Commentary

Persistent *Chlamydiae* and chronic arthritis

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Abstract

Urogenital infection with *Chlamydia trachomatis* can lead to development of an acute inflammatory arthritis, and this acute disease becomes chronic in some individuals. Research indicates that the organism is present in synovial tissue of patients with chronic disease in a persistent, rather than an actively growing, form. Importantly, metabolic and other characteristics of persistent *Chlamydia* differ from those of actively growing bacteria. Other studies suggest that *Chlamydia pneumoniae* can be found in a persistent state in the synovium and that it too may be involved in joint pathogenesis. These and other observations suggest a more complex role for the *Chlamydiae* in joint disease than previously recognized. This realization should engender a realignment of thinking among clinicians and researchers concerning both mechanisms of chlamydial pathogenesis in the synovium and design of new treatments for the disease.

Keywords: *Chlamydia pneumoniae*, *Chlamydia trachomatis*, inflammation, molecular genetics, pathogenesis, reactive arthritis

Introduction: the *Chlamydiae*

All *Chlamydia* species are obligate intracellular bacterial parasites, and all are pathogenic to their various hosts [1]. *Chlamydia trachomatis* and *Chlamydia pneumoniae* are human pathogens; the former being the etiologic agent for trachoma as well as a prevalent sexually transmitted bacterium, while *C. pneumoniae* is a respiratory pathogen responsible for community-acquired pneumonia. Both species disseminate from their sites of primary infection, and when they do so these organisms often take up long-term residence at distant anatomic locations. At sites of their dissemination, neither *C. trachomatis* nor *C. pneumoniae* produces any known toxins. Rather, both species may elicit a powerful immunopathogenic response that in turn can engender various diseases, one of which is inflammatory arthritis [2]. In some individuals with acute *Chlamydia*-associated arthritis, the disease can become chronic. This article focuses on chronic arthritides attributable, or potentially attributable, to disseminated, persistent infection by the *Chlamydiae*, and is intended primarily to stimulate discussion among researchers and clinicians regarding the unusual biology of these pathogens in relation to chronic disease. It is also intended to elicit discussion as to how knowledge of the biological attributes of chlamydial persistence might influence development of new therapies for treatment of that disease.

The chlamydial developmental cycle

According to classic microbiological description, all *Chlamydiae* undergo a biphasic developmental cycle [3]. In the first phase, the infectious extracellular form of the organism (the elementary body [EB]) locates and binds to an appropriate eukaryotic host cell. These host cells are usually epithelial or epithelia-like cells, although several

EB = elementary body; IFN = interferon; PCR = polymerase chain reaction; RB = reticulate body.

studies have indicated that other cell types can be infected. The host cell receptor to which *Chlamydiae* bind has not been firmly established. Following binding, the organism is brought into a membrane-bound cytoplasmic inclusion, within which it reorganizes into the metabolically active form, the reticulate body (RB). During this intracellular phase, the RBs undergo several rounds of cell division. At the termination of this active growth process, most dividing RBs reorganize back to the EB form and are released from the host cell via lysis or exocytosis. Released EBs then find new host cells to propagate infection. This standard view of the developmental cycle is derived primarily from *in vitro* studies using infected host cells 'permissive' for active chlamydial growth. *C. trachomatis* infecting HeLa cells requires approximately 50 hours to complete the developmental cycle. For *C. pneumoniae*, the standard *in vitro* host cell type is Hep-2, and the developmental cycle requires about 72 hours for completion.

Chlamydial persistence

Recent research results have modified this standard understanding of chlamydial growth characteristics in disease and other contexts. It is now clear that, following dissemination to distant anatomic sites, both *C. trachomatis* and *C. pneumoniae* can establish long-term infections at those sites; in this context, details of the organisms' passage through the normal developmental cycle and their overall metabolic characteristics differ from those of actively growing *Chlamydiae*. This modified biological state is referred to as 'persistence', a term intended primarily to indicate the changed metabolic and other characteristics that underlie the altered developmental cycle, while at the same time reflecting the organisms' tendency to engage in long-term infection at some sites of their dissemination [4]. *Chlamydiae* enter the persistent state *in vivo* in disease contexts, including chronic *Chlamydia*-associated arthritis, and they can be induced to persistence in *in vitro* model systems (for example [4–6]). Observations using infected 'permissive' host cell types in culture treated with IFNγ have proved to be important [4]. In these studies, it was shown that *C. trachomatis* cells in cytoplasmic inclusions within IFNγ-treated host cells assume an aberrant morphologic form. Similar aberrantly shaped forms have also been identified for this organism infecting normal human monocytes in culture in the absence of IFNγ treatment [6]. Persistent organisms show a suite of unusual transcriptional, and therefore presumably metabolic, characteristics (see later). Essentially identical attributes have been demonstrated for *C. pneumoniae* infecting IFNγ-treated Hep-2 cells, suggesting that these properties are common to all *Chlamydiae* in the persistent state (for example [7,8]).

