



The cellular paradigm of chlamydial pathogenesis

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Diseases caused by *Chlamydia* are based on intense and chronic inflammation elicited and maintained by reinfection or persistent infection. The traditional view in the field is that disease is mediated by antigen-dependent delayed-type hypersensitivity or autoimmunity. This immunological paradigm has served as the basis for years of chlamydial research but the mechanism or the antigen that causes pathology has yet to be unequivocally revealed. Recent research on responses elicited in *Chlamydia*-infected cells defines a new direction for our understanding of this microorganism–host interaction and provides the basis for a reassessment of disease mechanisms. *Chlamydia*-infected non-immune mammalian cells produce proinflammatory chemokines, cytokines, growth factors and other cellular modulators. This cellular response to infection supports an alternative hypothesis for chlamydial pathogenesis: the inflammatory processes of chlamydial pathogenesis are elicited by infected host cells and are necessary and sufficient to account for chronic and intense inflammation and the promotion of cellular proliferation, tissue remodeling and scarring, the ultimate cause of disease sequelae.

The processes of microbial infections are intimately linked to the immune responses of the host in its effort to resolve the infection. The ability of some pathogens to persist and elicit chronic immune responses or of other pathogens to elicit overzealous or misdirected immune responses can result in immunopathological disease. Diseases caused by chlamydiae are thought to be mediated by immunopathology; however, following 15 years of research neither the antigens that elicit immunopathology nor the mechanisms involved have been elucidated. Here, I examine the experimental basis for this perspective, propose rejection of this hypothesis and provide an alternative hypothesis that defines the origins of chlamydial pathogenesis as cellular responses evoked from non-immune cells that become infected with chlamydiae.

Chlamydiae are obligate intracellular bacteria that were originally thought to be protozoa and later viruses, but it ultimately became clear that chlamydiae had all the requisite properties of bacteria [1]. Chlamydiae have been placed in their own order, *Chlamydiales*, with one family, *Chlamydiaceae*. Molecular evaluation of rRNA sequences

confirms that chlamydiae are bacteria, but with only very distant relationships to other bacterial divisions [2–4]. Although it has been proposed that *Chlamydia trachomatis* and *Chlamydia pneumoniae* represent different genera [5], their gene content and genome organization are extremely similar [6], as are their structure and biology [7]. The newly proposed nomenclature has not been generally accepted, has caused confusion in the field, and has been contested because of scientific inconsistencies [8] and new evolutionary data based on genomics [9]. Thus, I have used the original designation, *Chlamydia pneumoniae* [10], for this review.

C. trachomatis is the most common notifiable infection in the USA [11] and *C. pneumoniae* is a very common respiratory pathogen that is probably involved in coronary artery diseases [12]. A subset of *C. trachomatis* strains cause trachoma, a leading preventable cause of blindness in the world [13]. Most *C. trachomatis* strains infect the genital tract and are the cause of important diseases in men and women; however, diseases in women, such as chronic pelvic pain, life-threatening ectopic pregnancy and pelvic inflammatory disease, that often result in involuntary sterility, reflect some of the most severe sequelae of infection in the USA. Infants are at risk for chlamydial eye infection and pneumonia if they pass through an infected cervix. Recent compelling evidence has demonstrated that *C. pneumoniae* is present and persistent at active sites of arterial disease [14] and thus contributes to coronary artery diseases [12], the leading cause of death of men and women in the developed world. Serological surveys have shown that virtually every human has been infected with *C. pneumoniae* [12]. The extremely high prevalence of *C. trachomatis* and *C. pneumoniae* infection reflects the long and successful adaptation of these organisms to persist in their human host population [9].

The damaging disease sequelae such as blindness and sterility caused by *C. trachomatis*, and *C. pneumoniae*-associated coronary artery disease, are caused by inflammation-based pathology. At sites of *C. trachomatis* infection, intense inflammation is characterized by redness, edema and mucopurulent discharge [15]. The ocular or genital tract histopathology in acute experimental and natural infection, or reinfection, consists of a profuse cellular infiltrate containing predominantly polymorphonuclear neutrophils and lymphocytes [15]. At later stages of infection plasma cells and macrophages are recruited and lymphoid follicles develop that contain populations of

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B cells and macrophages at their center and predominately T cells at the periphery [16,17]. Except for infant pneumonia, eosinophils are not a prominent feature. The intense and chronic inflammatory response and follicle necrosis cause damage to the epithelium that is followed by local epithelial cell proliferation and scar formation [15]. It is the physical consequence of scarring that ultimately leads to corneal damage and visual impairment, or tubal occlusion and infertility, many years after active infection. Animal models of *C. pneumoniae* infection show an analogous pattern of marked polymorphonuclear and mononuclear cell infiltration at focal sites of infection [18–20].

