OPINION

Chlamydia-induced ReA: immune imbalances and persistent pathogens

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Abstract | Reactive arthritis (ReA), an inflammatory arthritic condition that is commonly associated with *Chlamydia* infections, represents a significant health burden, yet is poorly understood. The enigma of this disease is reflected in its problematic name and in its ill-defined pathogenesis. The existence of persistent pathogens in the arthritic joint is acknowledged, but their relevance remains elusive. Progress is being made in understanding the underlying mechanisms of ReA, whereby an imbalance between type 1 and type 2 immune responses seems to be critical in determining susceptibility to disease. Such an imbalance occurs prior to the initiation of an adaptive immune response, suggesting that innate cellular and molecular mechanisms in ReA should be prioritized as fruitful areas for investigation.

Introduction

Reactive arthritis (ReA), previously known as Reiter's syndrome, is an inflammatory arthritis preceded by infection with enteric pathogens, such as *Salmonella* spp., *Yersinia* spp., *Shigella* spp. and *Campylobacter* spp., or the oculogenital pathogens, *Chlamydia* spp. By virtue of HLA-B27 association, the pattern of joint involvement, and the absence of rheumatoid factor, ReA is classified as part of the spectrum of spondyloarthritis (SpA). However, there are clear distinctions between ReA and classic SpA diseases, such as ankylosing spondylitis: notably, a weaker association with HLA-B27[1,2] and an established link between a previous infection with the aforementioned bacteria and the onset of arthritis. Moreover, it is becoming clear that post-enteric and post-chlamydial ReA are distinct entities, in that viable organisms can be detected in the joints of patients with *Chlamydia*-induced ReA (CiReA) but not in those with post-enteric ReA.[3]

Chlamydia trachomatis represents the most common single cause of ReA, whilst *Chlamydophila pneumoniae* has been implicated in ReA, albeit far less frequently than *C. trachomatis*.[4] As little study has been done in the field of *C. pneumoniae*-associated ReA, this article will focus on *C. trachomatis*-associated arthritis. Approximately 4–15% of those with genital *C. trachomatis* infections subsequently develop arthritis.[2] Given the high prevalence of chlamydial genital tract infections, it has been proposed that the incidence of CiReA might rival that of rheumatoid arthritis (RA).[3] Furthermore, *C. pneumoniae* and/ or *C. trachomatis* are detectable in 62% of patients with undifferentiated SpA, suggesting that ReA is hugely underdiagnosed.[5] Thus, CiReA represents a significantly understudied disease with a measurable physical and economic burden.[6]

In this article, we outline recent advances in our understanding of CiReA, with respect to both *Chlamydia* and host biology, and a focus on factors that might mediate CiReA susceptibility. We also introduce the field of macrophage polarization, an alteration of which is associated with susceptibility to both rheumatic and infectious diseases. The role of chlamydial persistence in the arthritic joint is also discussed, as is the indeterminate area occupied by CiReA between SpA and septic arthritis.

ReA: noncanonical septic arthritis

The term 'reactive' in ReA describes how this disease was traditionally viewed as a nonseptic, autoimmune reaction occurring in the joint in response to an extra-articular bacterial infection.[7] This notion was based on the fact that no pathogen could be cultured from the arthritic joint, despite detection of arthritis-associated bacteria at other sites in the body. Surprisingly, extensive searching for the bacterial antigens responsible for adaptive-immunity-mediated pathology has not yielded conclusive results to date. To further confound the auto-immunity-mediated hypotheses for ReA, it was discovered through the use of nucleic acid detection and electron microscopy that *Chlamydia* is present in the joint, albeit in an aberrant but viable state.[2]

The possibility of CiReA being driven not by a reactive autoimmune process, but rather by the infection of nonimmune cells *in situ*, has paralleled a paradigm shift in establishing the mechanism of tissue damage during chlamydial infections in general. Initially, the immunological paradigm proposed that tissue damage results from the aberrant activation of the adaptive immune system through delayed-type hypersensitivity or autoimmunity.[8] After a decade of research failed to prove this theory, an alternative cellular paradigm was proposed, postulating that chlamydial pathogenesis is driven by an inflammatory response propagated by sustained infection of nonimmune cells, whereby ongoing release of inflammatory mediators and subsequent recruitment of inflammatory cells causes tissue damage.[9] As *C. trachomatis* is known to infect both macrophage-like and fibroblast-like synoviocytes,[10] could the cellular paradigm hold true in CiReA?

