an inflammatory cytokine to evade the T cell response while at the same time avoiding any deleterious affects of TNFα by downregulating TNFR expression. Chlamydial infection of monocytes also affects lipid metabolism and adhesion molecule expression by these cells. This results in foam cell formation, increased adherence to vascular endothelium, and potentially increased numbers of cells able to cross the blood-brain barrier, which may play a role in CAD and neurodegenerative diseases.

Chlamydial infection of dendritic cells can also alter DC function, particularly cytokine secretion patterns and expression of surface markers such as MHC class II, CD40, CD80, and CD86, all of which are involved in activation of naive T cells. *Chlamydia*-infected BALB/c dendritic cells induce strong Th2 responses to a model allergen, OVA, in transgenic D10 cells whereas noninfected DCs induce a Th1 phenotype in these cells, and infected DC also exacerbated allergic airways disease in an animal model. This suggests that in atopic individuals, *Chlamydia* infection could elicit a nonprotective Th2 response that permits chlamydial persistence while at the same time exacerbating allergic responses to nonrelated environmental antigens. Although our studies used Th2-prone BALB/c mice, other investigators have recently shown that low dose respiratory *C. pneumoniae* infection and concomitant allergen exposure of C57BL/6 mice enhanced allergic sensitization, and these effects could be recapitulated by adoptive transfer of allergen-pulsed DCs from infected mice. Although this study did not determine if DCs were actually infected with *C. pneumoniae*, the data show that chlamydial respiratory infection, even in Th1-prone C57BL/6 mice, can affect DC function in a way that enhances allergic responses. Collectively, these studies may explain the epidemiological linkage between *C. pneumoniae* infection and asthma; however, future studies of *C. pneumoniae* infection of human dendritic cells from atopic and normal individuals will be required to confirm this association. The age of DC exposure to chlamydial infection is another factor that can influence the outcome of allergic airways disease in a mouse model, with exposure

to *Chlamydia* during the neonatal period having the greatest affect on subsequent airways disease. Thus, exposure of DCs to *Chlamydia* (and other pathogens) during the neonatal period may have a profound influence on immunity in adult life.

The mechanisms that allow *Chlamydia* to modulate host cell function are only beginning to be understood. *Chlamydia* do possess a type III secretion system that allows entry of chlamydial proteins into the host cell cytoplasm and this may provide a means of targeting host cell signaling and metabolic pathways. For example, *C. pneumoniae* secretes a protein (CP0236) that impairs IL-17–mediated signaling[160] by inhibiting NF-κB signaling via interaction of CP0236 with host cell Act1. *C. trachomatis* secretes a number of proteins including some that interact with NF-kB signaling pathways. Examples include the protease Tsp, which degrades p65,[161] and the deubiquitinase ChlaDub1, which deubiquitinates IkBa, preventing its degradation to release NF-κB.[162] It is tempting to speculate that *Chlamydia* may have evolved multiple mechanisms to target this important immune activation pathway as a means of surviving within immune cells in order to gain access to other target tissues and as a means of controlling the host immune response to ensure persistence.

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