Clinical persistence is a key concept in chlamydial pathogenesis. Microbial persistence is a state of infection during which the host immune response does not eliminate the pathogen, thereby resulting in continuing damage to the host [21]. Chlamydiae live in an unusual environment within eukaryotic cell vacuoles that is intimately partnered with the natural history and developmental biology of chlamydiae to facilitate microbiological persistence. For *Chlamydia*, there are no unequivocal data for persistent infection in humans; however, based on years of anecdotal clinical reports, evidence of chlamydial persistence in animals and birds, and murine model studies of reactivation of long-term quiescence of *C. trachomatis* [22] and *C. pneumoniae* infection [23,24], the understanding in the field is that chlamydial persistence is common and is maintained for years at least in a minority of individuals. Using a molecular epidemiological approach to type isolates genetically following a community-wide antibiotic treatment trial for trachoma, both reinfection and long-term (> 2 years) persistent infection in humans have been documented [25]. The persistence concept is also supported by *in vitro* cell culture models in which small amounts of interferon (IFN)- γ result in the arrest of the chlamydial developmental cycle for ocular strains of *C. trachomatis* [26] and for *C. pneumoniae* [27], but do not alter their intrinsic viability as the infection cycle can be restored by removal or neutralization of IFN- γ or by addition of excess tryptophan [28].

The immunological pathogenesis paradigm

Following an analysis of early vaccine trials in humans and data from animal models, Grayston and colleagues concluded that repeated infection by chlamydiae increases the severity of the inflammatory response and promotes chronic inflammation at focal sites of infection causing tissue damage and scarring [29]. Thus frequent reinfection and persistence of infection play central roles in providing stimulation leading to chronic pathological host responses. It was speculated that the chlamydial disease process is one of immunopathology in which an antigen shared among strains of chlamydiae immunologically primes the host leading to delayed-type hypersensitivity upon reinfection [29]. This concept has become a central dogma in the field. In one review, this hypothesis was called ‘the immunological paradigm’ [30] – a fitting assessment of the central role of the hypothesis in the field. However, the mechanism for the immunological paradigm is unclear as it has been described as either

delayed-type hypersensitivity or autoimmunity through molecular mimicry [30]. Significantly, in either case the eliciting factor is an antigen, in the strict immunological definition, that primes the host in an adaptive, chronic immune process that causes pathology.

If the disease process is delayed-type hypersensitivity, then it must meet the standard immunological criteria, including eliciting specific Th1 cell responses and memory. In contrast to autoimmunity, such a reaction is fed by the presence of an antigen and, once the antigen is removed, the response will resolve. To be reasonably considered autoimmunity it must be shown: (1) that there are epitopes from chlamydial antigens recognized by antibody or T cells and that these cross-react with defined epitopes from the (human) host following natural infection; (2) that disease progresses without the presence of chlamydiae; and (3) that the specific epitopes can, and are necessary to, elicit the disease without the presence of chlamydial infection [31].

Heat shock protein 60

The concept of a *Chlamydia*-specific antigen that induces delayed-type hypersensitivity is supported by the finding that triton X-100 extracts of chlamydial organisms elicit delayed-type hypersensitivity-like responses in the conjunctiva of *Chlamydia*-immune guinea pigs [32]. It was subsequently determined that the most likely active component in the triton X-100 extracts was a soluble 57 000 m.w. protein [33]. Testing the triton X-100 extract in the eyes of cynomolgus monkeys previously infected with *Chlamydia* revealed a marked inflammatory response notably within 24 h of a single administration of extract [34]. However, in this study, if there was more than one administration of extract, inflammatory responses were then observed in both the test and control group animals (i.e. triton X-100 buffer alone), apparently owing to toxicity of the triton X-100 that itself can cause ‘disease’. If *Chlamydia*-naive animals were primed with the chlamydial extract, they did not respond upon challenge 21 or 35 days post-inoculation, suggesting that antigenic stimulation alone was not sufficient to sensitize an animal and elicit a hypersensitivity response upon antigen challenge [34].

