Combination antibiotics for the treatment of *Chlamydia*-induced reactive arthritis: is a cure in sight?

The inflammatory arthritis that develops in some patients subsequent to urogenital infection by the obligate intracellular bacterial pathogen *Chlamydia trachomatis*, and that induced subsequent to pulmonary infection with *C. pneumoniae*, both have proved difficult to treat in either their acute or chronic forms. Over the last two decades, molecular genetic and other studies of these pathogens have provided a good deal of information regarding their metabolic and genetic structures, as well as the detailed means by which they interact with their host cells. In turn, these insights have provided for the first time a window into the bases for treatment failures for the inflammatory arthritis. In this article we discuss the biological bases for those treatment failures, provide suggestions as to research directions that should allow improvement in treatment modalities, and speculate on how treatment regimens that currently show promise might be significantly improved over the near future using nanotechological means.

KEYWORDS: antibiotics ■ chlamydial infection ■ clinical trials ■ inflammatory arthritis ■ nanocarriers ■ nanomedicine ■ reactive arthritis

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Chlamydia trachomatis, the most prevalent sexually transmitted bacterial pathogen in the USA, has been known for many years to engender an inflammatory arthritis in a small proportion of individuals who acquire a genital infection with the organism ([1–3] for review). Further, more recent studies from this group and those of others have demonstrated that a similar arthritis can result from infection with the related respiratory pathogen *C. pneumoniae* ([4–6] for review). Advances over the last 20 years in experimental molecular genetic and cell biologic methods have provided increasingly sophisticated understanding of critical aspects of the pathogenic process utilized by these organisms to elicit the inflammation that characterizes *Chlamydia*-induced arthritis. For example, and importantly for what follows, we now understand that the arthritis which can follow a primary genital infection with *C. trachomatis* involves metabolically active organisms residing over the long term in synovial tissues, in contrast to the clinically similar arthritis engendered by various species of enteric bacteria ([6–8] for review). Once established in the joint chlamydiae do not progress through the normal life or developmental cycle that is seen in standard genital tract infections [6,9]. Rather, they live for long periods within their monocytic cell hosts in synovial tissue, and in that context they display a panel of unusual metabolic, morphologic, and other characteristics, some of which contribute significantly to pathogenesis, as outlined in more detail below [10–12]. While we do not understand in full detail how the transcriptional and other peculiarities attributable to persistently infecting chlamydiae cause arthritis, some understanding of the complex pathogenic mechanisms involved is emerging from current research. Importantly for the topic at hand, the increased understanding of chlamydial biology and pathogenesis have suggested new therapeutic approaches for treatment of *Chlamydia*-associated arthritis, as well as providing at least initial understanding of why some treatments and treatment approaches have been ineffective.

In this review we discuss various aspects of the biology of *C. trachomatis*, and to a lesser extent *C. pneumoniae*, as they directly relate to the joint pathogenesis elicited by these bacteria. The overall intention is to initiate construction of a new foundation for understanding treatment failures and successes for the inflammatory arthritides that result from infection with these important organisms. Our committed view is that only within such a foundation will development of more effective therapies, and indeed development of altogether new treatment strategies, for the disease take place. In the process of constructing that foundation, we highlight research areas that in our opinion are likely to provide useful insights into those treatments and treatment strategies if they are given further investigation.

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A caution regarding diagnosis of *Chlamydia*-related joint disease

Before proceeding, we include a brief discussion of one issue relating to the current understanding of the epidemiology of *Chlamydia*-induced arthritis, which is well warranted in our opinion as prelude to what follows. At present, we do not understand why such a small proportion of the more than 1 million individuals who acquire a genital chlamydial infection each year in the USA develop acute arthritis, nor do we understand why only approximately half of those patients progress to chronic disease ([1–3,7,13] for review). New information, summarized below, regarding the biology of dissemination for *C. trachomatis* provides some explanation for this unexpectedly rare sequela to genital infection, as well as providing a suggestion for a means by which patients at increased risk for developing *Chlamydia*-induced arthritis might be identified. Regardless, we contend here that historically as well as currently the arthritis due to chlamydial infection is significantly underdiagnosed. As mentioned, *C. trachomatis* is the most prevalent sexually-transmitted bacterial pathogen in the USA, and we are convinced that a significant number of cases of *Chlamydia*-induced arthritis are being misdiagnosed or missed altogether [14]. Along the same line, epidemiologic studies indicate that infection with the respiratory pathogen *C. pneumoniae* is essentially ubiquitous in all populations studied to date, and as developed below this organism is now accepted as an etiologic agent for inflammatory arthritis ([6,15] and see below). At this point we have no information regarding what proportion of *C. pneumoniae*-infected individuals develop either acute or chronic arthritis, nor do we know whether particular strains of the organism are specifically arthritogenic. Research results outlined below point to a potentially effective therapy for the joint disease elicited by both these pathogens, and we therefore contend that it is critically important to improve the relevant diagnostic criteria and procedures related to *Chlamydia*-associated arthritis.