The biology of persistence

As indicated by the earlier discussion, many aspects of the biology of chlamydial persistence are significantly different from those of standard, active infection. Persistent *C. trachomatis* cells reside primarily within monocyte/macrophages in synovial tissue, and in this state they are metabolically active but morphologically aberrant [9,10]. Moreover, persistent *C. trachomatis* cells severely decrease production of new infectious EBs, reflecting the fact that the organisms are arrested at a late stage of the developmental cycle [4–6]. Studies have demonstrated that transcription of *omp1*, encoding the immunodominant major outer membrane protein of this organism, is attenuated in the persistent state, while expression of *hsp60*, encoding a highly immunogenic protein, is upregulated (for example [4,10,11]). Other transcriptional differences between actively growing and persistent *C. trachomatis* cells have been reported. For example, genes whose products are involved in DNA replication and partition are expressed during both active growth and persistence, but genes whose products are required for cytokinesis are downregulated during the latter state [12]. This attribute is also found in persistent *C. pneumoniae*, as are most other characteristics of persistence already discussed for *C. trachomatis* [4,8]. Interestingly, expression of genes encoding enzymes of the *C. trachomatis* glycolytic and pentose phosphate pathways are downregulated during persistence (Gérard *et al.*, manuscript submitted).

While we have some understanding of the detailed biology of chlamydial persistence, that understanding is rather minimal. Moreover, it is almost exclusively descriptive since we do not understand the molecular mechanisms underlying most biological characteristics shown by persistent *C. trachomatis* and *C. pneumoniae*. However, as already mentioned, those characteristics have been demonstrated for *C. trachomatis* both in *in vitro* models of persistence and *in vivo* in joint materials from patients with chronic *Chlamydia*-associated arthritis (for example [6,9,10]), and they unquestionably represent meaningful chlamydial adaptations for maintenance of long-term infection. Available data thus argue that persistence is a normal, perhaps the normal, state for *C. trachomatis* and *C. pneumoniae* during long-term infection *in vivo*. The data also suggest that active growth, as represented by the standard developmental cycle from EBs to RBs and back to EBs, may be primarily required for continued high level transmission of the organisms.

Chlamydia-associated arthritis

A relatively small proportion of individuals who acquire a genital infection with *C. trachomatis* develop acute inflammatory (reactive) arthritis, and only a portion of those patients proceed to chronic disease [13]. It has never been clear why some *Chlamydia*-infected individuals develop arthritis whereas others do not, and we do not understand why only a subset of patients who do develop acute disease proceed to chronicity. Clearly, both aspects are at least partly a function of host genetic background, but there may also be as yet unknown genetic or other characteristics of the infecting *Chlamydiae* that contribute.

Studies have shown that *C. trachomatis* reaches the joint from the urogenital system via circulating monocytes [2], and that monocytes/macrophages are the common host cells for persistent organisms during long-term infection of synovial tissue. It is not known how long a period is required for transit to the joint, whether *C. trachomatis* cells are already persistent on arrival in the joint, or whether that state develops only after the organisms are stably in place. We do know from *in vitro* studies, however, that *C. trachomatis* cells enter the persistent state within about 48 hours post-infection of normal human peripheral monocytes, that they then alter expression of several important genes, including increasing transcription of their *hsp60* gene [6,12], and that acute arthritis only develops after 1 week or more of post-genital infection [13]. These observations suggest that *Chlamydiae* are in fact in the persistent state as they reach the joint, in turn suggesting that even acute disease is elicited by persistent, rather than actively growing, organisms. The observations further suggest that the immunogenic hsp60 protein produced by persistent *Chlamydiae* is in large part responsible for eliciting synovial inflammation in both acute and chronic disease. We therefore argue that rheumatologists treating patients with chronic *Chlamydia*-associated arthritis, and probably also those with acute disease, must contend with persistent organisms rather than actively growing ones, and with the altered biology that characterizes the persistent state. Indeed, in our view, persistence should be considered the usual state of the organism in *Chlamydia*-associated arthritis.