With the recognition of viable *Chlamydia* in the joint, a contradiction arises regarding the classification and nomenclature of CiReA relative to underlying clinical and pathological features. This concept is illustrated in Figure 1, with ReA occupying a midpoint between SpA and septic arthritis. To support the concept of CiReA as a septic arthritis, a clinical trial in 2010 demonstrated that combination antibiotics could alter the course of CiReA.[11] In addition, the immunopathogenesis of CiReA recapitulates that seen in experimental septic arthritis with regard to mediators of susceptibility and the role of the immune system.[12,13]
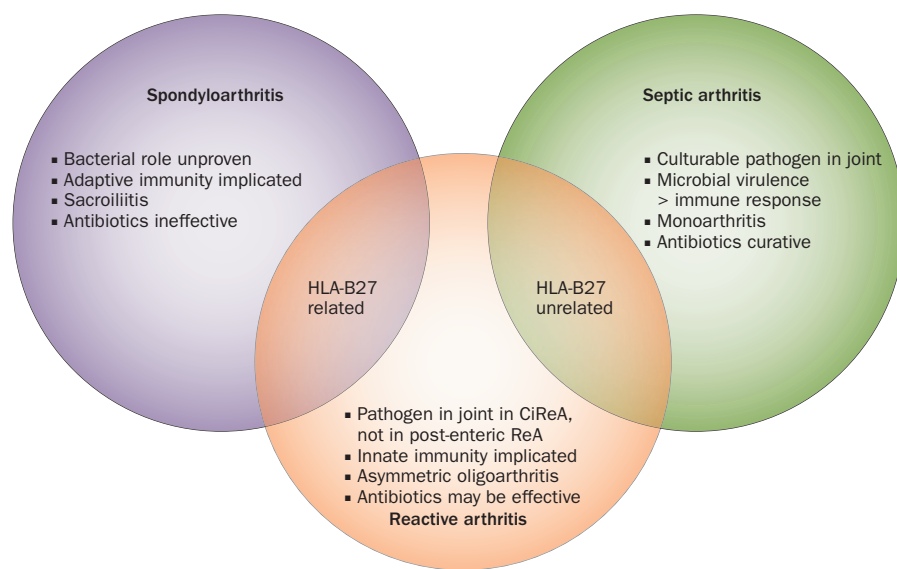
Figure 1 | The relationship between spondyloarthritis, reactive arthritis, and septic arthritis. Reactive arthritis shares clinical and immunopathogenic features with both spondyloarthritis, of which it is considered a subset, and septic arthritis. Enteric-pathogen-associated reactive arthritis better represents spondyloarthritis, whereas *Chlamydia*-associated reactive arthritis represents a noncanonical septic arthritis. Abbreviations: CiReA, *Chlamydia*-induced reactive arthritis; ReA, reactive arthritis.

The classification of CiReA as a septic arthritis necessitates a better understanding of bacterial colonization of the joint. After the primary infection, in both humans and animals, *Chlamydia* is known to spread throughout the body via monocytic cells[14] to a diverse range of tissues, including the spleen, liver, peritoneum and lungs.[15] *Chlamydia* is often detected long after the initial infection in selected tissues, such as the genital tract and synovium. In respect of the ability of *Chlamydia* to evade clearance in the synovium, the traditional hypothesis has been that the joint is a site of immune privilege; however, immune cells can be found in the joint in both healthy and arthritic states. One extension of this theory proposes that it is the hypoxic environment of the inflamed joint[16] that creates an immunoprivileged microenvironment. This idea stems from the fact that the natural site of infection for *Chlamydia*—the genital tract—is relatively hypoxic, and that *Chlamydia* thrives under hypoxic conditions.[17] *Chlamydia* might have adapted to growth under such conditions, given the organism's active manipulation of hypoxia inducible factor 1 (HIF-1).[18] Additionally, bacteriocidal cytokines, such as interferon γ (IFN-γ),[17] and antibiotics[19] have decreased efficacy under hypoxic conditions, providing the rationale for chlamydial escape from these control mechanisms within the joint.