Chlamydial biology circumscribes treatment options

Each of the several chlamydial species is an obligate intracellular parasite of eukaryotic cells, and within those host cells the organisms normally undergo a biphasic developmental cycle (Figure 1). The cycle involves initial attachment of the extracellular, metabolically inactive form of the organism, the elementary body (EB), to target host cells, upon which the organisms are taken into the host cytoplasm to reside in a membrane-bound vesicle. Within that cytoplasmic inclusion, each EB undergoes a transcriptionally-governed developmental process yielding the vegetative growth form of the organism, the reticulate body (RB), which undergoes 7–8 cell divisions. Near the end of the process approximately 80% of RB dedifferentiate back to the EB form, and for *C. trachomatis* those new extracellular forms are released to the external milieu by host cell lysis or exocytosis at approximately 48 h postinfection; *C. pneumoniae* requires approximately 72 h to complete the developmental cycle ([16–18] for review). *In vivo*, all chlamydial infections elicit a strong inflammatory response, although that response is often more clinically apparent in men than in women, at least for urogenital *C. trachomatis* infections [13,14].

Persistent bacterial infection is now recognized to be a general strategy utilized by many pathogens to promote their long-term survival in the host ([10,11] for excellent reviews). Along that line, it has been known for more than a decade that *Chlamydia*-induced arthritis is a function not of the normal processes of growth and cellular development for this pathogen. Rather, it is a function of this unusual infection state of the organism designated persistence (Figure 1) [3,6,7,13,14]. While the primary infection of the urogenital system is often cleared by the immune system, antibiotic treatment, or a combination of these, the initial inflammatory response in the genital system elicited by the infection attracts mononuclear cells; these cells become infected, are subsequently extravasated, and via the general circulation and/or lymphatics make their way to the joint to establish chlamydial infection of synovial tissue [6,19]. In earlier studies we reported that the primary, although not the sole, host cell type for *C. trachomatis* in synovial tissue is the monocyte [20,21].

Chlamydial persistence and its consequences have become critical foci of research, and new information relevant to the topic of this review has emerged from recent studies. For example, several aspects of persistent infection relating to gene expression for *C. trachomatis* in the joint mirror those known from persistence of *Mycobacterium tuberculosis*; important differences were identified as well, suggesting that development of a general antimicrobial strategy targeting different types of persistent bacterial infections will be challenging [22,23]. Further, one of the many surprises that emerged from sequencing the *C. trachomatis* and *C. pneumoniae* chromosomes was the finding of not one but three open reading

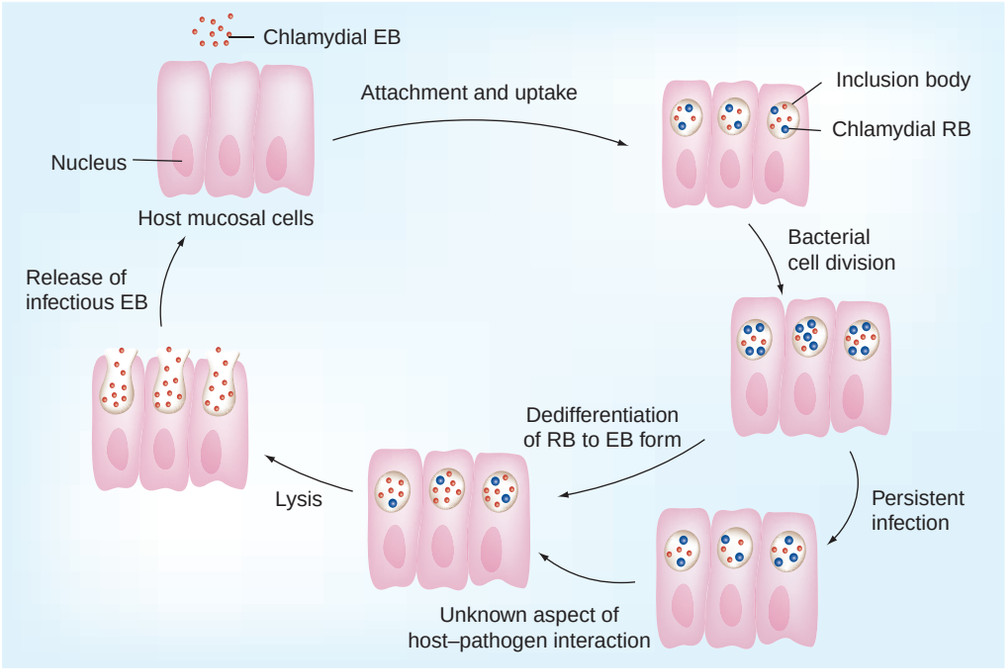

Figure 1. The chlamydial developmental cycle.
EB: Elementary body; RB: Reticulate body.
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frames encoding heat shock protein 60 paralogs in each organism [24,25]. The authentic hsp60 gene (*groEL*, designated Ct110 in the genome sequence of *C. trachomatis*) resides in an operon with *groES*, as in *E. coli* and other well-studied bacteria; the two additional paralogs, Ct604 and Ct755, are not identical to it and only distantly linked to *groEL* [24]. Production of the *groEL* gene product in chlamydiae and other bacterial pathogens elicits a strong immune response from the infected host and, interestingly, we showed in both an *in vitro* model of persistent infection (see below) and in patient samples that expression of the Ct604 gene is significantly increased, while expression of Ct755 is highly attenuated [26]. These observations certainly explain at least in part the continued elicitation of inflammation in patients with chronic *Chlamydia*-induced arthritis. They also suggest that the Ct604 gene product is involved in the transition from normal active to persistent infection as well as the maintenance of persistence; conversely the Ct755 gene product may function only during the former infection state. More study is required to elucidate the function(s) of these proteins in the pathogenesis of *Chlamydia*-induced arthritis and to assess whether they might serve as useful targets for therapeutic intervention.