Bavoil *et al.* [35] and Morrison *et al.* [36] determined that the *C. trachomatis* and *Chlamydia psittaci* heat shock protein 60 (Hsp60) or GroEL was readily extracted from organisms by detergents and concluded that the triton X-100 soluble 57 000 m.w. protein was Hsp60. Rank *et al.* [37] tested whether Hsp60 caused delayed-type hypersensitivity using a more rigorous experimental design. Rather than using animals that had been previously infected and then challenged with a triton X-100 extract, they subcutaneously primed guinea pigs by immunization with cloned chlamydial Hsp60 and tested whether this immune sensitization would exacerbate ocular disease following challenge with chlamydial organisms; however, no differences in pathology were observed. Thus it is questionable whether the inflammatory response observed with triton X-100-solubilized proteins is delayed-type hypersensitivity or, alternatively, that Hsp60 is the active component. Despite the potential significance of the Hsp60

hypothesis, this research has not been followed up using highly defined products without confounding detergent toxicity or by mapping of delayed-type-hypersensitivity-eliciting epitopes.

The search for immune correlates of pathology in humans was initiated by Brunham and colleagues [38] by testing sera from women with post-salpingitis sequelae using immunoblots of chlamydial lysates. Independent of total antibody titers to *C. trachomatis*, women who had severe disease, evidenced by tubal infertility or ectopic pregnancy, more often had antibodies to an unknown ~60 000 m.w. protein, as well as differential serological responses to several other antigens. Wagar *et al.* [39] compared the serological responses of two groups of women with high antibody titers to *C. trachomatis* and 80% of the sera from women with ectopic pregnancy (a very severe sequela) had reactivity to a ~60 000 m.w. chlamydial antigen compared with 31% of sera obtained from women with salpingitis. They further showed that this antigen was common to *Escherichia coli* and mycobacteria and it was characterized as Hsp60 using Hsp60-specific antibody. The *groEL* gene from *C. trachomatis* was cloned and protein was expressed from the entire gene, in addition to peptides from a family of gene truncations [40]. These recombinant proteins were tested for reactivity with the same sera used by Wagar *et al.* [39] and the sera were reactive to the recombinant proteins in precisely the same way as reported for their reactivity by immunoblotting [40]. The findings of elevated human serological responses to *C. trachomatis* Hsp60 in individuals with severe disease outcomes associated with chlamydial infection have been confirmed in a variety of different settings for both genital tract diseases and trachoma [41–49]. However, analogous evaluations of patients with coronary heart disease have not shown as definitive a relationship as those observed for *C. trachomatis* [50–52].

Despite the biased antibody responses to Hsp60 in diseased individuals, the hypothesis that Hsp60 is the antigen mediator of pathogenesis has been unclear from a functional and molecular perspective [30,53]. Is the delayed-type hypersensitivity effector a T-cell-sensitizing antigen, are the antibody responses merely a marker of persistent infection or is antibody playing a direct role in an as-yet-undefined way? Alternatively, some have proposed that the mechanism is autoimmunity by cross-recognition of *Chlamydia*-elicited immune responses to human mitochondrial Hsp60 [48–50,54–56], presumably because chlamydial and human mitochondrial GroEL share ~50% sequence identity. In a recent study of *C. pneumoniae* infection and coronary artery disease it was reported that serological reactivity to the chlamydial Hsp60 is an independent risk factor, and specifically independent of reactivity to human Hsp60 [57]. These data do not support the concept of Hsp60 cross-reactivity and autoimmunity as a mechanism of disease.

Delayed-type hypersensitivity

The role of Th1 and Th2 phenotypes and cell-mediated immunity in immune responses to chlamydiae is informative concerning the mechanism of chlamydial pathogenesis. This question has been addressed in studies of

trachoma by comparing immune responses of patients with either little or severe conjunctival scarring. Patients with severe scarring have high anti-chlamydial antibody responses but low cell-mediated proliferative responses, suggesting that strong Th1-type responses play a role in resolution of infection and Th2-type responses play a role in scarring [58]. Holland *et al.* [59] tested this hypothesis and found that trachoma patients with severe scarring more often produced Th2-type patterns of cytokines. These data support the hypothesis that strong cell-mediated Th1 responses are important for resolution of infection, not pathology. Indeed, such an association was found for patients that resolved ocular infection versus those in whom *Chlamydia* infection persisted [60].