We have no data to indicate how long infected monocyte/macrophages endure in synovial tissue, or how long the organisms can exist within them in the persistent state. It would be of interest, and also of clinical importance, to know the answer to these questions. Importantly, we also do not know whether *C. trachomatis* is fully cleared from the synovium in those patients with acute *Chlamydia*-associated arthritis who do not proceed to chronic disease, or whether organisms can remain at that site in a persistent but subclinical state. Another important question is why many patients with chronic *Chlamydia*-associated arthritis cycle between active and quiescent disease. For both clinical/therapeutic and scientific reasons, all these are critical issues.

Treatment of persistent synovial *Chlamydiae*

Antibiotic treatment of active cervical and urethral *C. trachomatis* infections is standard, and such regimens are effective in stopping growth of the organism [14]. However, antibiotic treatment of persistent organisms in the synovium has proved to be disappointing; while some groups have reported improvement in patients with *Chlamydia*-associated arthritis following antibiotic treatment (for example [15]), other groups have reported little or none (for example [16,17]). To a limited extent, such inconsistent outcomes can be traced to the antibiotics used, since not all such drugs have equal efficacy against *C. trachomatis*. Few data exist, however, concerning the synovial accessibility of any antibiotic following standard oral or other administration. While joints are well vascularized, suggesting little limitation on such accessibility, it would still probably be of value to determine effective concentrations of anti-chlamydial drugs in the joint for patients with *Chlamydia*-associated arthritis.

The efficacy of antibiotic treatment for *C. trachomatis* is usually assessed using *in vitro* systems in which drug and chlamydial EBs are added simultaneously, or nearly so, to permissive host cells in culture. Patients presenting with a urogenital infection by this bacterium, however, already have organisms well progressed into the developmental cycle, and those with chronic reactive arthritis have persistent organisms. This and other groups have suggested that drug determinations be carried out by addition to already-infected host cells in culture after inclusions have been formed [2,18]. Moreover, we have provided evidence that when such a system is employed, the effect of some antibiotics (ciprofloxacin and orofloxacin) on *C. trachomatis* growth is unexpected [19]. Specifically, in our study, treatment of already-infected permissive host cells with standard concentrations of either drug induced the persistent state, rather than clearing the organism. This result would not have been observed had the determination been carried out in the standard manner. *In vitro* definition of the minimal inhibitory concentration of any antibiotic for *C. trachomatis* or *C. pneumoniae* by the standard method thus appears inappropriate for patients with either acute or chronic *Chlamydia*-associated arthritis, and it may not even be optimal for individuals with active urogenital infection.

The question remains whether eradication of persistent *Chlamydiae* in the synovium can be achieved via antibiotic treatment alone. Even if such drugs function reasonably effectively for active infection of the urogenital or other systems, it does not necessarily follow that they can and will do so in the case of persistent organisms. We suggest that a useful approach to treatment of patients with *Chlamydia*-associated arthritis would be to define some means by which persistent organisms can be induced to return to the active developmental cycle, thereby making them more accessible to antibiotic activity.

C. pneumoniae and arthritis

Several laboratories have demonstrated the synovial presence of bacterial DNA and/or antigens from organisms not traditionally associated with joint disease (for example [20–22]). It is not clear whether joint pathogenesis is initiated or maintained by these organisms, but it is of interest that DNA from *C. pneumoniae* has been found in synovial tissues of a small but still significant number of patients