In persistent infection, both *in vivo* in synovial tissue and in various *in vitro* model systems of that state, the normal chlamydial developmental cycle is arrested at a late point, obviating production and release of new EB, and our group demonstrated that the block in the cycle which characterizes persistence is transcriptional (see below). The persistent infection state can be induced *in vitro* under certain growth conditions and/or within certain host cell types, the latter of which is immediately relevant to joint pathogenesis. Much of the early work on chlamydial persistence was based on studies of *C. trachomatis* infection of HeLa cells treated first with penicillin (Figure 2), later with low levels of IFNγ (e.g., [27]). In those studies, infected cultures so treated contain RB-like forms displaying aberrant morphology; supernatants from treated cultures contained no, or extremely low levels of, new EB (Table 1). Early studies also showed that aberrant chlamydial forms accumulate replicated/segregated copies of the bacterial chromosome in the absence of cell division [27]. Removing IFNγ from the medium releases *Chlamydia* from the block in completion of the cycle, resulting in return to normal morphology and EB production [27–29].

Another, in our view more immediately relevant, *in vitro* model of chlamydial persistence is that using normal human monocytes in culture [30,31]. Our group adopted this system since as mentioned above, our observations indicated that monocytic cells constituted the primary, although probably not the sole, host cell type for

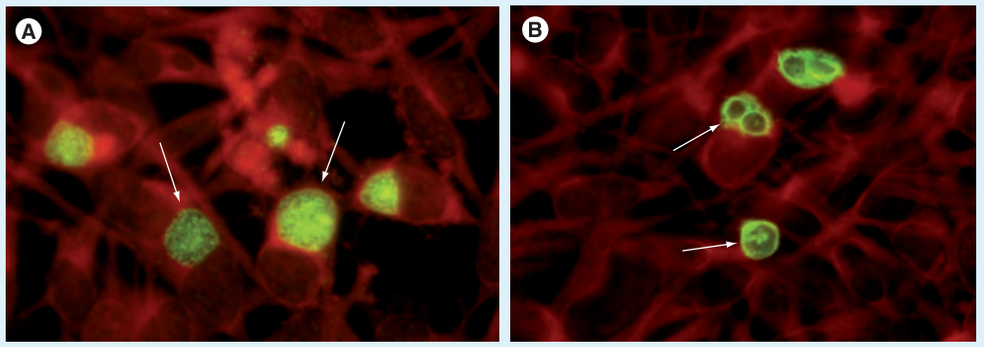

Figure 2. Morphology of chlamydial inclusions. (A) McCoy cells infected for 36 h with *Chlamydia trachomatis.* Fluorescent signal represents chlamydial inclusions which are filled with reticulate bodies and elementary bodies (arrows). **(B)** Cells infected with *C. trachomatis* for the same length of time but Penicillin G was added at 12 h postinfection to induce persistent infection. The large, aberrant reticulate bodies are representative of persistent infection. In both cases, cells were fixed with absolute methanol and stained with a fluorescein-isothiocyanate-conjugated anti-chlamydial lipopolysaccharide antibody (Pathfinder™, BioRad). Images at original magnification, 400×, were captured with ImagePro (MediaCybernetics, Bethesda, MD, USA).

the organism in synovial tissue [20,21]. It is not relevant to develop details of that model here, but study of the model over many years has demonstrated that the transcriptional block that initiates persistent infection occurs at the level of expression of genes whose products are necessary for cell division, including the genes *ftsW* and *ftsK* (Table 1) [28,32]. Expression of a number of other genes located on the chlamydial chromosome also is severely downregulated during the persistent infection state, of which the most important may be *omp1* (encoding the major outer membrane protein, MOMP), and others [33]. Interestingly, and as mentioned above, one study demonstrated that the panel of chlamydial genes involved in the transition to the persistent infection state from active infection, and those involved in maintenance of that state following transition, are similar to those which perform the same functions in *M. tuberculosis* [23]. We note in this context that both *C. trachomatis* and *M. tuberculosis* utilize many genes specifying products of unknown function in the transition to and maintenance of persistence. Elucidation of the functions of these proteins in chlamydiae well may provide new targets for therapy to eliminate persistence and the arthritis that this infection state underlies.

Early studies of chlamydial biology suggested that these organisms were energy parasites on their host cells; that is, that *C. trachomatis* and other chlamydial species possessed no genes specifying the enzymes required for a standard bacterial energy transduction system (see [9] for review). It was determined fairly early in the study of chlamydial biology, though, that the *C. trachomatis* genome does possess a gene (*adt1*) encoding a protein that is synthesized and inserted into the inclusion membrane to mediate the exchange of ATP and ADP with the host cytoplasm. The full genome sequence of *C. trachomatis*, however, demonstrated that the organism indeed does possess the enzymes required for glycolytic and pentose phosphate pathways, and others demonstrated that under conditions of normal active growth the enzymes are produced in quantity and that they function as expected [24,25,34]. Thus when the organism is

Table 1. Characteristics of persistent versus active chlamydial infection.