Chlamydial persistence in joints

Another incompletely explained aspect of the host–pathogen interaction during CiReA is the observation that *Chlamydia* adopts an atypical, persistent state within the joint. *Chlamydia* spp. are obligate intracellular pathogens that exist in two distinct states during their lifecycle: the extracellular, infectious elementary body, and the intracellular, replicative reticulate body. When appropriate stress is applied during the intracellular stage, such as IFN-γ exposure, antibiotic treatment or infection of monocytic cells, *Chlamydia* enters a nonreplicative and unculturable, yet viable, persistent state.[20] The persistent state differs from the normal intracellular state in that the reticulate bodies fail to divide or differentiate into infectious particles, have a reduced metabolism, and are immuno-evasive.[21]

Persistent *Chlamydia* were originally identified in CiReA through their detection using microscopy or PCR coupled with an inability to be cultured from synovial biopsy.[10] Accurate identification of *Chlamydia* cells with a persistent phenotype in CiReA is now possible using real-time PCR, as they display a unique gene expression profile.[22] The recognition of persistent *Chlamydia* in ReA represents an important advance in our understanding of this disease, but leaves key unanswered questions with respect to its pathogenesis.

Is persistent *Chlamydia* the cause of ReA, or the effect of host–pathogen adaptation? A mechanistic hypothesis to address *Chlamydia* as the instigator of ReA postulates that chlamydial persistence provides a continuous source of bacterial components that stimulate the immune system, resulting in chronic inflammation and tissue damage.[4] These components could be pathogen-associated molecular patterns (PAMPs) that stimulate host innate receptors, or microbial antigens that elicit adaptive immune responses. On the other hand, it is equally plausible that persistence represents the host's best attempt at controlling *Chlamydia*, which itself has developed specialized mechanisms to avoid the immune response, particularly the establishment of the immuno-evasive persistent state. In support of this theory, chronic chlamydial infection elicits little immune stimulation relative to that seen during acute infection.[23] This latter theory also provides the rationale for the arthritic flares that are seen commonly in ReA, in which chlamydial escape from persistence could result in an acute inflammatory event. The basic understanding of chlamydial persistence is in its early stages, and an emphasis must be placed on understanding the role of this phenomenon in the pathogenesis of CiReA. Thus, clinical ReA studies need to address the chlamydial state concurrently with joint inflammation, and animal models are needed to assess the temporal relationship between persistence and arthritis.

Mediators of CiReA susceptibility

Up to 80% of chlamydial infections are asymptomatic.[24] This statistic poses considerable hurdles in attempting to link clinical evidence of infection with subsequent ReA, but clearly, for reasons not yet defined, only a small proportion of infected individuals go on to develop ReA.