Chlamydial infection has been associated with eliciting reactive arthritis, a disease caused by persistent bacterial antigen. Simon *et al.* [61] characterized the pattern of cytokine production among patients with rheumatoid arthritis, an autoimmune disease, and found that they have a Th1 pattern of secreting cells whereas cells from reactive arthritis patients contained Th2-type secreting cells. Murine studies also consistently support the conclusion that Th1 cells and cytokines promote protection from, and resolution of, chlamydial infection and disease whereas Th2 cytokine patterns are associated with persistence and disease [62–64]. Cytokine profiles of susceptible and resistant mouse strains and of interleukin (IL)-10 knockout mice following chlamydial infection revealed that Th2-type cytokine production was associated with weaker delayed-type hypersensitivity, and decreased IL-10 was associated with Th1-type cytokine production and decreased granuloma formation, a hallmark of disease [65,66]. Taken together, these data do not strongly support a hypothesis of classical delayed-type hypersensitivity as the mechanism for chlamydial pathogenesis. By contrast, these data consistently and simply demonstrate that chlamydial disease is a function of the lack of resolution of infection (i.e. persistence) that confounds interpretations of immunological mechanisms of pathology.

Autoimmunity

The basic criteria for invoking autoimmunity as the mechanism of chlamydial pathogenesis also have not been met. Specific antibody-binding epitopes from chlamydial Hsp60 and human Hsp60 have been identified that cross-react at some level [67], but such epitopes have not been shown to elicit autoreactive antibodies or T cells and mediate disease, nor has it been shown that disease progresses following immunization, even with the entire Hsp60 molecule [56]. The data supporting the autoimmunity hypothesis in humans consist of showing reactivity of antibody to chlamydial and human Hsp60 in a subset of patient sera tested [49]. T cells reactive to chlamydial Hsp60 following experimental murine infection have been described but were not tested against human Hsp60 [68]. T cells characterized from *Chlamydia*-infected humans that were specific for chlamydial Hsp60 were not tested using human Hsp60 to show cross-reactivity [69,70] and these T cells predominantly produced IL-10 [70], which would be expected to downregulate Th1-mediated hypersensitivity responses. The IL-10 response is consistent

with murine models of chlamydial infection [66,71]. Immunization with chlamydial Hsp60 alone does not result in eliciting cross-reactive autoimmune T cells, but *Chlamydia*-specific T cells (probably Th2 cells) that produce an IL-10 cytokine response [56].

Evaluation of human serological responses to chlamydial Hsp60 epitopes reveals that among women with severe disease outcome most sera reacted to epitopes contained within the carboxy-terminal half of the protein [40,41,72]. Mapping epitopes by peptide scanning across the entire protein using rabbit and human immune sera revealed numerous reactive epitopes, but no consistent epitope was recognized by all the sera from humans with disease [67]. Several epitopes defined by peptide scanning were shown to be cross-reactive to analogous peptides from human Hsp60 although the reactivity to the human Hsp60 analog was always less, of uncertain affinity and the sequences were not very similar [67]. Thus, women with severe disease outcome have reactivity to a relatively broad repertoire of Hsp60 antigenic determinants in common, but they do not appear to share a common autoimmune molecular mimic, at least at the antibody level [40,41,67,72]. The observation that some *Chlamydia*-specific sera or T cells crossreact to host components is insufficient to implicate autoimmunity as the basis for chlamydial disease.