Attribute	Active infection	Persistent infection
Morphology	RB spherical, ~1 µm diameter	RB shape aberrant, >1 µm
omp1 expression	High level at all times	Highly attenuated
Expression of genes for DNA replication	*dnaA*, *mutS*, others high level	Same genes expressed but at lower level
Expression of genes for cell division	*ftsK*, *ftsW,* others high level	Same genes highly attenuated
Expression of genes for energy transduction	*tal*, *gnd*, *pyk*, others high level	Same genes highly attenuated
adt1 expression	High level	Expressed but at lower level
Metabolic rate	Normal rate	~10–100× lower

RB: Reticulate body.

undergoing its normal developmental cycle it is not fully dependent on its host cell for energy resources; that is, RB express not only *adt1* but also the gene panel specifying the enzymes required for energy transduction. Interestingly, during persistent infection the exchange protein is still expressed at high level, but the genes encoding the glycolytic and pentose phosphate pathway-related proteins are transcriptionally silent, forcing the organism to be an energy parasite during this infection state [35]. This observation suggested that the overall metabolic rate of persistently-infecting *C. trachomatis* would be lower than that of actively-growing chlamydiae, and this proved to be the case by roughly two orders of magnitude [35]. As developed in detail below, many groups have shown that both *C. trachomatis* and *C. pneumoniae* when in the persistent infection state are highly refractory to antibiotic treatment. That refractoriness is probably largely, although not completely, a result of a metabolic rate that is significantly slower than that of actively-growing chlamydiae. Again, elucidation of the genetic basis for transition to and maintenance of persistent infection will go a long way toward providing targets for obviating both active and persistent chlamydial infections.

Issues of strain & dissemination affect treatment options

In addition to its role as a genital pathogen, *C. trachomatis* is the etiologic agent for trachoma, a blinding disease that remains a highly prominent yet treatable illness in parts of the developing world [2,9,36]. Trachoma is caused primarily by ocular strains (serovars A, B, Ba, C) of the organism, while genital infections are caused primarily by a second group of strains (serovars D–K, and biovar lymphogranuloma venereum). As the word indicates, chlamydial strains were initially differentiated serologically; later a set of monoclonal antibodies targeting the MOMP, the product of the single copy chromosomal gene *omp1*, were developed for a microimmunofluorescence assay. While serovar determinations still are made using these monoclonal antibodies, the DNA sequence of the encoding gene is now frequently employed for that purpose [37]. Not surprisingly, studies have demonstrated that some DNA sequence variation in *omp1*, and thus the MOMP, is present within any given serovar; sequence variation also has been identified in other segments of the chlamydial chromosome [38,39]. In addition to differences at *omp1*, ocular and genital serovars have nonidentical deletions around the cytotoxin gene (*toxB*, Ct166) [40]. Further, while genital serovars have functional products from the *trpA* gene, encoding one component of the tryptophan synthase enzyme, ocular serovars have deletions in that gene that produce a nonfunctional product [41]. Importantly for the topic of this review, these and a few additional related differences are thought to account for tissue tropism and variable pathogenicity between the ocular and genital serovar groups (e.g., [42]). Other studies showed that differences in genomic structure among *C. trachomatis* ocular serovars result in varying IFNγ sensitivity, growth rate *in vitro*, virulence, and so on within that serovar group [43]. Importantly, studies from several groups have defined the mechanisms by which the alterations in chlamydial chromosome structure are generated [44,45].

Because *Chlamydia*-associated arthritis is by definition a sequela of genital infection, it has been assumed, and reasonably so, that the organisms which disseminate from the urogenital tract to the joint to cause the inflammatory arthritis belong to the infecting genital serovar group. As part of a recent study intended to evaluate DNA sequence diversity in persistent chlamydiae within synovial tissues of patients with inflammatory arthritis, we amplified, cloned, and sequenced the *omp1* gene from synovial tissue samples of 38 patients whom we knew from earlier studies to be PCR-positive for chlamydial DNA. We then compared the *omp1* sequences from each patient sample to congruent sequences in the databases to determine which serovar(s) were present. Contrary to expectation, we identified no clones at all of genital serovar *C. trachomatis*. Rather, we found only ocular serovar group chlamydiae in synovial biopsies from these arthritis patients; by far the most common ocular serovar identified in the samples was C serovar [46]. The ocular character of the samples studied was consistent with the published chromosomal structure of ocular serovars not only in terms of the *omp1* DNA sequences, but also with regard to deletions at/around *toxB* and *trpA*. Interestingly, previous epidemiologic studies have indicated that ocular serovars are identified only rarely in genital samples [38,47,48]; in studies that did identify a nongenital serovar in one or more genital samples, Ba was the most frequently found, with rare or no identification of C, A, or B [48]. Whether, and if so how, ocular serovar chlamydiae are uniquely arthritogenic as opposed to those of genital serovars remains to be established. Regardless, one likely explanation for the apparently exclusive presence of