Factors that contribute to the increased virulence of an infection include pathogen, host and environmental variation. The most commonly observed variability of *Chlamydia* relates to the outer membrane proteins, which dictate its biovar. This variation is associated with tissue tropism rather than virulence; however, a recent study did detect ocular biovars more commonly than genital biovars in ReA.[25] A limited number of studies have addressed non-biovar-related variance as a determinant of pathogenicity,[26] but the effect of this variance on human infection or CiReA remains to be determined. From a broader
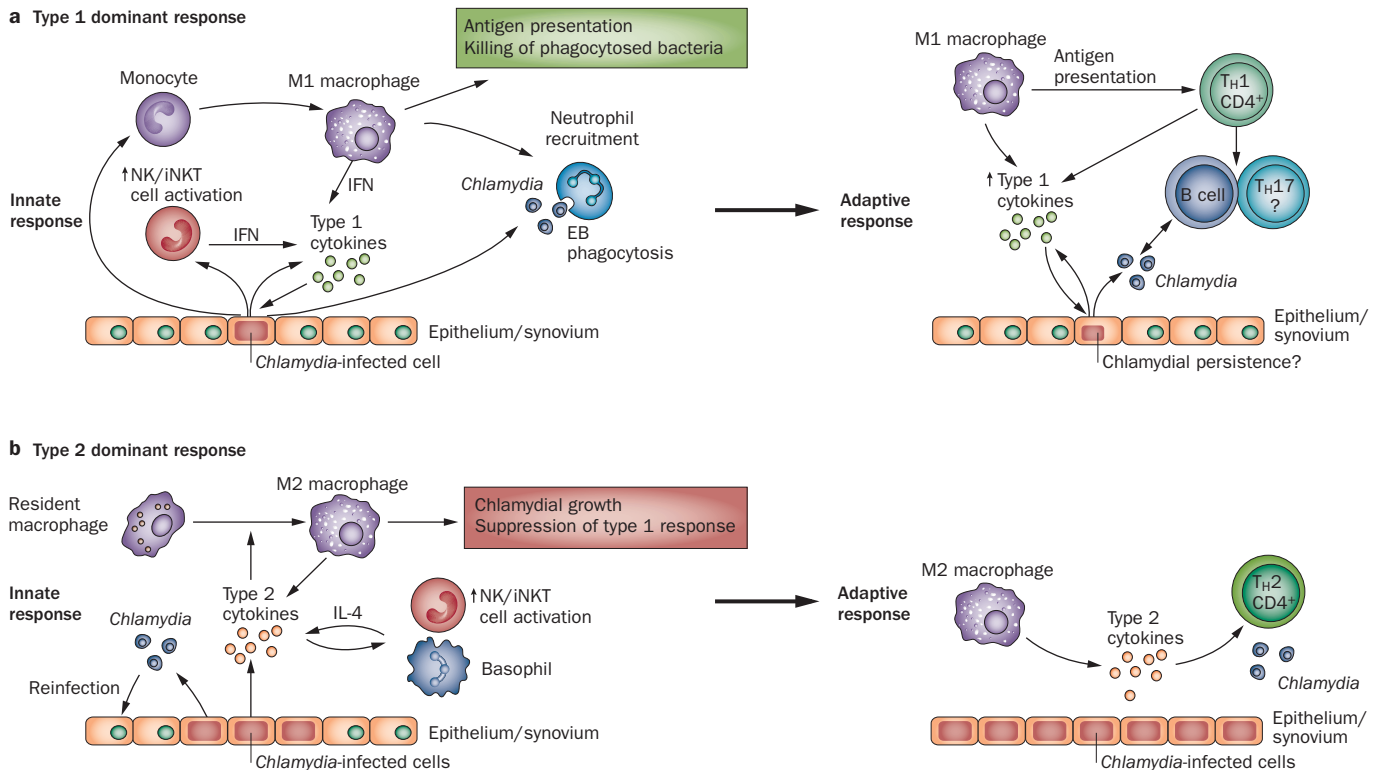
Figure 2 | Outcomes of a type 1 dominant versus type 2 dominant inflammatory response during chlamydial infection. These figures highlight the potential role of the macrophage, whereby a type 1 response may result in early activation of M1 macrophages and a type 2 response may reinforce M2 polarization. **a** | A type 1 skew is required for effective control of *Chlamydia* through early suppression of chlamydial growth and removal of extracellular chlamydial EBs during the innate phase. Subsequent effective control, which may involve the induction of persistence, requires adaptive immunity. **b** | A type 2 skew results in excessive chlamydial growth through an ineffective innate response and subsequently ineffective adaptive immune response. Abbreviations: EB, elementary body; IFN, interferon; NK cell, natural killer cell; iNKT cell, invariant natural killer T cell; T_H1, type 1 T helper (cell); T_H2, type 2 T helper (cell); T_H17, type 17 T helper (cell).

perspective, a stable host–pathogen interaction is indicated by the consistent rates of *Chlamydia*-related disease[27] in comparison to the fluctuating temporal incidence of highly mutable pathogens, such as influenza. This stability suggests that alterations in chlamydial pathogenicity are uncommon—an observation that is typical of the relationship seen during prolonged co-evolution of host and microbe, in which an increase in virulence would be disadvantageous to the long-term survival of an obligate intracellular pathogen.