One attempt to determine an autoimmune process at the molecular level used bioinformatics to identify epitopes for a 16-amino-acid cardiac myosin-specific peptide in proteins encoded in the chlamydial genome [73]. Hsp60 was not identified; however, Bachmaier *et al.* [73] identified a seven-amino-acid motif containing four identical residues in the *C. trachomatis* OmcB protein. Following immunization of mice with this *C. trachomatis* peptide, antibodies were produced that reacted with heart myosin and the immunization elicited myocarditis. However, the motif for the *C. pneumoniae* peptide contains only two amino acids in common, produced weak reactivity in only half of the mice, and this residual motif is present in 1334 peptides encoded in the *C. pneumoniae* genome (R.S. Stephens, unpublished). The peptide used in these studies is located within the leader signal peptide of OmcB and is cleaved from the mature protein during translocation [74] and thus would be expected to be immediately degraded and unavailable for eliciting antibody responses during a natural infection. Moreover, neither *C. pneumoniae* nor *C. trachomatis* is a common cause of myocarditis [12] and *C. trachomatis* is not associated with coronary artery diseases [12]. Demonstrating that the *C. trachomatis* peptide can cause myocarditis in mice falls profoundly short of showing that it does so during natural infection, and the lack of credible continuity with *C. pneumoniae* is inconsistent with the similarity of the fundamental disease processes among chlamydiae. Although autoimmune responses can be shown to occur in a minority of unique cases (e.g. HLA B27-related reactive arthritis), the data available do not support autoimmunity as a common basis for chlamydial pathogenesis.

The cellular pathogenesis paradigm

Given the lack of sufficient or compelling data to support delayed-type hypersensitivity or autoimmunity hypotheses,

especially for Hsp60, one can conclude that the pathogenesis data can only be awkwardly packaged into the immunological paradigm. Rasmussen *et al.* [75,76] approached the mechanism of pathogenesis from another perspective. As the influx of inflammatory cells is the singular hallmark of both acute infection and reinfection, they asked whether *Chlamydia* eliciting a response by its non-immune host cell might account for these observations. It was discovered that, following infection of epithelial cells *in vitro*, large amounts of proinflammatory chemokines, including IL-8, GRC α , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1 α and IL-6 were produced and secreted [75]. Moreover, unlike the short temporal responses typically observed for most other infectious disease systems *in vitro* [77], the proinflammatory responses elicited by chlamydiae are persistent. Such proinflammatory chemokines have been detected in murine models of chlamydial infection [78]. We have measured high levels of IL-8 in tears from children with trachoma, as well as in endocervical secretions obtained from women infected with *Chlamydia*, demonstrating that the cytokine patterns observed *in vitro* are also present *in vivo* during infection by chlamydiae (R.S. Stephens, unpublished). Consistent with the genus-level character of chlamydial pathology, IL-8 is also strongly upregulated following infection of endothelial cells by *C. pneumoniae* [79].

The upregulation and secretion of proinflammatory chemokines that promote immune cell migration and activation during acute infection readily accounts for the heavy cellular infiltrate observed *in vivo*. The proinflammatory chemokine response is sustained even when chlamydiae are prevented from continuing their developmental cycle using an *in vitro* model of chlamydial persistence [75]. The more rapid and intense inflammatory response observed upon reinfection can be attributed to the massive call for cells by the chemokine response of infected cells, but now the recruited infiltrate contains *Chlamydia*-specific immune cells that amplify the response.

Although the cellular pathogenesis model (Fig. 1) accounts for the inflammatory response observed in natural infection, can it also support the basis for the processes of cellular proliferation, tissue remodeling and scarring? Recent evidence suggests it can. Dessus-Babus *et al.* [80] showed that cells infected with chlamydiae produce large amounts of IL-11. IL-11 is a pleiotropic IL-6-family cytokine that can induce tissue fibrosis [81,82]. It has also been shown that *C. trachomatis* and *C. pneumoniae* infections elicit the externalization of phosphatidylserine on the surface of epithelial, endothelial, granulocytic and monocytic cells promoting phagocytosis, cell activation and plasma clotting [83]. Using microarrays of human genes, *C. trachomatis*-infected mammalian cells upregulate a variety of growth factors [i.e. heparin-binding epidermal growth factor, vascular endothelial growth factor, basic fibroblast growth factor, epidermal growth factor, connective tissue growth factor, tissue factor and transforming growth factor (TGF)- α], procoagulant mediators, and inhibitors of apoptosis (R.S. Stephens, unpublished; [84,85]) *C. pneumoniae* has also been shown to elicit tissue factor, tumor necrosis factor, proinflammatory chemokines and growth factors