organisms of ocular serovars in the synovia of arthritis patients may be that these organisms disseminate from the site of primary infection more efficiently than do genital serovar chlamydiae. In addition, the observations suggest that many, perhaps most, genital infections with *C. trachomatis* are not clonally initiated; that is, the infecting genital inoculum is not comprised always of a single chlamydial serovar. An issue that these data probably inform directly relates to the epidemiology of *Chlamydia*-induced arthritis. As mentioned above, it has never been clear why only a small proportion of individuals who acquire a genital chlamydial infection develop the acute arthritis. The explanation may lie in the composition of the initial infecting inoculum – if that inoculum includes some ocular serovar chlamydiae along with the predominant genital serovar group organisms, the infected patient is at risk for arthritis or other sequelae. If this indeed is the case, then monitoring cervical, urethral, or blood samples by PCR or other means for the presence of ocular serovar group chlamydiae in patients newly diagnosed with genital *C. trachomatis* infection may prove useful diagnostically.

One treatment issue raised by the unexpected presence of ocular chlamydial strains in the joints of arthritis patients centers on the initial antibiotic/antimicrobial sensitivity of these versus genital strains of the organism. That is, in the initial phases of acute genital infection will intervention by an alert physician abrogate, or at least significantly attenuate, dissemination of the organism to the synovium, assuming that a proper antibiotic choice is made? To our knowledge, no studies currently exist regarding differing antibiotic/antimicrobial sensitivity in ocular versus genital serovars of *C. trachomatis*. Such studies should begin with *in vitro* infection systems and then proceed to animal models, and in our view the results of these investigations may well provide useful initial guidance as to how therapeutic design should progress.

More importantly, however, the exclusive presence of ocular group *C. trachomatis* in the synovial tissue of patients with inflammatory arthritis raises the critical issue of whether the detailed molecular genetic, metabolic, and other characteristics of ocular chlamydiae differ significantly from those of genital group organisms during active infection, persistent infection, or both. A survey of the literature suggests that while some study of persistent infection using ocular serovar organisms in standard culture systems has been published, much more attention has been paid to genital serovar chlamydiae in this context, including in studies from our group; we, like other investigators, always have assumed that genital organisms are responsible for disease induction. As mentioned above and developed more extensively below, both *C. trachomatis* and *C. pneumoniae* are refractory to antibiotic treatment when in the persistent infection state, but in the case of the former organism those studies have focused primarily on genital strains. Interestingly in this context, our sense from the literature is that more *in vitro* studies of antibiotic sensitivity/refractoriness have been done on *C. pneumoniae* than on *C. trachomatis*, probably because of the association of this respiratory pathogen with a panoply of common, severe, and currently idiopathic diseases, including atherosclerosis [49–52]. We contend that if effective antibiotic and/or other therapeutic regimens are to be designed to treat *Chlamydia*-induced arthritis effectively, more detailed basic science information must be obtained regarding the biology of chlamydial dissemination and pathogenesis in the context of genital versus ocular serovar infections.

Antibiotic treatment of patients with *Chlamydia*-induced arthritis

It has been known for many years that infection with a relatively diverse array of bacterial pathogens can elicit the subsequent development of an inflammatory arthritis. The organisms include a variety of enteric pathogens, including several species of *Salmonella, Shigella, Campylobacter*, *Yersinia*, and others, in addition to chlamydiae [8,14]. Remarkably, disease phenotype in patients is congruent whether the trigger is chlamydiae or an enteric pathogen [8,14,53]. However, important differences exist in details of the biology of the organisms and the pathophysiology they engender in the joint. For present purposes the most important difference between the arthritis elicited by chlamydiae and that elicited by gastrointestinal pathogens centers on viability and metabolic activity of the organisms. Our observations and those of others indicate that both *C. trachomatis* and *C. pneumoniae* elicit the inflammation characterizing the disease when they are in the persistent infection state; that is they are viable and metabolically active; however, unusual characteristics of both might be compared to those of active infection. By contrast, the vast majority of reports investigating postenteric arthritis indicate that the organisms involved are not viable in synovial materials, with the possible exception of *Yersinia* [6,14,54]. The

fact that chlamydiae exist in the persistent infection state in the joint clearly suggests that they might be susceptible to antimicrobial therapy, while postenteric arthritis is unlikely to be so.

The current standard of care for patients with chronic *Chlamydia*-induced arthritis is adapted primarily from treatment strategies used for other chronic inflammatory arthritides, such as rheumatoid arthritis. These strategies include use of NSAIDS for acute disease, and use of various DMARDs, including sulfasalazine and methotrexate for chronic disease. Interestingly, a surprising lack of data exists concerning the efficacy and safety of DMARDs in the treatment of chronic bacterially induced inflammatory arthritis. Indeed, previous studies have suggested only modest improvement with sulfasalazine [13,55], and to our knowledge no prospective data analyzing methotrexate have been published. Recently, several studies utilizing various biologic modifiers targeting TNF-α, and a few targeting IL-6, have been reported [56–59]. Results of these studies, in both patients with postchlamydial and postenteric arthritis, have been mixed. In our view, however, use of biologic modifiers that blunt a major arm of the Th1 immune response in patients with metabolically active *C. trachomatis* or *C. pneumoniae* in their joints should be undertaken with great caution, and perhaps not at all [60]. In any case, all these treatment strategies, along with local steroids, aim at attenuating the inflammation, not treating the infection. In the instance of postenteric arthritis this is probably a reasonable strategy, since the eliciting pathogens seem not to be viable in the joint. In the case of postchlamydial arthritis, use of anti-inflammatory compounds does not address the fundamental cause of the inflammation.