Environmental factors are also known to affect chlamydial infections. Repeated chlamydial infections have been associated with increased genital tract pathology,[28] but whether repeated infections are required for the development of CiReA remains unknown. This issue is rarely addressed in practice, as diagnostic tests for *Chlamydia* do not distinguish between repeat infections or chronic infections with the same pathogen.[29] Another potential environmental factor is heavy-metal exposure, which has been shown in animal studies to modify the cytokine balance.[30] Experimentally, we have

shown that heavy metals sensitize animals that are otherwise resistant to CiRea;[31] however, clinical studies are required to validate this effect in humans. Heavy-metal-induced sensitization correlates with the suppression of the inflammatory cytokines IFN-γ and TNF, which raises the question of whether an immune imbalance acts as a mediator of CiReA susceptibility.

Most studies examining mediators of susceptibility to chlamydial sequelae focus on host genetic variability, in particular those determining the immune response. A robust type 1 (classical) inflammatory response is crucial for the control of *Chlamydia* infection, whereas an enhanced type 2 (alternative) inflammatory response is associated with susceptibility to infection (Figure 2). This distinction has been demonstrated in animal models, whereby a deficiency of type 1 cytokines, such as IL-12 and IFN-γ, influences susceptibility to chlamydial infection, whereas a deficiency of type-2-associated cytokines, such as IL-10, mediates resistance.[32] Furthermore, we have shown that high synovial levels of IFN-γ and TNF correlate with resistance to

experimental CiReA.[33] The protective effect of a type 1 inflammatory response during chlamydial infection in animals has been recapitulated in human studies, in that the sequelae are associated with stronger type 2 cytokine responses.[34] Additionally, an attenuated type 1 response is seen in patients with SpA,[35] manifested as an impaired IFN-γ-induced gene expression profile.[36] Although few such studies have been performed in CiReA, these trends seem to be consistent in this disease: those with prolonged ReA express lower levels of IFN-γ than those who rapidly overcome ReA.[37] Another study demonstrated the IL-10–IL-12 balance to be crucial in preventing ReA.[38]

The importance of innate immunity

If susceptibility to CiReA is determined by a disruption in the yin–yang balance of type 1 and type 2 inflammatory responses, at what point during an inflammatory response does such an imbalance have an effect? The immune system comprises two distinct arms: the germline-encoded, rapidly responding innate immune response; and the delayed, but highly specific, adaptive

immune response. Both systems can be polarized during a type 1 or type 2 dominant response. CD4+ T cells can be polarized into the well-defined T-helper type 1 (T_H1) and type 2 (T_H2) phenotypes, which have traditionally defined the type 1 and type 2 responses. T_H1 cells are crucial for eliminating chlamydial infections;[39] however, an effective immune response to *C. trachomatis*, and resistance to CiReA, is associated with a robust type 1 cytokine response as early as 3 days after infection.[33,40] These temporal kinetics exclude the adaptive immune system as the defining process in early resistance versus susceptibility to *Chlamydia*. But which cells of the innate immune response might cause a disrupted inflammatory balance, and how might such cells be subsequently affected?

Research into the innate immune response during *Chlamydia* infection has been somewhat neglected, as the primary focus has been on vaccine development. Nevertheless, many innate immune cells have been shown to have important roles. Natural killer cells have been implicated in providing an early protective effect through IFN-γ release,[41] an effect that is also mediated by natural killer T cells during CiReA.[42] Neutrophils, on the other hand, seem to have a dual role, in that they reduce early excessive chlamydial growth,[43] yet excessive activation can itself contribute to tissue damage, including CiReA.[44] Another innate cell type shown to be important in chlamydial infection is the macrophage; macrophage depletion greatly enhances infection progression.[45]