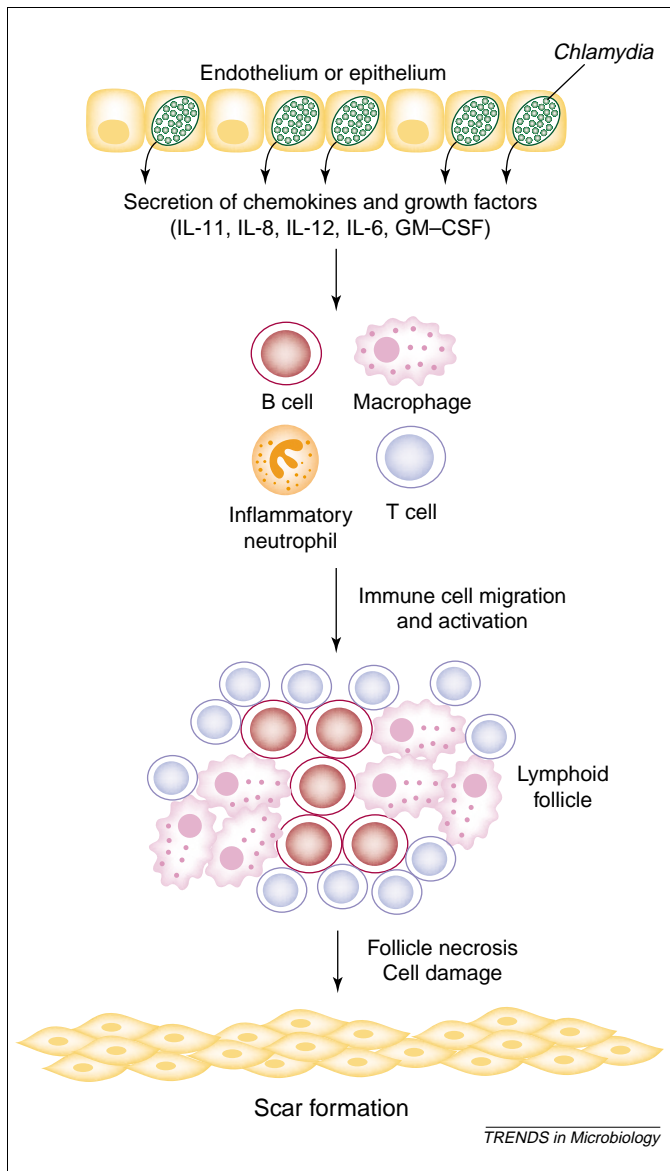


Fig. 1. The cellular pathogenesis model of *Chlamydia* infection.

(R.S. Stephens, unpublished; [86]). These *in vitro* data predict that within the nexus of infection, growth factors are produced that will promote cellular proliferation and tissue remodeling.

One can now begin to assemble the parts for a model of chlamydial pathogenesis. The cellular processes of non-immune cells elicited by chlamydial infection cause the influx of inflammatory neutrophils, T cells, B cells and macrophages that are stimulated by the proinflammatory cytokine and chemokine environment. These cells become activated in both antigen-nonspecific and, for reinfection, antigen-specific responses to produce their own repertoire of cytokines and growth factors. The cellular responses of epithelial and endothelial cells, the primary home for *C. trachomatis* and *C. pneumoniae*, respectively, will be reliably induced upon acute, chronic and persistent infection. The induction of host cell cytokines will promote foci of inflammatory responses in addition to promoting cellular proliferation, tissue remodeling and healing processes that, if they persist, result in scarring. Thus,

although there is surely a role for an adaptive immune-response component in chlamydial disease, it is secondary to the cellular responses of infected non-immune cells.