Over the past 20 years or more, several groups have reported studies of antibiotic therapy for postbacterial inflammatory arthritis. One early study utilized a 3-month course of lymecycline to determine its effects on the course of reactive arthritis [61]. The results indicated no long- or short-term improvement in patients with (presumed) postenteric arthritis, but a somewhat decreased disease duration in those with acute *Chlamydia*-induced disease; the overall judgment from the trial was that long-term lymecycline treatment did not alter the natural history or course of the disease. Further, follow-up on those patients who could be found some years after the end of the trial showed no, or extremely limited, improvement in those with chronic disease [62]. Another study asked whether long-term (4 month) treatment of patients with *Chlamydia*-induced arthritis was superior to short-term (10 days) treatment [63]. No advantage to the long-term treatment was identified. In still another trial, this one using long-term ciprofloxacin treatment in patients with bacterially induced inflammatory arthritis, reported a similar result [64]; this report did suggest, however, that such treatment might be of some value in those with *Chlamydia*-induced arthritis. We note with regard to the use of ciprofloxacin for treatment of chlamydial infections that an earlier report from our group demonstrated that giving actively-growing *C. trachomatis* in a standard *in vitro* culture system the MIC dose of this drug elicited persistent infection by the organism; persistence was reversed if the drug was removed from the culture medium [65]. In any case, these and other reports of antibiotic use for postchlamydial arthritis have not provided much encouragement in terms of efficacy.

Combination antibiotic therapy for *Chlamydia*-induced arthritis

As indicated above, the hallmark features of synovially based persistent chlamydiae include a transcriptionally aborted developmental cycle and differential upregulation of expression from the three chlamydial hsp-60 paralog genes. Because the developmental cycle of persistent chlamydiae is so severely attenuated, and because the metabolic rate in persistent organisms is extremely low, a prolonged antibiotic treatment course probably is necessary. Further, because persistent chlamydiae in the joint display a unique gene expression profile, a targeted approach also is warranted. In our view, the persistent synovial infection underlying *Chlamydia*-induced inflammatory arthritis will be most effectively treated with combination antimicrobials, as is the case with other persistent intracellular organisms including *Mycobacterium tuberculosis* and *Helicobacter pylori*. Further, the most successful approach to eradicating this persistent infection will employ a medication, such as rifampin, which is known to inhibit chlamydial gene transcription, in particular the hsp-60 paralogs [26,66]. Indeed, *in vitro* data suggest that successful synergistic eradication of cells infected with *Chlamydia* with rifampin and azithromycin can be achieved [67].

Our group completed an open-label trial in 2004, and data from that trial suggest that a 9-month course of doxycycline and rifampin is more efficacious at ameliorating the symptoms of suspected *Chlamydia*-induced ReA than is doxycycline monotherapy [68]. In this

trial, 30 participants with chronic inflammatory arthritis, and who fulfilled the European Spondyloarthropathy Study Group (ESSG) criteria without evidence of inflammatory bowel disease, psoriasis, ankylosing spondylitis, or preceding dysentery, were enrolled. The patients received doxycycline 100 mg by mouth twice daily or a combination of doxycycline 100 mg twice daily and rifampin 600 mg daily in an open label fashion for a total of 9 months. After 9 months of therapy, all six variables which were followed in the study improved more in the subjects receiving doxycycline and rifampin combination treatment compared with the doxycycline monotherapy group; improvement in four of these variables was statistically significant. Interestingly, there was no apparent improvement from months 6 to 9 in this trial. In spite of these positive results, this was an open label trial and thus did not provide definitive proof that chlamydiae were the trigger of the arthritis in these subjects.

More recently, we completed a double-blind placebo-controlled trial demonstrating that a 6-month course of combination antibiotics (either doxycycline and rifampin or azithromycin and rifampin) was superior to placebo not only at improving the clinical symptoms of chronic *Chlamydia*-induced arthritis, but also at clearing the synovial chlamydial infection underlying the disease [69]. This study protocol differed from previous trials assessing antibiotics as potential therapeutic agents for this arthritis in a number of ways. The first important difference was that study subjects could only be randomized to this combination antimicrobial strategy if they were PCR-positive for *C. trachomatis* or *C. pneumoniae* in synovial tissue or blood sample. All study subjects had to meet a modified ESSG at the screening visit, including minimum disease duration of 6 months (i.e., chronic patients). All study participants who met these screening criteria then had PCR analysis of their peripheral blood mononuclear cells, and some had PCR analysis of synovial tissue. 80 subjects were screened in all, and 42 were randomized to therapy. PCR analysis of synovial tissue in patients who meet the clinical criteria for *Chlamydia*-induced arthritis or undifferentiated spondyloarthritis currently represents the most accurate and specific means to identify chlamydiae as the trigger and etiology of their disease [70]. Thus, requiring study subjects to be PCR-positive for chlamydiae was the most definitive means to establish these organisms as etiologic agents, thereby ensuring that study participants did not have postenteric arthritis or another type of spondyloarthritis. Once the specific condition was established (i.e., *Chlamydia*-induced inflammatory arthritis) then study participants were randomized in a blinded fashion; both active treatment arms included rifampin as part of the combined antimicrobial strategy. All subjects were treated in a blinded fashion for 6 months and followed for 3 more months after cessation of therapy to ensure that symptoms did not 'rebound' after discontinuing treatment.