with various arthritides [23–25]. Our own analysis showed that when synovial tissue samples from individuals with various arthritides were screened, about 13% of patients were PCR-positive for the organism [24]; the organism is viable and metabolically active in joint tissue [26]. While it is well documented that this organism can induce a powerful inflammatory response at sites of its residence [27], no evidence available to date directly links synovial inflammation with the presence of *C. pneumoniae*, either in the actively growing or the persistent state. Interestingly, no clearly defined pattern of extra-articular clinical features similar to those known for *C. trachomatis*-associated arthritis has been identified in patients PCR-positive for *C. pneumoniae* in synovial tissue [24]. The question of whether this respiratory pathogen is responsible for initiation and/or exacerbation of joint disease thus remains open. In our opinion, however, it is difficult to argue that the organism is not involved in joint disease in at least some patients, given our understanding of the pathobiology of *C. pneumoniae* in other contexts. In any event, the relationship between *C. pneumoniae* and inflammatory or other arthritides is an issue that invites detailed investigation since, if an etiologic relationship can be demonstrated between synovial presence of the bacterium and inflammatory arthritis, the American College of Rheumatology criteria for reactive arthritis will have to be altered.

Chlamydial infection and other chronic arthritides

Beginning with the demonstration of *Helicobacter pylori* as an etiologic agent for ulcers, the notion that bacterial, viral, and probably fungal pathogens can play either an initiating or exacerbatory role in chronic disease has gained increasing credence. In the case of *C. trachomatis,* the sequelae of primary genital infection can be both chronic and severe; these include not only arthritis, but also fertility deficit in women and possibly cervical cancer [28,29]. Disseminated infection by *C. pneumoniae* has been linked to asthma, chronic obstructive pulmonary disease [27], atherosclerosis [30], multiple sclerosis [31,32], Alzheimer's disease [33], and other clinical entities including inflammatory arthritis, as already mentioned. Many of these associations are highly controversial, but they remain under active investigation.

Rheumatoid arthritis is a relatively common and often severe degenerative disease characterized by, among other things, chronic inflammation of the synovium and altered performance of the immune system. Several investigators have suggested that the inflammation, the break in immune self-tolerance, and other characteristics whose combined consequences include synovial degradation may be the common end point for a number of diverse starting points. As reviewed recently, several explanations have been advanced to account for the initiation of pathogenesis in rheumatoid arthritis [34]. One long-standing idea postulates an infectious etiology, perhaps operating in part through molecular mimicry, to elicit a host autoimmune response.

The idea that rheumatoid arthritis may have an infectious etiology is not new. Indeed, the disease was referred to as 'infectious arthritis' in the mid-20th century, and one recent editorial has suggested that this disease may be a form of reactive arthritis [35]. However, despite continuing interest in this idea, no viral or bacterial pathogen has been unequivocally shown to be responsible for initiation or maintenance of pathogenesis in any patient with rheumatoid arthritis. Interestingly, some recent studies have demonstrated therapeutic efficacy for antibiotics in patients with rheumatoid arthritis [36]; it is not clear from these studies, however, whether clinical improvement resulted from antimicrobial, anti-inflammatory, or other activity of the drugs used.

Earlier reports suggested a link between *Chlamydia* and rheumatoid arthritis (for example [37]), but to our knowledge this particular study has not been developed further. An important recent review did specify characteristics required of candidate pathogens for them to have a role in this disease [34]. These include persistence or dormancy, relatively low virulence, and ubiquitous distribution. All these characteristics are met in relation to the *Chlamydiae*. Moreover, it seems probable that the inciting organism should be present in the synovial fluid or tissue of patients with rheumatoid arthritis, at least in early disease, and this has been shown [38]. *C. trachomatis*, and possibly *C. pneumoniae*, may thus prove to be interesting candidates for the initiating agents in rheumatoid arthritis, at least in a subset of patients.

Conclusions

In this article, we have suggested some reasons for a realignment of thinking concerning *Chlamydia*-associated arthritis, based on research results regarding the basic biology of *C. trachomatis*, and to some extent that of *C. pneumoniae*. Our purpose has been to elicit discussion among rheumatologists and researchers as to how information regarding chlamydial persistence might refocus studies of mechanisms of synovial pathogenesis induced by these organisms in inflammatory arthritis, and perhaps other chronic arthritides. To a lesser extent, we have also tried to initiate discussion as to how these new insights might inform ideas for improvement of treatment of such joint diseases. In this brief article, it has not been possible to develop in detail all the bases supporting such realignments, of course, but if meaningful discussion results from this text then its purpose will have been served.

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