Heat shock protein 60 revisited

If the primary molecular basis of chlamydial disease is based upon cell responses to chlamydial infection of non-immune cells such as epithelial and endothelial cells, one can ask whether this hypothesis accommodates the data for the relationship of immune responses to chlamydial Hsp60 in patients with severe disease or is this merely a confounding finding of persistent infection? It has been shown *in vitro* that chlamydial Hsp60 alone can activate endothelial cells, smooth muscle cells and macrophages to produce adhesion factors and proinflammatory cytokines by activation of nuclear factor (NF)- κ B [87] and stimulate macrophage secretion of TNF- α [88]. Byrne and colleagues have shown that purified chlamydial Hsp60 is sufficient to elicit cellular oxidation of low-density lipoprotein [89]. The unifying mechanistic data are provided by two research groups who recently have shown that Toll-like receptors bind chlamydial Hsp60 and induce cellular signaling networks similar to the responses induced by *C. pneumoniae* organisms [90,91]. It is of interest that a *C. trachomatis*-specific Hsp60 was used in these investigations demonstrating that, despite only 90% sequence identity between the *C. trachomatis* and *C. pneumoniae* Hsp60, similar responses were apparently elicited. A recent report using *C. pneumoniae*-specific Hsp60 reported similar results for a predominant interaction of chlamydial Hsp60 and Toll-like receptors (TLRs) on dendritic cells [92]. TLRs are transmembrane proteins of mammalian cells with extracellular domains that recognize basic components common to microbial pathogens such as LPS, peptidoglycan and lipoproteins [93]. TLR induction is dependent upon a receptor-signaling pathway involving MyD88 and TRAF6 activating NF- κ B and mitogen-activated protein kinases (MAPKs) that regulate the expression of proinflammatory cytokines and other mediators of innate immune responses.

Chlamydiae produce abundant quantities of Hsp60 localized in the cytoplasm and intercalated in the outer membrane [35]. The current model for a mechanism of persistence for chlamydiae *in vivo* is the effect of IFN- γ in limiting intracellular pools of tryptophan resulting in the inhibition of chlamydial growth but the maintenance of viability [94,95]. One outcome is the ability to detect chlamydial Hsp60 with the diminishing ability to detect other proteins such as the major surface protein, OmpA [94,96]. This mechanism primarily applies for ocular strains of *C. trachomatis* [97] and for *C. pneumoniae* [6] that lack a functional tryptophan synthase. *C. trachomatis* strains that infect the genital tract appear to contain a functional tryptophan synthase [97,98] and, thus, will not be as susceptible to IFN- γ -mediated persistence [99], although they might have other undefined persistence mechanisms. Thus, the abundance of chlamydial Hsp60, even during chronic persistent infection, could promote cellular responses by engaging TLRs and promote Hsp60-specific immune T and B cells.

Concluding remarks

Based on the cumulative data from animal models, human infections and *in vitro* studies, one can propose an alternative to the immunological paradigm for chlamydial pathogenesis. The core of this hypothesis is not antigen-dependent adaptive immune responses, as in delayed-type hypersensitivity or autoimmune molecular mimicry; rather, pathogenesis is primarily based on the cellular responses elicited by non-immune cells infected with chlamydiae. Notably the non-immune cells include mucosal epithelial cells and vascular endothelial cells. We have observed that ~50% of the total cellular responses to chlamydial infection are dependent on chlamydial growth, suggesting that growth or persistence is required to enable expression of the full repertoire of host cell responses (R.S. Stephens, unpublished). These responses involve elaboration of proinflammatory chemokines, cytokines and growth factors. As immune cells are recruited to foci of infected cells they are activated to respond by production of their own repertoire of immune modulators, growth factors and immune responses that markedly augment host responses through the confluence of auto-crine and paracrine networks. Thus, this hypothesis embraces enhanced responses upon secondary infection as the adaptive immune cells will be recruited and augment the process more rapidly, with greater vigor and perhaps directed towards fibrogenic-promoting Th2 cytokine profiles [100]. This represents the interaction of the innate immune system with the adaptive immune system wherein the underlying mechanism of pathogenesis is based on the innate responses of infected non-immune cells – not on adaptive hypersensitivity or autoimmune mechanisms.

One prediction from the cellular pathogenesis hypothesis is there will be a range of cellular responses among individuals with chlamydial infection that correlates with differences in susceptibility to fibrosis, scarring and disease sequelae. Support for this prediction is an association with conjunctival scarring in trachoma patients and elevated levels of IL-10 [101], TNF- α , IL-1 β and TGF- β 1 [102,103]. The differences in IL-10 and TNF- α among individuals have been attributed to polymorphism in the IL-10 gene [101] and the TNF- α gene promoter, respectively [102]. Thus the cellular pathogenesis hypothesis can be tested experimentally using a combination of molecular and epidemiological experimental designs. Perhaps it is time for a paradigm shift for chlamydial pathogenesis, as understanding the fundamental cellular source of inflammation and disease permits us to consider new directions and dimensions for therapeutic approaches and strategies for disease prevention.

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