The results of this study clearly demonstrated that a 6-month course of combination antibiotics produced a significantly higher response rate in subjects with chronic *Chlamydia*-induced arthritis compared with placebo. Specifically, at month 6 (primary end point), 17/27 subjects (63%) randomized to combination antibiotics were responders compared with 3/15 (20%) on placebo (p-value = 0.01). Of the 27 subjects randomized to combination antibiotics, 11/27 (41%) were 50% responders, and 7/27 (26%) were 70% responders. Regarding the three responders in the placebo group, only 1/15 (7%) met the 70% response criteria. Finally, 22% (6/27) of the subjects randomized to combination antibiotics felt that their symptoms completely resolved, whereas no placebo-treated participant achieved remission. These clinical trial results are supported further by the observation that significantly more study subjects treated with combination antibiotics cleared their infection, as evidenced by PCR results on blood and synovial tissue samples after 6 months of therapy (p-value = 0.03). That is to say, PCR clearance of peripheral blood mononuclear cells and synovial tissue mirrored clinical response. Study participants randomized to combination antibiotics were not only more likely to clear their PCR infection, but responders also were more likely to achieve a negative PCR compared to nonresponders after 6 months of treatment. However, a number of questions remain. For example, it is not clear from these data which of the active treatment arms was more efficacious, since the study was not powered to answer that question [70–72]. Further, we do not know whether it is possible to achieve better clinical and PCR results with higher doses of antibiotics that block protein synthesis and chlamydial DNA/RNA metabolism. Other questions include: might 3 months of therapy be adequate to clear the etiologic synovial chlamydial infections? What effect will these or other combination antibiotic treatments have on normal bowel flora or other flora, in terms of potential downstream cytokine alterations? Could a change in background

cytokine levels effect chlamydial clearance from the joint or other organs? These and many other important questions must be answered in order to define the most efficacious treatment strategy with the least possible risk to the patient.

Options for more effective delivery of antibiotics to infecting chlamydiae

An important problem in treating chlamydial infections generally, especially those involving persistently infecting organisms such as *C. trachomatis*, centers on the concentrations of antibiotic or other antimicrobial compound which are achievable inside infected host eukaryotic cells. Azithromycin is a derivative of erythromycin produced by several detailed chemical modifications, including insertion of a methyl-substituted nitrogen at a specific position on the lactone ring, and others [73]. The advantage of these and other modifications to the parent molecule in terms of antimicrobial efficacy derives in part from an increased ability to enter the infected host cell; that is, the drug achieves a higher intracellular concentration than does erythromycin or many other antibiotics.

In spite of these improvements, achieving a significantly high intracellular concentration of azithromycin in chlamydiae-infected cells remains something of a challenge. That challenge is dictated by the biology of host and pathogen, in that antibiotic must pass not only the host cell membrane to accumulate a reasonable titer in the host cytoplasm, but it also must cross the inclusion membrane to have access to the organism, and cross the chlamydial (RB) membrane to perform its cidal function. A number of groups have experimented with nanotechnologic vehicles to deliver drugs to the interior of infected cells at high level, and some of these have been successful. For example, a recent study reported that nanoparticles comprised partly of poly(D,L-lactide-co-glycolide) (PLGA) showed promise for delivering antibiotics to pulmonary cells infected with *C. pneumoniae* [74]. Liposomes have also been studied for drug delivery in various contexts and with varying levels of success (see [75] for review).

Another option that is being explored for increasing efficacy in drug delivery centers on dendrimers. This is a relatively new class of nanoscale delivery vehicles which has the significant advantage of well-defined structure and controllable surface characteristics (see [76] for review), and studies from several groups have demonstrated that they can be effective delivery vehicles for small drug molecules [77,78], oligonucleotides [79], peptides, and other molecules of medical interest [80,81]. Indeed, our group has begun to explore delivery of azithromycin to *C. trachomatis*-infected cells, with some initial success [82]. Importantly, the linking chemistry between the dendrimer and the drug or other molecule can be used to tailor the release profile. In this context commonly used bonds between the drug and the dendrimer are ester and amide bonds, and more recently disulfide linkages have been used for conjugation of drug to dendrimer [83]. Also importantly, some studies have indicated that poly(amidoamine) (PAMAM) dendrimers home to cells in tissue regions undergoing an inflammatory response, even in the absence of specific targeting ligands [84]. This ability, of course, should prove valuable in designing and utilizing them as carriers for drugs for diseases associated with inflammation, such as chlamydial arthritis. It will be of considerable interest over the next several years to follow development of dendrimers and other nanoconstructs as delivery vehicles to increase the efficacy of antibiotic treatment for this disease, as well as for other diseases associated with chlamydial infection or infections by other intracellular pathogens.