Macrophages are a heterogeneous group of related cells ranging from aggressive, proinflammatory macrophages to passive, tissue-resident macrophages. Tissue-resident macrophages comprise up to 15% of all cells in healthy tissue, and up to 20% of synoviocytes.[46] Thus, the primary role of the macrophage should be viewed as that of a homeostatic local resident cell rather than an aggressive innate immune effector. Tissue-resident macrophages tend to be anti-inflammatory in nature, and have recently been shown to maintain their numbers by local proliferation.[47] Inflammatory macrophage populations, on the other hand, rarely exist in healthy tissue, and appear on the scene through recruitment and activation of blood monocytes.[47]

A classification system has been devised to delineate polarized macrophages on the basis of phenotype and function, encompassing a spectrum ranging from the classically activated or inflammatory M1 macrophage

to the homeostatic or alternatively activated M2 macrophage.[48] This classification system is based on analogy with the T_H1/T_H2 paradigm, whereby polarization to M1 is induced by a type 1 inflammatory environment, such as the presence of IFN-γ, and M2 is induced by an environment containing IL-10 and IL-4. Furthermore, the existence of FOXP3+ regulatory macrophages has recently been demonstrated, akin to the identification of FOXP3+ regulatory T cells.[49] A key distinction exists between macrophage and T_H cell polarization, in that macrophage polarization is highly plastic relative to the somewhat rigid differentiation of T cells.[50] This plasticity assists host defense, considering the role of the innate immune system, which is to rapidly respond to alterations in the immediate environment.

In many biological systems, a dynamic balance exists between opposing mechanisms, as is the case with macrophage polarization. Alterations to this balance, either endogenous or exogenous, are associated with certain pathological processes, but it can often be difficult to ascertain whether the polarized state is the cause or the effect. Differential macrophage polarization can be demonstrated in rheumatic diseases; for example, RA is associated with an M1 dominance, and SpA shows an M2 dominance.[35,51] Furthermore, a robust M1 response is essential for the control of intracellular pathogens, and it is recognized that a deficient M1 response facilitates uncontrolled acute intracellular bacterial infections.[52] Certain intracellular bacteria, such as *Francisella tularensis*, have been shown to induce M2 polarization in macrophages, resulting in prolonged bacterial survival.[53] *Chlamydia* is known to infect macrophages, but how *Chlamydia* can survive in differentially polarized macrophages or whether *Chlamydia* is capable of such immune subversion remains unknown.

A role for macrophages in CiReA?

Given the importance of the macrophage in chlamydial infections, and the association between susceptibility and a deficient early type 1 response, it is surprising that little is known about macrophage polarization in the setting of chlamydial infections, especially in the microenvironment of the joint during CiReA. However, there are indicators that M1 macrophages control *Chlamydia* infection,[54] and that M2 macrophages are permissive to chlamydial growth (Figure 2).[55] However, control of *Chlamydia* has traditionally been measured as a

reduction in culturable organisms, which is also the hallmark of persistence.

An important goal is to validate the observation of M2 macrophage dominance in SpA and to determine whether this alteration in macrophage phenotype is also present in CiReA. It is conceivable that individuals with an M2 dominance would be less able to control chlamydial infections, and thus be more susceptible to *Chlamydia*-associated inflammation of the joints. Additionally, it is plausible that joints are particularly sensitive to M2 polarization, given that IFN-γ has reduced activity under hypoxic conditions.

Conclusions

Current evidence suggests that CiReA is a noncanonical septic arthritis with viable, but nonculturable, persistent *Chlamydia* infection. Thus, our research in this disease should be tailored accordingly toward a better understanding of the articular immune response. As susceptibility to CiReA seems to be established during the innate immune phase, early cellular and molecular immune mediators should be examined closely. In addition, the ability of ocular chlamydial biovars to cause arthritis needs to be studied in more detail. Perturbations in the type 1–type 2 immune balance, which can be caused by both environmental and genetic factors, are likely to affect innate immunity. As macrophages have gained prominence as key gatekeepers of innate immunity, it might be that this role has also conferred on the macrophages a critical role in defining susceptibility to CiReA.

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