Summary & conclusion

It is reasonably clear at this point in the development of treatments for *Chlamydia*-induced arthritis that a cure for the disease, in either its acute or chronic form due either to *C. trachomatis* or *C. pneumoniae*, is not immediately at hand. However, to answer the question posed in the title of this article, we contend that the results of several recent and current studies bode extremely well for such a development in the foreseeable future. First and foremost among these is the observation that combined antibiotic therapy specifically targeting two different and critical aspects of chlamydial metabolism (transcription and translation) can eliminate chlamydiae in synovial tissue [68,69]. The initial clinical studies were relatively small in scope, though, and they must be extended to larger patient cohorts and to other antibiotic combinations. We suggest as well that repeat clinical trials for this approach to therapy also include use of dendrimers or other nanodevices for targeted delivery of the therapeutic molecules at increased efficiency.

A final comment regarding future research and the prospects for cure of *Chlamydia*-induced arthritis must center on our current insufficient understanding of chlamydial biology, especially in the sense of host–pathogen interaction at the

genetic level. One of the major messages of this article is that the relative lack of success over the years in developing a cure for this arthritis has been due to a general lack of understanding of chlamydial metabolic and other processes, especially as they relate to virulence within the infected host cell and tissue. To a large extent, that insufficient knowledge is a function of our somewhat limited experimental ability to define the functions of many chlamydial gene products, and to elucidate in a meaningful manner the detailed interaction between host cell and bacterium that ends in persistent synovial infection. Currently, no reliable, easily usable system for genetic manipulation of any chlamydial species is available (but see [85]); development of such a system should be given high priority since its use will unquestionably identify new therapeutic targets. Further, genome sequencing of multiple clinical isolates of *C. trachomatis* and *C. pneumoniae*, and indeed other chlamydial species as well, should provide important information concerning tissue tropism, relative levels of virulence, and many other aspects of pathogenesis that will influence the design and use of therapies currently under development.

Future perspective

As developed in this article, it is clear at this point that a straightforward and routinely effective treatment to cure *Chlamydia*-induced arthritis is not immediately at hand. However, recent reports provide strong encouragement that combination antibiotic therapy for either acute, or more importantly chronic, arthritis subsequent to chlamydial infection might be the basis for such a cure. It is our firm belief that, given ever increasing understanding of the basic biology of chlamydiae, including the molecular genetic and other details how this organism interacts with its host, the next decade will see the development of one or more such curative therapies based on that basic information. Importantly, it is also our view that simple oral treatment

Executive summary

Chlamydial biology circumscribes treatment options

- We relate treatment options and failures to what is known regarding the basic biology of *Chlamydia trachomatis*.
- The important issue underlying treatment failures with antibiotics has to do with the molecular genetics of persistent chlamydial infection of the synovium.
- A basic understanding has begun to emerge concerning how the organism in its persistent infection state in the joint elicits inflammation; however, future research must focus on elucidating the function(s) of gene products of currently unknown function(s) encoded on the chlamydial chromosome.
- These gene products unquestionably function in virulence and pathogenesis, and an understanding of their detailed role in those processes should provide new avenues to development of therapies to treat/cure the inflammatory arthritis.
- Molecules of some interest include but are not limited to the heat shock proteins of *C. trachomatis* and *C. pneumoniae*.

Issues of strain & dissemination affect treatment options

- Recent studies have indicated that the genital strains of *C. trachomatis* long thought to be the etiologic agents for *Chlamydia*-induced arthritis are in fact rarely or never present in synovial tissues of relevant patients; rather, ocular (trachoma) strains of the organism elicit disease.
- A major issue limiting development of new treatments centers on our lack of understanding concerning how the pathobiology of ocular strains differs from that of genital strains.
- A related issue is to determine whether the joint inflammation elicited by ocular chlamydial strains is a function of some difference in dissemination of these two groups of organisms from the urogenial system to the joint.

Antibiotic treatment of patients with* Chlamydia*-induced arthritis

- Many studies have demonstrated that standard antibiotic treatment of patients with *Chlamydia*-induced arthritis has limited and probably essentially no long-term value.
- The lack of efficacy clearly derives from the basic biology of chlamydial persistent infection, and a major direction for future research must be to elucidate the reasons for chlamydial refractoriness to antibiotic treatment during persistence in the joint and elsewhere.

Combination antibiotic therapy for* Chlamydia*-induced arthritis

- Recent studies from this group provide strong evidence that treatment of *Chlamydia*-induced arthritis in its chronic form using a combination antibiotic approach will be successful ultimately.
- However, those studies were relatively small and thus larger clinical trials must be designed and executed.
- Similarly, more combinations of antibiotics given variously over varying periods must be assessed for efficacy in such trials.

Options for more effective delivery of antibiotics to infecting chlamydiae

- A major limitation of any antibiotic or related treatment aimed at eliminating persistent chlamydial infections of the joint or elsewhere centers on the fact that it is difficult to achieve high and long-lasting concentrations of these molecules within infected cells.
- Studies from several groups using nanocarrier devices show promise in improving the ability to deliver high concentrations of cidal molecules to *Chlamydia*-infected cells in the joint and elsewhere, and development of this technology must form an important basis in future developments related to treatment of patients with the postvenereal arthritis.

with combination therapy will be replaced by, and significantly improved by, the use of medical nanodevices to deliver those or other antimicrobials to *Chlamydia*-infected synovial tissues, thereby improving efficacy of treatment.

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